Reproductive biology and genealogy in the endangered Iberian lynx:

Implications for conservation

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Abstract

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.

Keywords

Lynx pardinus; Mating patterns; Pedigree; Paternity analysis; Inbreeding.

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; Béréños 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.iberlynce.eu). Lynx populations have been subjected to close scrutiny during the last three decades, especially in the Doñana area (Ferrerías, 2004; Ferrerías 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 (Ferrerías et al., 1997).

In this study, we aim to infer the parental-offspring relationships of lynxes born from 1990 to 2013 in the 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°10' N, 6°23' W)), and their surrounding

107 areas, which played the role of sources within the Doñana lynx metapopulation (Gaona
108 et al., 1998). This metapopulation has been isolated since at least the 1950s and its
109 size has remained around 50 individuals in the interval 1985-2005 (Palomares et al.,
110 2011b). After several conservation actions, the total number of lynx in this area
111 reached 76 in the latest census (2015), and its range spans a wider area extending
112 from Aljarafe in the east to Mazagón in the west (Garrote et al., 2011; Simón et al.,
113 2012). Due to its isolation, this population harbors very low genetic diversity and is
114 highly inbred (Casas-Marce et al., 2013; Palomares et al., 2012).

115 116 Lynx data and sample collection

117 As a result of the intensive research carried out in this area since the 1980s, more than
118 120 lynx have been radio-tracked (Ferrerías, 2004; Ferrerías et al., 1997; López-Bao et
119 al., 2010; López-Bao et al., 2011; López-Bao et al., 2008a; Palomares, 2001). In
120 addition, multiple camera-trapping campaigns have been performed since year 2000
121 (e.g. Garrote et al., 2011; López-Bao et al., 2009). Whenever a lynx was captured for
122 collaring purposes, a sample (blood or buccal swaps) was collected. Tissue samples
123 were also taken from all dead lynx found. Occasionally, samples were obtained from
124 kittens (0 to 1 month-old individuals; commonly 3 weeks-old) located at their dens by
125 tracking females during the breeding season (López-Bao et al., 2010; Palomares et al.,
126 2005). Feces were also collected for some territories (Casas-Marce et al., 2013;
127 Palomares et al., 2011c). Iberian lynx is listed in Annex II and IV of the European
128 Habitats Directive (92/43/EEC). Handling and sample collection of lynx have been
129 specifically approved under permits of the Spanish Ministry of Environment and the
130 Andalusian Department of Environment, and all subjects were treated in accordance
131 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). 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 to 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.

160 Parentage analyses

161 We inferred parental-offspring relationships using the Mac version of Colony 2 (Jones
162 and Wang, 2010). Colony 2 uses a maximum likelihood approach to calculate
163 parentage for a given year/cohort, and allows the inclusion of *a priori* information. We
164 ran Colony three independent times with the following general parameters: three runs,
165 long run length, full likelihood method and medium precision. Besides, we included the
166 allelic frequencies of the population estimated for four different periods (1990-2006,
167 2007, 2008-2009, 2010-2013) to account for stochastic or known changes in the
168 population (i.e. translocations). We assumed a per locus genotyping error rate (i.e.
169 proportion of wrong genotypes due to allelic dropout, false alleles or other types of
170 errors) of 0.01 for all markers.

171

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

179

180 Preliminary analyses showed a limited power to estimate paternities correctly if field
181 information was disregarded, due to high inbreeding and low population diversity
182 (Appendix A). Therefore, we combined field information with molecular data to
183 maximize the number of reliable assignments. Field information was classified in two
184 groups: i) highly reliable (used as *a priori* in our analysis): animals being sampled at
185 dens and/or photographed with their mother before dispersion (between 0 and 6
186 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 incorporation of further assumptions as described below, but was not used as a priori information in parentage assignments.

We elaborated two different sets of candidate parents for each cohort based on reproductive age. The relaxed one comprised individuals from 2 to 12 years-old, while the strict included individuals from 3 to 10 years-old. The wider range may increase the number of miss-assigned parents, whereas the shorter may miss rare events of reproduction at extreme ages. We assigned parentage under both scenarios and used criteria based on genetic and field information to choose the scenario to apply for each cohort (Appendix A).

We also ran our analysis with and without assuming female monogamy. Assuming monogamy increases the probability of assigning paternity/maternity for the litter, but may prevent the detection of occasional polyandrous litters. Comparisons between assignments under monogamy vs. non-monogamy (Appendix A) showed that there was only one contradiction in a father assignment under the relaxed assumption. Unfortunately, field information for evaluating this case was scarce. However, a detailed analysis performed in those cases where field information was extensive and available showed that multipaternity must be a rare event in Iberian lynx (Palomares et al., 2017), and therefore we decided to include monogamy breeding system as a prior for the analysis to increase the power and reliability of the parentage assignments.

Finally, based on the preliminary analysis, we set a threshold of 0.9 for assigning a parental-offspring relationship (Appendix A). All the input data for Colony 2 was written

using a python script that extracted the information from SQL databases where we stored demographic and genotypic information (https://github.com/mlucenaperez/paternity_analysis).

Data analysis, pedigree representation and inbreeding estimation

We used parental-offspring relationships to reconstruct a genealogy of lynx in Doñana and to estimate lynx reproductive parameters (age of reproduction, breeding patterns, and inbreeding). For all descriptive statistics, we used only individuals which have at least one reproductive event detected. Firstly, we studied the distribution of breeding ages. Secondly, we determined the number of breeding events (i.e., confirmed reproduction events) per individual. Given that lynx reproduce once per year, these reproductive events could be higher than the number of seasons where males reproduce as they could have more than one event with different females in one reproductive season. As we are assuming monandry, this does not apply to females. We calculated the number of breeding events per reproductive season (year) for males, and explored the relation between the number of events and the number of accumulated reproductive mates during the studied period using Pearson correlations, in order to assess levels of polygamy between sexes. Differences in lynx reproductive parameters between sexes were evaluated using Mann-Whitney U tests. We also calculated pedigree-based individual inbreeding coefficients and recorded every mating with inbreeding above 0.1. All analyses were run in R software v. 3.4.1 (R Core Team 2017). To graphically represent the genealogy we used Pedigree Viewer (<http://www-personal.une.edu.au/~bkinghor/pedigree.htm>).

Results

Parentage assignment

Three independent runs yielded identical results, indicating full convergence of MCMC runs. Out of the 175 sampled individuals born in Doñana from 1990-2013, 95 (54%) were assigned to both parents with a posterior assignment probability ≥ 0.9 , while in 32 (18%) and 19 (10%) cases kittens were only assigned to a mother or a father, respectively. In 28 cases (16%), no parents were assigned; these would include cases in which their true parent was not sampled, but also cases where the parent was sampled but it did not reach the threshold probability for assignment (histogram of paternity/maternity assignments probabilities and comprehensive genealogy of the population in Appendix B, Fig. S1 and Table S3).

Age of reproduction

The average age of reproduction was significantly different between sexes (Mann-Whitney U test, $P = 0.045$). Female and male lynx reproduced at an average age of 5 years (median: 4, range: 2-11) and 4.2 years (median: 4, range: 2-10), respectively (Fig. 1). We found 8 cases of reproduction at 2 years in females and 13 in males, and these events were not homogeneously distributed throughout the study period (Fig. 2).

Breeding patterns

We did not detect any event of reproduction for 62 out of the 89 candidate males (70%) and 64 out of 92 candidate females (70%) genotyped. Although we cannot estimate lifetime reproductive success, this result evidences a heterogeneous demographic contribution of individuals to the population. The number of breeding events detected per individual followed a positively skewed distribution (Fig. 3), ranging from 0 to 7 cases in females (skewness=2.3 and kurtosis=8.4), and from 0 to 9 in males (skewness=3.2 and kurtosis=5.4). The number of events per breeding season was variable in males. While 2 males out of 28 showed, on average, 2 breeding events per

season, the majority of males (20; 74%) reproduced with just only one female per breeding season (Appendix B, Fig. S2). As an extreme case of successful male, Barro had 9 reproductive events (range 1–2 females per year) during 5 non-consecutive years between 1994 and 2000. The number of observed mates was correlated with the number of observed breeding events (Fig. 4), both for females (Spearman correlation analysis: $r_s = 0.778$, $P < 0.0001$, $n = 23$) and males (Spearman correlation analysis: $r_s = 0.789$, $P < 0.0001$, $n = 25$); the similar slopes indicate similar level of multiseasonal polygamy in the two sexes. Although we did not follow all animals during their whole reproductive lifetime, the average number of mates for males was 1.6 (median = 1, range: 1-5). Barro bred with 5 different mates in 5 years (9 events) while Baya reproduced during 3 years with 4 different mates (5 events). For females, the average number of mates was 1.6 (median = 1.5, range: 1-5).

Inbreeding

The average pedigree-based individual inbreeding coefficient was 0.012 (see the constructed multi-generational pedigree for the Iberian lynx Doñana population in Fig. 5a). This value represents the inbreeding accumulated in the population from 1990 to 2013 due to mating between relatives, but it can be an underestimate due to undetected relationships. More importantly, we found 4 crosses between close relatives (Fig. 5b). As a result, 10 individuals showed an accumulated pedigree inbreeding coefficient equal or above that expected for matings between first grade cousins (0.125): 4 individuals of 0.125, 2 of 0.140, and 4 of 0.148.

Discussion

Getting reliable parentage information is difficult or directly unfeasible for cryptic and elusive species, like most solitary felids. Most importantly, parentage information

relaying on field observations is often limited to females and may consistently overlook cryptic mating (Coltman et al., 1999; Gottelli et al., 2007). Molecular markers, such as microsatellites, present a powerful tool for inferring paternity. However, they present limitations if the genetic diversity of the population or the proportion of the population sampled is low, which is usually the case for endangered species. In these scenarios, the combination of field observation and molecular markers can alleviate these limitations (Harrison et al., 2013). In our case, we could assign a high proportion of individuals (83%) to at least one of its parents with high confidence. Due to the high proportion of individuals sampled (Appendix B, Table S2), this analysis comprises the majority of the reproductive events in Doñana in the last 25 years. Our study provides the first description of mating patterns in Iberian lynx in the wild for both males and females.

While assessing the parental relationships we dealt with several limitations. Firstly, despite its relatively large size (N=36), the marker panel we used has relatively low power due to the extremely low diversity of the population, especially in years preceding the translocation of animals from the other population in Sierra Morena (Appendix A). Second, parent assignments are further complicated by a high proportion of closely-related candidates. Third, the incorporation of genotyping error allows the inference of real relationships that otherwise might be categorical excluded, but, as it increases the number of potential genotype combinations to be considered it can also lower our power to detect real relationship in the population. To circumvent these limitations and maximize the power and reliability of the results, we opted for assuming the broadly used 0.01 genotyping error rate, incorporating all highly reliable sibship and maternal field information available as a prior, and assuming female monogamy.

Studies of the reproductive parameters within the Lynx genus are scarce. Most of the parameters analyzed in this study, except the age of first reproduction, have not been studied in other lynx species, to our knowledge. The age of first reproduction in lynx genus ranged from 1-2 (*Lynx rufus*), 2 (*Lynx canadensis*), and 2-3 (*Lynx lynx*) (Axnér et al., 2009; Fritts and Sealander, 1978; Göritz et al., 2009; Kvam, 1991; Nilsen et al., 2010). This estimation is inferred from physiology and thus must be considered as the age when an individual is potentially fertile. Although this physiological age could act as an indication of the age of reproduction in nature, the last one has higher ecological relevance and conservation implications.

Mismatches between the physiological age and the age of actual reproduction in the wild may reflect the importance of behavioral influences on fitness. In our case, the Iberian lynx is extremely territorial and the acquisition of territory seems to modulate the occurrence of actual breeding. This acquisition is in turn influenced by population demography (Ferrerías et al., 1997) and suitable-territory availability (Ferrerías et al., 2010; Simón et al., 2012). Previous observational studies covering the period 1992-2001 established the earliest breeding event at 3-year-old and the latest one at 10-year-old (Palomares et al., 2005). After, Palomares et al. (2012) found successful breeding of one 2-year-old male and several 2-year-old females in the period 2005–2008 when lynx were expanding outside of the most protected area by the Doñana National Park (Simón et al., 2012). In this study we also observed an increase in 2 years-old breeding success after 2007 (Fig. 2), when high mortality rates due to the outbreak of feline leukaemia virus in the adult reproductive lynx population (Meli et al., 2010; Meli et al., 2009) yielded numerous high quality empty territories available for younger individuals (Meli et al., 2009; Palomares et al., 2012; Palomares et al., 2011a). Therefore, lynx that reproduced at 2 year-old held territories either in areas where there was leukemia, or in new suitable areas where there was an improvement in the trophic

conditions and/or a reduction of human pressure (Ferrerías et al., 2010; Simón et al., 2012). Male reproduction at 2 years should now be incorporated into the viability models of Iberian lynx, that, so far, took as 3 years the earliest age of reproduction of males (Revilla and Wiegand, 2008). The reproduction of 1-year-old female, reported during the editorial revision of this manuscript (Garrote et al., 2017), must be considered anecdotal and would not change our recommendation to reduce the minimum age of reproduction to 2 years in population models. On the other hand, maximum age of reproduction could be risen up to 11 years for females and 10 years for males instead of 9 years (Revilla and Wiegand, 2008). The expansion of the reproductive ages for lynx would allow being, slightly more optimistic on the viability of lynx populations than previously reported. In a context of reintroductions, we expect that individuals that encounter no competition in a new reintroduction area would breed from 2 years to 10 or 11 years, thus contributing longer to population growth.

The observed individual reproductive contribution was highly variable among both males and females. However, our temporally and spatially heterogeneous sampling, resulting in variable opportunities to detect reproduction for different individuals, does not allow a proper estimation of variance in lifetime reproductive success. For example, reproduction is more likely to be detected for males that are candidates at the beginning of the study period than for those that become candidates in the last years of the study. Therefore, even though these results must be taken with caution, we found that close to 30% of the individuals in the population reproduce, which is fully consistent with our estimate N_e/N_c ratio of 31% for the period 1995-2007 (Casas-Marce et al., 2013).

Although, we could not assess female monogamy because we were assuming it in our parentage assignments, another study combining territoriality and genetic data found

no evidence of multiple paternity in Iberian lynx litters (Palomares et al., 2017). However, most males reproduced with a single female in a single season, which is in agreement with observational studies suggesting a breeding system close to monogamy, with occasional polygyny, for Iberian lynx (Ferrerías et al., 1997).

The extension and exhaustiveness of our study allowed the reconstruction of a pedigree of this wild population. Pedigrees of wild populations have been used for studying inbreeding (Liberg et al., 2005), dispersion (Janečka et al., 2006), evaluation of reintroductions and population diversity (Vonholdt et al., 2008) or genetic rescue (Åkesson et al., 2016). Here, using the genealogy, we estimated an average inbreeding of 0.012 in our pedigree, which is an underestimate of the real inbreeding of the population. First of all, founder individuals (individuals lacking parentage information) are assumed to be unrelated. Besides, some parent-offspring relationships may have been missed due to unsampled individuals, or lack of power to assign. Actually, the molecular estimation of the population inbreeding accumulated since the isolation of Doñana population is 0.5, the inbreeding expected for the offspring of a self-fertilization (Casas-Marce et al., 2013). Such high inbreeding levels could be lowering the fitness of individuals and probably the population average fitness. Inbreeding seems to be negatively affecting semen quality (Ruiz-López et al., 2012), and reproduction and survival in the captive lynx population (Cuenca et al, in preparation), and may be behind the significant reduction in the litter size and the increase in non-traumatic mortality from 2002-2007 compared to the 1983-1997 (Palomares et al., 2012). The translocation of divergent individuals from genetically differentiated populations is expected to reduce the accumulation of inbreeding in the population and mitigate its detrimental consequences (Simón et al., 2012). Besides, we provide definitive evidence for matings between close relatives in Doñana. This process is expected to occur even under random mating in small isolated populations,

due to lack of inbreeding avoidance mechanism or unavailability of unrelated individual in the area. Therefore, reintroduction programs should avoid the reintroduction or translocation of close relatives to the same areas, and avoid the use of individuals with high individual inbreeding. To be able to respond to this challenges, extant and new populations should be subjected to an active genetic management.

Conclusion

For endangered species, the availability of genealogies and a good knowledge of mating patterns are valuable resources for conservation purposes. Here we report similar mating patterns between male and female and the reconstruction of the pedigree of a population of the endangered Iberian lynx. This study was only possible thanks to the combination of genetic markers and field information. We assess that only 30% of the reproductive individuals were able to successfully reproduce, and for those that did, the number of breeding event followed a positively skewed distribution. 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 a high turnover of the territories. Lack of competition allows young individuals to access territories and reproduce. Viability models for lynx could now incorporate this data to evaluate present and future risks and to design optimal management strategies for the species. Thanks to the reconstruction of the pedigree, we detected crosses between close relatives (eg. full-sibs). The lack of an absolute inbreeding avoidance mechanism in the population should be taken into account for reintroduction strategies. Moreover, the reconstructed pedigree will also be a valuable resource to trace the inheritance of genetic diseases and to investigate their genetic bases, eventually facilitating their management. These challenges make of the

428 genetic management of the specie a fundamental component of conservation
429 purposes.

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Figure and table captions.

Fig. 1 Histogram of breeding events detected for different age classes in males (grey) and in females (black) for the Iberian lynx population between 1990 and 2013. Dashed lines represent the mean for males and females.

Fig. 2 a) Census of total Doñana population (circle) and territorial females (triangle). Census information taken from annual reports of the LIFE10NAT/ES/570 project (http://www.iberlince.eu/images/docs/3_InformesLIFE/Informe_Censo_2015.pdf). The arrow indicates the outbreak of leukemia virus, which was followed by the translocation

of an individual from the other remnant population (Andújar, Sierra Morena). b) Number of mating of individuals with 2 years (black) related to the total mating events (grey), for females (top) and males (bottom) for each year.

Fig. 3 Histogram of breeding events per individual, including 0 (no reproduction detected). Males are represented in grey and females in black. The dash line represents the median for both males and females.

Fig. 4 Relationship between the number of breeding events detected for an individual and number of partners registered for that each individual. The size of the shape represents the frequency and ranges from 1-11 in males and 1-7 in females. A regression line was added with the corresponding 95% confidence interval. Males are represented in grey and females y black.

Fig. 5 a) Pedigree of Doñana population from 1990 to 2013. Orange lines represent paternity relationships while grey lines represent maternity relationships. b) Four crosses producing offspring with high inbreeding coefficient ($F \geq 0.125$).

638 **Appendix A**

639 **Supplementary Methods.**

- 640 1. Statistical power of the marker panel
- 641 2. Preliminary parentage analyses.
- 642 3. Selection of relaxed or strict assumption per cohort
- 643 4. Convergence analyses in Colony
- 644

Appendix B

Supplementary figures and table.

Fig. S1 Histogram of the paternity/maternity assignment probabilities for Doñana population between 1990 and 2013 given by Colony (binwidth=0.05). Paternity assignments are represented in black and maternity in grey.

Fig. S2 Average number of breeding events per breeding season. Dashed line represents the median for males (in grey) and females (in black).

Table S1 Detailed information about field data available for each individual. When several sources of information are available for the same individual only the most informative one used for age determination is listed (e.g. capture neonates > capture juvenile).

Table S2 Comparison of the number of offspring and candidate parents used for this study with the census of the population for the years 2002 to 2013. Census information was recovered using camera traps or other direct evidence of living individuals in the frame of LIFE10NAT/ES/570 project

(http://www.iberlince.eu/images/docs/3_InformesLIFE/Informe_Censo_2015.pdf).

Female census refers only to territorial females. We identified parent candidates in a particular year based on demographic information, i.e., birth and death year). As the census is based on evidence of living and we rely on lack of evidence of death, the percentage of sampled individuals is often higher than 100%.

Table S3 Genealogy of Doñana population between 1990-2013. Individuals with at least one of the parent assigned with a probability above 0.9 are shown. Name of

672 offspring, birth year and the assigned father and mother (and its probabilities) are
673 included. NA accounts for unassigned individuals or below our threshold (0.9).
674

a **b**