JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH

Accepted on 1 October 2013
J Zoolog Syst Evol Res doi: 10.1111/jzs.12049

[1]*Université de Lyon, F-69000, Lyon; Université Lyon 1; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622, Villeurbanne France;* [2]*MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College - School of Public Health, London UK;* [3]*Office National de la Chasse et de la Faune Sauvage, Montfort, Birieux, France*

How reliable are morphological and anatomical characters to distinguish European wildcats, domestic cats and their hybrids in France?

Sébastien Devillard[1], Thibaut Jombart[2], François Léger[3], Dominique Pontier[1], Ludovic Say[1] and Sandrine Ruette[3]

Abstract

Phenotypic variation in hybridizing species or subspecies is a prerequisite for allowing conservation ecologists and wildlife managers to identify parental populations and their hybrids in the field. We assessed the reliability of a set of eight morphological (body size and pelage characters) and four anatomical criteria (skull and intestine morphometric measurements) to distinguish between 302 French specimens classified as wildcat, domestic cat or hybrid on the basis of a Bayesian analysis (STRUCTURE) of their multilocus microsatellite genotypes. This aim was achieved by performing a set of multivariate analyses on morphological, anatomical and genetic data sets (Hill and Smith's analysis, co-inertia analysis and discriminant analysis of principal components). Wildcats and domestic cats were very satisfactorily distinguished, even when using simple non-invasive morphological criteria easily usable in the field like the morphology of the tail, dorsal line or flank stripes. Using anatomical instead of morphological characters slightly increased the discriminating power. Many more difficulties arose when we tried to distinguish hybrid specimens from both wildcat and domestic ones. Anatomical characters performed better than morphological ones in recognizing hybrids, but the assignment success rate remained very low, about 31.6% and 1.5%, respectively. Overall, the most discriminating characters were two continuous, derived anatomical characters: the cranial index followed by the intestinal index. Classification of specimens in three classes based on their microsatellite genotypes appeared to be inadequate for identifying hybrid specimens, as hybrid specimens seemed to be distributed along an anatomical continuum. With this observation in mind, we assessed the linear relationships between a proxy of the individual level of hybridization (q_{ik}) and the cranial and intestinal indices, respectively. Both relationships were highly significant. The greatest correlation was found with the cranial index (R^2 = 60.4%). Altogether, our results suggest that future work should be geared towards enhancing the measure of hybridization using more discriminating molecular markers and improving morphometric skull measurements through the use of modern geometric morphometric methods, using landmarks rather than skull dimension.

Key words: Hybridization – multivariate analysis – morphometry – wildcat – *Felis silvestris silvestris*

Introduction

The role of hybridization in the evolution of living organisms has been extensively discussed among evolutionary ecologists (e.g. Arnold 1992; Dowling and Secor 1997; Barton 2001; Fitzpatrick 2004). Strikingly, interspecies hybridization can facilitate evolutionary diversification in both plants and animals, including the origin of new species (Rieseberg 1997; Arnold 2004; Grant et al. 2005) so that hybridization can lead to evolutionary innovation and even speciation, especially via the production of novel genotypes/phenotypes (Anderson and Stebbins 1954; Barton and Hewitt 1985; Allendorf et al. 2001; Rieseberg et al. 2003). However, when hybridization is driven by anthropogenic changes (e.g. invasive species, domestication, habitat loss and fragmentation), that is, human-induced hybridization, it might become a conservation concern. Non-natural hybridization may have little effect on the genetic integrity of wild populations when it occurs in a narrow zone between two common, geographically widespread species (Barton and Hewitt 1985). However, in cases of already rare or endangered populations, hybridization can also result in the genetic swamping of the rare population by the main one (Rhymer and Simberloff 1996). Understanding this phenomenon is of prime importance not only to assess its evolutionary relevance, but also as a conceptual basis for designing adequate conservation strategies for endangered populations showing signs of admixture with other taxa. It is still unclear how threatening the human-induced hybridization really is and whether some zoological groups or biogeographical regions may be more prone to foster such processes.

Several cases of hybridization threats involving terrestrial carnivores have been reported in canids (American wild canids, Lehman et al. 1991; Wayne and Jenks 1991; Roy et al. 1994; Reich et al. 1999; Miller et al. 2003; Fredrickson and Hedrick 2006; Hailer and Leonard 2008; and domestic dogs *Canis familiaris*/wild canids, Gottelli et al. 1994; Vila and Wayne 1999; Randi and Lucchini 2002; Elledge et al. 2008; Hindrikson et al. 2012), in mustelids (Davison et al. 1999; Lodé et al. 2005; Cabria et al. 2011) and in felids (Beaumont et al. 2001; Lecis et al. 2006; Oliveira et al. 2008a,b; O'Brien et al. 2009; Schwartz et al. 2004; Homyack et al. 2008; Trigo et al. 2008) involving or not the domestic cat *Felis silvestris catus*. The hybridization complex between the domestic cat and the European wildcat *F. s. silvestris*, as well as some cases involving domestic dogs, polecats *Mustela putorius* (Lodé et al. 2005), American mink *Neovison vison* (Kidd et al. 2009; Tamlin et al. 2009), red fox *Vulpes vulpes* (Sacks et al. 2011) or arctic fox *Alopex lagopus* (Noren et al. 2009), describes situations in which the hybridizing populations are conspecific, that is, domestic and wild forms of the same species. Such situations are especially difficult to assess because genetic and morphological differences between subspecies or domestic and wild forms are less clear-cut than in separate species, hindering the detection of hybrids.

The European wildcat is widely distributed in Europe, ranging from Eastern Europe to Portugal and from Scotland to Italy, with the notable exception of Scandinavia (Nowell and Jackson 1996), but its detailed geographical distribution is only known in France (Say et al. 2012). For 25 years, its hybridization pattern

Corresponding author: Sébastien Devillard (sebastien.devillard@univ-lyon1.fr)
Contributing authors: Thibaut Jombart (t.jombart@imperial.ac.uk), François Léger (francois.leger@oncfs.gouv.fr), Dominique Pontier (dominique.pontier@univ-lyon1.fr), Ludovic Say (ludovic.say@univ-lyon1.fr), Sandrine Ruette (sandrine.ruette@oncfs.gouv.fr)

with feral domestic cats had been assessed through molecular approaches in numerous places within its distribution area (French et al. 1988; Hubbard et al. 1992; Daniels et al. 1998; Beaumont et al. 2001; Daniels and Corbett 2003; Pierpaoli et al. 2003; Kitchener et al. 2005; Lecis et al. 2006; Oliveira et al. 2008a,b; Hertwig et al. 2009; O'Brien et al. 2009; Eckert et al. 2010). Varying degrees of hybridization have been found, suggesting that the risk of hybridization is not uniform throughout the continent or across habitat types. Unfortunately, these studies used different methods, making a formal comparison of hybridization patterns difficult. Overall, it seems that the concept of 'pure' wildcat should be ruled out mainly due to the old sympatry and interbreeding history of these two subspecies (Daniels and Corbett 2003), but that some genetically distinct populations of cats having the European wildcat phenotype and functional ecology persist in Europe (Daniels and Corbett 2003; Germain et al. 2008, 2009; O'Brien et al. 2009; Say et al. 2012). As a prerequisite for the conservation of such populations, relevant diagnostic tools for the correct identification of the three morphs are needed (domestic, hybrid and wildcat, Reig et al. 2001). Ideally, these tools should be based on simple, non-invasive morphological criteria easily usable and recordable in the field rather than on anatomical criteria, needing corpses to be analysed in the laboratory. Morphological and anatomical characteristics have been historically used to describe the 'typical' wildcat, more recently in combination with molecular data, for identifying wild, domestic and hybrids forms in museum collection or in field work (Schauenberg 1969, 1977; Piechocki 1990; Puzachenko 1996; Yamaguchi et al. 2004; Kitchener et al. 2005; Krueger et al. 2009; Platz et al. 2011). However, both morphological (e.g. pelage shape and colour) and anatomical criteria (e.g. skull morphometry) appeared variable across study sites, and the main pitfall remains the identification of hybrids (e.g. Krueger et al. 2009).

Here, we used a sample of 302 specimens (O'Brien et al. 2009; Say et al. 2012) for which we compiled both the multilocus microsatellite genotype and a combination of morphological characters (pelage, body size), anatomical characters (cranial and intestinal morphometry) and their derived indices to assess the reliability of these criteria to identify domestic, hybrid and wildcat specimens. The overall approach was to use as a reference the genetic classification established on multilocus genotyping for comparing the power of morphological and anatomical characters to assign specimens in these reference groups, as for example, Krueger et al. (2009) did, instead of defining reference groups with morphological and anatomical characters (e.g. Platz et al. (2011)). More precisely, we investigated two different topics:

1) Assessing the correlation between morphological, anatomical and genetic data, as a prerequisite of the general approach described above;

2) Investigating the power of morphological and anatomical characters for assigning specimens into a well-established and genetically based three-group classification.

Material and methods

Sampling and genetic classification of specimens

Specimens (n = 341, entire corpses, skulls or tissue samples only) were collected as road-killed free-living tabby cats (possibly *F. s. s.* and *F. s. c.*) in north-eastern France (covering an area of 142.000 km² from 1994 to 2006 by the French National Agency for Wildlife (Office National de la Chasse et de la Faune Sauvage, ONCFS, Say et al. 2012). The genetic classification of these n = 341 specimens as *wild*, *domestic* and *hybrid* cats based on multilocus microsatellite genotypes (12 microsatellite loci) was made following the approach previously described in detail in the study by O'Brien et al. (2009) and used after in the study by Say et al. (2012). This approach allowed us to attribute to each specimen a q_{ik} value ranging from 0 to 1 and quantifying the proportion of the multilocus microsatellite genotype of each specimen belonging to the wildcat genetic cluster determined by the STRUCTURE software (Pritchard et al. 2000). q_{ik} values can thus be interpreted as a measure of the individual levels of hybridization (Supporting Information SM1). As we added 75 new specimens, and hence genotypes, in the data set compared with Say et al. (2012), we redid the overall analysis of the genetic classification, including the assessment of the power of admixture analysis and the threshold determination. From this new analysis, specimens having a q_{ik} value higher than 0.870 were classified as wildcats, those having a q_{ik} value lower than 0.086 were classified as domestic cats, and intermediate values of q_{ik} defined hybrid specimens (thresholds obtained from simulation study, Supporting Information SM1).

Morphological and anatomical characterization of specimens

For n = 39 specimens, we only recovered tissue sample, and these specimens were excluded from subsequent analyses due to the lack of morphological and anatomical information. Consequently, the initial data set for this study was n = 302 specimens (117 females, 174 males and 11 unsexed). These n = 302 specimens were genetically classified as follows: 190 wildcats, 36 domestic cats and 76 hybrids. Whenever possible, specimens were characterized on eight morphological (i.e. external) and five anatomical (i.e. internal) characters (Daniels et al. 1998; Yamaguchi et al. 2004; Krueger et al. 2009; Table 1). From these anatomical characters, we first computed the cranial index *ci*, which has been proven to be a reliable discriminating variable (e.g. Schauenberg 1969, 1977; Krueger et al. 2009). Second, we corrected the intestinal length for differences in body size across specimens by dividing it by the head + body length *hbl* to derive the standardized intestinal index *ii* (Schauenberg 1977). Specimens were thus characterized by four anatomical characters in subsequent analyses (Table 1). Note that sometimes the state or the unavailability of the entire corpse (i.e. only the skull was available) did not allow us to record all characters for all the 302 specimens used here. Consequently, the initial data set for this study (n = 302 specimens) had to be reduced in size depending on the analysis. The complete list of specimens is given in Supporting Information SM2.

Statistical analysis

Relating morphological and anatomical characters to multilocus genotypes using co-inertia analysis

Because of the substantial number of correlated explanatory variables (eight morphological characters and four anatomical characters, Table 1), we used multivariate analyses to characterize specimens. To test for a relationship between morphological/anatomical and genetic data, two separate co-inertia analyses (COIA, Dolédec and Chessel 1994; Dray et al. 2003) were used: (1) morphological characters and genetic data ($COIA_{morpho/genet}$, n = 270) and (2) anatomical characters and genetic data ($COIA_{anat/genet}$, n = 68). For that purpose, we performed a Hill and Smith' analysis (HSA, Hill and Smith 1976), allowing us to combine quantitative and qualitative morpho-anatomical variables in a principal component analysis (PCA), on both morphological (HSA_{morpho}, n = 270 specimens) and anatomical (HSA_{anat}, n = 68 specimens) data sets. As *Felis silvestris* species are sexually dimorphic (Krueger et al. 2009), we removed a possible 'sex' effect by taking the residuals of the ANOVA of each variable predicted by sex. We also performed a principal component analysis (PCA) on allelic frequencies on the corresponding genetic data set ($PCA_{genet/morpho}$ and $PCA_{genet/anat}$). We then linked HSA and PCA through COIA (Dray et al. 2003). The global relationships between the two matrices in COIAs were quantified using the RV coefficient (i.e. a multivariate equivalent of R^2, Robert and Escoufier 1976). All multivariate analyses were performed using the packages *ade4* (Chessel et al. 2004) and *adegenet 1.3* (Jombart 2008) of the R 2.15 software (Ihaka and Gentleman 1996; R Development Core Team 2012; http://www.r-project.org).

Assessing the discriminating power of morphological and anatomical characters

We used discriminant analysis of principal components (DAPC, Jombart et al. 2010) to evaluate how the morphological and anatomical characters

Table 1. Morphological and anatomical characters recorded on specimens (FC: domestic like, FS: wildcat like). When we were unable to confidently diagnose phenotypes, we attributed the modality 'D: doubtful' to specimens

Character	Type	Name abbreviation	Unity/modalities/formula	Comment/reference
Morphological	Body size	Weight *w*	kg	
		Head+body length *hbl*	mm	
		Tarsus length *tarl*	mm	
		Tail length *tail*	mm	
	Pelage	Pelage colour *fc*	light-tawny (FS)-/grey-coloured pelage (FC)	
		Tail shape *ts*	Large with a large, rounded, black tip and at least two black bands that completely encircled the tail (FS) /narrow with a tip tapered to a point, black bands that do not completely encircle the tail (FC)	
		Dorsal line *dl*	Stops at root of the tail (FS)/ continues onto tail (FC)	
		Flank stripes *fs*	Lateral stripes that are not pronounced and not linked to the backline (FS)/pronounced lateral stripes and linked to the back line	
Anatomical	Internal Organs	Intestinal length *il*	mm	Not used in the analysis
	Cranial	Greatest length of skull *gls*	mm	Not used in the analysis
		Cranial volume *cv*	cm^3	Not used in the analysis
		Shape of parietal suture *sps*	Sinuous (FS)/straight (FC)	
		Mandible *m*	Equilibrated (FS)/disequilibrated (FC)	
Derived	Internal Organs	Intestinal index *ii*	*ii* = *il* / *hbl*	Schauenberg (1977)
	Cranial	Cranial index *ci*	*ci* = *gls* / *cv*	Schauenberg (1969)

can be used to classify the individuals with regard to the genetic classification determined using STRUCTURE. In both cases, the three reference groups defined with STRUCTURE (wildcats, domestic cats and hybrids, O'Brien et al. 2009; Say et al. 2012) were used as *a priori* groups.

We performed DAPC on both HSA_{morpho} and HSA_{anat} individual scores. DAPC generates discriminant functions (DF) representing the linear combinations of the original variables that most differ across groups. We conducted two DAPCs: $DAPC_{morpho}$ ($n = 270$) and $DAPC_{anat}$ ($n = 68$) on the eight and four components of HSA_{morpho} and HSA_{anat}, respectively, for morphological and anatomical data. The percentage of correctly classified cases indicates how effective the DF are in identifying group differences and, hence, how the variables used in DAPCs are relevant to distinguishing between wildcats, domestic cats and their hybrids.

Results

COIA on morphological characters and allelic frequencies

When performing the HSA on morphological characters (HSA_{morpho}, eight variables on 270 specimens), we retained the two-first axes explaining 35.49% and 21.69% of the observed variation in morphological data. The first HSA_{morpho} axis F1 is clearly linked to the distinction between domestic and wildcat morphs (Fig. 1a): the typical wildcat pelage characters (*fs*: lateral stripes are not pronounced and not linked to the back line; *dl*: dorsal line stops at root of the tail; *ts*: large tail with a large, rounded, black tip and at least two black bands that completely encircled it, Table 1) grouped together. The second axis is the size and body mass axis (variables *w*, *hbl*, *tarl* and *tail*). Note that the 'doubtful' modality of the flank stripes, pelage colour and dorsal line characters (Table 1) are more correlated with the second axis, whereas the 'doubtful' modality of the tail shape character grouped with wild characters (Fig. 1a). However, sample sizes are very low for these 'doubtful' modalities (between 4 and 17 specimens depending on variables) so that no strong conclusion from this pattern can be drawn. For the $PCA_{genet/morpho}$ on allelic frequencies, we also retained the two-first axes of the 153 existing explaining a total of 6.33% of the observed variation in allelic frequencies.

The overall similarity in the structure of the morphological data set and the genetic data set highlighted by the $COIA_{morpho/genet}$ resulted in a RV coefficient of 0.254. Most of this costructure between the two data sets was accounted for by the first axis (F1, 79.43%) of the $COIA_{morpho/genet}$. The correlation between F1 individual scores of HSA_{morpho} and $PCA_{genet/morpho}$ was highly significant (Spearman $\rho = 0.47$, $p < 10^{-4}$, $n = 270$), suggesting that the morphological characters, mainly pelage characters, capture the essential features of the genetic variability (Fig. 2). Indeed, the comparative distribution of HSA_{morpho} and $PCA_{genet/morpho}$ F1 individual scores for the three preclassified STRUCTURE groups (wild, domestic and hybrid cats) showed that the F1 axis of HSA_{morpho} allows us to clearly identify wild and domestic cats, whereas the hybrid group showed a greater variance in F1 coordinates and seemed to be separated into two subgroups with intermediate phenotype: hybrid with wildcat morphological characters and hybrids with domestic characteristics (Fig. 2). The use of binary qualitative variables broke the continuous gradient from domestic to wildcat captured using genetic features. Nonetheless, hybrids with wildcat phenotype are those having the highest $PCA_{genet/morpho}$ scores compared with hybrids with domestic phenotype. Note also that two genetically defined wildcats had a HSA_{morpho} F1 score similar to the domestic specimens (FS0317 and FS6812).

COIA on anatomical characters and allelic frequencies

We also retained the first two axes of HSA_{anat} explaining 68.14% and 16.87% of the observed variation in anatomical data (four variables on 68 specimens). On the first axis, low values of both cranial and intestinal indices grouped together with the sinuous modality of the shape of the parietal structure, a typical character of wildcats (Fig. 1b). On the contrary, the mandible character

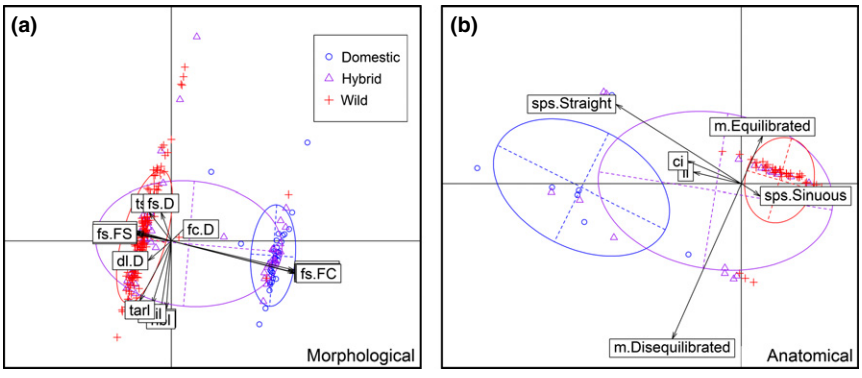

Fig. 1. Bivariate plot of the two-first scores generated by a Hill and Smith analysis performed on (a) morphological characters HSA_{morpho} and (b) anatomical characters HSA_{anat}. The three groups, wild, hybrid and domestic cats, are genetically determined. Crosses, triangles and circles represent individuals, and inertia ellipses of each group are displayed by different colours.

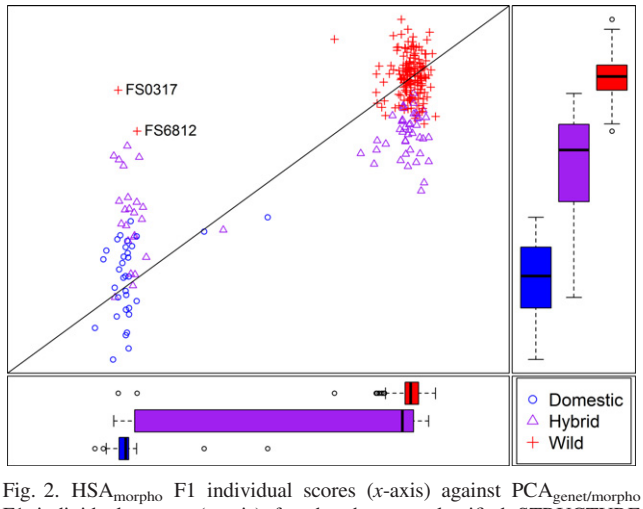

Fig. 2. HSA_{morpho} F1 individual scores (x-axis) against $PCA_{genet/morpho}$ F1 individual scores (y-axis) for the three preclassified STRUCTURE groups (wild, domestic and hybrid cats, $n = 270$). Identity line $y = x$ as well as box plots for each STRUCTURE group and F1 scores are also displayed.

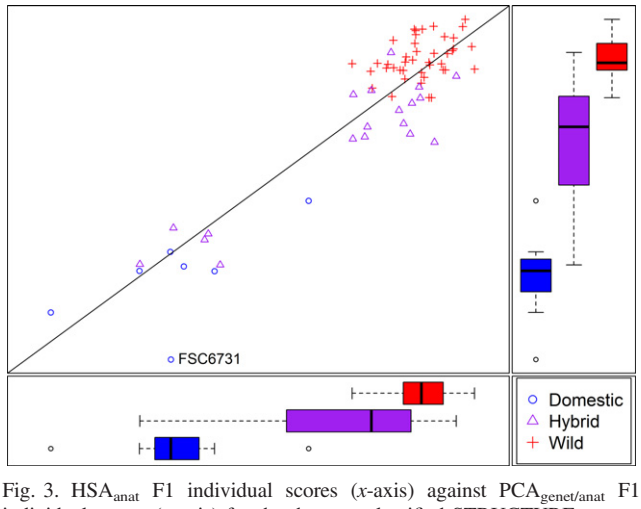

Fig. 3. HSA_{anat} F1 individual scores (x-axis) against $PCA_{genet/anat}$ F1 individual scores (y-axis) for the three preclassified STRUCTURE groups (wild, domestic and hybrid cats, $n = 68$). Identity line $y = x$ as well as box plots for each STRUCTURE group and F1 scores are also displayed.

seemed to be accessory in differentiating specimens (Fig. 1b). The first two of the 68 existing $PCA_{genet/anat}$ axes explained a total of 10.23% of the observed variation in allelic frequencies. $COIA_{anat/genet}$ resulted in a RV coefficient of 0.328. The first axis F1 of $COIA_{anat/genet}$ accounted for 85.72% of the costructure. The correlation between F1 individual scores of HSA_{anat} and $PCA_{genet/anat}$ was highly significant (Spearman $\rho = 0.51$, $p < 10^{-4}$, $n = 68$) so that the anatomical characters, but mandible shape captured the essential features of the genetic variability as well if not better than morphological characters (Fig. 3).

Sample size for domestic specimen is very low ($n = 7$) so that it is difficult to comment the distribution of HSA_{anat} F1 scores for the domestic group despite its variance appeared to be higher than for the HSA_{morpho} F1 scores (Figs 2 and 3). For hybrid and wildcat specimens, the same conclusions as for the $COIA_{morpho/genet}$ can be drawn: anatomical characters led to separate hybrid specimen in two subgroups, whereas they identified well the wildcat specimens (Fig. 3).

Reassignment of specimens using discriminant analysis

Both $DAPC_{morpho}$ and $DAPC_{anat}$, performed on morphological and anatomical characters, respectively, produced one discriminant axis. We used the DF associated with this discriminant axis to reassign specimens into the three reference groups established by the STRUCTURE analysis.

The first axis of $DAPC_{morpho}$ corresponds almost exactly to the first principal component of HSA_{morpho} so that the DF are essentially based on pelage characters. The overall reassignment success rate (RSR) is 73% (197 of 270), and the main error type almost fully concerns hybrid specimens (67 of 68) reassigned to both domestic and wildcat groups with a predominance of false reassignment to the wildcat group (43 of 67). As previously mentioned, two wildcats were assigned to the domestic group based on morphological variables (FS6812 and FS0317) as they typically have domestic pelage phenotype (Table 2). If FS0317 appeared to be clearly a wildcat based on genetics (*qik* = 0.959), FS6812 had a *qik* value very close to the threshold (0.892 slightly higher than the 0.870 threshold), suggesting that we cannot rule out the fact it was a hybrid. In addition, two genetically defined wildcats were reassigned to the hybrid class (FS0301 and FS1813) because they showed doubtful modalities for two of four pelage characters (Table 2). Overall, wildcats were mostly well reassigned (RSR = 165/169 = 97.6%).

The first axis of $DAPC_{anat}$ is clearly associated with the first axis of HSA_{anat} so that the DF mainly used *ci*, *ii* and *sps* to discriminate. The overall reassignment success rate is slightly higher

Table 2. Assignment errors in discriminant analysis of principal components (DAPC) analysis: individual ID; sex; age class (Juvenile/Adult); classification with genetic, morphology and anatomy; individual q_{ik} scores; and morphological and anatomical values (FC: domestic like, FS: wildcat like, D: doubtful)

ID	Sex	Age	Genetic classification	Morphological classification	Anatomical classification	q_{ik}	*fc*	*ts*	*dl*	*fs*	*m*	*sps*	*ci*	*ii*
FS0317	M	J	Wild	Domestic	-	0.959	FC	FC	FC	FC	Equilibrated	Sinuous	-	2.59
FS6812	M	A	Wild	Domestic	-	0.892	FC	FC	FC	FC	-	-	-	-
FS0301	M	A	Wild	Hybrid	Wild	0.986	FC	FS	D	D	Equilibrated	Sinuous	2.31	2.91
FS1813	F	A	Wild	Hybrid	-	0.917	D	FS	D	FS	-	-	2.10	2.70
FS39I	M	A	Wild	Wild	Hybrid	0.932	FS	FS	FS	FS	Disequilibrated	Sinuous	2.33	2.51
FS45C	F	A	Wild	Wild	Hybrid	0.977	FS	FS	FS	FS	Disequilibrated	Sinuous	2.25	2.58

'-': no data due to skull or intestine deterioration

than for $DAPC_{morpho}$ and equals 76.5% (52 of 68), and the main error type concerns again hybrid specimens reassigned to both domestic and wildcat groups, but with a less magnitude than for $DAPC_{morpho}$ (14 of 19). Unfortunately, the two previous wildcats assigned as domestic by $DAPC_{morpho}$ were not in the anatomical data set due to missing values (Table 2). Nonetheless, the FS0317 specimen showed at least two typical anatomical criteria of wildcat (intestinal index *ii* = 2.59; shape of the parietal suture *sps* = sinuous, Fig. 1b), suggesting that it would have been well classified by the $DAPC_{anat}$. Of the two wildcats assigned to hybrids by $DAPC_{morpho}$, FS1813 was unequivocally classified as wildcat by $DAPC_{anat}$, and FS0301 clearly showed both *ci* and *ii* values typical of wildcat albeit been not in the anatomical data set (Table 2). $DAPC_{anat}$ also misclassified two wildcat genotypes (FS39I and FS45C, Table 2) due to their disequilibrated mandible *m* even if it was not really a discriminating character (Fig. 1b), and these two wildcats were well assigned by $DAPC_{morpho}$ (Table 2). For these two individuals, the posterior probability of assignment to the hybrid class was very low and almost equal to the probability of assignment to the wildcat class (0.509 versus 0.489 for FS39I and 0.543 versus 0.454 for FS45C) so that we cannot interpret their assignment based on $DAPC_{anat}$ confidently. $DAPC_{anat}$ performed better than $DAPC_{morpho}$ to reassign the hybrid specimens. Finally, genetically hybrids wrongly assigned to the domestic class by the $DAPC_{anat}$ (n = 4, median = 0.126 [0.105–0.268]) showed lower *qik* values (pairwise Wilcoxon test, p = 0.01) than genetically hybrid wrongly assigned to the wildcat class [n = 10, median = 0.708 (0.194–0.860)]. This underlined that only highly introgressed hybrids (low *qik* values) are misclassified as domestic cats. On the contrary, no difference was observed (pairwise Wilcoxon test, p = 0.86) between genetically hybrid correctly assigned [n = 5, median = 0.58 (0.102–0.736)] and genetically hybrids classified as wildcat, so that highly introgressed hybrids (low *qik* values) might be identified as wildcats using anatomical characters. Nonetheless, these results underlined the existence of a continuum from wildcat to domestic in anatomical metrics, especially *ci* and *ii*, as these two continuous characters are clearly associated with the DF used in $DAPC_{anat}$.

Performance of the cranial and intestinal indices to disentangle hybrid from wildcat specimens

Disentangling hybrid from wildcat and domestic specimens proved difficult in both COIAs and DAPCs. From the genetic point of view, hybrids are distributed more or less continuously through the q_{ik} gradient. The only two continuous variables with a high discriminating power are the cranial and the intestinal indices. We thus linearly regressed both indices on q_{ik} values to assess their reliability. Both indices were highly negatively correlated with q_{ik} (cranial index: R^2 = 60.7%, n = 127; intestinal index: R^2 = 44.3% n = 232; all p-values < 10^{-4}, Fig. 4). For the cranial index, no wildcat was misclassified, whereas five were misclassified using the intestinal index (Fig. 4). Error rates are similar when classifying domestic cats. We counted two and five misclassified domestic cats, respectively, for the cranial and intestinal indices. Despite the negative relationships between these two indices and q_{ik}, variation around the regression line is high, and even specimens with low q_{ik}, that is, values lower than 0.5, showed cranial and intestinal indices typical of wildcats, based on the thresholds of 2.67 and 3.17 defined by Schauenberg (1969, 1977). Hence, these threshold values above which the indices classify specimens as wildcat are predicted from the regression line for rather low values of q_{ik}: 0.473 and 0.466,

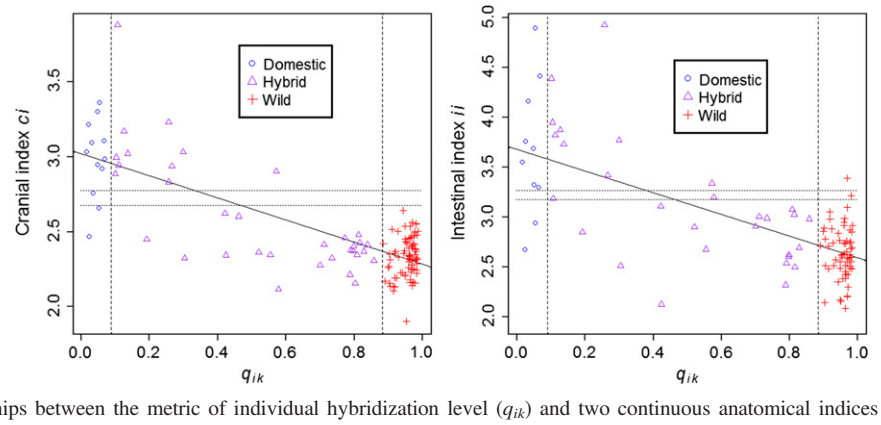

Fig. 4. Linear relationships between the metric of individual hybridization level (q_{ik}) and two continuous anatomical indices (cranial *ci* and intestinal *ii* index). Vertical dashed lines are for the thresholds used to classify STRUCTURE groups (wild, domestic and hybrid cats). Horizontal dashed lines are for the thresholds used to classify wild, domestic and hybrid cats based on cranial index *ci* and intestinal index *ii* (Schauenberg 1969, 1977).

respectively, for the cranial and the intestinal indices. No specimen showed a cranial index in the range of the expected values for hybrids (between 2.67 and 2.77, Schauenberg 1969, 1977), so that based on the cranial index, hybrids are again strictly split into both domestic and wildcat reference groups, whereas only five specimens (four hybrids and one domestic) had intestinal index typical of hybrids (between 3.17 and 3.26). Compared with multivariate analysis, the power of cranial and intestinal indices for distinguishing hybrid from wildcat specimens fell between the power of $DAPC_{morpho}$ and $DAPC_{anat}$. The median q_{ik} value of hybrids specimens erroneously assigned to the wildcat reference group is similar in all analytical approaches, and for example, all approaches fail to detect as hybrid a specimen with a $q_{ik} = 0.210$.

Discussion

Conservation plans for European wildcats need a reliable tool for identifying wildcats from hybrids and domestic cats in the field. In a comprehensive sample of road-killed animals collected over the main area of presence in France (Say et al. 2012), we recorded a set of morphological and anatomical characters that we confronted to a genetic classification of individuals into wild, hybrid and domestic cats based on microsatellite markers (O'Brien et al. 2009; Say et al. 2012). Based on previous studies (e.g. Yamaguchi et al. 2004; Kitchener et al. 2005; Krueger et al. 2009; Platz et al. 2011) and on our own study (O'Brien et al. 2009), those morphological and anatomical characters were expected to provide good discrimination as they described body size, pelage, cranial and intestinal anatomy. The first step of our approach was to validate this by investigating, through co-inertia analysis, the correlation between morphological and anatomical data and multilocus microsatellite data. As expected, both morphological and anatomical data set were well correlated with the multilocus microsatellite data set, suggesting that those characters capture the essential features of the genetic variability, anatomical characters performing slightly better than morphological ones. In addition, the way those characters captured the genetic variability is in agreement with previous results (O'Brien et al. 2009) so that the derived genetic classification performed with STRUCTURE is well grasped by the first HSA axes (Figs 2 and 3) and can be used as reference groups to investigate the reliability of both morphological and anatomical characters in identifying wildcat and domestic cats. The mean discriminating power of the morphological and anatomical characters was nearly equivalent, with about 75% of correctly assigned specimens, with again a slightly better performance of anatomical characters. However, large disparities exist between the reassignment success rates calculated for the different genetic classes. The key findings are that both morphological and anatomical set of characters correctly reassigned almost all the wildcats (RSR = 165/169 = 97.6% and RSR = 40/42 = 95.2%, respectively, for morphological and anatomical characters), whereas the discrimination of hybrids is dramatically low (RSR = 1/68 = 1.5% and RSR = 6/19 = 31.6%, respectively, for morphological and anatomical characters).

On the ability to separate wildcat from domestic cats

As in previous recent morphological studies (e.g. Krueger et al. 2009; Platz et al. 2011), we identify a set of morphological and anatomical characters that allowed to distinguish very well domestic cats from wildcats. Except for a few cases (Table 2), when a specimen is genetically a wildcat, we are able to assess it based on either morphological, mainly pelage characters, or anatomical characters (*ii*, *ci* and *sps* mainly). Size effect between wild and domestic cats was negligible, even after accounted for sexual dimorphism, a pattern already shown in the study by Krueger et al. (2009). Interestingly, discrimination was achieved using morphological and anatomical characters easily measurable in the field or in the laboratory by trained experts. However, some classification errors occurred (genetically wildcat classified as either domestic or hybrid by morphological and anatomical characters, see results Table 2). For some of these specimens, the q_{ik} threshold value used to genetically classify specimens might be the main issue. Moreover, the first axis of $DAPC_{anat}$ is associated with *ci* and *ii*, two continuous characters. From a conservation point of view, the main type of classification error is more insidious, however. Both morphological and anatomical characters may indeed classify some specimens as wildcat while being genetically hybrids (Table 2). This point clearly underlined the difficulty we, as other authors previously mentioned (Yamaguchi et al. 2004; Krueger et al. 2009), have in distinguishing wildcat from hybrids based only on morphology and anatomy.

On the difficulty to identify hybrid specimens

Hybrid identification based on morphology was poor, mainly because the doubtful modality in pelage characteristics is underrepresented: by far and large, specimens were forced to be classified as wild or domestic using the typical wildcat pelage as a reference. Clearly, the pelage characteristics we recorded in the field are not precise enough to allow the discrimination of hybrids. It would be interesting to establish a precise description of the pelage on known wild and hybrid specimens to identify possible key pelage characteristics.

Anatomical characters provided slightly better discrimination than morphological characters (RSR = 31.6% versus RSR = 1.5%, Fisher's exact test p-value = 0.001), with cranial and intestinal indices being the most discriminating characters (Krueger et al. 2009; Platz et al. 2011). Taking advantage of the greater number of genetically hybrids we had when compared with previous studies (Krueger et al. 2009), we showed that both indices were significantly and linearly related to the continuous proxy of the individual levels of introgression (Fig. 4). Schauenberg's thresholds (Schauenberg 1969, 1977) were used satisfactorily to discriminate between wild and domestic specimens. However, despite rather high R^2 values, a large variability occurred that might correspond to highly introgressed specimens having index values typical of wild specimens. We did not control for age (except juveniles less than 6 months), body mass and sex in this analysis, and a part of the unexplained variability might be attributed to these factors. In addition, this linear relationship implicitly implied that hybrids are morphologically halfway between both parents, but how hybridization and introgression average parental phenotypes on hybrids or lead to new morphologies remains largely unknown, especially in traits following a complex multigenic determinism such as skull shape (Leamy et al. 1999). Again, part of the unexplained variability might be attributed to that point. Overall, the linear relationship between a proxy of the individual level of introgression and a continuous anatomical metrics of skull dimension is promising.

On the geographical variability in the reliability of using phenotype to infer genotype

Platz et al. (2011) underline that the strength of the morphological and anatomical differences between wild and domestic specimen is geographically variable, likely due to the spatial variability in the level of introgression as well as to an east-western morphological continuum. We calculated the coefficient of difference (Mayr et al. 1953) for both the cranial index *ci* and the cranial volume *cv* to compare with those published in Krueger et al. (2009) and Platz et al. (2011). Our estimates were lower

(CD_{ci} = 1.65 and CD_{cv} = 1.52 versus 2.28 and 2.44 in Slovakia and 1.8 and 2.1 in Germany, respectively), strengthening the idea of a large spatial variability in reliability of anatomical characters for disentangling between classes. The discriminating value of the cranial volume *cv* was difficult to identify due to the overlap between its distribution in wild and domestic specimens, but should be around 36 cm^3 (result not shown), a value closer to the one in Slovakia than to the one in Germany. This particular point is not in agreement with the east-western continuum suggested by Platz et al. (2011) and calls for pan-European comparison of specimens.

Perspectives and conclusion

The reliability of anatomical and morphological characters for distinguishing between domestic and wildcat specimens in the laboratory and more interestingly in the field was quite good. However, our ability to identify genetically hybrid specimens was very poor using this set of characters. This key point in conservation remains largely unsolved. The most promising direction we identify comes from the relationship we found between a continuous proxy of the individual level of introgression, which avoids the problem of defining genetic threshold to classify specimens in three classes, and a continuous anatomical metrics of skull dimension. Enhancing the estimation of both variables in this relationship through the use of more stringent and efficient molecular markers (e.g. diagnostic SNPs on nuclear DNA, Nussberger et al. 2013) together with modern geometric morphometric methods, using landmarks rather than skull dimension (Corti 1993; Slice 2007) will be our next step. This approach would shed light on how cranial morphological variation arises due to hybridization; that is, do hybrids show a 'middle shape', halfway between both parents, dampening subspecies delimitation or a transgressive shape, not merely intermediate between parental ones, generating new phenotypes (e.g. Renaud et al. 2012). In addition, from diagnostic SNPs, we can expect a better identification of hybrids. This would allow to extent our multivariate approach, coupling co-inertia analysis and DAPC, to more than two data sets. Relating anatomical and genetic variability together with habitat variables, life-history traits and/or parasite load would provide important insights into the fitness consequences of hybridization and introgression.

Our study confirms that the reliability of phenotypic information, that is, cranial index *ci* and cranial volume *cv*, to discriminate between the three groups of specimens is geographically variable at the European scale. Indeed, their discriminating power is itself geographically variable, and such variability is likely due to different levels of introgression. Implication of this result is important because managers should be cautious in transferring thresholds or variables proven to be relevant in one place to their own study site. Such geographical variability of discriminating power is likely to occur in a broader taxonomic range. In that general context, the multivariate approach used here is particularly useful in quickly identifying the most discriminating morphological and anatomical variables from a large data set in local studies. Focusing on those characters only would help to grasp more efficiently the processes underlying hybridization and introgression.

Finally, our sampling covered a vast geographical space (north-eastern quarter of France), and this likely contained some degrees of geographical variability, especially considering that the wildcat is distributed in two subpopulations within this area (Say et al. 2012). Ideally, geographical scales of studies need to be the same for comparison and at the population scale, that is, at a scale where behavioural interactions can arise. Despite these local studies are needed, they often mean low sample size in rare and elusive carnivores. In that broad context, it is of a prime importance for ecologists and wildlife managers involved in wildcat conservation to think about a common analytical approach using the same molecular markers, the same set of morphological and anatomical characters and a sampling design (definition of parental population, recovery of dead animals, design for hair trapping) that could be applied in different places in future studies.

Acknowledgements

We thank Estelle Germain, Luc Baudot, Olivier Hubert, Emmanuel Lienard, Amandine Sager, Audrey Szymanowicz and all the technicians and officers, especially Jean-Luc Wilheim, for their help in the collection of cats and in the laboratory. Many thanks also to Béatrice Nussberger for her very helpful comments on a previous draft. This study was supported by the Office National de la Chasse et de la Faune Sauvage and the University of Lyon-CNRS.

References

Allendorf F, Leary R, Spruell P, Wenburg J (2001) The problems with hybrids: setting conservation guidelines. Trends Ecol Evol **16**:613–622.

Anderson E, Stebbins G (1954) Hybridization as an evolutionary stimulus. Evolution **8**:378–388.

Arnold M (1992) Natural hybridization as an evolutionary process. Annu Rev Ecol Syst **23**:237–261.

Arnold M (2004) Natural hybridization and the evolution of domesticated, pest and disease organisms. Mol Ecol **13**:997–1007.

Barton N (2001) The role of hybridization in evolution. Mol Ecol **10**:551–568.

Barton N, Hewitt G (1985) Analysis of hybrid zones. Annu Rev Ecol Syst **16**:113–148.

Beaumont M, Barratt E, Gottelli D, Kitchener A, Daniels M, Pritchard J, Bruford M (2001) Genetic diversity and introgression in the Scottish wildcat. Mol Ecol **10**:319–336.

Cabria MT, Michaux JR, Gomez-Moliner BJ, Skumatov D, Maran T, Fournier P, Lopez de Luzuriaga J, Zardoya R (2011) Bayesian analysis of hybridization and introgression between the endangered european mink (*Mustela lutreola*) and the polecat (*Mustela putorius*). Mol Ecol **20**:1176–1190.

Chessel D, Dufour A-B, Thioulouse J (2004) The ade4 package-I- one-table methods. R News **4tj311**:5–10.

Corti M (1993) Geometric morphometrics: an extension of the revolution. Trends Ecol Evol **8**:302–303.

Daniels M, Corbett L (2003) Redefining introgressed protected mammals: when is a wildcat a wild cat and a dingo a wild dog? Wildlife Res **30**:213–218.

Daniels M, Balharry D, Hirst D, Kitchener A, Aspinall R (1998) Morphological and pelage characteristics of wild living cats in Scotland: implications for defining the 'wildcat'. J Zool **244**:231–247.

Davison A, Birks J, Griffiths H, Kitchener A, Biggins D, Butlin R (1999) Hybridization and the phylogenetic relationship between polecats and domestic ferrets in Britain. Biol Conserv **87**:155–161.

Dolédec S, Chessel D (1994) Co-inertia analysis - an alternative method for studying species environment relationships. Freshw Biol **31**:277–294.

Dowling T, Secor C (1997) The role of hybridization and introgression in the diversification of animals. Annu Rev Ecol Syst **28**:593–619.

Dray S, Chessel D, Thioulouse J (2003) Co-inertia analysis and the linking of ecological data tables. Ecology **84**:3078–3089.

Eckert I, Suchentrunk F, Markov G, Hartl GB (2010) Genetic diversity and integrity of German wildcat (*Felis silvestris*) populations as revealed by microsatellites, allozymes, and mitochondrial DNA sequences. Mamm Biol **75**:160–174.

Elledge AE, Allen LR, Carlsson BL, Wilton AN, Leung LKP (2008) An evaluation of genetic analyses, skull morphology and visual appearance for assessing dingo purity: implications for dingo conservation. Wild Res **35**:812–820.

Fitzpatrick B (2004) Rates of evolution of hybrid inviability in birds and mammals. Evolution **58**:1865–1870.

Fredrickson RJ, Hedrick PW (2006) Dynamics of hybridization and introgression in red wolves and coyotes. Conserv Biol **20**:1272–1283.
French D, Corbett L, Easterbee N (1988) Morphological discriminants of Scottish wildcats (*Felis silvestris*), domestic cats (*Felis catus*) and their hybrids. J Zool **214**:235–259.
Germain E, Benhamou S, Poulle ML (2008) Spatio-temporal sharing between the European wildcat, the domestic cat and their hybrids. J Zool **276**:195–203.
Germain E, Ruette S, Poulle ML (2009) Likeness between the food habits of European wildcats, domestic cats and their hybrids in France. Mamm Biol **74**:412–417.
Gottelli D, Sillero-zubiri C, Applebaum G, Roy M, Girman D, Garcia-Moreno J, Ostrander E, Wayne R (1994) Molecular-genetics of the most endangered canid - the Ethiopian wolf *Canis simensis*. Mol Ecol **3**:301–312.
Grant P, Grant B, Petren K (2005) Hybridization in the recent past. Am Nat **166**:56–67.
Hailer F, Leonard JA (2008) Hybridization among three native north-American *Canis* species in a region of natural sympatry. PLoS ONE **3**: e3333.
Hertwig ST, Schweizer M, Stepanow S, Jungnickel A, Boehle UR, Fischer MS (2009) Regionally high rates of hybridization and introgression in German wildcat populations (*Felis silvestris*, *Carnivora*, *Felidae*). J Zool Syst Evol Res **47**:283–297.
Hill MO, Smith AJE (1976) Principal component analysis of taxonomic data with multi-state discrete characters. Taxon **25**:249–255.
Hindrikson M, Maennil P, Ozolins J, Krzywinski A, Saarma U (2012) Bucking the trend in wolf-dog hybridization: first evidence from Europe of hybridization between female dogs and male wolves. PLoS ONE **7**:e46465.
Homyack JA, Vashon JH, Libby C, Lindquist EL, Loch S, McAlpine DF, Pilgrim KL, Schwartz MK (2008) Canada lynx-bobcat (*Lynx canadensis* x *L. rufus*) hybrids at the southern periphery of lynx range in Maine, Minnesota and new Brunswick. Am Midl Natur **159**:504–508.
Hubbard A, Mcorist S, Jones T, Boid R, Scott R, Easterbee N (1992) Is survival of European wildcats *Felis silvestris* in Britain threatened by interbreeding with domestic cats. Biol Conserv **61**:203–208.
Ihaka R, Gentleman R (1996) R: a language for data analysis and graphics. J Comput Graph Stat **5np93**:299–314.
Jombart T (2008) *adegenet*: a R package for the multivariate analysis of genetic markers. Bioinformatics **24**:1403–1405.
Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet **11**:1–15.
Kidd AG, Bowman J, Lesbarreres D, Schulte-Hostedde AI (2009) Hybridization between escaped domestic and wild American mink (*Neovison vison*). Mol Ecol **18**:1175–1186.
Kitchener AC, Yamaguchi N, Ward J, Macdonald D (2005) A diagnosis for the Scottish wildcat (*Felis silvestris*): a tool for conservation action for a critically-endangered felid. Anim Conserv **8**:223–237.
Krueger M, Hertwig ST, Jetschke G, Fischer MS (2009) Evaluation of anatomical characters and the question of hybridization with domestic cats in the wildcat population of Thuringia, Germany. J Zool Syst Evol Res **47**:268–282.
Leamy LJ, Routman EJ, Cheverud JM (1999) Quantitative trait loci for early- and late-developing skull characters in mice: a test of the genetic independence model of morphological integration. Am Nat **153**:201–214.
Lecis R, Pierpaoli M, Biro Z, Szemethy L, Ragni B, Vercillo F, Randi E (2006) Bayesian analyses of admixture in wild and domestic cats (*Felis silvestris*) using linked microsatellite loci. Mol Ecol **15**:119–131.
Lehman N, Einsenhawer A, Hansen K, Mech L, Peterson R, Gogan P, Wayne R (1991) Introgression of coyote mitochondrial DNA into sympatric north-American gray wolf populations. Evolution **45**: 104–119.
Lodé T, Guiral G, Peltier D (2005) European mink-polecat hybridization events: hazards from natural process? J Hered **96**:89–96.
Mayr E, Linsley E, Usinger R (1953) Methods and Principles of Systematic Zoology. London McGraw-Hill Book Company, New York, Toronto.
Miller C, Adams J, Waits L (2003) Pedigree-based assignment tests for reversing coyote (*Canis latrans*) introgression into the wild red wolf (*Canis rufus*) population. Mol Ecol **12**:3287–3301.
Noren K, Kvaloy K, Nystrom V, Landa A, Dalen L, Eide NE, Ostbye E, Henttonen H, Angerbjorn A (2009) Farmed arctic foxes on the Fennoscandian mountain tundra: implications for conservation. Anim Conserv **12**:434–444.
Nowell K, Jackson P (1996) Wild Cats, Status Survey and Conservation Action Plan. IUCN/SSC Cat Specialist Group, Gland, Switzerland.
Nussberger B, Greminger M, Keller L, Wandeler P (2013) Development of SNP markers identifying European wildcats, domestic cats, and their admixed progeny. Mol Ecol Resour **12**:447–460.
O'Brien J, Devillard S, Say L, Vanthomme H, Leger F, Ruette S, Pontier D (2009) Preserving genetic integrity in a hybridising world: are European wildcats (*Felis silvestris silvestris*) in eastern France distinct from sympatric feral domestic cats? Biodivers Conserv **18**:2351–2360.
Oliveira R, Godinho R, Randi E, Alves P (2008a) Hybridization versus conservation: are domestic cats threatening the genetic integrity of wildcats (*Felis silvestris silvestris*) in Iberian peninsula? Philos Trans R Soc Lond B Biol Sci **363**:2953–2961.
Oliveira R, Godinho R, Randi E, Ferrand N, Alves PC (2008b) Molecular analysis of hybridisation between wild and domestic cats (*Felis silvestris*) in Portugal: implications for conservation. Conserv Genet **9**:1–11.
Piechocki R (1990) Die Wildkatze. A. Ziemsen Verlag, Wittenberg Lutherstadt.
Pierpaoli M, Biro Z, Herrmann M, Hupe K, Fernandes M, Ragni B, Szemethy L, Randi E (2003) Genetic distinction of wildcat (*Felis silvestris*) populations in Europe, and hybridization with domestic cats in Hungary. Mol Ecol **12**:2585–2598.
Platz S, Hertwig ST, Jetschke G, Krueger M, Fischer MS (2011) Comparative morphometric study of the Slovakian wildcat population (*Felis silvestris silvestris*): evidence for a low rate of introgression? Mamm Biol **76**:222–233.
Pritchard J, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics **155**:945–959.
Puzachenko A (1996) Variability of some cranial characters in *Felis silvestris*, *Felis libyca* and *Felis catus* (*Mammalia*, *Felidae*). Zool Zhurnal **75**:1078–1085.
R Development Core Team (2012). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/
Randi E, Lucchini V (2002) Detecting rare introgression of domestic dog genes into wild wolf (*Canis lupus*) populations by Bayesian admixture analyses of microsatellite variation. Conserv Genet **3**:31–45.
Reich D, Wayne R, Goldstein D (1999) Genetic evidence for a recent origin by hybridization of red wolves. Mol Ecol **8**:139–144.
Reig S, Daniels M, Macdonald D (2001) Craniometric differentiation within wild-living cats in Scotland using 3D morphometrics. J Zool **253**:121–132.
Renaud S, Allibert P, Auffray J-C (2012) Modularity as a source of new morphological variation in the mandible of hybrid mice. BMC Evol Biol **12**:141.
Rhymer J, Simberloff D (1996) Extinction by hybridization and introgression. Annu Rev Ecol Syst **27**:83–109.
Rieseberg L (1997) Hybrid origins of plant species. Annu Rev Ecol Syst **28**:359–389.
Rieseberg L, Raymond O, Rosenthal D, Lai Z, Livingstone K, Nakazato T, Durphy J, Schwarzbach Z, Donovan L, Lexer C (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. Science **301**:1211–1216.
Robert P, Escoufier Y (1976) A unifying tool for linear multivariate statistical methods: the RV-coefficient. Appl Stat **25**:257–265.
Roy M, Geffen E, Smith D, Ostrander E, Wayne R (1994) Patterns of differentiation and hybridization in north-American wolflike canids, revealed by analysis of microsatellite loci. Mol Biol Evol **11**:553–570.
Sacks BN, Moore M, Statham MJ, Wittmer HU (2011) A restricted hybrid zone between native and introduced red fox (*Vulpes vulpes*) populations suggests reproductive barriers and competitive exclusion. Mol Ecol **20**:326–341.

Say L, Devillard S, Leger F, Pontier D, Ruette S (2012) Distribution and spatial genetic structure of European wildcat in France. Anim Conserv **15**:18–27.

Schauenberg P (1969) L'identification du chat forestier d'Europe *Felis. s. silvestris* Schreber, 1777, par une méthode ostéométrique. Rev Suisse Zool **78**:433–441.

Schauenberg P (1977) Longueur de l'intestin du chat forestier *Felis. s. silvestris* Schreber 1977. Mammalia **41**:357–360.

Schwartz M, Pilgrim K, McKelvey K, Lindquist E, Claar J, Loch S, Ruggiero L (2004) Hybridization between Canada lynx and bobcats: genetic results and management implications. Conserv Genet **5**:349–355.

Slice DE (2007) Geometric morphometrics. Annu Rev Anthropol **36**:261–281.

Stahl P, Artois M, Aubert M (1988) The use of space and the activity pattern of adult European wild cats (*Felis silvestris* Schreber 17777) in Lorraine. Rev Ecol Ter Vie **43**:113–132.

Tamlin AL, Bowman J, Hackett DF (2009) Separating wild from domestic American mink *Neovison vison* based on skull morphometrics. Wildlife Biol **15**:266–277.

Trigo TC, Freitas TRO, Kunzler G, Cardoso L, Silva JCR, Johnson WE, O'Brien SJ, Bonatto SL, Eizirik E (2008) Inter-species hybridization among Neotropical cats of the genus *Leopardus*, and evidence for an introgressive hybrid zone between *L-geoffroyi* and *L-tigrinus* in southern Brazil. Mol Ecol **17**:4317–4333.

Vila C, Wayne R (1999) Hybridization between wolves and dogs. Conserv Biol **13**:195–198.

Wayne R, Jenks S (1991) Mitochondrial-DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. Nature **351**:565–568.

Yamaguchi N, Driscoll C, Kitchener AC, Ward J, Macdonald D (2004) Craniological differentiation between European wildcats (*Felis silvestris silvestris*), African wildcats (*F. s. lybica*) and Asian wildcats (*F. s. ornata*): implications for their evolution and conservation. Biol J Linn Soc **83**:47–63.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

SM1. STRUCTURE analysis, power of the analysis and estimation of the individual levels of introgression with q_{ik}

SM2. Specimen list: sex, age, and raw data for morphological and anatomical characters are provided for each specimen together their genetic classification and q_{ik} scores (STRUCTURE analysis), their morphological classification ($DAPC_{morpho}$ analysis) and their anatomical classification ($DAPC_{anat}$ analysis). 'Location' indicates owners and places were specimens are deposited. 'NA' denotes that no data are available.