Original investigation

Reproductive biology and genealogy in the endangered Iberian lynx: Implications for conservation

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A B S T R A C T

For endangered species, the availability of genealogies and a good knowledge of mating patterns are valuable resources for conservation purposes. Here we studied mating patterns in the endangered Iberian lynx, *Lynx pardinus*, and reconstructed a partial genealogy of the intensively monitored population in Doñana from 1990 to 2013. Using microsatellites in combination with field information we assigned 146 out of the 175 analysed individuals to at least one parent with confidence above 0.9. We detected breeding events for 30% of individuals in the population (n = 181), which follows a positively skewed distribution (maximum: 7 breeding events for females, 9 for males). On average, individuals reproduce with approximately 1.6 mates in their life, from 2 years-old to a maximum of 10 years for males and 11 years for females. This broadening of the previously reported breeding age is likely due to stochastic changes in the demography that resulted in lack of competition and a high turnover of the territories. We identified several crosses between close relatives (e.g. full-sibs) which resulted in highly inbred offspring. To our knowledge, this is one of the most comprehensive studies on reproductive patterns of an endangered felid in the wild. This novel information highlights the importance of both field and genetic data to broaden the knowledge of the species and to improve conservation programs.

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Introduction

Parentage information provides insights into mating patterns and thus on individual fitness, sexual selection, and social and genetic structure (Gopurenko et al., 2006; Harcourt et al., 2007; Serbezov et al., 2010; Ursprung et al., 2011; Vonholdt et al., 2008). When parentage information is available for most individuals and expands several generations, a genealogy can be reconstructed, which can then be used to estimate relatedness, coancestry and inbreeding, and evolutionary and quantitative genetic parameters like heritability, fitness, or inbreeding depression (Åkesson et al., 2016; Bérénos et al., 2014; Charpentier et al., 2006; Garant and Kruuk, 2005; Pemberton, 2008; Vonholdt et al., 2008). A full description of mating patterns and the reconstruction of a reasonably complete and accurate genealogy is of especial relevance for endangered species, as it can greatly assist genetic management

programs (Fernández et al., 2005). For example, incorporation of relatedness among founders of a captive population can improve the retention of genetic diversity and the accumulation of inbreeding in management programs, which often assume unrelated founders. A complete genealogy can also be used to assess the mode of inheritance of deleterious traits and facilitate an active management of such traits (Ralls et al., 2000). Moreover, a robust estimation of reproductive parameters is desirable for modeling the viability of extant or reintroduced populations (Manlik et al., 2016).

The Iberian lynx, *Lynx pardinus*, is one of the most endangered carnivores in the world (Rodríguez and Calzada, 2015) with a censused population of only 52 mature individuals in 2002. Since the 1980s, numerous conservation actions have been implemented (Palomares et al., 2011b; Simón et al., 2012) in order to reverse its precarious status (Simón et al., 2012). At present (2015), population size has increased to ca. 400 individuals distributed between two remnant lynx populations (Doñana and Sierra Morena) and several reintroduction areas (www.iberlince.eu). Lynx populations have been subjected to close scrutiny during the last three decades, especially in the Doñana area (Ferreras, 2004; Ferreras et al., 1997;

López-Bao et al., 2010; López-Bao et al., 2014; López-Bao et al., 2008b; Palomares, 2001; Palomares et al., 2012), providing extensive knowledge on the ecology and demography of the species on which to base conservation actions (López-Bao et al., 2008b). However, lynx reproductive parameters are still poorly understood, and the available information is almost exclusively based on field observations of females (Ferreras et al., 1997).

In this study, we aim to infer the parental-offspring relationships of lynxes born from 1990 to 2013 in Doñana population, using molecular markers in combination with available field information. We reconstructed a pedigree and estimated lynx reproductive parameters. In particular, we focused on: age of reproduction and breeding patterns (i.e. number of breeding events per individual, number of successful reproductive seasons per individual, number of breeding events per reproductive season for males, number of partners per individual, and number of breeding events vs. number of reproductive mates in a lifetime). Due to the severe isolation and small size of the population (Casas-Marce et al., 2013; Palomares et al., 2012), we also assessed the occurrence of crosses between highly related individuals and estimated the pedigree-based inbreeding coefficient for the population.

Material and methods

Doñana population

We focused our study in the Iberian lynx population of Doñana. In this population, lynx have traditionally been restricted to the nuclei of Doñana Biological Reserve and Coto del Rey (both in Doñana Natural Reserve (37.110° N, 6.123° W)), and their surrounding areas, which played the role of sources within the Doñana lynx metapopulation (Gaona et al., 1998). This metapopulation has been isolated since at least the 1950s and its size has remained around 50 individuals in the interval 1985–2005 (Palomares et al., 2011b). After several conservation actions, the total number of lynx in this area reached 76 in the latest census (2015), and its range spans a wider area extending from Aljarafe in the east to Mazagón in the west (Garrote et al., 2011; Simón et al., 2012). Due to its isolation, this population harbors very low genetic diversity and is highly inbred (Casas-Marce et al., 2013; Palomares et al., 2012).

Lynx data and sample collection

As a result of the intensive research carried out in this area since the 1980s, more than 120 lynx have been radio-tracked (Ferreras, 2004; Ferreras et al., 1997; López-Bao et al., 2010; López-Bao et al., 2011; López-Bao et al., 2008a; Palomares, 2001). In addition, multiple camera-trapping campaigns have been performed since year 2000 (e.g. Garrote et al., 2011; López-Bao et al., 2009). Whenever a lynx was captured for collaring purposes, a sample (blood or buccal swab) was collected. Tissue samples were also taken from all dead lynx found. Occasionally, samples were obtained from kittens (0–1 month-old individuals; commonly 3 weeks-old) located at their dens by tracking females during the breeding season (López-Bao et al., 2010; Palomares et al., 2005). Feces were also collected for some territories (Casas-Marce et al., 2013; Palomares et al., 2011c). Iberian lynx is listed in Annex II and IV of the European Habitats Directive (92/43/EEC). Handling and sample collection of lynx have been specifically approved under permits of the Spanish Ministry of Environment and the Andalusian Department of Environment, and all subjects were treated in accordance with Animal Care Guidelines and international regulations (Sikes and Gannon, 2011).

When a sample was available, DNA was extracted and genotyped for up to 36 microsatellite loci as described in Casas-Marce et al. (2013). All samples were genotyped for a minimum of 16 loci,

and all but five for 24 loci or more. Most samples were good quality blood or tissue samples, and the few low quality (five fecal) samples were genotyped a minimum of four times and the consensus genotype was used to minimize the incidence of genotyping error rates (Ramon-Laca et al., 2015). For this study we used 196 previously genotyped individuals. 175 (90%) were born in the Doñana population between 1990 and 2013, and were used as offspring and candidate parents when they reached 2 years-old in parentage analyses. 16 animals born in Doñana before 1990 and 4 translocated from the Sierra Morena population were only used as candidate parents. Overall, our dataset contained genotypic information for 98 females, 94 males and 4 individuals with no sex information.

The age of the individuals was established based on different types of field information (Appendix B, Table S1 in Supplementary file). Firstly, the birth dates of many individuals were known because their mothers were radio tracked (López-Bao et al., 2010; Palomares et al., 2005). Starting on 1983, individuals belonging to different age categories have been captured, including neonates (<3 months) (n = 45), juveniles (<1 year) (n = 60) and subadults (n = 27) since 1983. Transponders were implanted from 1993 onward, Secondly, from 1987 to 1999, the age of some captured adults (n = 6) was determined using canine radiographs and cementum annuli enumeration (Zapata et al., 1997). The age of some untagged lynx was determined on the basis of when they were first recorded in camera traps as juveniles (<1 year) (n = 39) or subadults (1–2 years) (n = 3) and later identified from their unique pattern of spots (López-Bao et al., 2009). Finally, for a few individuals that were captured as adults (n = 9), age was estimated based on dentition and general body condition.

Parentage analyses

We inferred parental-offspring relationships using the Mac version of Colony 2 (Jones and Wang, 2010). Colony 2 uses a maximum likelihood approach to calculate parentage for a given year/cohort, and allows the inclusion of *a priori* information. We ran Colony three independent times with the following general parameters: three runs, long run length, full likelihood method and medium precision. Besides, we included the allelic frequencies of the population estimated for four different periods (1990–2006, 2007, 2008–2009, 2010–2013) to account for stochastic or known changes in the population (i.e. translocations). We assumed a per locus genotyping error rate (i.e. proportion of wrong genotypes due to allelic dropout, false alleles or other types of errors) of 0.01 for all markers.

For every cohort, we delimited the offspring and a set of potential candidate parents based on age (see details below). In order to establish the percentage of the population that was sampled, we compared our number of candidate parents with the estimated census size for each year (Appendix B, Table S2 in Supplementary file). The former exceeded the latter by 10–51%, indicating that we were probably including the majority of possible parents, together with some that were likely dead. Still, we set the proportion of sampled parents as 90% to account for any unsampled candidate parent.

Preliminary analyses showed a limited power to estimate paternities correctly if field information was disregarded, due to high inbreeding and low genetic diversity (Appendix A in Supplementary file). Therefore, we combined field information with molecular data to maximize the number of reliable assignments. Field information was classified in two groups: i) highly reliable (used as a priori in our analysis): animals being sampled at dens and/or photographed with their mother before dispersion (between 0 and 6 months-old); and, ii) an expanded list of pre-assigned offspring-mother pairs, based on more uncertain field information such as a juvenile (<1 year) photographed alone in a known maternal territory, and any other relevant information on social status or localization. This information allowed us to evaluate the incorpo-