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# Range expansion as an explanation for introgression in European wildcats

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## ABSTRACT

Introgression between domestic and wild taxa is a conservation issue because it can lead to the genetic extinction of wild taxa. Understanding the causes of introgression is thus a crucial task for conservation biologists. Here we provide evidence from biparentally, paternally and maternally inherited genetic markers in hybridizing European wildcats (*Felis silvestris silvestris*) and domestic cats (*Felis silvestris catus*) that one cause of introgression can be range expansion of the threatened species. We analyzed 68 autosomal, two Y-chromosomal and four mitochondrial diagnostic single nucleotide polymorphisms, and a sequence of 384 base pair of mitochondrial DNA, in 224 wild- and domestic cats from the Jura region of eastern Switzerland and western France. Using Bayesian estimation approaches, we found more gene flow from domestic cats to wildcats than vice versa (0.017 and 0.003 migrants per generation). Introgression of maternally inherited markers was higher than of paternally inherited markers. To test if these observed introgression patterns might be explained by wildcat expansion, we simulated neutral genetic data under various models of hybridization including spatial features such as range expansion. The most likely scenario represented an expansion of wildcats into domestic cat range. We also explored the geographic distribution of wildcats and hybrids. In comparison to wildcats, hybrids were found closer to the edge of the wildcat distribution range. Overall, the patterns we observed are compatible with the hypothesis that introgression is caused by wildcat range expansion, rather than by domestic cat invasion of wildcat habitat. That the threatened European wildcat is expanding is a positive sign, but careful monitoring of introgression and its fitness consequences is needed to ensure that the wildcat does not go genetically extinct in the generations to come.

## 1. Introduction

Understanding the causes and consequences of hybridization and introgression is crucial to conservation biology. Introgression can threaten species (Rhymer and Simberloff, 1996), especially when domesticated forms are involved (Allendorf et al., 2001). Negative consequences of introgression from domestic taxa can include outbreeding depression and swamping of the genome in the wild taxon (Ellstrand et al., 1999). Conservationists thus generally consider signs of introgression as indications of risk (Barilani et al., 2007; O'Brien et al., 2009; Hindrikson et al., 2012; Meilink et al., 2015).

Extent and patterns of introgression depend crucially on spatial dynamics. When a species expands its range into an area occupied by a local, closely related taxon hybridization and introgression can occur (Currat et al., 2008). In the absence of selection, the expanding taxon is predicted to be more introgressed than the local one, especially at genes experiencing reduced gene flow (e.g. mtDNA and genes on sex chromosomes) and in the less-dispersing sex (Petit and Excoffier, 2009). These predictions are based on the different effective population sizes among taxon and sex: At the expansion front, the expanding taxon has usually lower densities than the local one, thus resulting in the directionality of introgression from local to expanding taxon. Similarly, the less-dispersing sex has locally lower effective population size than the more-dispersing sex, thus the asymmetry of introgression between both sexes. Consequently, when conservation biologists observe directional and asymmetric introgression, one can expect the taxon with more introgression, especially in the less-dispersing sex, to be the one expanding – at least if introgression is influenced by spatial dynamics. Thus, in the case of an endangered taxon hybridizing with a common taxon, the direction and asymmetry of introgression reveals whether it is the common species which is invading the endangered taxon or vice versa. Observing more introgression in the endangered taxon than in

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the common one could then be a positive sign of demographic recovery of the endangered taxon – irrespective of any potentially negative consequences of this introgression. Here we focus on range expansion as a possible cause of introgression and highlight that introgression in an endangered species can be caused by a positive demographic recovery of this species using data from the endangered European wildcat (*Felis silvestris silvestris*) and its domesticated and abundant relative, the domestic cat (*Felis silvestris catus*).

Wildcats and domestic cats are known to hybridize and produce fertile offspring (Beaumont et al., 2001; Randi et al., 2001; Lecis et al., 2006; Oliveira et al., 2008; Hertwig et al., 2009; O'Brien et al., 2009). The International Union for Conservation of Nature (IUCN) thus considers hybridization with domestic cats to be a major threat to European wildcats (Driscoll and Nowell, 2010; Oliveira et al., 2008; Hertwig et al., 2009) and wildcats are listed as endangered in the Red Lists of several countries (e.g. Germany, Portugal, Switzerland). In Switzerland, wildcats were once present throughout the Jura Mountains, a subalpine mountain range (up to 1700 m. a.s.l.) mainly covered with deciduous forests, and the Swiss Plateau, a flat landscape of low elevation (400–700 m. a.s.l.) between the Jura and the Swiss Alps. Wildcats became extremely rare in the middle of the 20th century, due to persecution and habitat loss during the previous centuries (von Tschudi, 1890; Nussberger et al., 2007). Legal protection of wildcats under the Swiss hunting law in 1962, under the Berne Convention (Appendix II) in 1979 and under the European Habitat Directive 92/43/EEC (Appendix IV) in 1992 have since led to a recovery of wildcat populations across Europe (Streif et al., 2012; Say et al., 2012; Weber et al., 2010). At the same time, based on data of the pet food industry (www.fediaf.org, www.vhn.ch), the number of domestic pet cats has also been increasing. It is generally assumed that such an increase and global dispersion of domestic cats has led to more contacts between domestic cats and wildcats (Oliveira et al., 2008; O'Brien et al., 2009; Beugin et al., 2016). Note that in this article, we always include feral cats when we refer to domestic cats, since we are not able to distinguish between feral cats living without any human contact and free-ranging house cats. Domestic cats may colonize wildcat habitats (Sarmento et al., 2009). However, some studies revealed quite low simultaneous habitat overlap between the two subspecies (Germain et al., 2008; Gil-Sanchez et al., 2015). Introgression could thus be due to both wildcat expansion into areas occupied by domestic cats or to domestic cat expansion into areas occupied by wildcats.

In the present study, we provide evidence that observing introgression in an endangered species can be an indication of range expansion of this endangered species. We hypothesized that introgression might reflect range expansion of wildcats, if we observe a directional introgression where wildcats are more introgressed than domestic cats, a sexually asymmetric hybridization pattern where the less-dispersing females are more introgressed than the more-dispersing males (Devillard et al., 2004), and a geographic hybridization pattern where hybrids are mostly found at the periphery of wildcat distribution range. Thus, we (I) assessed the extent, direction and asymmetry of introgression between European wildcats and domestic cats with biparentally, paternally, and maternally inherited genetic markers, (II) tested the likelihood of different introgression scenarios, including wildcat or domestic cat expansion, using spatially explicit simulations, and (III) explored the geographic pattern of wildcat and hybrid occurrence.

## 2. Material and methods

### 2.1. Data collection

We combined 224 haphazardly and systematically collected tissue and hair samples from European wildcats and free ranging domestic cats from the Franco-Swiss Jura region from 1990 to 2015 (Supplementary data 1; for subspecies identification see also Table 2). Systematic data were collected during national wildcat monitoring programmes in France (Say et al., 2012) and in Switzerland (Weber et al., 2010). Previous genetic analyses showed that the wildcats of this area represent a genetically fairly homogeneous population (Ponta, 2012).

### 2.2. Assessing introgression

To assess introgression patterns of biparentally, maternally, and paternally inherited markers, we genotyped 68 autosomal nuclear SNP-markers, four mtDNA SNP-markers and two SNP-markers on the SRY region of the Y chromosome (Nussberger et al., 2013). All these SNPs are highly differentiated between wildcats and domestic cats and thus allow the identification of introgression with high certainty (Nussberger et al., 2013). We genotyped these diagnostic SNP-markers using 96.96 Fluidigm SNP genotyping arrays (SNP chips). DNA extraction methods, SNP-markers and genotyping methods followed Nussberger et al. (2013, 2014a, 2014b).

#### 2.2.1. Assessing individual nuclear introgression

We assessed nuclear introgression in each of the 224 samples using a Bayesian model and data from the 68 autosomal nuclear markers. We used NewHybrids 1.1 beta (Anderson and Thompson, 2002) to determine the nuclear genealogical category of each individual, allowing for wildcat (Wc), domestic cat (Dc), first generation hybrid (F1), and three second generation hybrid classes: F2 (offspring of F1 × F1), backcross into wildcat (BxW) and backcross into domestic cat (BxD). We also ran NewHybrids with the same sample set allowing for third generation backcrosses into Wc (BxWxW) and Dc (BxDxD). A great strength of NewHybrids is that it determines the genealogical categories based on the allele frequencies in the sample set, without the need for reference samples of parental groups (Anderson and Thompson, 2002).

#### 2.2.2. Assessing directionality of introgression

We estimated the rate of nuclear gene flow between wildcats and domestic cats with the Bayesian model implemented in BayesAss 3.0.3 (Wilson and Rannala, 2003). We used the mixing parameters $dM = 0.05$, $dA = 0.15$, $dF = 0.15$ to assure proper mixing. The number of MCMC iterations was 1,000,000, with Burn-in = 10,000 and Sampling interval = 1000. We defined the domestic cat group as those individuals categorized by NewHybrids as Dc or BxD and the wildcat group as the ones categorized as Wc or BxW. The programs Convert 1.3.1 (Glaubitz, 2004) and Formatomatic 0.8.1 (Manoukis, 2007) were used to convert formats of the input files between softwares.

#### 2.2.3. Assessing asymmetry of introgression

The genotypes at the four diagnostic mtDNA SNP-markers and the two SNP-markers on the Y chromosome indicated if a cat had mtDNA or Y haplotypes of the wild or domestic type. Individuals were considered introgressed on the female line when they were Wc or BxW based on the nuclear markers and had a domestic mtDNA haplotype or when they were Dc or BxD and had a wild mtDNA haplotype. Similarly, males that were Wc or BxW based on nuclear markers but with domestic Y-markers, or Dc or BxD with wild Y-markers, respectively, were considered as introgressed on the paternal line.

To explore mtDNA haplotype diversity not revealed by the mtDNA SNPs, we sequenced mtDNA haplotypes in a subset of 194 samples. We used the two primer pairs Lf15926 - Hf3 and Lf4 - DLH (Eckert et al., 2010) with annealing temperatures of 50° and 56 °C, respectively. These two primer pairs yielded two non-overlapping sequences of the mitochondrial control region of about 350 and 200 bp, respectively. Sequencing of the PCR products was performed using Big Dye Terminator v3.1 chemistry on a 3730 DNA Analyzer (Applied Biosystems). The sequences obtained were base called using Sequencing Analysis v5.1 (Applied Biosystems) and subsequently edited in Geneious Pro v5.5.6 (Drummond et al., 2009). All sequences were trimmed to the