Distribution and spatial genetic structure of European wildcat in France

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Abstract

Given the problem of hybridization with domestic cats, there is a growing need to identify populations of the European wildcat *Felis silvestris silvestris* in order to protect the genetic integrity of this subspecies. In this paper, we use known locations of observations of wildcats or recovered carcasses to reassess the distribution of the wildcat in France and, in cases where carcasses were collected, we use both phenotypic and molecular genetic analyses to distinguish wildcats from hybrids with domestic cats. Spatially explicit multivariate analysis of wildcat' genotypes was then performed to define genetic units. Our study confirms the presence of wildcats in a large area of *c.* 155 000 km^2, suggestive of a range of expansion, and divided into two clearly distinct and unconnected areas – the Pyrenees and the north-eastern part of France. However, European wildcat populations may be decreasing in the French Pyrenees, whereas the north-eastern part represents the main area (MA) of wildcat presence. This extension does not appear to be primarily due to hybrids, as both wildcats and hybrids were located throughout the MA. In addition, we found that genetic diversity of wildcats in the MA is remarkably high, suggesting that French populations are not threatened by a lack of genetic diversity. Furthermore, wildcats of the MA are structured into two genetically distinct populations that are contiguous and probably extend into Germany to form the largest area of wildcat presence in Europe and an area of major interest for their conservation. Our study calls for localized examination of the feasibility and usefulness of wildlife corridors to enhance connectivity between the different populations, thereby allowing sufficient levels of immigration and gene flow within the regional meta-population to ensure the long-term viability of these populations.

Introduction

The European wildcat *Felis silvestris silvestris* is one of the last living wild felids in Europe, but its conservation status is somewhat paradoxical. It is the most common and widely distributed wild cat species, ranging from Russia to Portugal and from Scotland to the Near East, with the notable exception of Scandinavia (Nowell & Jackson, 1996). Consequently, it is listed as Least Concern by the IUCN (2008). However, annotation of the species in Appendix II of CITES (UNEP-WCMC, 2006) underscores the need for its protection. Human-mediated habitat disturbance and large-scale hunting in the early 20th century have led to severe local declines and extirpations in Europe (Stahl & Léger, 1992; Sunquist & Sunquist, 2002), resulting in a fragmented distribution (Stahl & Artois, 1991; Nowell & Jackson, 1996; Peichocki, 2001). Subsequent legal protection, under the Bern Convention (Appendix II, 1979) and the European

Habitat Directive 92/43/EEC (EUROP, 1992), has reduced or even eliminated the causes of this decline and has led to a spontaneous recovery of European wildcat populations in some parts of Europe (Stahl & Artois, 1994). However, wildcats continue to face the significant threat of hybridization with feral domestic cats *F. s. catus*. Hybridization takes place across almost the entire range and is particularly pervasive in Scotland and Hungary (Pierpaoli *et al.*, 2000; Beaumont *et al.*, 2001). It has been suggested that hybrids may be responsible for the apparent population recovery because of their greater behavioural plasticity, whereas genetically pure wildcats may be in decline (Macdonald *et al.*, 2004; Battersby, 2005; Herrmann & Vogel, 2005; Lecis *et al.*, 2006).

This discrepancy between global and local management recommendations suggests the need for a reassessment of the conservation status of the European wildcat. To devise

adequate conservation and management strategies, it is important to incorporate a reliable understanding of the history and population structure of European wildcats and to characterize their genetic diversity.

The European wildcat has been poorly studied (Bashta & Potish, 2005) and information regarding its current status and trends in population size are lacking (Macdonald *et al.*, 2004). France is expected to harbour large wildcat populations and, consequently, the study and conservation of French wildcat populations should be a priority for protecting the species. In France, where the species has been protected since 1976, recent work has shown that hybridization with feral domestic cats occurs, but that a pool of genetically pure wildcats exists, which deserves protection (O'Brien *et al.*, 2009). Recent ongoing work has focused on the factors contributing to hybridization by comparing the ecological needs of wildcats, feral domestic cats and their hybrids (Germain, Benhamou & Poulle, 2008). Little information exists on the distribution of wildcats in France, which prevents the development of effective conservation measures. Acquiring data on the distribution of this species is difficult because of their elusive behaviour (Stahl & Artois, 1991; Daniels *et al.*, 1998; Wolsan & Okarma, 2001; Bashta & Potish, 2005; Germain *et al.*, 2008) and because it is difficult to distinguish European wildcats from hybrids on the basis of coat colour and morphology (Daniels *et al.*, 1998; Yamaguchi *et al.*, 2004; Krüger *et al.*, 2009). The most complete data on the distribution of this species in France dates from over 20 years ago (SFEPM, 1984). Two areas, the Pyrenees mountains along the border of France and Spain and the north-eastern portion of France, were identified as population strongholds, and a possible connection between the two areas has been posited. More recent studies have been regional (e.g. Lustrat & Vignon, 1991; Fournier, 1994; Bas, 1996; Ariagno, 1999; Léger, 1999; Bourand, 1999). These do not allow the establishment of the geographical distribution of the species, nor do they give an account of recent changes in wildcat populations in France. In this paper, we sought to (i) reassess the distribution of the European wildcat in France and provide more information on the possible connectivity between the Pyrenean and the north-eastern populations; (ii) compare and contrast the distribution of wildcats based on phenotypic measures versus genetic classification; (iii) describe the distribution of hybrids using genetic approaches and assess whether hybrids are preferentially found in newly colonized areas; (iv) describe the genetic diversity of the European wildcats in France.

Material and methods

Sample collection

Sampling occurred between 1995 and 2006, covering the distribution of the European wildcat as established in 1984 (SFEPM, 1984; Fig. 1a), and a broad peripheral zone. The total area investigated covered 51% of metropolitan France.

This area was divided into grid cells of $10 \times 7 \, \text{km}^2$ ($n = 4019$), as recommended by the French Natural History Museum. Details about the methods and the sampling effort are described in Léger *et al.* (2008). Grid cells were surveyed multiple times, but sampling effort may have changed from one grid cell to the other. Surveys were performed in each grid cell by officers of the National Hunting and Wildlife Agency, professionals of various hunting associations and by naturalists specifically trained to recognize the wildcat coat pattern. Probable presence of wildcat in a given grid cell was assumed when an individual was observed by a trained observer or when a cat carcass was collected ($n = 465$ carcasses, Table 1), mainly as road kill, by the National Hunting and Wildlife Agency, and then examined by the authors. Presumed wildcats corresponded to cats that exhibited all typical wildcat pelage characteristics: (i) a tail with a large, rounded, black tip and at least two black bands that completely encircled the tail; (ii) one thin, straight, dorsal stripe, interrupted at the root of the tail; (iii) lateral stripes that are not pronounced and not linked to the back stripe; (iv) light-tawny or grey-coloured fur. When possible, cranial indices, calculated as cranial volume divided by the greatest length of the skull (Schauenberg, 1969), and intestine indices, calculated as intestine length divided by body length (head included; Schauenberg, 1977), were also measured in adults. Cranial indices <2.65 and intestine indices <3.1 were considered characteristic of wildcats. When recent observations over the study period were lacking, we also took into account some sporadic data recorded before 1995, but only data confirmed by examining crania and furs preserved by regional museums or by the authors were retained for inclusion in the distribution map.

Hair samples were used to establish the genotype of 266 individuals, all from the north-eastern area, except one individual from the Pyrenees (57 cats in addition to the 209 previously used in the study of O'Brien *et al.*, 2009; Table 1), using molecular protocols described in O'Brien *et al.* (2009). We used STRUCTURE (Pritchard, Stephens & Donnelly, 2000) following the methodology in O'Brien *et al.* (2009) to identify hybrid and feral domestic cats and remove them from subsequent analyses to avoid a bias in the estimation of wildcat genetic diversity. A Bayesian clustering method was used to calculate individual qi/wild values, that is, the proportion of each individual genome representative of a wildcat parental cluster, and simulation was used to determine a threshold proportion above which a given individual was determined to be a wildcat (supporting information Appendix S1 and O'Brien *et al.* (2009) for more details). We analysed six new microsatellite markers (Fca24, Fca37, Fca85, Fca547, Fca577, Fca675) in wildcats and hybrids only, in addition to the 12 markers used in O'Brien *et al.* (2009; Table 2).

Spatial genetic structure of wildcats in north-eastern France

As a prerequisite in seeking for spatial genetic structure using spatial principal component analysis (sPCA), we
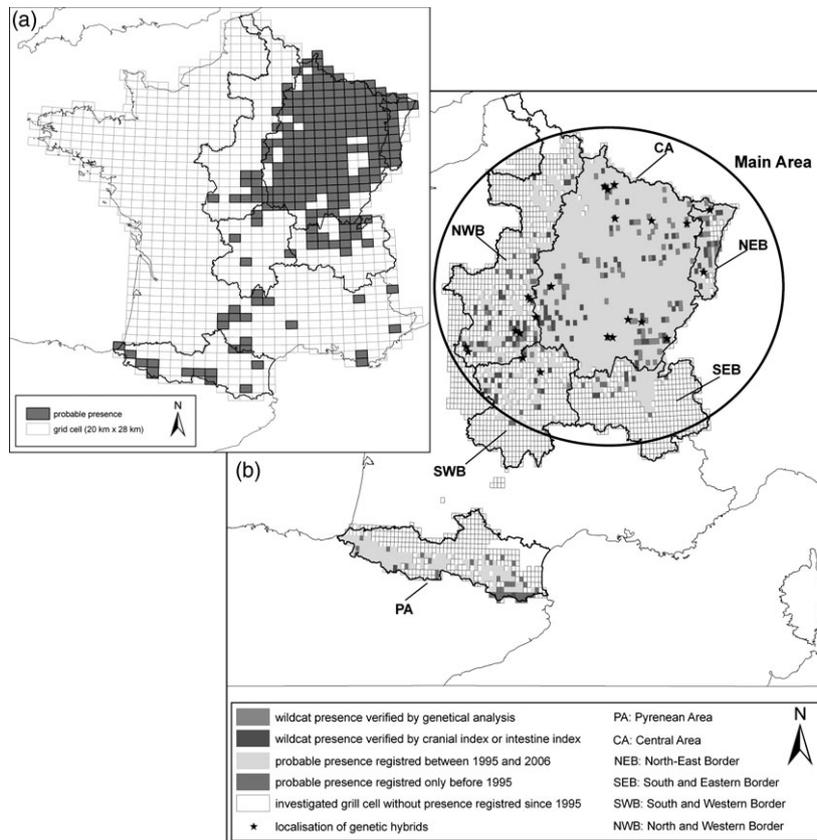
Figure 1 (a) Distribution of European wildcat in France in 1984 (SFEPM 1984); (b) updated (1995–2006) distribution of the European wildcat in France and location of genetically confirmed wildcats and hybrids. Five zones within MA were identified based on historical and continuous presence of wildcats. Each zone is roughly homogenous in term of topography and vegetal cover. Their limits were arbitrarily chosen as the borders of the departments which constitute the administrative divisions of the French territory and are given only for graphical display. Each grid cell (n=4019) corresponds to a $10 \times 7\,km^2$ area.

tested for isolation by distance (IBD) by calculating the coefficient of correlation of Mantel (R, Mantel, 1967) between two distance matrices. The matrix of genetic dissimilarities between individuals, estimated by the pairwise *a*-distances of Rousset (2000) calculated using SPAGEDI (Hardy & Vekemans, 2002), was thus regressed against the matrix of pairwise Euclidean geographical distances (after ln-transformation) using a Mantel test implemented in the package ADEGENET (Jombart, 2008) for the software R (R development Core team, 2009). Significance of the Mantel's R was assessed using permutation test ($n = 9999$).

We then used sPCA (Jombart *et al.*, 2008), implemented in the package ADEGENET (Jombart, 2008) to investigate more precisely the spatial genetic structure of wildcats in the north-eastern part of their range. sPCA seeks principal components that optimize the product of the variance of allelic frequencies and of Moran's I (Moran, 1948; Cliff & Ord, 1981), ensuring that both the genetic diversity and the spatial patterns are taken into account. sPCA is appropriate for our study because the distribution of the wildcat is more or less continuous (see 'Results'), borders are difficult to establish and wildcat assignment to a given *a priori* area may be problematic. The sPCA allows us to identify 'global structures' (*sensu* Jombart *et al.*, 2008) that correspond to a positive spatial auto-correlation between genotypes, indicating the occurrence of patches or clines in the spatial distribution of genetic diversity. Graphical displaying of the global spatial structure onto a geographical map helps us in disentangling patches and clines, and thus, in refining the interpretation of the IBD evidenced in the IBD analysis. As in other multivariate analyses (Jombart, Pontier & Dufour, 2009), such global structures are typically detected graphically by an abrupt decrease in positive eigenvalues. Jombart *et al.* (2008) provide non-parametric randomized Monte–Carlo tests (global test) that facilitate assessment of the strength of the inferred structures. Spatial information is provided to the sPCA through a matrix of pairwise spatial proximity between individuals, that can be either a neighbourhood graph or pairwise matrix of Euclidean distances (Jombart *et al.*, 2008). Here, we used the inverse of Euclidean distances between each pair of individuals as spatial weights because the spatial scale at which the wildcats were sampled was very large (relative to wildcat home range diameter, Germain *et al.*, 2008), preventing us from defining two wildcats as neighbours based on potential behavioural interactions.

Table 1 Spatial partitioning of carcasses (*n* = 465) and of tissue samples for STRUCTURE analysis (*n* = 266)

Geographical zone	Sub-zones	No. of carcasses (no. presumed wildcat)	No. of genotypes	No. of wildcat/ hybrids/domestic	No. of wildcat/hybrids with geographic location
North-eastern France	NEA	245 (203)	161	92/42/27	82/17
	NEB	61 (37)	34	16/14/4	15/1
	NWB	101 (60)	45	19/20/6	19/7
	SEB	13 (8)	5	3/1/1	2/-
	SWB	41 (31)	20	13/5/2	13/2
Pyrenean zone		4 (4)	1	1/-/-	1*/-
Total		465 (343)	266	144/82/40	266 (132*/27)

*This one individual from the Pyrenean area was not included in spatial genetic analysis.

The result of the STRUCTURE assignment procedure and the numbers of geo-referenced genotypes are also given

Table 2 Genetic diversity indices calculated for each locus and globally for wildcats (*n* = 131)

Locus	No. of alleles	H_o	H_e	HW test
Fca8	14	0.814	0.821	0.377
Fca23	10	0.638	0.587	0.146
Fca24	4	0.475	0.605	0.008*
Fca26	13	0.846	0.837	0.157
Fca37	6	0.706	0.706	0.015*
Fca43	12	0.612	0.627	0.044*
Fca45	9	0.520	0.719	<0.0001*
Fca58	8	0.362	0.413	0.065
Fca77	10	0.752	0.814	0.150
Fca78	10	0.732	0.781	0.065
Fca85	34	0.847	0.931	<0.0001*
Fca96	17	0.843	0.828	0.344
Fca124	11	0.817	0.791	0.338
Fca 126	9	0.780	0.743	0.009*
Fca 547	12	0.724	0.762	0.355
Fca 577	11	0.664	0.711	0.296
Fca 668	13	0.692	0.740	0.050
Fca 675	9	0.686	0.742	0.107
All loci	11.78	0.695	0.731	<0.0001*

*indicate significance (*P* < 0.05). *P* values for exact tests of Hardy-Weinberg equilibrium are also provided for each locus.

Locus name, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity.

Genetic diversity

To investigate the degree of genetic diversity in wildcat samples from the putative genetic 'patches' identified by the sPCA, we estimated multi-locus mean number of alleles, mean expected heterozygosity (H_e) and mean observed heterozygosity (H_o) using the ADEGENET package (Jombart, 2008). Departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were estimated using the Markov chain method implemented in GENEPOP 3.4 (Raymond & Rousset, 1995) with 8000 dememorization, 800 batches and 8000 iterations per batch. Exact tests of HWE were performed for each locus in each patch. We used the false discovery rate (FDR) control for *P*-values of the HWE tests on individual loci and for linkage disequilibrium

tests with the R package FDRTOOL (Strimmer, 2008*a*, 2008*b*). We computed pairwise values of F_{st} following Weir & Cockerham (1984). Significance of genetic differentiation was tested among all pairs of patches using the *G*-test (Goudet *et al.*, 1996), implemented in FSTAT (Goudet, 2001). We also calculated the *M*-statistic for each locus and each patch to detect recent genetic bottlenecks. The *M*-statistic is the ratio of the number of alleles to the total range in allele size (in number of repeats). An average *M* was computed across loci for each patch and compared to the patch-specific critical values (M_c). The M_c were calculated using critical_M software (Garza & Williamson, 2001) based on 18 loci, $\theta = 10$ and the conservative parameters of 90% one-step mutations and a mean size of 3.5 for non-one-step mutations (Garza & Williamson, 2001).

Results

Distribution of the European wildcat in France

Wildcat presence was established in 55.2% (2217/4019) of the grid cells examined; the total area was *c*. 155 000 km². For 77 of these grid cells (3.5%), wildcat presence could be determined only before 1995. Of the 465 carcasses examined, 343 (73.8%) were classified as presumed wildcat, based on pelage, cranial and/or intestine indices (Table 1). Those presumed wildcats were distributed over 182 grid cells (8.2% of grid cells ascribed to wildcat presence, Fig. 1). Similarly, wildcats confirmed using STRUCTURE (*n* = 131, supporting information Appendix S1) were distributed over 113 grid cells [5.1% of wildcat-inhabiting grid cells (Fig. 1b)] throughout the total potentially inhabited area. The correspondence between phenotypic and genetic classification was good, as 71% of the presumed wildcats were assigned genetically as wildcats and 29% as hybrids using STRUCTURE.

We confirmed the existence of two main areas of wildcat presence in France. They were clearly distinct with no apparent connectivity: we did not record any specimen of putative European wildcat between these two areas, despite investigation in 167 grid cells between the two areas (vacant grid cells shown in Fig. 1b). The Pyrenean zone (PA) represents an area of *c*. 13 000 km². The presence of wildcats was recorded in 37% of the grid cells investigated, mainly in

the piedmonts, with the number of observations decreasing with increasing altitude; no wildcat was observed or recorded above 1800 m. However, recent observations are rare so that the number of grid cells with probable presence (i.e. data ascertained only before 1995) is large (41/77, i.e. 53% of grid cells with presence ascertained only before 1995 are in the PA, Fig. 1b). Only one individual from this area was genetically confirmed as a wildcat.

The second area (MA) is larger and covers almost the entire north-eastern quarter of France (Fig. 1b). Five zones within the MA were delineated based on historical and continuous presence of wildcats. Each zone is roughly homogenous in terms of topography and vegetal cover. The limits of these zones were arbitrarily chosen as the borders of the departments that constitute the administrative divisions of the French territory. The Central Area (CA) is of greatest conservation value for the species in France, with continuous woodland forest representing more than 30% of the habitat and wildcat presence registered in almost all investigated grid cells. The CA extends to the north-east border (NEB) region, where presence of wildcats has been known for years, but they are absent from the Alsace plain, a 30 km wide and 170 km long lowland between the Rhine River and the Vosges. Wildcat presence was recorded in 82% (110/134 of grid cells ascribed to wildcat presence) of grid cells in the NEB. Wildcats are mainly found in woodland areas of low altitude (up to 600 m) and their presence is rare above 800 m. Presence of wildcats is less regular in the north and western Border (NWB) where wildcats were recorded from only 36% (356/989 of grid cells ascribed to wildcat presence) of grid cells. However, this area represents the main extension zone of the species in France when compared to data published in 1984 (SFEPM 1984, Fig. 1a). Finally, other populations can be found in the pre-Alpine area (south-eastern Border) and in the Massif Central [south-western Border (SWB)] where wildcat presence is sporadic (12%; 56/450 of grid cells ascribed to wildcat presence; and 20%; 111/553 of grid cells ascribed to wildcat presence; of grid cells, respectively).

Spatial genetic structure in the MA of France

STRUCTURE analysis allowed us to classify 144 individuals as European wildcat, but only 131 of them had a geographical location (Lambert II extended coordinates, Table 1). Similarly, of 82 hybrids, only 27 with a geographical location were analysed (Table 1). Genetically determined wildcats ($n = 131$) and hybrids ($n = 27$) were distributed throughout the entire MA area (Fig. 1b), and especially in the NWB, thereby excluding the hypothesis that hybrids are mainly responsible for geographic expansion.

The regression of Rousset's *a*-distance estimator on the logarithm of the Euclidean distance using the Mantel test provided an estimate for R equal to 0.096. Permutation test indicated a significant IBD ($P < 0.0001$, 9999 permutations) among genetically determined wildcats, a prerequisite for the sPCA analysis.

The barplot of the eigenvalues of the sPCA on genetically identified wildcats ($n = 131$) (Fig. 2, bottom right) suggested that one global (i.e. the first positive eigenvalue) principal component should be retained. The global randomized Monte–Carlo test indicated that this global structure was unlikely to arise from random spatial distribution of the sampled genotypes ($P = 0.009$, 9999 permutations). The first global component clearly showed an east-west differentiation within the MA area, mainly contrasting the NEB and the north-east portion of the CA, called hereafter the French north-eastern patch, with the SWB and the southern part of the NWB, hereafter called the French central patch (Fig. 2). Between these two patches, individual genotypes appeared to be distributed more or less continuously with a mixture of positive and negative scores, suggestive both of a weak cline in genetic differentiation from east to west and of a likely contact zone between the two patches. Interestingly, the highly human-dominated Paris–Lyon axis through Dijon and Auxerre seems to be a likely boundary separating the two patches.

Genetic diversity of the European wildcat in the MA of France

To investigate the degree of differentiation between the two definite patches and to take into account the cline in genetic differentiation, we selected in the subsequent analyses 44 individuals in each patch (i.e. a third of the samples). These individuals had the 44 most positive and the 44 most negative scores, respectively, on the first principal component of the sPCA. This maximized the genetic differentiation between the two patches, but our objective was to use a standard metric of genetic differentiation, allowing future comparisons. Maximum genetic differentiation between these two patches was thus moderate ($F_{st} = 0.041$, *G*-test $P < 0.001$). Tests for genotypic disequilibrium revealed no significant linkage between loci within each patch after correcting for multiple comparisons. Genetic diversity was very similar between the French north-eastern and central patches (Table 3). Exact tests of HWE showed that 2/18 and 5/18 loci significantly deviated from HWE after FDR correction in the French north-eastern and central patches, respectively. Finally, the mean *M* values were 0.49 [0.29-0.64] ($M_c \approx 0.72$) and 0.46 [0.24–0.70] ($M_c \approx 0.75$), respectively, in both cases indicative of recent bottlenecks.

Discussion

Distribution of the European wildcat in France

Our study confirms the presence of wildcats in an area of *c.* 155 000 km^2 of metropolitan France. Wildcats live along the borders of continuous, open, deciduous or mixed forests of plains, hills and low or medium mountainous areas of a large north-eastern quarter of France (MA) and in the Pyrenean piedmonts (PA). Contact between wildcats from these two areas is highly unlikely because they are separated by ~300 km wide strip, where no
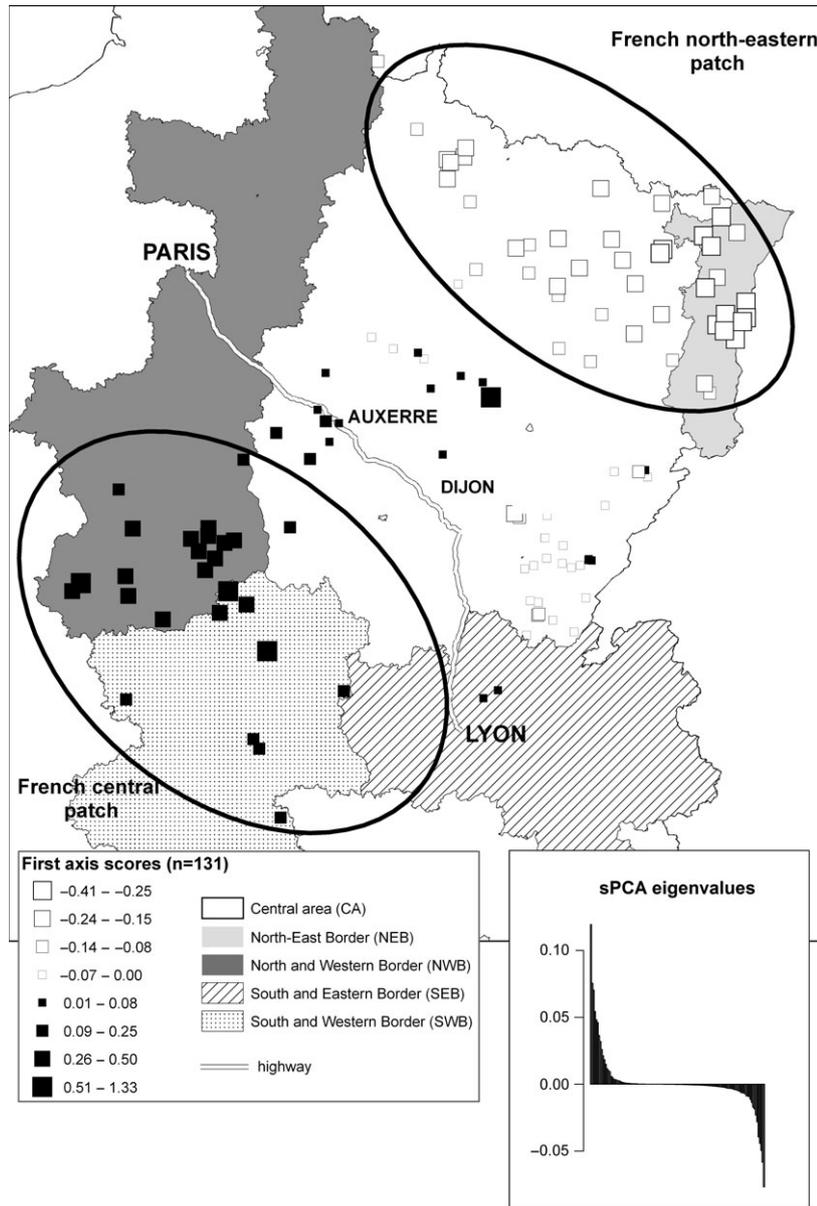
Figure 2 Spatial genetic structure of wildcats in the MA assessed by sPCA. Black and white squares represent individual scores for the first principal component: large white squares corresponding to individuals with high negative scores concentrated in the French central patch versus large black squares corresponding to individuals with high positive scores concentrated in the French north-eastern patch, together illustrating the main spatial genetic structure in our microsatellite dataset. Bottom right: bar plot of the sPCA eigenvalues.

wildcats were found. The large MA extends into Germany (Raimer, 1994), Luxembourg (Moes, 1994) and Belgium (Le Proux de la Rivière & Libois, 2006), whereas the PA population may extend into Spain and Portugal (Stahl & Artois, 1994).

The species might have been broadly distributed from the Holocene to the Middle Ages (Sommer & Benecke, 2006) when populations likely started to decline, bringing the species close to extinction in the early 20th century because of the destruction and fragmentation of their habitat (Stahl

& Léger, 1992; Sunquist & Sunquist, 2002). More recently, wildcat populations have slowly recovered across Europe (e.g. Klar *et al.*, 2008). In fact, the distribution reported here represents a range increase of around 30% in comparison to data published in 1984 (SFEPM, 1984, Fig. 1a). Since specimens detected in the newly colonized area are both wildcats and hybrids, *sensu* O'Brien *et al.* (2009), the extension of the wildcat range does not appear to be solely due to hybrids, as previously suggested (e.g. Lecis *et al.*, 2006). Other species of small carnivores have recovered and

Table 3 Mean number of alleles N_a, mean expected (H_e) and observed (H_o) heterozygosities, M values within the French north-eastern and the French central patches

	N_a	H_e	H_o	M
French north-eastern patch	8.23 [3–21]	0.718 [0.525–0.927]	0.714 [0.474–0.889]	0.49 [0.29–0.64]
French central patch	8.67 [4–24]	0.708 [0.405–0.925]	0.680 [0.333–0.884]	0.46 [0.24–0.70]

Ranges of values are given in brackets.

expanded in France following legal protection in the 1970s (Robitaille & Laurence, 2002 for the European otter *Lutra lutra*; Vandel & Stahl, 2005 for the Eurasian lynx *Lynx lynx*; Gauber *et al*., 2008 for the common genet *Genetta genetta*), although the trigger of such expansions may be sometimes attributed to active or accidental release of individuals in France or in adjacent foreign countries (e.g. lynx in Switzerland). The European wildcat appears as a noteworthy exception as its expansion cannot be attributed to any direct human intervention.

In France, the wildcat recovery masks some disparities across regions. Wildcat populations may be decreasing in the French PA, as in the Spanish Pyrenees (Stahl & Artois, 1991), whereas the NWB appears to be the main region of range extension. However, some of the geographic range extension we describe may be attributable to different survey intensities between studies. The SFEPM (1984) study primarily relied on opportunistic sightings, whereas we specifically trained agents to locate putative wildcats. In addition, biometric measurements and genetic analysis confirmed visual identification in 10% of our grid cells. Finally, the wildcat is an elusive species and the risk of visual misidentification is real (Beaumont *et al*., 2001; Yamaguchi *et al*., 2004; Krüger *et al*., 2009). Thus, we acknowledge that wildcats and hybrids cannot be readily distinguished visually, so the distribution presented here undoubtedly represents a mixture of hybrids and wildcats. Moreover, even if specimens exhibit both the wildcat phenotype and genotype, which clearly differentiates them from free-ranging sympatric feral cats, some domestic cat ancestry cannot be ruled out (O'Brien *et al*., 2009). However, the range and the distribution of genetically determined wildcats are concordant with the range and distribution based on visual indices, suggesting that our large temporal- and spatial-scale study captures the contemporary distribution of the European wildcat in France.

Genetic diversity and structure of European wildcat in north-eastern France

It is widely accepted among conservation geneticists that understanding levels of genetic diversity and population genetic structure is a prerequisite for conservation programmes for endangered species (e.g. Frankham, Ballou & Briscoe, 2002). Genetic diversity of French wildcats is remarkably high (mean number of alleles and observed heterozygozity similar to those found in sympatric feral cats, O'Brien *et al*., 2009), suggesting that the French populations, at least in the MA, are not threatened by a lack of genetic diversity. Lower levels of genetic diversity were found in Scotland and Hungary (Daniels *et al*., 1998; Beaumont *et al*., 2001), whereas our results are similar to those reported for other European populations (Pierpaoli *et al*., 2000; Randi *et al*., 2001; Lecis *et al*., 2006; Oliveira *et al*., 2008). Nevertheless, sampling and type and number of genetic markers used in the different studies vary greatly and may partly explain differences in reported genetic diversities and genetic structures.

Wildcats of the MA appeared to be split into two genetically distinct populations, the French north-eastern and the French central patches, separated by a likely contact zone showing a weak cline in genetic differentiation. The maximum genetic differentiation between these two French populations, while significant ($F_{st} = 0.041$) and maximized by our sampling approach to define patches (lower and upper one-third quantiles), seems lower (but see cautions stated above for inter-study comparison) than that between western and eastern German populations ($F_{st} = 0.19$, Eckert & Hartl, 2005), and is similar to the low genetic differences reported from Portugal ($F_{st} = 0.02$–0.04, Oliveira *et al*., 2008). This pattern in the MA may be consistent with a once continuous population, separated into two distinct bottlenecked units due to increasing 20th century industrial development (e.g. urban growth, highway network expansion, etc.; Fig. 2) along the Paris–Lyon axis, but later becoming re-connected because of effective expansion, most likely due to protective measures since 1976. The signature of past bottlenecks demonstrated by the M value in both populations supports this hypothesis. Finally, deviation from HWE appears to be more frequent in the central population. This may be due to the more fragmented habitat found in this area, leading to putative subpopulation structuring and, hence, to a more intense Wahlund effect. Only a fine-scale spatial study can clarify this. Whether wildcats from the MA of France belong to a larger population extending into Germany where wildcats are also expanding (Stubbe & Stubbe, 2002; Klar *et al*., 2008), or are a distinct, well-established population remains in question. In addition, we were unable to assess the degree of differentiation between the Pyrenean population and those in the northeast, because there was an insufficient number of carcasses from the Pyrenees to describe genetic diversity in this area (Table 1).

Our findings show that the European wildcat is likely recovering in France and that there are three populations of interest (Pyrenean, central and north-eastern). This recovery is not due to hybrids. Both wildcats and hybrids were located throughout the MA and hybrids were not more

frequent in the expansion areas. Nevertheless, this recovery is not equal everywhere. Compared with historical data, we describe a recently colonized area in the NWB and suggest a decrease in their distribution within the Pyrenees. From a management point of view, the central and north-eastern populations, while genetically distinct, are connected and probably extend as far as south-western Germany. This whole area appears to be the area of major interest for conservation of the European wildcat. Linkage between the central and north-eastern populations ought to be promoted to allow sufficient levels of immigration and gene flow within the regional meta-population, thereby ensuring the long-term viability of these populations. Following the example of amphibians and reptiles (e.g. Woltz, Gibbs & Ducey, 2008), the use of wildlife corridors could be examined locally (e.g. Mata *et al.*, 2008; Glista, DeVault & DeWoody, 2009 for a review), particularly at the level of the highly human-dominated Paris–Lyon axis through Dijon and Auxerre, which seems to be the main barrier to genetic exchange between the French central and north-eastern populations of European wildcat. An experimental corridor study would also allow us to assess locally whether other carnivore species such as martens *Martes martes* and *Martes foina*, Eurasian badgers *Meles meles* and European polecat *Mustela putorious* might benefit from such enhanced functional connectivity.

An important and peculiar difficulty in protecting European wildcats is their capacity to hybridize with sympatric domestic cats. Identification of genetic units of wildcats may help focus management effort on control of feral cat populations in areas of major interest. For example, investigations using maternally inherited mitochondrial DNA markers could be undertaken to examine both sex-specific patterns of dispersal and colonization, and directionality of gene flow between these species (Gill, 1997) to develop local and specific sterilization measures for the sex responsible for introgression.

The apparent decrease in wildcats in the Pyrenees and the loss of connectivity with the central patch are worrying. More specific studies will be necessary to confirm our distributional data and assess the level of genetic diversity in this population. Protecting the genetic integrity of the European wildcat is essential. Enhancing our ability not only to distinguish European wildcats from feral cats and hybrids but also to identify spatial and genetic units at a broader scale remain ambitious but necessary challenges for the future.

Acknowledgments

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Appendix S1. Hybrids identification

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