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Quantifying genetic distance between wild and captive strains of the grey partridge *Perdix perdix* in France: conservation implications

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Abstract

The grey partridge *Perdix perdix* is an important gamebird in Europe. Its numbers have decreased dramatically during the XXth century and releases are commonly undertaken for the conservation of the populations and/or hunting purposes in Western Europe. However, this practice that generally involves birds from commercial farms raises several concerns, among which a potential hybridization between farmed and wild individuals. Herein, based on microsatellite markers, we characterize the genetic patterns of farmed birds in view of wild birds of the two French subspecies (*P. p. armoricana* in central-northern France and *P. p. hispaniensis* in the Pyrenees). Hence, we estimate the risk of genetic introgression between wild and farmed birds. Our results highlight a genetic divergence between both subspecies—in accordance with the known evolutionary history of the grey partridge during the Quaternary. In central-northern France, a slight but significant difference in the genetic signature between wild and farmed partridges is detected. This difference however does not seem prone to alter the gene pool of wild birds if farmed birds are released in the wild and reproduce. On the contrary, in the Pyrenees, the large and significant genetic difference between wild and farmed birds represents a real risk of genetic introgression. This threat should be taken into account in population management.

Keywords

Perdix perdix · Conservation · Hybridization · Genetic introgression ·

Introduction

To face biodiversity erosion, release programs are commonly employed to reinforce the effective size and consequently the resilience of collapsing populations of threatened species (Armstrong et al. 2015; IUCN/SSC 2013). In such programs genetic considerations play a crucial role (Frankham et al. 2010). Indeed, genetic diversity represents the raw material for adaptive evolutionary changes, notably because its loss can lead to reduction in reproductive fitness, as it can be observed in populations suffering from inbreeding depression (Frankham et al. 2010). Even if inbreeding effects on wild populations are complex, there are now ample evidences that inbreeding depression can reduce population's viability via a decrease in survival and/or reproduction abilities of individuals (Frankham et al. 2010). Thus, releases of individuals carrying potentially new alleles are expected to increase population fitness by restoring adaptive variations and removing possible inbreeding effects. However, the use of individuals from non-local stocks might also cause losses of local genetic adaptations (i.e. genetic swamping), resulting from an outbreeding depression. In such case, hybrid descendants have a lower fitness than their parents (Randi 2007; Sutherland et al. 2006). This outbreeding depression can appear when genetic differences between the source and target populations are too important. Such differences can result from the combined effect of a genetic drift and the disappearance of specific adaptations that have occurred during the evolutionary process (Randi 2007). Insofar as possible, a compromise should be found between the maintenance of the genetic diversity, on the one hand, and the risk of outbreeding depression, on the other hand, in order to the genetic criteria may be prone to play a crucial role for the success of release programs. These considerations are now part of the risk assessment that should be considered before a release program is implemented (i.e. "gene escape", (IUCN/SSC 2013). Thus, the challenge lies in finding a "source population" sufficiently different to rehabilitate the genetic diversity of the target population, without being inconsistent and causing an outbreeding depression into the hybrid offspring. This compromise may be a special challenge when released individuals come from conservative breeding program centers or commercial farms. Indeed, captive stocks are generally founded with few individuals whose genetic pattern is not well characterised. In addition, whatever the origin of founders, inbreeding and genetic drift increase with successive generations and are likely to modify the genetic signature of captive stocks.

Such genetic effects have already been highlighted as potential concerns for the grey partridge (Blanc et al. 1986; Effenberger and Suchentrunk 1999). France supports almost 50% of the European stock of this species (BirdLife_International 2015). It mainly occurs in central northern plains (Bro and Crosnier 2012) and in the Pyrenees above 1000 m above sea level (OGM

2011), respectively, as two biogeographical and genetic subspecies (*Perdix p. armoricana* and *P. p. hispaniensis*, Martin et al. 2003). Both subspecies experienced an important decline since World War II, that still goes on nowadays (BirdLife_International 2015; EBCC 2015; Reitz and Mayot 2017). This ongoing trend is both observed in intensively cultivated lowlands as well as in Pyrenean mountain tops due to an array of environmental causes among which the global climatic and habitat changes (Bro and Reitz 2014; Godin and Bro 2017; Novoa 1998; Reitz and Mayot 2017). As a consequence, both subspecies have been red listed as “*Near threatened*” in some French regions (CSRPN 2013; UICN-France et al. 2016). In turn, releases are commonly performed for conservation or hunting purposes, using birds from conservative breeding programs or commercial farms (Bro et al. 2016, 2017; SNPGC—Syndicat National des Producteurs de Gibier de Chasse 2019). This practice raises several concerns, among which a potential genetic introgression resulting from hybridizations between farmed and wild individuals, as already highlighted in the subspecies *P. p. hispaniensis* (Bech et al. 2012; Martin et al. 2003) and in *P. p. lucida* (Liukkonen 2006). However, this conservation concern may also be raised with the European western subspecies, the so-called *P. p. armoricana* since farmed birds may evolve in strains/lineages presenting some specific genetic profiles due to selection and some degree of drift/inbreeding (Blanc et al. 1986; Liukkonen 2006).

In this context, we examine herein the genetic structure of farmed grey partridges from a diversity of conservative breeding program centers and commercial farms and compare it to several French wild populations.

Materials and methods

Sample

We collected 753 wings of harvested wild birds from both subspecies of grey partridge: *Perdix perdix hispaniensis* occurring in the Pyrenees (n = 604, from 1986 to 2008) and *P. p. armoricana* occurring in the Central northern France (n = 149, from 2007 to 2014). We geolocated each individual according to the coordinates of the municipality where the bird was hunted (longitude and latitude in the World Geodetic System 1984 (WGS 84)). Based on geographical distances, individuals from the Pyrenees were gathered into 17 groups for analyses conducted at the scale of the population, and those from Central northern France into 5 groups (Table 1; Fig. 1).

We also collected samples from 230 harvested farmed individuals released in the wild (ringed birds): 41 in the Pyrenees from three different farms, 189 in central northern France from nine different farms. Moreover, we also analysed individuals from two conservative breeding programs, one: in the

Pyrenees [i.e. “Pallars Sobirà”, 57 individuals (F1/F2)] and one in central northern France [11 individuals (F1)].

Hence, our total of 1051 individuals were gathered into 36 groups representing either a wild population, a farm or a conservative breeding program center (hereafter “groups”). This large and diversified sample allowed us to robustly picture the genetic signature of both wild and farmed individuals.

Molecular markers

We extracted DNA from all samples using *silica* columns (e.Z.N.A from OMEGA BIOTEK), according to the manufacturer protocol. Because mitochondrial markers have only a maternal inheritance and thus the signal of the genetic introgression is lost when wild females mate, we genotyped all samples using 12 nuclear molecular markers: microsatellites: MNT412, MNT12, MNT477, MNT404, MNT467, MNT408 (Bech et al. 2010), Aru1G4, Aru1F25 (Ferrero et al. 2007), ADL142, LEI31, LEI319 (Mautner 2001) and BG15 (Piertney and Höglund 2001). We amplified the relevant DNA fragments using the Polymerase Chain Reaction (PCR) and the QIAGEN multiplex kit. The PCR amplifications were carried out according to the manufacturer standard microsatellite amplification protocol in a final volume of 10 μ L and at 57 °C for annealing temperature. Moreover, the PCR amplifications of loci MNT412, MNT12 and BG15 were grouped in the multiplex 1; loci MNT477, MNT404, ADL142, LEI31 and LEI319 were grouped in the multiplex 2; loci Aru1G4, Aru1F25 and MNT408 were grouped in the multiplex 3 and finally, the last microsatellite marker MNT467 was amplified alone. We added 1 μ L of PCR product to 9 μ L formamide and 0.5 μ L 600 LIZ standard (Life Technologies) and visualized it by electrophoresis on an ABI PRISM 3130 Genetic analyser. We determined amplification product size using Genemapper software (Applied Biosystems), followed by visual verification.

We used the software Micro-Checker (Oosterhout et al. 2004) to identify the presence of null alleles or scoring errors due to stuttering. The level of significance was adjusted using a Bonferroni correction as implemented in the software.

Departures from Hardy–Weinberg expectations as well as linkage disequilibria were assessed using exact tests (1200 permutations) implemented in Fstat v.2.9.3.2. (Goudet 2001). The level of significance for multiple testing was adjusted using the Chandler correction because Bonferroni’s procedure is considered too conservative when strictly applied. We used an error rate of 10% as suggested by Chandler (1995).

Analysis of the global genetic structure

Genetic diversity

We estimated the polymorphism of each of the 36 groups of birds. For this purpose, we computed over all loci the allelic richness (AR), the expected heterozygosity (He) (Weir and Cockerham 1984) and the Fis using FSTAT v.2.9.3.2. We performed Anova tests for AR and He between each pairwise groups to detect potential differences in genetic variations.

Genetic structure

We examined the global genetic structure of the 1051 individuals using the Bayesian approach implemented in Structure v.2.0 (Pritchard et al. 2000). We chose the admixture model and the option of correlated allele frequencies among groups. This software assigns each individual to a given genetic cluster, the number of genetic clusters varied from 1 to 5. Each simulation was replicated 20 times as recommended (Evanno et al. 2005), with 10 000 burn-in iterations followed by 500 000 sample iterations.

The likely number of independent genetic clusters (K) was obtained using Structure Harvester software version 0.6.1 (Earl and vonHoldt 2012). It compares the likelihood of the dataset for the different values of K. We also described the distribution of microsatellite variability using a Factorial Correspondence Analysis (FCA) based on the birds' genotypes. Finally, to estimate the genetic differentiation between all pairwise groups, we used the software Fstat v.2.9.3.2. to calculate Fst values according to Weir and Cockerham (1984). The level of significance of the tests was adjusted for multiple testing using the Chandler correction and an error rate of 10% (Chandler 1995).

Genetic differences between wild and farmed individuals

We selected 103 individuals assigned to the "wild" genetic cluster and 16 to the "farmed" one, using the proportions of ancestry inferred from the Bayesian approach implemented in Structure v.2.0 (i.e. qi-value > 0.97).

The genotypes of these selected individuals were implemented as 'parent genotypes' in the software Hybridlab v1.0 (Nielsen et al. 2006) which simulates random matings and supplies the genotypes of artificial hybrids. By this way we built 100 artificial F1 hybrids and 100 artificial F2 hybrids (i.e. F1 × F1).

The genetic signatures of these 200 artificial hybrids were pooled with the ones of the 1051 biological birds and implemented into the Newhybrids software (Anderson and Thompson 2002) to assign each "individual" to one of the four categories: each pure subspecies (i.e. "Pure_*P.p.hispaniensis*" and "Pure_*P.p.armoricana*") and hybrids (i.e. "F1 hybrids" and "F2 hybrids"). NewHybrids was run with 10 000 burn-in iterations followed by 1 000 000 Markov chain Monte Carlo iterations. Analyses did not use prior allele

frequency information but considered uniform prior distributions for θ and p parameters (Anderson and Thompson 2002). This procedure allowed us (1) to test the reliability of our approach, i.e. by checking the inference of our 200 known artificial hybrids to the suitable hybrid category, and (2) to estimate the frequency of the four genotypic categories with true birds.

Genetic structure of groups of wild Pyrenean birds

We only report here the genetic structure of groups of wild grey partridges collected in the Pyrenees, as exactly the same work has already been published with wild birds from central northern France (Bech et al. 2014).

We tested the occurrence of isolation-by-distance (IBD) using a regression analysis of the genetic distance (F_{st} values of pairwise wild groups, see above) against the Euclidian distance (simple Mantel test implemented in GENALEX software, Peakall and Smouse 2006). Moreover, we used Structure software in the same conditions as previously in order to estimate the likely number of genetic clusters corresponding to our wild birds collected in the Pyrenees.

Results

Validation of molecular markers

33 of the 432 tests exhibited null alleles but they were randomly distributed across loci and groups. Only two groups revealed deviations from Hardy–Weinberg expectations (P-value threshold after Chandler correction: $P = 0.0002$) (Supplementary file 1). Moreover, no evidence of linkage disequilibrium was detected (P-value threshold after Chandler correction: $P = 0.001$). As deviations from Hardy–Weinberg and null alleles were inconsistent across loci, we retained all loci in the analyses.

Global genetic structure

All microsatellite markers were polymorphic within all groups. The allelic richness ranged from 1.767 to 6.379 and the genetic diversity ranged from 0.232 to 0.944 (Supplementary file 1). Genetic diversities were similar across all groups ($P = 0.34$ for H_e and $P = 0.11$ for AR). F_{is} values revealed that two groups had a heterozygosity excess, and one a heterozygosity deficit (P-value threshold after Chandler correction: $P = 0.0002$).

Number of genetic clusters of wild birds

The highest posterior probability for the number of genetic clusters was given for $K = 2$, corresponding to the two subspecies. Indeed, individuals collected in wild populations in the Pyrenees revealed high proportions of ancestry into the genetic cluster 1 (mean \pm SD: 0.847 ± 0.001) while individuals collected in wild populations in central northern France revealed high proportions of ancestry into the genetic cluster 2 (mean \pm SD: 0.838 ± 0.001) (Fig. 2). This

clear-cut genetic differentiation between both subspecies can be visualised in the FCA output (Fig. 3) and is quantified by a mean F_{st} value = 0.072 (Table 2; Supplementary file 2).

Genetic difference between farmed and wild birds

F_{st} values comparing wild and farmed birds revealed a significant genetic differentiation, both in central northern France (mean F_{st} value = 0.026) and in the Pyrenees (mean F_{st} value = 0.108). The FCA depicts a distinct distribution between the centroids of the groups of wild Pyrenean birds and the groups of farmed birds, with farmed birds being located among centroids of the groups of wild birds sampled in central northern France (Fig. 3). This suggests that farmed birds released in the Pyrenees belong to the subspecies *P. p. armoricana* or to a close related subspecies.

Detection and repartition of hybrids

In the Pyrenees

We selected genotypes from 103 wild and 16 ringed released individuals to depict the wild and farmed genetic signatures and then to artificially generate hybrids. We had some difficulties to infer genotypes of artificially generated hybrids using Newhybrid software. Indeed, only 29 over 100 F1 hybrids and 12 over 100 F2 hybrids have been inferred to suitable predefined F1 and F2 genetic categories, respectively.

However, as far as wild birds are concerned, 504 birds over 604 were inferred into 1 of the 4 predefined genetic categories. Accurately, 472 birds were inferred into the “Pure_*P. p. hispaniensis*” category, 19 into the “Pure_*P. p. armoricana*” and 13 into a hybrid classes (i.e. 2 into F1 class and 11 to the F2 class). The 100 other birds showed ambiguous genotypes making inferences difficult.

39 over 41 farmed birds released in the Pyrenees were inferred into the “Pure_*P. p. armoricana*” category. Finally, among the 57 individuals collected from Pyrenean conservation breeding programs, 8, 9, 1 and 10 were inferred into the “Pure_*P. p. hispaniensis*”, “Pure_*P. p. armoricana*”, “F1” and “F2” categories, respectively. The other birds showed ambiguous genetic profiles making inferences difficult to any class.

In central northern France

We could not discriminate wild and farmed birds as the posterior probabilities inferred all of them to a same genetic cluster. In this way, we have not been able to infer hybrids within sampling localities.

Genetic structure in Pyrenean wild populations

The mean F_{st} values indicated a significant genetic differentiation across the groups revealing a substructure in the Pyrenees (mean F_{st} : 0.040 ± 0.024 ; $P < 0.0004$). This was corroborated at the individual scale by a significant positive correlation between genetic and Euclidian distances ($r^2 = 0.0092$; $P = 0.0001$). This result indicates a strong isolation by geographical distance. At the individual scale, results from both Structure and Structure-Harvester software revealed some difficulties to converge toward a defined number of genetic clusters K (as Delta K value < 20), suggesting a unique genetic cluster ($K = 1$) and so a unique genetic signature of wild grey partridge in the Pyrenees.

Discussion

Two distinct genetic subspecies of grey partridges in France

Although already highlighted with mitochondrial markers (Martin et al. 2003), we quantified the differences between *P. p. hispaniensis* and *P. p. armoricana* wild birds using our set of 12 nuclear microsatellite markers. We both deepened the genetic characterization of these two subspecies and calibrated the scale for the genetic distance between wild and farmed birds.

The results presented herein support a divergence between both subspecies, estimating a mean F_{st} of 0.072. This significant genetic differentiation is explained by the evolutionary history of the grey partridge during the Quaternary. The species experienced changes in its geographic distribution in Europe during ice ages (Birkan and Jacob 1988). In France, these changes resulted in both subspecies, *P. p. armoricana* occurring in central northern France and *P. p. hispaniensis* occurring southward in the high-mountain zones of the Pyrenees (i.e. the meridional refuge to survive to quaternary ice caps) (Liukkonen-Anttila et al. 2002).

Genetic structure across subspecies populations

Within each subspecies, the genetic differentiation between groups of birds revealed lower rates of differentiation than between both subspecies. However, the genetic structure of wild populations is twice stronger in the Pyrenees than in central northern France, with mean F_{st} values of 0.040 and 0.019, respectively (Bech et al. 2014). This result is likely to emerge from habitat structure. Indeed, even if central northern France represents a landscape largely fragmented by human activities, the level of fragmentation of suitable habitat for the grey partridge is higher in the Pyrenees. Indeed, for species restricted to high-mountain zones, habitat is often referred as 'sky islands' illustrating patches of suitable habitat surrounded by inappropriate landscape (i.e. valley) (Haila 2002). Whatever its level, the habitat fragmentation combined with the intrinsic low dispersal abilities of grey partridge [maximum distance of 1.5–2 km, with few cases > 10 km as recorded

by telemetry (Bro et al. 2016)] could explain the high isolation by the distance detected herein in the Pyrenees compared to what occurs in central northern France (Bech et al. 2014). Such a genetic structure is often found in Galliform populations, in mountain landscapes [capercaillie *Tetrao urogallus* (Segelbacher et al. 2003), rock ptarmigan *Lagopus muta pyrenaica* (Bech et al. 2009)] as well as in plains (greater sage grouse *Centrocercus urophasianus* (Oyler-McCance et al. 2005)).

Differences between wild and farmed partridges

In central northern France, even if the genetic differentiation between farmed and wild partridges is statistically significant, it is quantitatively weak (i.e. mean F_{st} value = 0.026) and of the same order of magnitude that the genetic differentiation observed in the field between wild populations (mean F_{st} value = 0.018). So, farmed birds—such as randomly sampled in our study—are genetically closed to the native *P. p. armoricana*. Thus, their genetic background does not seem prone to alter significantly the gene pool of wild birds occurring in this area.

On the contrary, in the Pyrenees, the genetic difference between wild and farmed partridges is much larger. Farmed individuals released into the Pyrenean wild populations are likely to belong, or to be genetically very closed, to the subspecies *P. p. armoricana*. Thus, there is an important risk of genetic introgression if the farmed birds released in nature reproduce with wild individuals. This risk was already documented in previous studies based on mitochondrial data (Martin et al. 2003) and is supported herein by the admixed genetic profiles. Indeed, even if our methods have not accurately inferred these admixed genotypes to a predefined hybrid class, we suppose they likely represent hybrid individuals. Moreover, we have obtained similar results with hybrids generated in silico. This difficulty to infer admixed genotypes to a predefined hybrid class can originate from either a weak genetic resolution of our microsatellite marker panel or a low frequency of hybrids in populations. A low frequency may result from the combination of a low survival of partridges released on the field (< 30% a few months after release, Bro et al. 2016), a lower reproductive success of the released partridges (Buner et al. 2011)—that could be due to a less effective anti-predator behaviour (see Mayot et al. 2012) as also observed in the quail (Capdevila et al. 2016)—or from the rapid disappearance of the hybrid genetic signature as first back-crosses occur.

Conservation issues

The genetic difference we found in the Pyrenees between wild and farmed partridges has important consequences with regard to conservation actions of the emblematic *P. p. hispaniensis*. Indeed, due to the genetic specificity of this

subspecies and the detection of introgression in wild populations (Bech et al. 2012), releases have been forbidden above 1000 m asl to avoid further hybridizations. Some conservation release programs use birds from conservative breeding centers (from Esterri d’Aneu). However, the genetic pattern of these birds is finally only slightly closer to the one of wild birds (mean F_{st} value = 0.096), compared to farmed birds found in commercial farms in the Pyrenees (mean F_{st} value = 0.108). So, it is better, but not the best yet. The great difficulty to breed wild-born partridges in captivity, even birds born in captivity from rescued clutches (Millot et al. 2012; Vannesson et al. 2017), may explain the low availability of partridges genetically suitable. The significant genetic differentiation across wild Pyrenean populations may also raise the question whether local strains should be considered or not.

On the contrary, farmed partridges found in commercial farms* that are released in central northern France display a genetic pattern close to the one of wild birds (considered as such from a genetic point of view, knowing that quite a lot of partridges were released in the past, Bro et al. 2016). This result might be explained by local origins of founders used in commercial farms (Birkan 1979; Blanc et al. 1987) and/or regular backcrosses of captive strains with wild birds if any. In this context, the plus-value of conservative breeding centers seems quite less crucial, the genetic risk arisen by conservation or hunting releases** may be considered as weak.

* we did not detected strains of partridges said from “eastern Europe” that display a distinct genetic signature (East vs. West clades, Liukkonen-Anttila et al. 2002).

** hunting releases may however have adverse demographic effects on wild populations if harvest quotas are not strictly managed (Bro and Mayot 2006; Mauvy et al. 1992).

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This study was conducted in accordance with the principles and specific guidelines of the French current legislation for animal welfare and wildlife regulations. No animal was killed for the purpose of this study. All the biological samples were collected from dead animals, by taking the opportunity of hunting bags.

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Table 1 List of the 36 groups of individuals (N = effective size) of grey partridges in the Pyrenees and Central northern France

Groups of individuals		N	He	Ar	Fis	
Pyrenees	Wild (n = 604)	1	21	0.64	3.34	0.01
	<i>P. p. hispaniensis</i>	Canigo	51	4	0	9
		u 2		0.64	3.32	0.05
	Puigma	1	1	8		
	l					
	3 Carlit	203	0.67	3.55	0.05	
			9	6	2	
	4 Cerdagne	46	0.68	3.60	0.11	
			3	1	0	
	5 Andorre	22	0.68	3.54	0.06	
			2	0	0	
	6 Madres	16	0.61	3.15	0.01	
			8	5	6	
	7 Donezan	12	0.63	3.31	0.05	
			8	2	2	
	8 Mont Tabe	30	0.67	3.47	–	
			5	5	0.014	
	9 Aston	32	0.66	3.39	0.09	
			5	9	3	
	10 3	11	0.67	3.47	0.02	
	Seigneurs		0	0	1	
	11 Ustou	24	0.62	3.32	0.09	
			0	3	6	
	12	41	0.64	3.48	0.07	
	MellesLuchon		9	4	0	
	13 Vallée du	36	0.64	3.33	0.11	
	Gave		2	7	3	
	14 Aubisque	19	0.66	3.61	0.10	
			9	6	6	
	15 Lescun	7	0.60	3.10	–	
			4	2	0.084	
	16 Pallars	25	0.64	3.43	0.02	
	Sobirà		6	1	9	
	17	8	0.60	3.14	0.11	
	AltaRibagorça		1	0	6	
	Conservative breeding program	Pallars Sobirà	57	0.67	3.52	–
			2	9	0.007	

Farmed individuals (n = 41)	Andorre	14	0.63	3.31	–	
			4	1	0.032	
	Aubisque	11	0.58	2959	–	
			3		0.099	
	Cerdagne	16	0.62	3.17	0.05	
Central norther n France	Wild (n = 149) <i>P. p. armoricana</i>	BP1-sau	39	0.66	3.45	0.07
		BP2-sau	52	5	2	6
		BP3-sau	18	0.67	3.49	0.02
			1	4	7	
				0.71	3.81	0.04
			1	0	6	
		BP4-sau	8	0.62	3.33	0.11
			1	9	4	
		BP5-sau	32	0.66	3.44	–
			9	4	0.021	
Conservative breeding program	Farmed individuals (n = 189)	BP-cons	11	0.66	3.51	0.13
			9	4	6	
		BP-EI1	20	0.65	3.42	0.08
			7	6	1	
		BP-EI3	24	0.69	3.74	0.08
			0	5	0	
		BP-EI4	11	0.63	3.24	–
			0	0	0.106	
		BP-EI6	30	0.67	3.55	0.01
			7	1	5	
		BP-EI7	29	0.65	3.39	0.01
			6	7	9	
		BP-EI8	27	0.68	3.60	0.03
		3	7	7		
	BP-EI9	26	0.66	3.44	–	
		1	0	0.056		
	BP-EI11	10	0.66	3.56	–	
		3	9	0.031		
	BP-EI13	12	0.67	3.50	0.04	
		3	9	0		
	Total	105				
		1				

Genetic indices for sampling localities: the allelic richness (AR), the expected heterozygosity (He) and Fis values. In italic: the Fis values significantly different from 0 (p-value threshold after Chandler correction $p = 0.0002$)

Table 2 Mean and range of the F_{st} values for each pairwise group of individual comparisons

	Pyrenees wild	Pyrenees farmed	Pyrenees con - servative breeding programs	Central northern France wild	Central northern France farmed	Central northern France breeding programs
Pyrenees wild	0.040[0-0.106]	0.108[0.049-0.213]	0.096[0.045-0.167]	0.072[0.023-0.151]	0.082[0.028-0.193]	0.096[0.045-0.167]
Pyrenees farmed	-	0.020[0-0.037]	0.058[0.047-0.067]	0.055[0.015-0.090]	0.023[0-0.076]	0.072[0.053-0.100]
Pyrenees conservative breeding programs	-	-	-	0.051[0.046-0.058]	0.047[0.030-0.084]	0.065
Central northern France wild	-	-	-	0.018[0.002-0.040]	0.026[0.008-0.071]	0.025[0.018-0.037]
Central northern France farmed	-	-	-	-	0.005[0-0.033]	0.330[0.023-0.046]

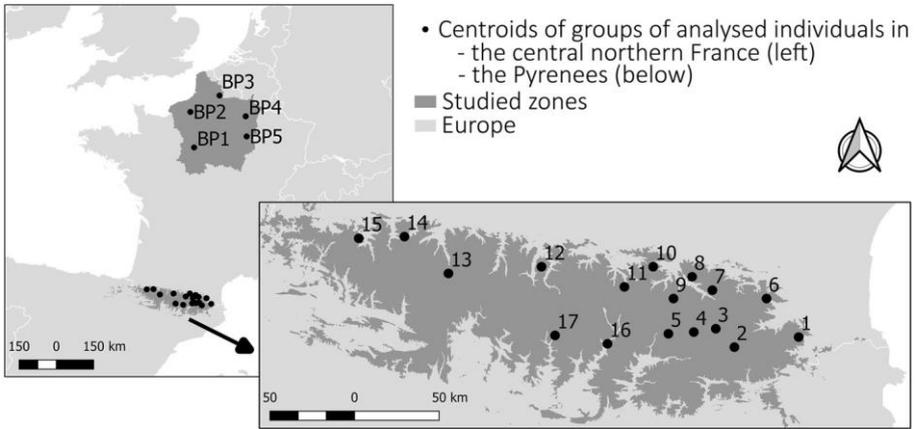


Figure 1

Maps of the studied zones (i.e. central northern France and Pyrenees)

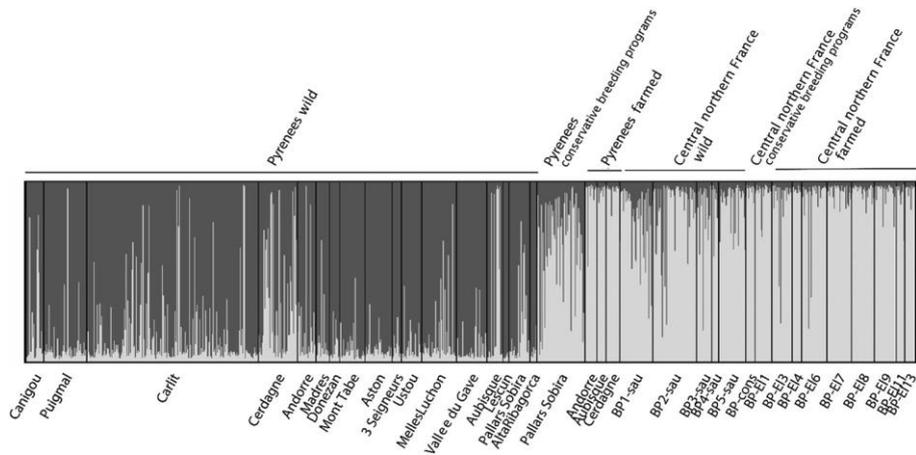
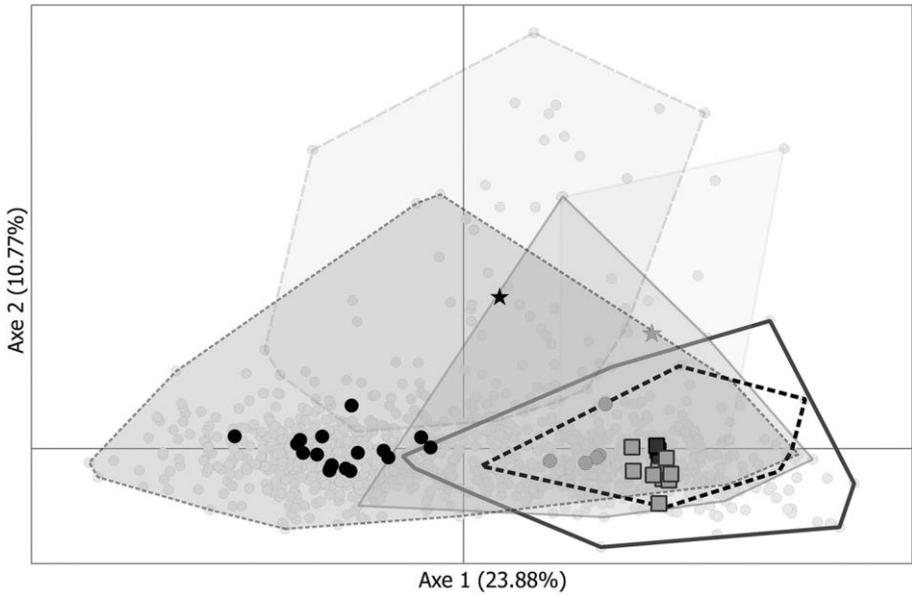


Figure 2

Results from Structure software: proportions of ancestry of each individual to the two inferred genetic clusters. Each individual is represented by a vertical bar, each color represents a genetic cluster and black lines separate the individuals from different groups



Legend

● Individuals (n=1051)

Centroids of Individuals

● Central northern France Wild (5 groups ; n=149)

● From the field (Pyrenees) (17 groups ; n=604)

★ From conservative breeding programs in Central northern France (n=11)

★ From conservative breeding programs in Pyrenees (n=57)

■ Farmed Central northern France (9 farms; n=189)

■ Farmed Pyrenees (3 farms; n=41)

Polygons showing the individuals distribution from

■ Wild from Central northern France

■ Wild from Pyrenees

■ From conservative breeding programs in Central northern France

■ From conservative breeding programs in Pyrenees

■ Farmed Central northern France

■ Farmed Pyrenees

Figure 3

Results of Factorial Correspondence Analysis (FCA) based on individuals' genotypes. This FCA was conducted on all individuals from both central northern France as well as Pyrenees and using Genetix software v.4.05.2. While individuals were characterised by light grey circles, we represented here groups' centroids in order to facilitate interpretations