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Conservation Genetics

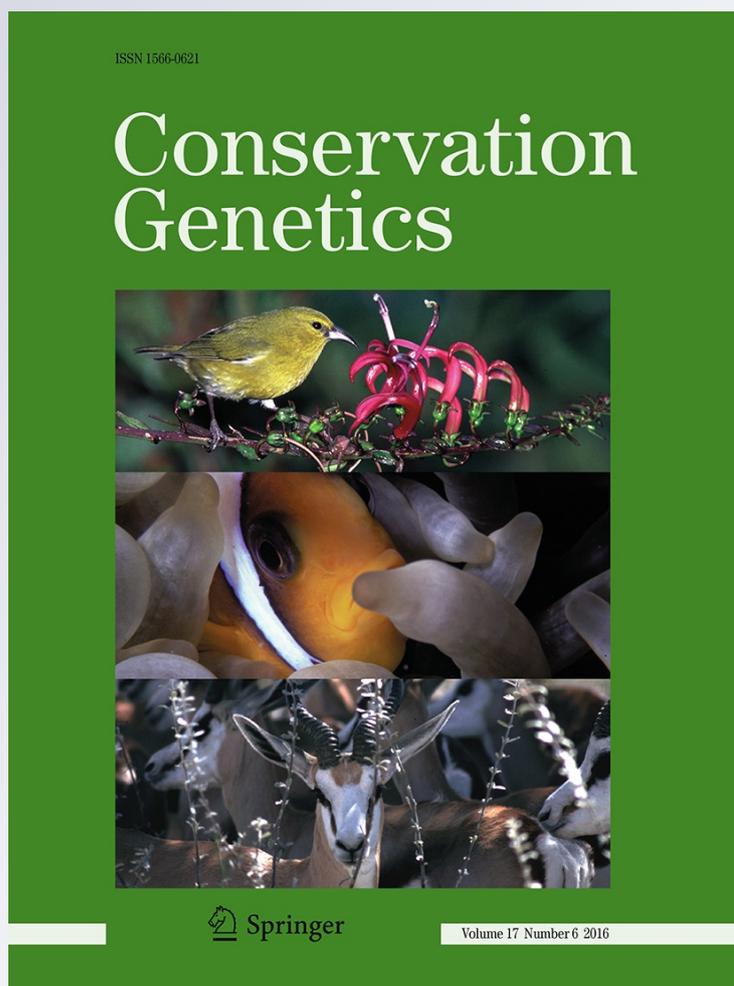
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RESEARCH ARTICLE

Female in the inside, male in the outside: insights into the spatial organization of a European wildcat population

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Abstract Hybridization between the European wildcat, *Felis silvestris silvestris*, and the domestic cat, *Felis silvestris catus*, has been found in several European countries with different landscape structures and in various proportions. In this study, we focus on a local population of European wildcats in forests fragmented by agricultural lands in northeastern France. Our aim is to better understand how the spatial organization of the wildcats in this particular type of environment might impact the proportion of hybridization. We combined radio-tracking and genetics through the use of microsatellite markers in order to assess both the spacing pattern and the level of hybridization of this wildcat population. Hybridization is rare in this wildcat population with only one putative hybrid (most likely backcrossed) detected out of 42 putative wildcats. We found that most females were concentrated inside the forest while males stood in the periphery or outside the forest. Furthermore, many males and females resulted related. Such a spacing pattern might limit contacts between male

domestic cats and female wildcats and can be one of the causes that explain the low level of hybridization in the wildcat population in this environment. We could not exclude the possibility of hybrid presence in the neighboring domestic cat populations. Our results yield new insights on the influence that the landscape configuration and the spacing pattern can have on genetic flow between the populations of the two subspecies.

Keywords *Felis silvestris silvestris* · Hybridization · Microsatellites · Kinship · Habitat fragmentation · Radio-tracking

Introduction

The European wildcat is a medium-sized carnivore that belongs to one of the five *Felis silvestris* sub-species, with a wide geographical distribution (Driscoll et al. 2007). For long time, humans have had conflictual relationships with the European wildcat (Stahl and Artois 1994; Inskip and Zimmermann 2009) that led to the reduction of their number. Nowadays, its range goes from Portugal (Oliveira et al. 2008) to Bulgaria (Petrov et al. 1992; Randi 2008) passing through Scotland (Daniels et al. 2001), and the species is classified as ‘Least Concern’ by the International Union for the Conservation of Nature (Driscoll and Nowell 2010). The most threatening species for the wildcat is the domestic cat *Felis silvestris catus*, which descends from the African wildcat *F. s. lybica* (Vigne et al. 2004; Driscoll et al. 2007). The domestic cat is increasingly appreciated as pet and always for its ability to control rodents (Loss et al. 2013) in rural environment, as it was in the past. In France, it is estimated that eleven millions of domestic cats are owned in 2012 (FACCO 2012) for 65 million inhabitants.

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This estimation does not take into account feral and stray cat populations. Both are domestic cats, the former are born in or have reverted to a wild state, while the latter have not had socialization with humans but live around rural properties (Bradshaw et al. 1999; Medina et al. 2014). The growing popularity of the domestic cat as pet increases the population of feral and stray cats directly through uncontrolled births that in turn increase uncontrolled adult feral and stray cats (e.g., Hellard et al. 2012). In rural habitats, both stray and feral cats might favor hybridization between the wild and the domestic cat.

Hybridization between these two sub-species has been found in several European countries in different proportions (Beaumont et al. 2001; Pierpaoli et al. 2003; Lecis et al. 2006; Oliveira et al. 2008; Hertwig et al. 2009; Say et al. 2012; Nussberger et al. 2014) and has probably led to the extinction of 'true' European wildcats in Scotland (Beaumont et al. 2001; Daniels et al. 2002). The heterogeneity of hybridization importance suggests that genetic compatibility is not enough to explain the occurrence of hybridizing events and that environmental parameters such as the landscape configuration might play a substantial role.

For hybridization to happen, individuals from the two sub-species have to meet. In France, the areas of distribution of the two sub-species are considered to overlap almost entirely (Léger et al. 2008). Encounters and hybridization are expected to occur during the mating season when both feral cats and wildcats have been observed to roam outside their usual home range in order to find a sexual partner (Corbett 1979; Ferreira et al. 2011). However, the assessment of the sub-species areas does not contain information on their spacing pattern and/or on the spatial relationships between males and females (and on the resulting mating system; Corbett 1979). Moreover, most studies focused on individual movements in their environment (Biró et al. 2004; Germain et al. 2008; Jerosch et al. 2010) but few considered the whole population and investigated how individuals interact (e.g., Klar et al. 2008).

Here, we analyzed the spacing pattern of a population of wildcats at a fine geographic scale in Lorraine (France) where the landscape is structured with fields and forests and which constitutes the historical area of distribution of the European wildcat in France (Léger et al. 2008). We combined genetic analyses and radio-tracking in order to, firstly, distinguish wildcats, domestic cats and hybrids and, secondly, to analyze the spacing pattern of the wildcats. Furthermore, we analyzed the genetic relationships of wildcats in order to understand if the kinship influenced their spatial distribution. Then, matching these results our aim was to assess whether the spacing pattern of this wildcat population may explain the observed rate of hybridization.

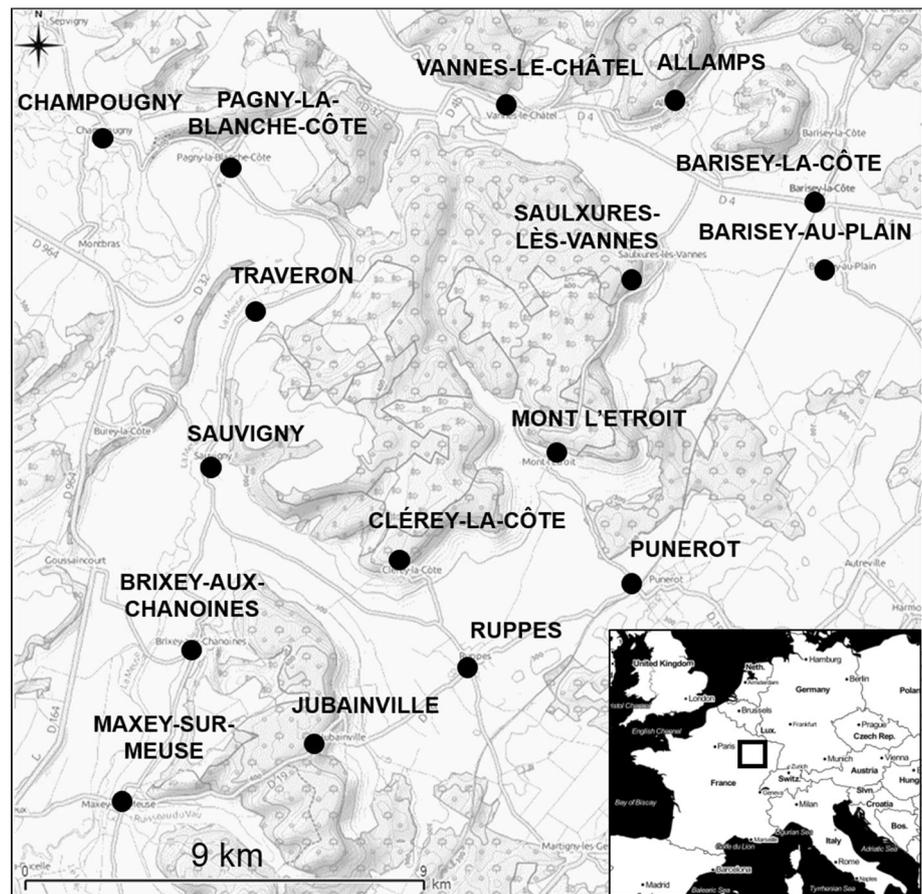
Materials and methods

Study area and data collection

The study took place in Northeastern France (5°45'51.0''E, 48°31'04.3''N) between April 2008 and May 2011. All the cats of the study were trapped and radio-tracked in an area of 130 km². We further used this area to assess the density of the wildcat population. The landscape consists of an alternation between forests, agricultural fields and permanent grasslands with an altitude comprised between 300 and 400 m in forest and between 250 and 300 m in the villages surrounding it. A total of sixteen villages, with a mean density of 35 inhabitants per km² (less than 600 inhabitants per village, 228 on average), were in direct proximity with the central forest where wildcats were sampled (Fig. 1). The fragmentation in this region was estimated as being substantial (Cemagref et al. 2010). The local climate is semi-continental. The precipitations are abundant and regular all along the year. The average temperature in summer is 18.5–19 °C. The average winter temperatures approach 1 °C in January.

The fieldwork has been conducted by qualified people according to current French legislation. Accreditation has been granted to the UMR-CNRS5558 (accreditation number 692660703) for the program. Cats were captured using trapping cages containing crushed valerian roots (*Valeriana officinalis*), a common attractant for cats. As it mimics cats' sexual secretions, valerian's use is bound to the mating season (Kilshaw et al. 2015). All captures were then made from November–February of each year. Trapped individuals were anaesthetized with ketamine chlorohydrate (Imalgène 1000, 15 mg/kg, Merial) and aceprozamine (Vétranquil 5.5 %, 0.5 mg/kg, Ceva). A permanent subcutaneous electronic device (transponder Trovan, AEG & Telefunken Electronic, UK) was injected in each cat to aid subsequent identification of each individual. Sex, age, pelage and morphological characteristics including body weight (using a hanging scale, ±0.3 %) and cranial measurements were assessed. A blood and hair samples were collected for further genetic analysis. We determined wildcats' age based on teeth and morphological characteristics. All cats presenting the typical wildcat phenotype (specific coat color; Leger et al. 2008) were classified as wildcats and this classification was confirmed or rejected based on the result of the genetic analysis. Individuals were released at the site of capture. Thirty-two wildcats (20 females, 12 males) were caught alive and the eighteen first caught individuals (14 females, 4 males) were equipped with VHF radio transmitters (Biotrack, UK). The dead bodies of ten wildcats (3 females, 7 males) were collected on the road following car crush accidents. Thirty cats with

Fig. 1 Distribution of the villages surrounding the wildcat population (*forests in grey*) where all the domestic cats were trapped (data from QGIS)



a typical domestic phenotype were captured using baited traps in the villages surrounding the forests. All cats were feral ($n = 19$, 14 females, 5 males) or domestic with free access to the outside ($n = 11$, 6 females, 5 males). The same protocol followed for wildcats was applied for domestic cats.

Genetic analysis: subspecies characterization and kinship determination

The forty-two wildcats were genotyped using thirty-one microsatellites and the thirty domestic cats using twenty-five (Menotti-Raymond et al. 1999; O'Brien et al. 2009; see Supplementary Table S1). DNA extraction was performed using a purification column kit (Nucleospin 96 Tissue kit, Macherey–Nagel). DNA was then amplified and analyzed using an ABIPRISM 3130XL Applied Biosystem DNA sequencer. Results of sequencing were read using GeneMapper v.4.1 (Applied Biosystem/Life Technology).

The genetic identification of the species to which each individual belonged was performed by employing the Bayesian analysis implemented in STRUCTURE v.2.3 (Pritchard et al. 2000; Falush et al. 2003) based on the twenty-five microsatellites genotyped for both the wild and

domestic cats. The analysis was performed under a model allowing admixture and using no prior information of phenotypic classification. Individuals were then assigned to the *F.s. silvestris* or to the *F.s. catus* clusters according to the posterior probabilities obtained. We performed twenty independent runs, each with values of K assumed genetic groups ranging from 1 to 4. The expected value of K was 2 but we ran K to higher values to discard the eventuality of a third potential hybrid cluster. According to Gilbert et al. (2012)'s suggestions, we performed 500,000 Markov Chain Monte Carlo iterations after a burn-in period of 100,000. The convergence of the algorithm was checked visually using STRUCTURE run-time plots. We determined the number of clusters following the method of Evanno et al. (2005) using STRUCTURE HARVESTER online web 0.6.94 (Earl and VonHoldt 2011) after running CLUMPP v.1.1.2 (Jakobsson and Rosenberg 2007) in order to obtain an average matrix over the twenty simulations. We considered as hybrid any individual for which the posterior assignment probability was lower than 0.91 according to the maximum threshold used for the detection of hybrids in wildcats (between 0.80 and 0.91; Mattucci et al. 2013; O'Brien et al. 2009; Oliveira et al. 2008). Since our aim was to check whether individuals morphologically

classified as wildcats were truly wildcats, we chose the highest threshold in order to be conservative. For each population, deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were tested using FSTAT v.2.9.3.2 (Goudet 1995). The presence of null alleles or other scoring errors was evaluated using MICRO-CHECKER v.2.2.3. (Van Oosterhout et al. 2004). We estimated F_{st} values according to Weir and Cockerham's (1984) version of Wright's F-statistic using GENETIX v.4.05.2 (Belkhir et al. 1996).

We used ML-RELATE (Kalinowski et al. 2006) software and estimated genetic relationships between all dyads of wildcats, based on 31 microsatellites. This program uses the maximum likelihood estimate of relatedness between pairs of individuals to discriminate between the relationships: Unrelated (U), Parent-Offspring (PO), Full sib (FS), Half sib (HS). We tested all assignments to one of these categories compared to the alternative ones using 1000 simulations, which gave us a probability that the determined category is the correct one (see supplementary Table S2). We discarded any pairwise relationship for which the p value of the determined category against unrelated (U) was higher than 5 %. When two individuals were categorized as FS and when their age differed by at least one year, kinship was assumed to be PO. In order to test whether relatives are geographically closer together compared to non-relatives, we calculated the pairwise geographic distance between individuals using the program SpaGeDi (Hardy and Vekemans 2002). Then, we statistically assessed if the pairwise distance between relatives was significantly different from the pairwise distance between non-related individuals using a Mann–Whitney test. For this spatial analysis, we discarded seven individuals out of forty-two for which we did not record the coordinates with a GPS.

Spatial organization and home-range analysis

We located each of the 18 equipped wildcats at least once per week (mean 1.24, SD 0.18) by triangle procedure using hand-held antenna. Individuals were monitored all along the year, including during the mating season (November–February). For each individual, we had a number of locations over a year comprised between 52 and 81. Locations were positioned on a map using Quantum GIS (Quantum GIS Development Team 2012) and the plugin open layers available in the software. In order to assess the minimal distance between each village and the locations recorded, we calculated the distance matrix with QGIS between each location and the periphery of urban areas. Urban areas were previously defined by building a polygon circling all the infrastructures inside each village. A random distribution of 1546 locations was simulated. A distance matrix was

also calculated and further compared to the distances obtained with our dataset with a Mann–Whitney test. We also estimated the number of locations monitored within the forests. Only the forests, and not the wooded local areas such as field borders, were taken into account for this calculation. We used the package adehabitatHR (R Development Core Team 2010; Calenge 2011) to estimate the annual home-range size using the minimum convex polygon estimator (MCP). We set the percentage parameter to 95 % as usually recommended in order to overcome possible bias due to an occasional displacement of the individual outside its home-range. Only individuals spotted in the same area all along their monitoring were taken into account. We did not have enough locations to establish the accurate home-range size, which would be comparable with other studies, except from one male (M2, see Supplementary Fig. S3). However, the estimations obtained allowed us to compare home-range sizes of individuals within this study. We chose to compare only adults over a period of one year (see Supplementary, Fig. S4).

Results

Genetic characterization of the two subspecies

More than 50 % of the loci was amplified in all domestic and wildcats, consequently they were all included in STRUCTURE analysis. The optimal value of K was 2 (see Supplementary, Figs. S5, S6). All individuals classified as wildcats according to their external phenotypic characteristics were assigned to one cluster with posterior probabilities greater than 0.8 while all presumed domestic cats were allocated to the second cluster with probabilities greater than 0.97. Then, the phenotypic classification coincided to the wildcat genotype. The two clusters were highly differentiated with an F_{st} value of 0.16 (CI at 95 % 0.12–0.2). All domestic cats were trapped outside the forest, at the edge or inside the villages (see Supplementary, Fig. S7).

Among the forty-two wildcats, only one was identified as hybrid (F9C) with a posterior probability of 0.82 for the wild cluster in STRUCTURE. This individual was discarded from the following analyses (see supplementary, Table S8 and Table S9 for detailed information about individuals used in the different analyses). Therefore, this population of wildcats was mostly constituted of 'true' wildcats and was not a swarm of hybrids. We did not detect neither departure from Hardy–Weinberg equilibrium for any of the loci nor linkage disequilibrium both in the domestic and wildcat populations. Analysis with MICRO-CHECKER did not indicate the presence of null alleles in the wildcat population, but it suggested the presence of null alleles at 3 loci (Fca45, Fca96 and Fca577 with estimated

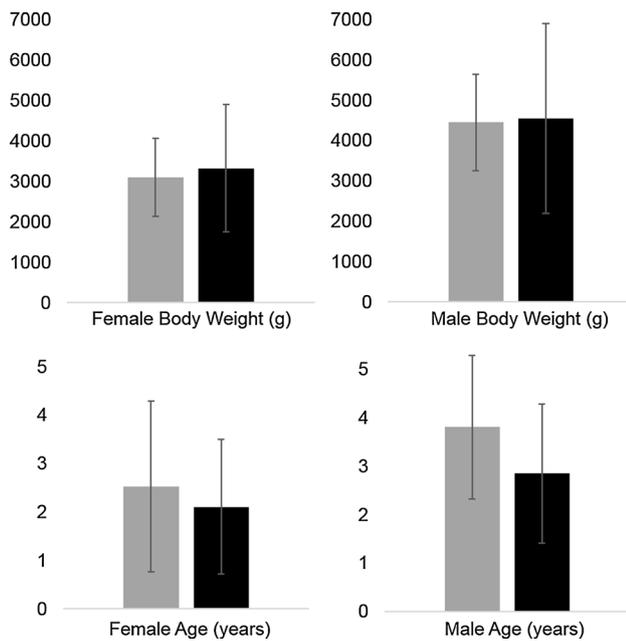


Fig. 2 Body weight and age according to the sex of individuals and their sub-species (*Grey Felis s. catus*; *Black Felis s. silvestris*). Errors bars stand for standard deviation

null allele frequencies of 0.077, 0.13 and 0.10, respectively) in the domestic cat population.

We found no significant difference in body weight between domestic and wildcats (Fig. 2). The age of trapped domestic cats ranged between 1 and 7 years old while for wildcats, the estimated age varied between 1 and 4. These two variables, body weight and age, were strongly correlated (Spearman rank correlation test, $Rho = 276.46$, $p = 0.00085$).

Kinship analysis

We found that 90 % of the wildcats were related to at least another individual (Table 1). Most of the pairwise relationships identified (79.2 %) were poorly defined (corresponding to HS) and the remaining ones were equally distributed between PO and FS relationships. Fifty per cent of the pairwise kinships involved two females, 34.9 % a male and a female, and 15.1 % involved two males. Males were related with less than two individuals on average (mean 1.89, SD 1.633) while females had three relatives on average (mean 3.23, SD 1.66). The lower number of relatives for males was significant (Mann–Whitney test: $w = 309.5$, $p = 0.0075$). We found three males (M22, M29, M33) and one female (F13) who were not related to any other individual.

Relatedness appeared to be correlated with the pairwise geographic distance only when considering female-female related couples. The existence of parentage between two females (PO, FS and HS taken altogether) was indeed correlated with a smaller geographic distance between those

females ($w = 935.5$, $p = 0.0029$) while it was not significant when considering related couples involving males ($p = 0.69$). This resulted in the occurrence of ‘related units’ made of related females with neighboring home-ranges (see Fig. 3a). Two individuals belonged to the same related unit when, in addition to being geographically close, they were strongly related (PO/FS). HS relationships allowed an individual to be considered as part of a related unit when the HS kinship was retrieved for several individuals of the unit. No related units that included adult males were observed.

Spatial organization of the population

The density in our area was estimated to 0.32 cats per km². Wildcats were not homogeneously distributed across the massif. They remained significantly further from the villages than by chance ($w = 167$, $p < 0.0001$). On average, the minimal distance between a cat and a village was of 1 km (SD 0.73, min 52 m, see Supplementary, Fig. S10). Also, 92 % of the locations were monitored inside the forests, forests that occupied about 28 % of the area of study. All the fourteen females radio-tracked had settled inside the forest with some overlapping between their home ranges; some of the home-ranges included the ecotone between the forest and the crops (Fig. 3b). Only one female (F23) was caught clearly outside the forest (see Fig. 3c). Home ranges of males were systematically on the border of the forest, between two forests (M2) or outside the forest (M11). No male wildcat was captured in the heart of the forest. During the time of the study two males (M4, M17, see Fig. 3d) changed their home range from year to year because they were probably dispersing.

One male over the four died during the monitoring so only three equipped males had enough locations to allow home-range size estimation, but two of them (M4, M17) were dispersing during the study. For male M2, both a global home-range (over the whole period of radio-tracking) and an annual home-range (year 2009) were calculated, the latter for comparison with those of females. Eight females matched the requirements defined (i.e., enough locations over a complete year, see Supplementary S8, S9). The annual home-range size estimated for the male M2 (422.11 ha) was larger than all estimated female annual home-range sizes (mean 130.82, SD 39.82, see Fig. 3b). The global home-range size of the male M2 was 347.57 ha.

Discussion

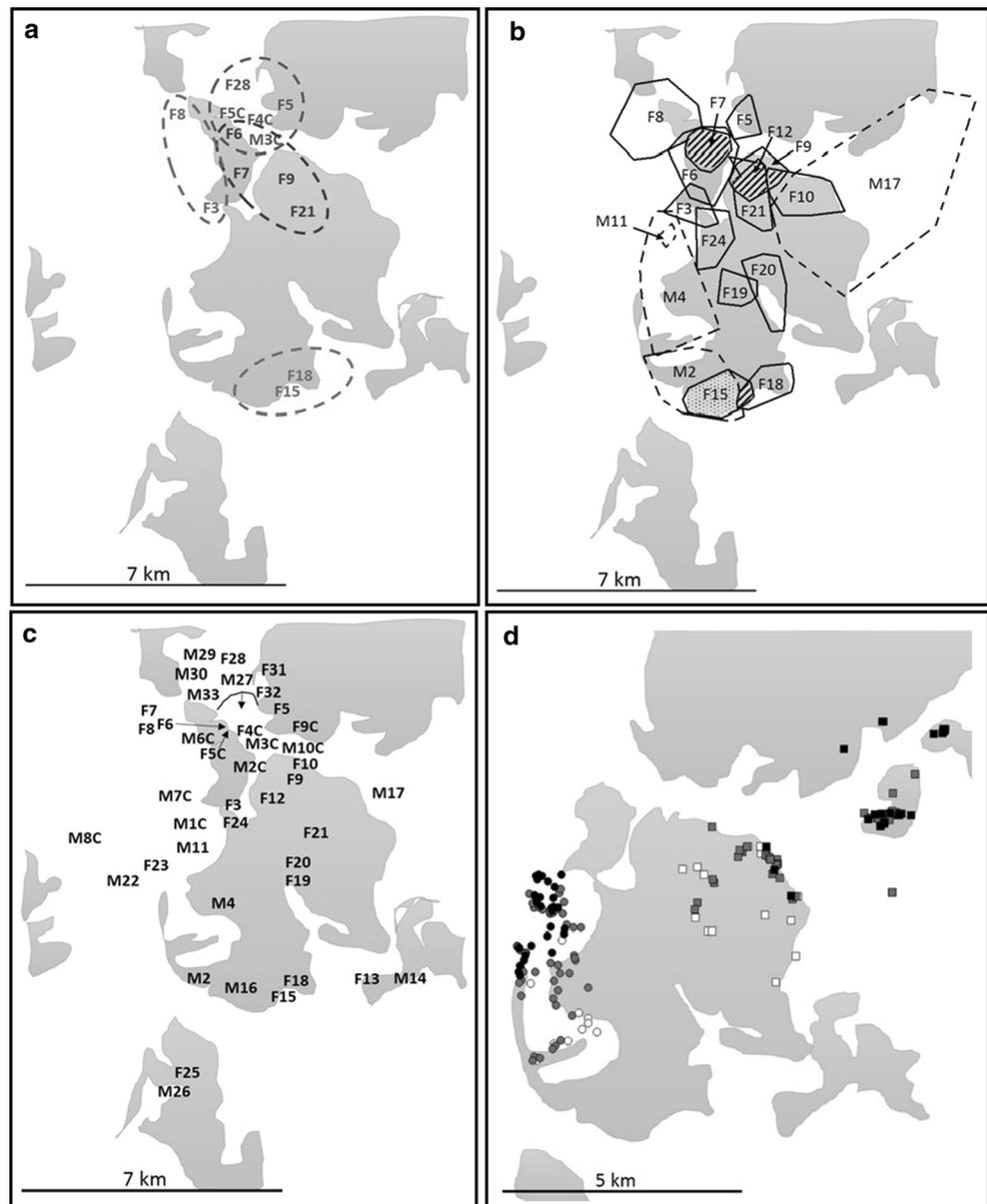
The combination of genetic analyses and radio-tracking of the wildcats allowed us to estimate the level of hybridization in this environment and shed light on

Table 1 Related individuals classified depending on the kind of kinship relation for each wildcat by the software ML_RELATE

Ind.	PO	FS	HS
F4C	F5C (P)	M3C	F28, F5, F7, M11
F5C	M3C (C), F4C (C)		F28, F5, M26
F3		F8	M11
F5			M2, M3C, F4C, F5C, F28, F32
F6	F7(C), F9(?)		F20, F21
F7	F6 (P)		F9, F4C, F31
F8		F3	F24
F9	F6(?)		F7, F21, F31
F10			F15, F28, F31
F12	M17 (P)		F28
F13			
F15	F18 (C)		F20, F10
F18	F15 (P)		F24,
F19			M2C, M26
F20			F19, F6, F15
F21			F6, F9
F23			F25, M27
F24			F8, F18
F25			M30, F23, M14
F28			F5C, F5, F10, F12, M3C, F4C, M8C
F31		M2	F7, F9, F10
F32			F5, M2, M26
M1C		M4	
M2C			F19
M3C	F5C (P)	F4C	F28, F5, M11
M6C			M14
M7C		M10C	M8C
M8C			F28, M10C, M7C
M10C		M7C	M8C
M2		F31	F32, F5
M4		M1C	
M11			M3C, F4C, F3
M14			M6C, M27, M30, F25
M16			F24
M17	F12 (C)		
M22			
M26			F5C, F32, F19
M27			F23, M14
M29			
M30			F25, M14
M33			

The sex of each individual is indicated by F for females and M for males. For each individual, we indicate if he is linked by a PO, FS or HS relationship with any individual. The direction of the PO relationships is given in parenthesis: C mean "Child", P means "Parent", "?" means that the determination of the age was not precise enough to draw any conclusion. F15 and F18 individuals were initially assigned to FS by ML_RELATE, we set their kinship to PO as one was fully adult (F15) and the other one juvenile (F18)

Fig. 3 Spatial organization of the wildcat population. Forests are represented in grey. White areas are composed of fields and urban areas. **a.** Representation of the four main related units identified by the kinship analysis. Only the thirteen females or kittens defining these units are represented. Each unit is shown by the *dotted circle* that encloses the individuals of the unit. The four units are: F3-F8, F6-F7-F9-F21, F5-F28-F5C-F4C-M3C, F18-F15. **b.** Home-range represented by 95 % minimum convex polygons for individuals (15 individuals) that did not die during the monitoring for males (*dotted lines*) and females (*solid lines*). Substantial overlapping between females (*hatching areas*) and between a male (observed only for the male M2) and females (*dotted areas*) are represented. **c.** Trapping locations of all the wildcats. **d.** Movement per semester of the two migrating males. One (M4) is shown by *filled circles*, the other one (M17) with *filled squares*. For both the monitoring lasted three semesters. *White spots* represent the first semester, *grey spots* the second semester and *black spots* the last one



remarkable features of the spacing pattern that might explain the low proportion of hybrids.

Density and individual spacing pattern

Our wildcat population density ($0.32 \text{ cats km}^{-2}$) falls within the values found in other areas ($0.16\text{--}0.44 \text{ cats km}^{-2}$, Dimitrijevic 1980; $0.17\text{--}0.25 \text{ cats km}^{-2}$, Heller 1992; Okarma et al. 2002, $0.1\text{--}0.13 \text{ cats km}^{-2}$) and it is coherent with studies carried out in similar landscapes in France ($0.2\text{--}0.5 \text{ cats km}^{-2}$; Stahl and Léger 1992) and in Switzerland ($0.35 \text{ cats km}^{-2}$; Weber et al. 2008). This density might be underestimated, as it was not possible to trap all individuals in spite of an important capture effort.

Our results showed substantial differences in the spacing pattern between females and males. We found that all the females concentrate inside the forest. Females are known to be highly territorial (Biró et al. 2004) and the rarity of cases of overlapping in the population studied here confirms this statement. Most female home ranges allow direct access to agricultural fields or grasslands (see Fig. 3b, Fig.S7). While forests provide a shelter against many different threats, fields are food-rich areas attracting rodents that cats can hunt. Such alternating between resting sites and hunting grounds has been previously proposed to be the optimal environment for wildcats (Lozano et al. 2003).

On the contrary, we found that all the males were trapped and/or located with telemetry outside or in the

periphery of the forest all year round. Unfortunately, we do not have enough data on male home ranges, but the fact that no males were captured or detected within the forest raises questions about the observed population spatial structure. The greater dependence of females on forest compared to males has also been reported in a quite similar habitat in Germany by Klar et al. (2008). The pattern we observe might be related to different requirements between sexes. Availability of prey might be more important in males than the protection provided by the forest cover. On the opposite, females that do all the rearing of offspring might privilege shelters and forest ecotones often richer in small prey abundance and diversity than interior forest (Doyle 1990; Gomez and Anthony 1998; Osbourne et al. 2005). Finally, as expected (Daniels et al. 2001; Biró et al. 2004), males have probably larger home-range than females and may include several female home-ranges, but the shortage of data on males does not allow us to confirm this pattern (see Fig. 3b). The home-range (347.57 ha over the whole monitoring period-422.11 ha over one year) of the only male (M2) included in our analyses was three times larger than that of females and overlapped part of the home-ranges of two females (F15, F18). Its size falls within the range (from 170 to 1000 ha) found in the literature (Corbett 1979; Stahl 1986; Daniels et al. 2001; Biró et al. 2004; Piñeiro and Barja 2011; Kilshaw et al. 2015).

Kinship structure

The wildcat population exhibited strong relatedness but the structure differed in males and females. If most females resulted to be related among them and to males, males were not related among them. In addition, related females tended to remain in the same area while kinship did not affect the spatial distribution of males: related and unrelated males were found at comparable distances. This pattern might reflect a tendency in males to disperse more and over larger distances than females (if they disperse). The hypothesis is reinforced first by the high percentage of males (70 %) killed from collisions with vehicles, then by the fact that the two individuals who changed their home range year after year were both males. Such a male-biased dispersal behavior is a common feature in felids (Pusey and Packer 1987; Janečka et al. 2007; Croteau et al. 2010) and in particular in domestic cats (Devillard et al. 2004; Hansen et al. 2007).

We did not find a clear pattern between relatedness and home ranges overlapping in females. We observed four cases of substantial overlapping between females. In one case the two females (F9 and F10, four and three years old, body weight of 4 kg and 3.1 kg, respectively) were not related. The overlapping lasted only one spring during which two other neighboring females were located outside the usual area where they had been monitored until then.

This might reflect a temporarily environment disturbance more than a relevant behavioral pattern. In the three other cases home range overlap was observed between related females. Two of them occurred most probably between a mother and her daughter (F6–F7, F15–F18) but the kinship for the last one was unclear (F9–F21). In each case the daughter was a young adult (1 year old) and a kitten (not trapped) was observed close to one of the daughters. These observations suggest that prey might be abundant enough in this habitat resulting in greater tolerance for overlap between females and their relatives.

Hybridization

We found only one putative hybrid, most probably back-crossed, out of the 42 putative wildcats, suggesting a negligible impact of hybridization on this area (2.3 %). No hybrid individuals were detected in the 30 domestic cats. Our local hybridization rate confirmed what was found at a larger scale in France (O'Brien et al. 2009), and is among the lowest rates found in Europe (from 2.1 % in Italy, Randi et al. 2001, to 26.1 % in Hungary, Pierpaoli et al. 2003). Such a low hybridization rate has been found in similar environments in eastern Germany (4 %, Hertwig et al. 2009) and Italy (8 %, Lecis et al. 2006). Previous studies have found various levels of differentiation between wildcats and domestic cats (F_{st} varying from 0.11 to 0.12: Hertwig et al. 2009; Beaumont et al. 2001; Pierpaoli et al. 2003; Randi et al. 2008; to 0.20–0.22: Oliveira et al. 2008; Mattucci et al. 2013). The differentiation ($F_{st} = 0.16$) we found in our study between wildcats and domestic cats is then substantial when compared to what has been observed in various locations and confirms that hybridization is rare in our study area.

In this type of environment, crops act as “corridors” between the forest and the villages, allowing physical meeting between domestic and wildcats. During the same three-year study (2008–2011), 474 domestic feral and stray cats have been captured in the different villages surrounding the forests (Hellard et al. 2012). Both subspecies share the same diet based on rodents (Sarmiento 1996; Malo et al. 2004; Germain et al. 2009; Piñeiro and Barja 2011) and both utilize agricultural fields as hunting grounds (G. Leblanc, personal observations). Furthermore, since the breeding season of the two sub-species partially overlap (November–February for the wildcats; February–September for the domestic cats, Condé and Schauenberg 1974; Gagnon and Dantzer 2013), conditions for extensive hybridization would be met in this environment.

The specific wildcat population spacing pattern, with a sex-biased distribution of the wildcats inside/outside the forest (females inside and males on the edges) might limit mating between female wildcats and male domestic cats. In

addition, male wildcats are known to be strongly territorial with large territories encompassing smaller female ones (Klar et al. 2008). Although we did not have enough male wildcats monitored to confirm this pattern, male wildcats can be effective to limit the possibility of mating between male domestic cats and female wildcats living in their territory. On the contrary, we cannot exclude that male wildcats can mate with female domestic cats. Such asymmetry in the hybridization pattern has been suggested for wildcats in Swiss Jura (Nussberger et al. 2014) based on mtDNA analysis. No wildcat phenotype was recognized in the captured 474 domestic cats, but genetic analyses are going to be achieved to test for this hypothesis in the near future (Beugin et al. in preparation).

Conclusion

To conclude, the radio-tracking of a local population of wildcats combined with genetic analysis allowed us to shed light on the spacing pattern of the wildcat in a mixed forest/crop habitat, never described previously. Males' kinship was uneasy to trace, probably due to their high dispersal over long distances, while we identified related females living in close proximity, suggesting a phylopatric behavior. Although the data is not sufficient to draw definitive conclusions, the pattern found can partly explain why the level hybridization is low in France, despite the claimed complete overlapping of wild and domestic cat areas. Our results suggest that to understand the underlying mechanisms responsible for hybridization in a species it is essential to analyze the situation at a fine geographic scale, which put in evidence possible barriers between the subspecies due, for example in this case, to habitat choice. More studies combining genetics and animal radio-tracking should be led in order to assess how unique this spatial organization is, how much it depends on the landscape structure and to investigate further if it limits the risk of hybridization in the wildcat.

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Compliance with ethical standard

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

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