Phylogenetic and Phylogeographic Analysis of Iberian Lynx Populations


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Abstract

The Iberian lynx (Lynx pardinus), one of the world’s most endangered cat species, is vulnerable due to habitat loss, increased fragmentation of populations, and precipitous demographic reductions. An understanding of Iberian lynx evolutionary history is necessary to develop rational management plans for the species. Our objectives were to assess Iberian lynx genetic diversity at three evolutionary timescales. First we analyzed mitochondrial DNA (mtDNA) sequence variation to position the Iberian lynx relative to other species of the genus Lynx. We then assessed the pattern of mtDNA variation of isolated populations across the Iberian Peninsula. Finally we estimated levels of gene flow between two of the most important remaining lynx populations (Doñana National Park and the Sierra Morena Mountains) and characterized the extent of microsatellite locus variation in these populations. Phylogenetic analyses of 1613 bp of mtDNA sequence variation supports the hypothesis that the Iberian lynx, Eurasian lynx, and Canadian lynx diverged within a short time period around 1.53–1.68 million years ago, and that the Iberian lynx and Eurasian lynx are sister taxa. Relative to most other felid species, genetic variation in mtDNA genes and nuclear microsatellites were reduced in Iberian lynx, suggesting that they experienced a fairly severe demographic bottleneck. In addition, the effects of more recent reductions in gene flow and population size are being manifested in local patterns of molecular genetic variation. These data, combined with recent studies modeling the viability of Iberian lynx populations, should provide greater urgency for the development and implementation of rational in situ and ex situ conservation plans.
Fossil records suggest that lynx species originated in North America (MacFadden and Galiano 1981; Martin 1989). The bobcat appears to represent the earliest lineage to diverge (Johnson and O’Brien 1997), but subsequent evolutionary history is less clear. The Iberian lynx and Eurasian lynx may be derived from an ancestral lynx species (*Lynx isidorensis*) whose remains have been found in China (Wederlin 1981). The Canadian and Eurasian lynx have been hypothesized to be sister taxa (Wederlin 1981). The Iberian lynx and Eurasian lynx were both found in central Europe during the Pleistocene (Kurten 1968; Kurten and Grandqvist 1987), but may never have had significantly overlapping geographic ranges.

The distribution and population sizes of the Eurasian and Iberian lynx have been reduced significantly during the last two centuries. The Eurasian lynx was extirpated from most of central and southern Europe during the 19th century (Breitenmoser and Breitenmoser-Wursten 1990). Similarly, by the beginning of the 20th century the Iberian lynx was rare in northern Spain and by the 1960s its range was essentially limited to isolated populations in the southwestern portion of the peninsula (Rodriguez and Delibes 1990). By the 1990s, it was estimated that no more than 1000 lynx were restricted to fewer than 50 disjunct breeding areas that could be grouped into less than 10 distinct subpopulations (Castro and Palma 1996; Rodriguez and Delibes 1992). Only two of these subpopulations, Sierra Morena and Montes de Toledo (Figure 1) inhabit areas larger than 2000 km$^2$, which is of major concern since between 1960 and 1988 Iberian lynx are presumed to have disappeared from most of the remaining small habitat patches (from 91% of the areas smaller than 1000 km$^2$). This corresponds to an 80% reduction in occupied range (Rodriguez and Delibes 1990). However, current geographical limits and population sizes are not well known and continued loss of habitat and the disappearance of lynx from previously occupied areas suggests that fewer than 500 individuals may now remain (Beltrán and Delibes 1994; Palomares et al. 2000; Pires and Fernandes 2003).

The best known of the Iberian lynx populations inhabits Doñana National Park, Andalusia, where field data on the species have been collected since the 1950s and radio-tracking studies have been conducted for more than 15 years (e.g., Ferreras et al. 1997; Gaona et al. 1998; Palomares et al. 1991, 2001). The Doñana metapopulation (1500 km$^2$) of approximately 40–50 individuals currently is distributed in at least four distinct areas that were last completely connected during the middle of the last century. Within the Doñana metapopulation, the population occupying the protected parkland areas is relatively stable, while mortality in the lesser-protected areas is higher, especially among dispersing animals (70–85% mortality rate) (Ferreras et al. 1992; Gaona et al. 1998). Because of its small numbers and isolation, the Doñana metapopulation would be susceptible to low levels of genetic variation (Beltrán and Delibes 1993).

The Doñana metapopulation has been isolated from other Iberian lynx populations for at least 50 years by an expanse of more than 50 km of croplands to the north and almost 30 km of comparatively dense human settlements to the west (Rodriguez and Delibes 1992). The nearest lynx populations are found in the Sierra Morena Mountains (Figure 1), where several of the largest remaining lynx metapopulations are suspected to still exist. This mountain range, perhaps one of the keys to the future preservation of the Iberian lynx, has received increasing attention by conservationist and resource managers.

The objectives of this study were to quantify the evolutionary relationship of the Iberian lynx with the other *Lynx* species using mtDNA sequence variation, describe patterns of lynx mtDNA variation across the Iberian peninsula, and compare patterns of microsatellite size variation and estimate gene flow between the important lynx populations of Doñana and Sierra Morena. An improved understanding of patterns of Iberian lynx molecular genetic variation and recent evolutionary history is necessary to develop rational management plans for the species (Vargas 2000) and provide further insights into the biogeographical history of the Iberian peninsula.

**Methods**

**DNA Extraction**

Lynx DNA was extracted from blood and tissue samples from 20 wild animals captured or found dead during capturing and radio-tracking of lynx from 1985 to 2000, and from 35 tissue and skin samples in scientific collections from seven metapopulations throughout the range of the Iberian lynx (Table 1). Blood samples were stored in four volumes of lysis buffer (0.1 M Tris-HCl pH 8.0; 0.1 M Na-EDTA; 0.01 M NaCl, 0.5% SDS) and tissue samples were kept frozen or at room temperature in a dimethyl sulfoxide (DMSO)-salt solution (20% DMSO, 0.25M Na-EDTA, and NaCl to saturation, pH 8.0). From museums specimens, approximately 1 cm$^2$ of skin was cut with a sterile scalpel after superficial cleaning with 10% commercial bleach, distilled water, and 70% ethanol. DNA extractions of museum materials were conducted in a dedicated room along with extraction blanks to monitor for contamination. DNA was extracted following standard proteinase K/phenol chloroform protocols (Sambrook et al. 1989); for museum skin extractions, several prewashes with NTE pH 9.0 (NaCl 10 mM; Tris base 50 mM; EDTA 20 mM) were included to remove possible enzyme inhibitors. For comparative purposes we used DNA extracted using similar techniques in previous studies from three European lynx (Lly 12, 15, and 16), two Canadian lynx (Lca 3 and 7), five bobcat (Lru 6, 26, 48, 68, and 73), two marbled cats (*Pardofelis marmorata*) (Pma 4 and 5), and one clouded leopard (*Neofelis nebulosa*) (Nne 80) (Johnson and O’Brien 1997).

**Mitochondrial DNA Markers**

Sequence variation in portions of five mtDNA genes (ATPase-8, 16S rRNA, 12S rRNA, NADH-5, and cytochrome b) was assessed in four Iberian lynx, three European lynx, two Canadian lynx, and five bobcat, along with two
marbled cats and a clouded leopard as outgroups, to assess the uniqueness of the Iberian lynx and to determine the evolutionary relationships among *Lynx* species. A larger set of Iberian lynx from across the Iberian peninsula (43 individuals from seven metapopulations; Table 1) was used to characterize patterns of mtDNA sequence variation in three mtDNA fragments: 191 of ATPase-8 gene, and 195 and 89 bp of two noncontiguous fragments of the hyper-variable segment 1 of the control region (Kim et al. 2001).

DNA amplification reactions contained 67 mM Tris-HCl pH 8.0, 16 mM (NH₄)₂SO₄, 2.5 mM MgCl₂, 0.01% Tween-20, 0.2 mM dNTPs, 1 µM of each primer, 0.5 U of *Taq* polymerase and 50–100 ng of total DNA for blood samples or 5 µl of museum skin extracts as template. Polymerase chain reaction (PCR) primers and primer conditions have been published previously (Janczewski et al. 1995; Johnson and O’Brien 1997; Johnson et al. 1998; Palomares et al. 2002). Bovine serum albumin (BSA) was included at a concentration of 0.1 µg/µl for amplification of blood DNA and 0.8 µg/µl for museum and skin samples. Amplification reactions were performed in an MJ Research (Boston) thermocycler, model PTC-100, at an initial denaturation cycle of 94°C for 2 min, followed by 35 cycles of denaturation at 92°C for 30 s, annealing at 55–67.5°C (depending on primers) for 30 s, extension at 72°C for 30 s, and were completed with a final extension at 72°C for 5 min. Positive and negative DNA controls were included with each set of PCRs. Amplification products were separated by

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**Figure 1.** Geographic distribution of the Iberian lynx populations sampled in the study modified after Rodriguez and Delibes (1992) and Castro and Palma (1996). In Spain, the data represent estimated distributions from the 1980s and in Portugal the data are from 1987–1996. The distribution of mtDNA haplotypes in each of the major populations (which are labeled following Table 1) are represented in pie charts, along with the number of individuals sampled from each population.
Table 1. Sample identifier, sample type, population and metapopulation of origin, nucleotide residue at positions 8672 and 16,804 (domestic cat sequence, López et al. 1996) and mtDNA haplotype for gene segments of ATP-8, control region 1 (CR1), and control region 2 (CR2) combined

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Metapopulations are also depicted on the map in Figure 1. Samples used in microsatellite analyses are marked with an asterisk (*) and samples from the Museo de Ciencias Naturales de Madrid (MCNM), Estacion Biologica de Doñana (EBD), Parque Nacional de Doñana (PND), and the Instituto da Conservação da Natureza (ICN) are noted. The additional samples were collected by several of the authors during field studies.
electrophoresis in 2–3% agarose gels in TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA) in the presence of 0.5 mg/L EtBr. Gels were visualized under ultraviolet light and photographed with a digital image system (Eastman Kodak).

Polymerase chain reaction products were cleaned by ultrafiltration through Centriprep-100 (Millipore Corp.) and sequenced on an automated DNA sequencer (ABI 377) using the BigDye Terminator Cycle Sequencing Kit following the manufacturer’s instructions (Applied Biosystems). Sequences were edited, assembled, and aligned using the program Sequencher (Gene Codes Corp.) and submitted to GenBank (accession numbers AY499248–AY499337).

Microsatellite Markers

Twenty-eight microsatellite loci (Fca43, 71, 75, 80, 82, 90, 96, 97, 98, 102X, 117, 161, 132, 193, 232, 272, 369, 391, 424, 441, 453, 476, 493, 519, 547, 566, 571, 698) from 11 of the 19 domestic cat chromosomes were characterized in 20 presumably unrelated lynx from two populations (Vera and Coto del Rey) in Doñana and in Valquemado from Sierra Morena (Table 1) following previously described PCR amplification conditions (Menotti-Raymond et al. 1997, 1999). All microsatellites were dinucleotide repeats except FCA391, FCA441, and FCA453, which had tetranucleotide repeats. Of the 28 loci, 22 were unlinked or at least 20 cm apart in the domestic cat and are presumed to be unlinked in Iberian lynx (Menotti-Raymond et al. 1999; submitted). Three pairs of loci were linked at distances of 9 cm (Fca75 and Fca96), 8 cm (Fca90 and Fca566), and 1 cm (Fca132 and Fca369). The dye-labeled PCR products of the microsatellite primer sets were pooled and diluted together based on size range and fluorescent dye so that microsatellite loci could be multiplexed and electrophoresed and subsequently analyzed in an ABI 377 automated sequencer. Microsatellite allele sizes were estimated by comparison with a GS350 TAMRA (ABI) internal size standard. Data were collected and analyzed using the ABI programs GENESCAN (version 1.2.2-1) and GENOTYPER (version 1.1). PCR product length was used as a surrogate for actual repeat length (Ellegren et al. 1995).

Phylogenetic and Population Analyses

Phylogenetic comparisons among lynx species were conducted with sequence variation from five mtDNA gene fragments (ATPase-8, cytochrome b, 12S rRNA, 16S rRNA, NADH-5). Marbled cat and clouded leopard sequences were included for outgroup comparisons. Sequences from each of the mtDNA gene fragments were combined into a contig of 1613 bp after separate analysis of each gene fragment (Huelsenbeck et al. 1996). Phylogenetic relationships among the haplotypes were estimated using minimum evolution (ME), maximum likelihood (ML), and maximum parsimony (MP) methods using PAUP* (Swofford 2001). An MP analysis was conducted using a heuristic search, with random addition of taxa and tree-bisection-reconnection branch swapping. The ME approach employed a neighbor-joining tree (Saitou and Nei 1987) constructed from Kimura two-parameter distances, with the proportion of variable sites estimated to be 0.3687 from the empirical data and the rate for variable sites assumed to follow a gamma distribution. After testing and comparing several models, ML analysis was done using the HKY85 model (Hasegawa et al. 1985) with parameters estimated from the dataset. The reliability of the nodes in each of the analyses was assessed by 100 bootstrap iterations (Hillis and Bull 1993).

Mitochondrial DNA sequence variation across Iberian lynx was assessed in 452 bp from three mitochondrial gene fragments (ATP-8 and two control region segments). Measures of mtDNA sequence variation were estimated using MEGA 2.1 (Kumar et al. 2001). The divergence date among European lynx, Iberian lynx, and Canadian lynx was estimated by averaging all pairwise (p) distances among haplotypes. Feline-specific mtDNA divergence rates of 1.39% (ATPase-8), 0.97% (cytochrome b), 1.22% (NADH-5), 0.88% (12S rRNA), and 0.97% (16S rRNA), as developed by López et al. (1997), were weighted based on the number of base pairs used for each gene to obtain a composite divergence rate of 1.04% per million years.

Estimates of microsatellite size variation, such as average expected heterozygosity, average variance, number of unique alleles, and average number of repeats, were derived from the program MICROSAT (version 1.5) (Minch et al. 1995). Deviations from Hardy-Weinberg equilibrium, following the procedure of Guo and Thompson (1992), were estimated using the program POPULATION SUBDIVISION, FST, and RST analogs (Michalakis and Excoffier 1996; Slatkin 1995; Weir and Cockerham 1984) were derived using ARLEQUIN (Schneider et al. 2000).

Pairwise genetic distances among individuals using the composite microsatellite genotypes were estimated using the proportion of shared alleles (Dps) algorithm with a (1 – M) correction as implemented in the program MICROSAT (version 1.5) (Minch et al. 1995). A phylogenetic tree was constructed from the Dps distance matrix using the Neighbor option of the program PHYLIP (version 3.572) (Felsenstein 1993) and was drawn using the program TREEVIEW (version 1.5) (Page 1996).

Results and Discussion

Phylogenetic Analyses

Analysis of the evolutionary relationships among the four Lynx species from 1613 bp of sequence from five mtDNA genes (ATPase-8, 16S rRNA, 12S rRNA, NADH-5, and cytochrome b) confirmed the taxonomic status of the Iberian lynx as a unique species with a relatively long evolutionary history (Beltrán et al. 1996). Among the four Lynx species, there were 158 variable sites, of which 141 were parsimonious informative (Figure 2). There were two Iberian lynx haplotypes from four individuals, two haplotypes from three European lynx, two haplotypes from two Canadian lynx, and three haplotypes from five bobcats, along with two marbled
cat haplotypes (Figures 2 and 3). Among the Lynx species, using the marbled cat and clouded leopard as outgroup species, there was strong support (96–100% bootstrap support from MP, ME, and ML phylogenetic analyses) that the bobcat was the most basal lineage, or the first to diverge (Figure 3). The relative relationships among the Iberian lynx, Eurasian lynx, and Canadian lynx were less well defined, with bootstrap support varying depending upon the method of analysis. However, each analysis suggested that the Iberian lynx and Eurasian lynx were sister taxa. The pairwise genetic distances among these three species were low, ranging from 53 to 58 bp (of 1639 bp), or 3.2–3.5%.

Figure 2. Variable sites among Iberian lynx (Lyp), Eurasian lynx (Lly), Canadian lynx (Lca), Bobcat (Lru), and Marbled cat (Pma) for 12S, 16S, Atp8, NADH-5, and CytB mtDNA gene fragments. Sample codes refer to the individual Iberian lynx (Lyp), European lynx (Lly), Canadian lynx (Lca), bobcat (Lru), and marbled cat (Pma) depicted in the phylogenetic tree of Figure 3. Position numbers correspond to the complete domestic cat mtDNA sequence (López et al. 1996).
These results confirm that the Iberian lynx is a unique species and that the Eurasian lynx, Canadian lynx, and Iberian lynx all speciated or diverged into monophyletic lineages around the same time. Assuming an mtDNA divergence rate of 1.04% per million years, based on a feline gene-specific mtDNA mutation rate (Culver et al. 2000; Johnson and O’Brien 1997), the rapid divergence among the three lynx species occurred around 1.53–1.68 million years ago.

Mitochondrial DNA Diversity

Mitochondrial DNA sequence variation across 46 Iberian lynx from throughout most of their distribution on the Iberian Peninsula (from seven metapopulations) was assessed in 452 bp from three mitochondrial gene fragments (positions 8657 to 8818, as numbered in the complete Felis catus mtDNA sequence of López et al. [1996] and two control region segments). Because many of these samples were hides from museums, not all individuals amplified for each of the three gene segments. MtDNA diversity among Iberian lynx was low (Figure 2 and Table 2). Among the 46 Iberian lynx sequenced, there were two variable sites in 452 bp from three mtDNA fragments that defined three haplotypes (A, B, and C). At position number 8672 (nucleotide numbers from the reference domestic cat sequence; López et al. 1996) in the ATP-8 gene, there was either a T (haplotypes A and C) or a C (haplotype B). At position number 16,804 of the control region, two individuals from the Sado-Algarve metapopulation had a C (haplotype C), compared with T for the other lynx (haplotypes A and B). All eight Iberian lynx from the southernmost metapopulation of the Doñana National Park area had haplotype B, as did one lynx from eastern Sierra Morena, two lynx from Sado-Algarve, Portugal, and four lynx from Sierra Gata Malecata, Portugal. Haplotype A was found in the easternmost metapopulations of eastern Sierra Morena and Montes de Toledo. Haplotype C was restricted to the two samples from the western Sierra Morena metapopulation (Figure 1).

The Iberian lynx displayed among the lowest levels of mtDNA diversity that have been documented for a feline species, as is apparent from a comparison of the sequence variation across the roughly 880 bp from the mtDNA gene fragments of NADH-5, 16S, and ATP-8 among several cat species (Table 2). This overall pattern suggests that Iberian lynx is descended from a recent founder effect or a population bottleneck. In addition, the disjunct distribution of the haplotypes (Figure 1) suggests that recent isolation of populations and reduced population sizes may have led to haplotype fixation. All three haplotypes were found between

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample size</th>
<th>Number of variable sites</th>
<th>Number of haplotypes</th>
<th>π × 100</th>
<th>Reference</th>
</tr>
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<tr>
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<td>44</td>
<td>14</td>
<td>1.12</td>
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<td>Geoffroy’s cat</td>
<td>38</td>
<td>48</td>
<td>32</td>
<td>1.25</td>
<td>Johnson et al. (1999)</td>
</tr>
<tr>
<td>Kodkod</td>
<td>6</td>
<td>7</td>
<td>3</td>
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<tr>
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<td>20</td>
<td>3</td>
<td>3</td>
<td>0.05</td>
<td>This study</td>
</tr>
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Figure 3. Phylogenetic relationships among Lynx species and outgroup Felid species from 1639 bp of sequence from six combined mtDNA gene fragments. Depicted is a maximum likelihood phylogenetic tree constructed with the HKY85 model using empirical nucleotide frequencies, a transition/transversion ratio of 12.6, an assumed proportion of invariable sites of 0.295, and a shape parameter (q) of 0.206. Above the branches are bootstrap values (100 iterations) for maximum parsimony/minimum evolution/maximum likelihood analyses and below the branches are the number of base substitutions/number of homoplasies from the maximum parsimony analyses. Maximum parsimony trees were obtained via a tree-bisection reconnection algorithm with starting trees obtained by stepwise addition. Minimum evolution trees were depicted using the neighbor-joining algorithm using Kimura two-parameter distances.

Table 2. Measures of mtDNA sequence variation in the same combined fragments of NADH-5 (positions 12,647 to 12,946 from the complete Felis catus mtDNA sequence of López et al. 1996), 16S (positions 2904 to 3285), and ATP-8 (positions 8657 to 8818) (a total of about 880 bp) among eight felid species.

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the eastern and western Sierra Morena metapopulations, in south-central Spain, in the middle of the historic distribution of lynx.

Microsatellite Diversity

The amount of microsatellite allele variation among Iberian lynx from the two Doñana populations was very similar (Table 3). Average observed heterozygosity was 26.7% in Coto del Rey and 29.4% in Vera and the average range in allele sizes in both populations was 1.57. Estimates of microsatellite size variation were slightly higher in Sierra Morena than in Doñana (Table 3), including a larger percentage of polymorphic microsatellite loci (75% versus 71.4%). Overall, this amount of microsatellite variation is less than or comparable to that seen in felids such as cheetahs and North American pumas (Table 4), which experienced demographic bottlenecks around the time of the Pleistocene ice ages (Culver et al. 2000).

The populations of Coto del Rey and Vera each had three unique alleles that were not observed in the other. In contrast, the metapopulation of Doñana had 19 unique alleles not observed in Sierra Morena, and Sierra Morena had 26 that were not seen in Doñana (Table 3). These differences were reflected in the analyses of population structure. \(F_{ST}\) values among all three populations were significant (\(P<.05\)), but were highest between Sierra Morena and the two Doñana populations (0.378 with Coto del Rey and 0.227 with Vera; 0.132 between Coto del Rey and Vera). \(R_{ST}\) values were only significant between Sierra Morena and Coto del Rey (\(R_{ST} = 0.576\)). However, several loci were significantly out of Hardy-Weinberg equilibrium in the Doñana metapopulation and in the Vera population when analyzed separately. In each case there was a deficiency of heterozygotes that may reflect inbreeding, disproportionate reproductive success of some individuals, or some degree of allele dropout.

The pattern of differentiation among populations can be visualized in the dendrograms resulting from the phylogenetic analyses of individual composite genotypes (Figure 4). The six lynx from Sierra Morena are separated from Doñana individuals with high bootstrap support (93%). In comparison, lynx from the two Doñana populations were intermixed, although animals from the same population tended to be most closely linked with another individual from the same population. During the last 15 years there has been only one documented instance of a lynx that moved from Vera to Coto del Rey and another three cases of lynx moving from Coto del Rey to Vera (Ferreras 2001). Of these, only the lynx that emigrated to Coto del Rey established a territory and successfully bred.

Evolutionary Implications

Our estimation, based on mtDNA sequence variation and a felid-specific divergence rate for these genes, that \(L.\) \(pardinus\) diverged as an unique species 1.53–1.68 million years ago is compatible with paleontological evidence that the \(Lynx\) species inhabiting Europe during the late Pliocene and early Pleistocene was probably a common ancestor (frequently called \(L.\) \(issiodorensis\)) of three current \(Lynx\) species, \(L.\) \(lynx\), \(L.\) \(pardinus\), and \(L.\) \(canadensis\) (Kurten 1968; Werderlin 1981). The earliest paleontological evidence of \(L.\) \(pardinus\) has been found in France from around several hundred thousand years ago, in the Middle Pleistocene and later (Kurten and

<table>
<thead>
<tr>
<th>Population</th>
<th>Sample size</th>
<th>Loci typed</th>
<th>Observed heterozygosity</th>
<th>Mean microsatellite variance</th>
<th>Number of alleles/locus</th>
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<tr>
<td>Asian lion</td>
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<td>12</td>
<td>48.2</td>
<td>3.732</td>
<td>3.50</td>
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<td>10</td>
<td>11</td>
<td>53.0</td>
<td>5.067</td>
<td>4.09</td>
<td>Driscoll et al. (2002)</td>
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<td>26.0</td>
<td>3.908</td>
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<td>Puma, Idaho</td>
<td>10</td>
<td>11</td>
<td>61.7</td>
<td>11.651</td>
<td>3.91</td>
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<td>Puma, South America</td>
<td>10</td>
<td>11</td>
<td>81.3</td>
<td>31.667</td>
<td>7.82</td>
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<td>11</td>
<td>38.0</td>
<td>1.540</td>
<td>2.27</td>
<td>This study</td>
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</tbody>
</table>

Table 3. Measures of variation in 28 microsatellites in three Iberian lynx populations

Table 4. Measures of microsatellite size variation at 12 loci among populations of four felid species
Cooper et al. 1995) and some shrews of the Sorex araneus Hewitt 1996), such as the grasshopper Chorthippus parallelus occurred with numerous other species (Bennet et al. 1991; Iberia during one or more of the frequent ice periods, as Iberian lynx was restricted to a glacial refugium in southern levels of genetic variation (Johnson et al. 2001).

If current trends continue, it is very likely that much more active management of some populations will be necessary in order to maintain sufficient population sizes and existing levels of genetic variation (Johnson et al. 2001).

We recommend that any future movement of animals in the wild or the establishment of captive programs be accompanied by more thorough analysis of the population sizes and levels of microsatellite variation in the other lynx metapopulations in addition to those of Doñana and Sierra Morena. This will necessitate an increased emphasis on coordinated conservation action plans, both in situ and ex situ, among the numerous jurisdictions encompassing lynx distributions in Portugal and Spain (Vargas 2000).

References


Granqvist 1987; Werderlin 1981). These lynx, often referred to as cave lynx (L. p. speleus), were larger than current L. pardinus.

Ecological and biogeographical data suggest that the Iberian lynx was restricted to a glacial refugium in southern Iberia during one or more of the frequent ice periods, as occurred with numerous other species (Bennet et al. 1991; Hewitt 1996), such as the grasshopper Chorthippus parallelus (Cooper et al. 1995) and some shrews of the Sorex araneus group (Taberlet et al. 1994). The relatively low levels of mtDNA sequence and microsatellite size variation relative to other cat species in evidence today may have resulted from at least one demographic bottleneck during this time period.

Granqvist 1987; Werderlin 1981). These lynx, often referred to as cave lynx (L. p. speleus), were larger than current L. pardinus.

These molecular genetic results suggest that there is modest genetic differentiation among metapopulations between Doñana versus Sierra Morena metapopulations. However, the differences are slight and inapparent with mtDNA. These results suggest that the overriding genetic concern of Iberian lynx populations may be their small effective population sizes, which are at risk for extinction and further genetic reduction. If current trends continue, it is very likely that much more active management of some populations will be necessary in order to maintain sufficient population sizes and existing levels of genetic variation (Johnson et al. 2001).

Figure 4. Phylogenetic relationships among Iberian lynx individuals from three lynx populations based on the proportion of shared alleles among the combined genotypes of 28 microsatellite loci. Among the individuals from the Doñana metapopulation, those from the Coto del Rey population are underlined and those from the Vera population are not. Bootstrap support (100 iterations) between populations is listed.

Conservation Implications

There currently are no recognized subspecies of Iberian lynx. Most of the conservation programs that have been envisaged for the Iberian lynx have been based on metapopulations. These molecular genetic results suggest that there is modest genetic differentiation among microsatellites between Doñana versus Sierra Morena metapopulations. However, the differences are slight and inapparent with mtDNA. These results suggest that the overriding genetic concern of Iberian lynx populations may be their small effective population sizes, which are at risk for extinction and further genetic reduction. If current trends continue, it is very likely that much more active management of some populations will be necessary in order to maintain sufficient population sizes and existing levels of genetic variation (Johnson et al. 2001).
Genetic individualization of domestic cats using feline STR loci for forensic


