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Pedigree Reconstruction Sheds Light on the Mating System and Social Dynamics of Urban Bobcats (*Lynx rufus*)

A thesis submitted in partial satisfaction of the

requirements for the degree Master of Science

in Biology

by

Shaelynn Alise Sleater-Squires

2016

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2016

ABSTRACT OF THE THESIS

Pedigree Reconstruction Sheds Light on the Mating System and Social Dynamics of Urban Bobcats (*Lynx rufus*)

by

Shaelynn Alise Sleater-Squires

Master of Science in Biology

University of California, Los Angeles, 2016

Professor Robert Wayne, Chair

Wildlife pedigrees are crucial to addressing a broad range of fundamental ecological and evolutionary questions related to population dynamics, mating systems, trait heritability, and inbreeding levels. Pedigree analyses also have important implications for the conservation and management of threatened species. In this study, we reconstructed a multigenerational pedigree for 196 urban bobcats (*Lynx rufus*) sampled from 1996–2015 in the Simi Hills of southern California using 21 microsatellite loci. We determined that both sexes exhibit moderate to high mate fidelity between breeding seasons, but did not detect multiple paternity among litters, which adds support to the long-standing hypothesis that bobcats are polygynous within breeding seasons. Our results shed light on the social dynamics and mating system of an elusive, solitary mesopredator and will provide an invaluable tool for future research related to spatial social organization, trait heritability, and mating patterns in this population.

The thesis of Shaelynn Alise Sleater-Squires is approved.

Daniel T. Blumstein

Peter Nicholas Nonacs

Seth Dunmore Riley

Robert Wayne, Committee Chair

University of California, Los Angeles

2016

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INTRODUCTION

Evaluating the genealogical relationships among individuals, both within and between generations, reveals their lifetime reproductive success, which in turn facilitates the investigation of myriad questions related to the evolutionary trajectory of populations over time. Thus, wildlife pedigrees provide researchers with a powerful tool to investigate a broad range of fundamental biological questions related to population dynamics, mating systems, trait heritability, inbreeding levels, and even speciation (Kruuk & Hill 2008; Pemberton 2008; Jones & Wang 2010a), all of which are key to understanding basic ecological and evolutionary processes. Furthermore, a better grasp of these processes can inform conservation planning and management strategies for the ever-growing number of threatened wildlife populations.

Although the value of pedigrees has long been recognized, pedigree reconstruction was slow to gain traction until the last few decades, during which individual-based studies amassed long-term datasets spanning several generations (Clutton-Brock & Sheldon 2010), access to informative molecular markers flourished (Garant & Kruuk 2005), and sophisticated statistical methods were developed (Blouin 2003; Jones et al. 2010). Among other advances, the rise in molecular marker-based pedigree analyses has revolutionized our understanding of mating systems in numerous vertebrate species. In birds where monogamy was thought to be the norm historically, genetic studies revealed the reverse: 86% of the avian species surveyed were promiscuous with up 18.7% of broods having multiple sires in socially monogamous species (reviewed by Griffith et al. 2002). Similarly, several mammals traditionally thought to be monogamous, such as alpine marmots (*Marmota marmota*: Goossens et al. 1998), red foxes (*Vulpes vulpes*: (Baker et al. 2004; Iossa et al. 2009), African wild dogs (*Lycaon pictus*: Girman et al. 1997; Moueix 2006), and white-handed gibbons (*Hylobates lar*: Reichard 2009; Barelli et

al. 2013), exhibit extra-group paternities. Moreover, even purportedly polygynous mammals have turned out to be sexually polygynandrous or promiscuous within breeding seasons in some populations (e.g., Gottelli et al. 2007; Delgado et al. 2008; Hawkins & Racey 2009; McEachern et al. 2009; Poteaux et al. 2009; Lyke et al. 2013; reviewed by Wolff & Macdonald 2004). Clearly there is much more variation in vertebrate mating systems than was expected before the advent of molecular-marker based pedigrees.

Recent years have seen further improvement in the techniques for generating pedigrees, even for populations in which sampling is incomplete and comprehensive records of social interactions, mating events, and movement patterns are unavailable (Richards-Zawacki et al. 2012; Aykanat et al. 2014; Cope et al. 2014; Wang 2014). Thus pedigrees continue to offer the opportunity for insight into fundamental biological questions. Only in the past few years, for example, a knowledge of genealogical relationships has shed light on inbreeding risks and costs in capuchin monkeys (*Cebus capucinus*; Godoy et al. 2016), the mating system and sexual selection of secretive rattlesnakes (*Crotalus atrox*; Clark et al. 2014), the heritability of methylation marks in gray wolves (*Canis lupus*; Janowitz Koch et al. 2016), adoption and monozygotic twinning phenomena in polar bears (*Ursus maritimus*; Malenfant et al. 2016), and the dynamics of a mountain lion (*Puma concolor*) population threatened by habitat fragmentation (Riley et al. 2014). Despite these advances and instructive studies, investigating questions related to the social dynamics and mating behavior of solitary carnivores remains challenging due to their low population densities and elusive nature. As a result, such studies continue to be rare. Moreover, much remains to be discovered regarding lifetime fitness in natural populations in addition to how human-induced and natural environmental change influences social

organization and mating systems over extended periods of time (Clutton-Brock & Sheldon 2010; Andrew et al. 2013).

In this study, we sought to contribute to this current knowledge gap by constructing a multigenerational pedigree for a well-studied bobcat (*Lynx rufus*) population found in the Simi Hills in southern California. Bobcats in the Simi Hills and surrounding Santa Monica Mountains have been continuously monitored via trapping and radio-telemetry by National Park Service (NPS) and University of California, Los Angeles (UCLA) biologists since 1996. In the Simi Hills alone, approximately 200 individuals have been sampled through live captures of adults and yearlings, kitten sampling at dens, and opportunistic mortality tissue collection. Additionally, observation of survival, health, movement patterns, and female reproduction has been ongoing for the past 20 years.

This focal population has broad significance for questions related to human-induced environmental change because the study area comprises large expanses of contiguous habitat with low levels of urban development in addition to highly fragmented regions that overlap with urban Los Angeles County and intersect several secondary roads and a major freeway (US-Route 101). Two foundational genetic studies have revealed urbanization as a driver of evolution in this bobcat system over short timescales (Riley et al. 2003, 2006). The 101 freeway in particular has been shown to be a significant barrier of gene flow not only for bobcats, but two other mammalian carnivores *(Canis latrans, Puma concolor*), three lizards (*Uta stansburiana*, *Plestiodon skiltonianus, Sceloporus occidentalis*), and even one bird species (*Chamaea fasciata*) (Riley et al. 2003, 2006, 2014; Delaney et al. 2010).

The Simi Hills bobcats also experienced a precipitous population decline due to disease, which has had a profound and rapid impact on genetic change at both neutral and functional

(immune-related) regions (Riley et al. 2007; Serieys et al. 2015a; b). Starting in 2002, a large number of bobcats and mountain lions found in the Simi Hills region contracted notoedric mange (Riley et al. 2007; Uzal et al. 2007), which is an ectoparasitic disease that typically only occurs in isolated cases among wild felids globally (e.g., Penner & Parke 1954; Pence et al. 1982, 1995; Ryser-Degiorgis et al. 2002); although there was a more widespread outbreak in a feral cat (*Felis silvestris catus*) population in Florida (Foley 1991). At the peak of the mange epizootic, the annual survival rate for radio-collared bobcats in the Simi Hills population fell from greater than 75% to less than 30%, with the mange mortality rate reaching a high of 51% in 2003 (Riley et al. 2007; Serieys et al. 2013). Researchers discovered that concurrent exposure to anticoagulant rodenticides was associated with increased susceptibility to advanced mange in bobcats (Riley et al. 2007; Serieys et al. 2015a), which is further evidence of the pervasive anthropomorphic challenges faced by this population. Moreover, Serieys et al. (2015b) found that a genetic bottleneck occurred between 2002 and 2005, which produced greater genetic differentiation between the pre- and post-mange Simi Hills population than that between populations that have been separated by major freeways for more than 60 years in the broader Santa Monica Mountains area.

Although there was local extinction of bobcats in some habitat patches throughout the Simi Hills region during the mange-induced population decline (Riley et al. 2007), the population appears to be in strong recovery in recent years (NPS, unpublished data). Thus, this thoroughly and continuously studied system presents a rare opportunity to compare social dynamics before, during, and after a severe genetic bottleneck, and evaluate the population's patterns of reproductive behavior as the landscape has been repopulated. In addition, kitten sampling at dens where the mother was known provides key data on parentage and preliminary

information on reproductive success in the system that can be compared to the genetic results to assess pedigree reconstruction accuracy.

In this study, we sought to construct a multigenerational pedigree that spans 19 years of sampling of bobcats in the Simi Hills in order to (1) describe the mating system of bobcats, including the occurrence of multiple paternity and incestuous mating events, (2) shed light on individual life histories and lifetime reproductive success, and (3) provide a valuable tool for future studies in this unique population of bobcats that is undergoing demographic change associated with human-induced environmental challenges.

MATERIALS AND METHODS

Focal organism

Bobcats are small felids that are roughly twice the size of domestic cats (*Felis catus*) and exhibit sexual dimorphism in size with males (9.6 kg) being slightly larger than females (6.8 kg) on average. Bobcat diets are strictly carnivorous and largely comprise lagomorphs, although they also feed on insects, rodents, birds, reptiles, fish, and even deer. They are broadly-distributed across North America, ranging from southern Canada to central Mexico and California to Maine and are habitat-generalists, populating a wide array of environments from deserts and prairie-woodland mixes to subtropical wetlands and temperate forests. Male home ranges are typically two to three times larger than those of females, but average home range sizes vary considerably with latitude, which is most likely due to lower prey densities present in northern regions (reviewed by Provost et al. 1973; McCord & Cardoza 1982; Larivière & Walton 1997; Whitaker & Hamilton 1998; Sunquist & Sunquist 2002; Anderson & Lovallo 2003; Hansen 2007; Riley et al. 2010).

Aside from interactions among females and her dependent offspring, bobcats are generally solitary and territorial with adult males and females only interacting during the breeding season. Little is known about the bobcat mating system beyond the belief that they are polygynous (Provost et al. 1973; McCord & Cardoza 1982; Anderson & Lovallo 2003). Bobcats typically have a well-defined breeding season which varies with latitude, but generally occurs from December to July. However, females are seasonally polyestrus, and thus females who fail to reproduce or lose their litter early in the season may breed later in the year. Most males are capable of breeding during their second winter whereas females reach sexually maturity at 9–12 months of age, but rarely breed before their second year. Bobcat gestation periods range from 63 to 70 days and kittens typically emerge from their dens when they are 33–42 days old. Between the ages of three and five months, young bobcats begin accompanying their mothers on hunts and typically remain dependent on her for an additional two to four months, although some mother-offspring associations last a year or more. Once offspring reach independence, they may disperse long distances (greater than 150 km in some populations), although dispersal distances vary among individuals and populations (reviewed by Provost et al. 1973; McCord & Cardoza 1982; Larivière & Walton 1997; Whitaker & Hamilton 1998; Sunquist & Sunquist 2002; Anderson & Lovallo 2003; Hansen 2007; Riley et al. 2010).

Study area and sample collection

Our target bobcat population is located in and around the Simi Hills, which are part of Transverse Ranges System in the Greater Los Angeles Area in southern California. The coastal sage scrub, mixed chaparral, grassland, and oak woodland natural habitat of this area is interrupted by varying levels of residential, commercial, and recreational development, including

an 8–10 lane freeway to the south (United States Route 101), two 4–6 lane freeways to the north and west (California State Routes 118 and 23, respectively), and numerous secondary roads that intersect the Simi Hills (Figure 1). Due to the extensive urban development surrounding the area, it is considered to be a crucial habitat corridor linking the Santa Monica Mountains to the south with more extensive natural habitat, including the Santa Susana Mountains, to the north (Penrod et al. 2001). In addition to bobcats, this area supports mountain lions (*Puma concolor*), coyotes (*Canis latrans*), gray foxes (*Urocyon cinereoargenteus*), and raccoons (*Procyon lotor*) among other native and non-native mesopredator species (Crooks 2002).

A total of 196 bobcats were included in this study. Bobcat blood, tissue, and buccal swap samples were collected as part of a long-term National Park Service (NPS) urban bobcat ecology study as previously described (Riley et al. 2003, 2006, 2007, 2010; Serieys et al. 2013, 2015a; b). Briefly, biologists from the NPS and UCLA captured bobcats with padded foothold traps from 1996 to 1998 and with box traps from 2000 to 2015. Captured animals were immobilized with ketamine hydrochloride and xylazine hydrochloride (5:1 ratio) and then a blood, tissue, or buccal swab sample for DNA was taken as the animal was aged, sexed, weighed, measured, and ear-tagged before being released at the capture site. A subset of adults were also fitted with very high frequency (VHF) radiotransmitters (Telonics Inc., Mesa, AZ; Telemetry Solutions, Concord, CA; Advanced Telemetry Systems, Isanti, MN) before release as part of the larger study. Postmortem tissue samples were collected opportunistically from carcasses found throughout the research area. After extensive review to minimize animal stress and suffering, all animal capture, handling, and sample collection protocols were approved by the Office of Animal Research Oversight of UCLA (Protocol ARC#2007-167-12). The California Department of Fish and Wildlife (SC-9791) authorized scientific collecting permits.

Microsatellite genotyping

DNA extraction and microsatellite genotyping protocols have changed over the course of the 19-year study. DNA was extracted from blood, tissue, and buccal swabs using a standard phenol-chloroform protocol until 2003, after which we used QIAGEN kits (QIAamp DNA Mini Kits or DNeasy Blood and Tissue Kits, Valencia, CA, USA) according to the manufacturer's instructions. We genotyped each individual at 28 microsatellite loci (Table 1), including seven immune-linked loci (DRA1, DRB1, DRB3, DRB4, FLA1, TLR3, and TLR4) developed in the bobcat by Serieys et al. (2015b) along with 21 putatively neutral microsatellite loci developed by Faircloth et al. (2005) for the bobcat (BC1AT, BCD1AT, BCE5T, and BCH6T), Carmichael et al. (2000) for the Canada lynx (*Lynx canadensis*: LC109, LC110, and LC111), and Menotti-Raymond et al. (1999, 2005) for the domestic cat (*Felis catus*: FCA008, FCA023, FCA026, FCA031, FCA043, FCA045, FCA077, FCA082, FCA090, FCA096, FCA132, FCA149, FCA559, and FCA742). All 28 loci were previously confirmed to be polymorphic in bobcats by other studies (e.g., Carmichael et al. 2000; Ernest et al. 2000; Faircloth et al. 2005; Reding et al. 2013; Serieys et al. 2015b).

Microsatellite genotypes were obtained by polymerase chain reaction (PCR) amplification methodologies adapted from Boutin-Ganache et al. (2001) using QIAGEN Multiplex PCR Kits (QIAGEN, Valencia, CA, USA). For each of the 28 loci, the reverse primers were used as published, whereas the forward primers were modified to contain the 16-bp M13 sequence (–20: 5'–GTA AAA CGA CGG CCA G–3') at the 5' end. The PCR products were fluorescently labeled during the PCR reaction using a second forward primer comprised of the above M13 sequence along with a dye-label (1996–2006: D4, Beckman Coulter, Brea, CA; 2007–2015: 6-FAM or NED, Applied Biosystems, Foster City, CA). PCR reactions were

performed in 10 μL volumes, which consisted of 1.0 μL M13 hybrid primer mix, 2.1 μL distilled water, 0.4 μL 10 mg/mL bovine serum albumin, 5.0 μL QIAGEN multiplex PCR master mix, and 1.5 μL (20–50 ng) sample DNA. Seventeen of the microsatellite loci were amplified individually while 12 loci were grouped into three multiplex amplifications based on PCR product size and primer compatibility (Table 1) after extensive testing and optimization from a pool of 19 putatively neutral microsatellites used in previous bobcat studies (e.g., Carmichael et al. 2000; Ernest et al. 2000; Faircloth et al. 2005; Janečka et al. 2006; Reding et al. 2013). Although the remaining seven markers successfully amplified in individual PCR reactions among a subset of samples, their performance in the multiplex assays was not as robust, and thus they were excluded from the analyses herein (Table 2).

PCR amplifications were implemented under the following thermocycling profile: initial activation at 95°C for 15 min; followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 90 s, and elongation at 72°C for 60 s; then 15 cycles at 94°C for 30 s, 53°C for 90 s, and 72°C for 60 s with a final extension step of 60°C for 30 min. PCR products were sized on a CEQ 2000XL DNA Analysis System (Beckman Coulter, Brea, CA) from 1996–2006. Beginning in 2007, 9.7 μL Hi-Di formamide and 0.3 μL GeneScan™ 500 LIZ® Size Standard were added to a 1:20 dilution of the PCR products before being run on a capillary 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). For the eight immune-linked loci, two PCR products with different fluorescent labels (6-FAM and NED, Applied Biosystems) were run together. Allele sizes were scored from the resulting electropherograms using PEAK SCANNER 2.0 software (Applied Biosystems). Any samples with ambiguous allele calls or failed amplifications were re-genotyped. Additionally, numerous positive and negative controls were used to ensure that genotype calling remained consistent over time.

Population genetics and statistical analyses

For each locus, we calculated allele frequencies, estimated expected and observed heterozygosity (H_E and H_O, respectively), determined polymorphic information content (PIC), computed the probability of identity (PID) for unrelated individuals and among siblings (PID_{sibs}), and tested for deviations from Hardy-Weinberg equilibrium (HWE) in CERVUS 3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007). Both pedigree programs used in this study, CERVUS and COLONY, are robust to slight deviations from HWE (see www.fieldgenetics.com and www.zsl.org/science/software/colony); thus significant deviations from HWE did not preclude any loci from the pedigree reconstruction analyses.

Population structure and incomplete sampling can lead to an overestimation of homozygosity known as the Wahlund effect (Selkoe & Toonen 2006; Chapuis & Estoup 2007). To account for any potential underlying population structure resulting from the sharp population decline and incomplete sampling of our elusive species, tests for null alleles, stuttering, large allele dropout, and linkage disequilibrium (LD) were performed in two ways: first, across all individuals without any a priori subpopulation assignments and second, assuming that the samples comprised two subpopulations (before/during mange, BDM and post-mange, PM) based on whether the individual was sampled before and during the peak of notoedric mange-induced population decline (1996–2005) or after (2006–2015) as described above and in both Riley et al. (2007) and Serieys et al. (2015b).

We screened the microsatellite data for genotyping errors due to null alleles, stuttering, and large allele dropout at all loci in MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). Null alleles were detected in LC109 when the samples were assessed both with and without a priori temporal subpopulation assignments, and, therefore, we removed it from further analyses (see

Results). To test for LD between pairs of microsatellite loci, we performed exact G-tests with a Markov chain algorithm (10,000 dememorizations, 1000 batches, and 5000 iterations) in GENEPOP 4.3 (Raymond & Rousset 1995; Rousset 2008). We used a Bonferroni-adjusted p-value (Rice 1989) corresponding to $\alpha = 0.05$ to correct for multiple testing (378 tests; $\alpha = 0.00013228$). Twelve microsatellite pairs tested positive for linkage disequilibrium and the locus in each pair with the lowest polymorphic information content (PIC) was eliminated from further analyses (FCA026, DRA1, DRB1, DRB3, DRB4, and FLA1; see Results). As a result, 21 of the 28 loci were included in the subsequent relatedness and pedigree reconstruction analyses.

Unless otherwise noted, all descriptive statistics calculations along with pairwise comparisons were conducted in R 3.3.1 (R Core Development Team 2016). To test whether the data met normality and homogeneity assumptions, we performed Shapiro-Wilk and Levene's tests ($\alpha = 0.05$), respectively, and transformed the data when necessary. The appropriate nonparametric test (Wilcoxon signed-rank or Mann-Whitney-Wilcoxon) was used instead of a one- or two-sample t-test when the data did not meet the assumption of normality. If the data failed to meet the homogeneity of variance requirement even after data transformation attempts, violations were reported (see Results) and statistical tests were performed with this caveat.

Relatedness and pedigree reconstruction

The pedigree data set comprised 196 individual bobcats genotyped at 21 microsatellite loci. We reconstructed the pedigree using a consensus of outputs from ML-RELATE (Kalinowski et al. 2006), CERVUS 3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007), COLONY 2.0.6.1 (Wang 2004; Wang & Santure 2009; Jones & Wang 2010b), and field observations (NPS unpublished data).

ML-RELATE finds the maximum likelihood estimate of pairwise relatedness (r) using the downhill simplex method of optimization. In addition, it uses a maximum likelihood approach to distinguish among four pedigree relationships: unrelated (U), half-siblings (HS), full-siblings (FS), and parent-offspring (PO). ML-RELATE also accommodates specific hypothesis testing of relationships with simulations of random genotype pairs. For example, if the program finds that a FS relationship between two individuals has the highest maximum likelihood but that the likelihood for U is not much lower, a researcher can use this function to determine whether both relationships fit the data or if the alternative hypothesis (U in this case) can be rejected.

CERVUS estimates parentage with a pairwise likelihood-based approach. In brief, CERVUS runs simulations based on population allele frequencies, the number of candidate parents, and population sampling rate to estimate critical values for log likelihood (LOD) scores. The program then uses the estimated critical LOD score from the simulations to assign parentage at either a relaxed confidence of 80% or strict confidence of 95% to the candidate parent with the largest, positive LOD score.

In contrast to CERVUS, COLONY2 infers both parentage and sibship using a full-likelihood method based on group clustering. The program partitions the sample into three sub-samples: offspring, candidate mothers, and candidate fathers. Offspring are then assigned to maternal and paternal clusters based on their multi-locus genotypes. The offspring within a cluster are assumed to be either full- or half-sibs whereas offspring between clusters are assumed to be unrelated. Finally, COLONY2's algorithm infers the best pedigree configuration based on maximum likelihood.

To generate critical LOD scores in CERVUS, we simulated 10,000 offspring with 20 candidate mothers and 20 candidate fathers and assumed that 30% of the candidate parents were

unsampled with a 1% genotyping error rate. In COLONY2, we also allowed for 30% of the candidate parents to be unsampled and assumed class II genotyping error rates of 0.5% per allele, which is approximately equivalent to CERVUS's corresponding 1% genotyping error rate (Hadfield et al. 2006).

Parentage was not known *a priori* for most individuals; therefore, the pool of candidate parents for each individual was based on known demographic parameters such as age and mortality. For kittens and yearlings, birth year was known or could be reasonably estimated, and all adult animals known to be alive during that time period were included as candidate parents. For adults, all other adults were included as possible parents while kittens and yearlings born later were excluded. A subset of individuals were captured as kittens in dens with known or suspected mothers and/or siblings. For these individuals, analyses were first run without assuming known parentage or sibship, as described above, to determine whether the genetic data matched the field observations. Relationships were considered resolved based on the output from all three programs and field observations along with manual genotype comparisons of putative parent-offspring-sibling relationships. Pedigree figures were created in the web-based MADELINE 2.0 PEDIGREE DRAWING ENGINE service (Trager et al. 2007).

RESULTS

Sampling and genetic results

From 1996–2015, we obtained blood, tissue, or buccal swab samples from 186 live-captured bobcats and postmortem tissue from 10 deceased bobcats for a total of 196 Simi Hills individuals, including 93 females, 98 males, and 5 individuals of unknown sex. Seventy individuals were classified as adults at first capture, 42 as yearlings, 71 as kittens, and 13 of

unknown age class. We obtained complete 28-locus genotypes for 175 (89.3%) individuals, 27-locus genotypes for 10 (5.10%) individuals, and 26- and 25-locus genotypes for four (2.04%) individuals each. The three remaining individuals had 24-, 21-, and 17-locus genotypes, respectively.

For the full, 28-locus dataset, all markers were polymorphic with between three and 12 alleles per locus ($\bar{x}$ = 6.71, s = 2.09). Polymorphic information content (PIC) ranged from 0.27–0.82 but was high on average ($\bar{x}$ = 0.64, s = 0.14). At the population level, the average multi-locus expected heterozygosity (H_E) was 0.68 (s = 0.13, range =0.31–0.84) while the mean observed heterozygosity (H_O) was 0.65 (s = 2.09, range = 0.33–0.82). Two loci, LC109 and FCA045, showed significant deviations from HWE (Table 1). LC109 was ultimately excluded for exhibiting null alleles (see below); however, we retained FCA045 since both CERVUS and COLONY2 account for deviations from HWE in their models.

We did not detect genotyping errors due to stuttering or large allele dropout in any of the 28 markers. However, homozygote excess, which suggested the presence of null alleles, was observed at 10 loci when the data were analyzed as a single population along with six in BDM and four in PM when we assumed temporal substructure (Tables 3a–c). Locus LC109 showed evidence of null alleles across both testing scenarios, which led us to exclude it from subsequent relatedness and pedigree analyses. Given that no other loci displayed consistent results in the two testing scenarios, we attributed the remaining positive outcomes to underlying population structure due to the sharp mange-induced population decline in our study sample and retained the other loci for subsequent evaluation.

When tested as one population, 61 of 378 pairwise comparisons showed significant LD after Bonferroni correction. When temporal population substructure was assumed, 34 tests in

BDM and 35 tests in the PM yielded significant LD p-values after Bonferroni correction. Twelve pairs involving eight loci (Table 4) exhibited significant LD under both conditions, which led us to drop the following six loci from the relatedness and pedigree analyses: FCA026, DRA1, DRB1, DRB3, DRB4, and FLA1. Some significant LD was expected in the immune-linked markers since DRA1, DRB1, DRB3, DRB4, and FLA1 are located within 18.3 MB of each other on chromosome B2. Given that the other significant LD results were not consistent among the two testing scenarios nor in several other bobcat populations (e.g., Janečka et al. 2006; Croteau et al. 2010, 2012; Lee et al. 2012; Reding et al. 2013), we assumed that the remaining significant outcomes were likely the result of population structure and the number of related individuals in our dataset rather than true physical linkage. Accordingly, we included the remaining 21 loci in our relatedness and pedigree analyses.

For the relatedness and pedigree datasets where seven loci were excluded, we obtained complete 21-locus genotypes for 182 (92.8%) individuals, 20-locus genotypes for nine (4.59%) individuals, and 19-locus genotypes for two (1.02%) individuals. The three remaining individuals had 18-, 15-, and 12-locus genotypes, respectively. The probability of identity (PID) and PID among siblings (PID_{sibs}) in the 21 markers used in the relatedness and pedigree analyses were 3.56×10^{-18} and 6.00×10^{-08}, respectively, which indicates that the possibility that our set of loci failed to differentiate between both two-randomly selected individuals and between two full-siblings was highly unlikely in our study population. This result implies that our panel of loci had sufficient power to perform pedigree reconstruction analyses.

Pedigree results

We assigned full or partial parentage to 128 (65.3%) of the 196 individuals (Table 5). Specifically, both maternity and paternity were resolved for 86 (43.9%) individuals, maternity alone for 23 (11.7%), paternity alone for 19 (9.70%), and neither maternity nor paternity for 68 (34.7%) individuals. For 30 of the 110 individuals with partial or unsresolved parentage, sibship reconstruction in ML-RELATE and COLONY2 predicted 10 maternities from four unsampled females where paternity was known, 13 paternities from four unsampled males where maternity was known, and seven maternities and paternities from three unsampled females and three unsampled males for a total of 14 unsampled parents (seven dams and seven sires). Altogether we resolved 133 full- and 291 half-sibling relationships.

We presumed to know the mother's identity in 65 of the 71 sampled kittens based on field observations: 63 kittens from 28 litters were captured and sampled at their respective den sites and two deceased kittens were sampled from their pregnant mother who had been hit by a car. The 29 litters ranged in size from one to five kittens ($\bar{x}$ = 2.24, s = 1.12) with most having one (eight litters) or two (11 litters) kittens. The genetic and maternity findings coincided with field observations in 26 out of the 29 sampled litters. In one of these conflicting cases, the field-based mother (B099) was a maternal half-sister to the kittens (B100, B101) and B078 was the genetically assigned mother. For the other two litters (B120, B121; B122), a mother-daughter pair (B075-B071) with overlapping home ranges were presumed to be the mother of each other's litters. Field observations placed the field-assigned mothers at each of these three den sites (NPS, unpublished data), which points to the possibility of den-visiting by a grandmother in one instance and a half-sister in the other two.

Paternity was successfully resolved for 46 of the 65 kittens in 22 of the sampled 29 litters. Of the remaining 19 kittens, 17 were from five litters with more than one kitten. Within each of these five litters, both ML-RELATE and COLONY2 indicated that the kittens were full-siblings sired by four different unsampled males. We also found four yearling cohorts, and those that shared a mother were sired by the same male. Thus, we did not detect multiple paternity in any of the 21 litters with more than one kitten or in the four cohorts of more than one yearling.

Thirty-four of our 93 (36.6%) sampled females produced at least one litter with between one and 10 kittens per dam over her lifespan ($\bar{x}$ = 3.21, s = 2.61). Of the three most prolific dams sampled before and during the mange outbreak, B069 and B078 produced seven offspring each while B132 produced nine kittens. In the post-mange years, B171 and B255 were the most successful with nine and 10 offspring, respectively. Of the 34 females who successfully reproduced, 11 (32.4%) had more than one litter (range = 2–5), and among these 11 females, seven (63.6%) reproduced with the same male across multiple breeding seasons. Most dams reproduced with one male in two different years, but two females (B078 and B171) reproduced with two different males (B058, B115; B179, B181, respectively) twice each. In addition, one female (B132) seems to have bred with the same male (B094) on three separate occasions.

Of the 98 males sampled during our study, 27 (27.6%) successfully reproduced with between one and 11 kittens each ($\bar{x}$ = 3.89, s = 3.21). The three most successful males before and during the mange epizootic were B058, B064, and B094 with 10, eight, and nine kittens, respectively. B179 and B181 were the most prolific in the post-mange years with 10 and 11 offspring, respectively. Of the 27 males who reproduced in our sample, 14 (51.9%) sired more than one litter (range = 2–6), and nine (64.3%) reproduced with the same female in at least two breeding seasons. For 13 of the 14 males with more than one litter, offspring birth year was

known or could be reasonably estimated, and found that at least six (46.2%) of these males sired more than one litter within a breeding season while 12 (92.3%) sires produced offspring across multiple years.

Figures 2–7 display pedigree relationships among a selection of notable family lines that were uncovered during our pedigree analysis. The lineages of B051/B052, B075 and B119 with three, four, and three generations, respectively, feature three family lines whose demise seems to be due in part to the mange epizootic (Figures 2–4). In contrast, B132 and B155 top two lineages that intertwine and extend through five and six generations, respectively, with descendants that largely comprise the bobcats sampled in the post-mange sampling period (Figures 5–7).

Relatedness

On average, relatedness among the 196 genotyped bobcats was r = 0.064 (s = 0.109, median = 0.003, range = 0.000–0.802). The average relatedness among the 93 females of r = 0.073 (s = 0.119, median = 0.006, range = 0.00–0.734) was slightly greater than that of the males (r = 0.06, s = 0.105, median = 0.002, range = 0.00–0.717; Mann-Whitney-Wilcoxon test: W = 10554000, p = 0.00088). However, this significant difference might be spurious since the female and male relatedness values showed statistical evidence of unequal variances (Levene's test: $F_{1,9029}$ = 30.09, p <0.001).

For the 214 identified parent-offspring dyads, the mean relatedness of r = 0.518 (s = 0.071, median = 0.50, range = 0.208–0.764) was marginally higher than the expected value of r = 0.50 (Wilcoxon signed-rank test: W = 5744.5, p = 0.00012). We did not find evidence that the average relatedness among the 133 full-sibling dyads of r = 0.503 (s = 0.121, median = 0.50,

range = 0.146–0.802) differed from the r = 0.50 expectation (one-sample t-test: t_{132} = 0.244, p = 0.808). The mean relatedness among the 291 half-sibling dyads of r = 0.28 (s = 0.141, median = 0.28, range = 0.00–0.62) was close to but significantly higher than the expected value of r = 0.25 (Wilcoxon signed-rank test: W = 26112, p = 0.0007).

Parentage analyses indicated that there were at least 46 independent mating events among 36 pairs of our sampled individuals since some pairs mated more than once during different breeding seasons. Pairwise relatedness among the 36 mated pairs ranged from r = 0.00 to 0.495 ($\bar{x}$ = 0.069, median = 0.009, s = 0.107), which suggests that breeding typically occurred between unrelated pairs. However, two of 36 mated pairs had relatedness values indicative of a half-sibling, aunt, uncle, or grandparent relationship of r = 0.25 (B168–B154: r = 0.219; B078–B115: r = 0.289) while the relatedness of another pair suggested a parent-offspring or full-sibling breeding event of r = 0.50 (B255–B178: r = 0.495). Unfortunately, the pedigree relationships of these three potential incestuous mating events remains unresolved. However, COLONY2's parentage analysis and genotype reconstruction of unsampled individuals suggested that B255 and B178 could be half-siblings with the same unsampled father. In our resolved pedigree (Table 5), we did uncover an instance of one male (B181) who bred with both his half-niece (B171, r = 0.118) and his half-great-niece (B171's daughter B295, r = 0.174).

DISCUSSION

Insights into bobcat mating behavior and social dynamics

Over the past two decades, multiple paternity has been reported in a growing number of carnivore species from dwarf mongooses (*Helogale parvula*: Keane et al. 1994), badgers (*Meles meles*: Dugdale et al. 2007), raccoons (*Procyon lotor*: Nielsen & Nielsen 2007; Hauver et al.

2010), and gray foxes (*Urocyon cinereoargenteus*: Weston Glenn et al. 2009) to African wild dogs (*Lycaon pictus*: Girman et al. 1997; Moueix 2006), black bears (*Ursus americanus*: Schenk & Kovacs 1995), brown bears (*Ursus arctos*: De Barba et al. 2010), and polar bears (*Ursus maritimus*: Zeyl et al. 2009). When surveying felids specifically, two populations of mountain lions did not exhibit evidence of multiple paternity (Onorato et al. 2011; Riley et al. 2014) nor did an opportunistically sampled jaguar (*Panthera onca*) that was killed by a car while pregnant with three kittens (Pinho et al. 2014). However, it has been observed in a few other felid species including Eurasian lynx (*Lynx lynx*: Jewgenow et al. 2006), cheetahs (*Acinonyx jubatus*: Gottelli et al. 2007), tigers (*Panthera tigris altaica*: Liu et al. 2013), and African lions (*Panthera leo*: Lyke et al. 2013), though the Eurasian lynx and tigers were in captivity. Thus, a survey of mating systems in a wider array of natural felid populations would be instructive.

Unlike many of the various carnivore studies mentioned above, we did not find evidence of multiple mating in female bobcats given that we did not detect multiple paternity in any of the litters with more than one kitten nor in the yearling cohorts with resolved parentage. On the other hand, nearly half of the males who sired multiple litters (n = 13) did so within one breeding season. Thus, our pedigree results support the long-standing hypothesis that bobcats are polygynous within breeding seasons (Provost et al. 1973; McCord & Cardoza 1982; Anderson & Lovallo 2003) rather than polygynandrous or promiscuous. Between breeding seasons, however, we found that females are polyandrous and males are polygynous. Intriguingly, mate fidelity in both females and males was moderately high with nearly two-thirds of both sexes reproducing with the same individual in more than one breeding season. Bobcats tend to show strong home range fidelity over their lifespans (reviewed by Anderson & Lovallo 2003). Thus, combining our pedigree results with movement pattern information from radio-telemetry data collected by the

NPS during our study period in a future project would shed light on how home range dynamics influence mate pairings and fidelity across the landscape and over time.

There are numerous reasons why we may not have detected multiple paternity in our study. For instance, female bobcats might not mate multiply. This seems unlikely, however, given that female bobcats seem to have the opportunity to mate with multiple males within a breeding season. Several males may follow a female while she is in estrus (Whitaker & Hamilton 1998), and once courtship begins between a pair, copulation may occur up to 16 times per day (reviewed by Provost et al. 1973; McCord & Cardoza 1982; Larivière & Walton 1997; Anderson & Lovallo 2003; Hansen 2007). In addition, despite being two to three times smaller than those of males, female home ranges may overlap with those of several males (Anderson & Lovallo 2003), which could make it difficult for males to monopolize individual females during the breeding season. On the other hand, competition for breeding opportunities can be high, and male-male aggression among bobcats has been observed during the breeding season (Provost et al. 1973; Anderson & Lovallo 2003; Benson et al. 2004). Thus, investigating whether there is some level of mate guarding along with a male dominance hierarchy among bobcats would be an instructive avenue for future researchers to pursue.

Another potential explanation is that variation in mating systems may be density-dependent (Kokko & Rankin 2006). In support of this hypothesis, investigators have found that the rates of multiple paternity are positively correlated with density in several mammalian species, including domestic cats (*Felis catus*: Say et al. 1999, 2002), red foxes (*Vulpes vulpes*: Baker et al. 2004; Iossa et al. 2009), swift foxes (*Vulpes velox*: Kamler et al. 2004), European water voles (*Arvicola territris*: Aars et al. 2006), and gray-sided voles (*Clethrionomys rufocanus*: Ishibashi & Saitoh 2008). If rates of multiple paternity in Simi Hills bobcats followed this same

pattern, we would expect to have observed higher rates of multiple paternity among the individuals sampled before and during the notoedric mange outbreak. Unfortunately, kitten sampling at dens was initiated in 2002, the same year that the mange epizootic began. Subsequently, population densities declined dramatically as mortality rates increased sharply (Riley et al. 2007, 2010; Ruell et al. 2009). Thus low densities may have prevented females from mating multiply in our study population. It will be interesting to follow Simi Hills bobcats as the population recovers to determine whether multiple paternity is detected in the future. In addition, a comparison with a population of bobcats with higher resident density would be informative.

Sperm competition in conjunction with male quality might also contribute to the reason why we did not observe multiple paternity in our study population. Jewgenow et al. (2006) studied semen quality, testis size, and testosterone levels in four captive male Eurasian lynx. They then performed mating experiments over three years during which ten females were paired with two males over two consecutive days. They discovered multiple paternity in three of the resulting 14 litters, and, intriguingly, 26 of 31 cubs (84%) were sired by the same male, independent of whether he was the first or second mating partner in the mating experiments. Moreover, that particular male exhibited both the most developed reproductive tract and had the highest semen quality. Thus, an investigation into how sperm competition and male quality might influence mating patterns in wild felid populations would be illuminating.

Apart from associations between mothers and their dependent offspring, sociality is rare among wild felids. Notable exceptions include coalitions among male cheetahs and group-living in African lions (reviewed by Bradshaw 2016). We observed three instances of surprising social interaction in this population where a female visited the den of her grandoffspring or half-

siblings in the Simi Hills bobcat population. Although uncommon, female range overlap has been recorded in bobcats (Kitchings & Story 1984; Chamberlain et al. 2003; Janečka et al. 2006). In the Simi Hills population, the available habitat is heavily fragmented by urbanization, which, before the notoedric mange-induced population decline at least, forced the females in this population to live at high densities and increased overlap in their home ranges (Riley et al. 2010). This unique setup might explain this unusual phenomenon of den-visiting by second degree relatives in these three instances, all of which occurred prior to the precipitous population decline. Additional confirmation such as the re-extraction of sample DNA and re-genotyping of each individual is required to improve confidence in these results. These observations are remarkable and intriguing, however, and, should they survive further scrutiny, open up the possibility that social dynamics among solitary bobcats are more complicated than previously thought.

Future directions

The pedigree established herein lends itself to numerous future projects in our study population that will contribute to our knowledge of bobcat ecology and behavior in an urban environment in addition to shedding light on fundamental evolutionary and conservation questions related to social organization, trait heritability, and mating patterns. We intend to combine pedigree information established in this study with other data collected as part of the NPS's larger study on bobcat ecology to improve our understanding in these areas of research.

The NPS has tracked the movement patterns of the majority of individuals included in this pedigree analysis using radio-telemetry, which will allow us to investigate dispersal patterns, the potential for kin-biased spatial associations, and how home range size and habitat quality

influence fitness in our Simi Hills population. Bobcats are thought to display high levels of dispersal but their solitary and elusive nature make research in this area difficult. Janečka et al. (2007) found evidence of male-biased dispersal in population of bobcats in Texas but their sample size was small (n = 21); thus further research is warranted and our large sample size coupled with our knowledge of pedigree relationships will be instrumental in shedding further light in this area.

In general, the paradigm of bobcat spatial organization depict females as intolerant to intra-sexual conspecifics in their home ranges with the exception of dependent offspring while males have larger home ranges and overlap with both females and other males (Bailey 1974; McCord & Cardoza 1982; Packer 1986; Larivière & Walton 1997); however, other studies have found that female home ranges do overlap in some populations (Kitchings & Story 1984; Chamberlain et al. 2003; Janečka et al. 2006), including our study population in the Simi Hills (Riley et al. 2010). Kin-biased spatial associations have been posited as a potential explanation for this phenomenon (Benson et al. 2004), and researchers have found that females in other carnivore species including mountain lions (Logan & Sweanor 2001), black bears (Rogers 1987), and tigers (Smith et al. 1987) seem to allow philopatry and shift their home ranges to accommodate their female offspring, at least when resources are abundant. In a bobcat population found in Texas, Janečka et al. (2006) found evidence that female offspring established home ranges in or near their mothers' home ranges although their sample size was small (n = two of three sampled yearlings). Moreover, they could not distinguish between full- and half-sibling relationships because the number of microsatellite loci was insufficient. Data from our extensive pedigree will allow us to determine whether these patterns are apparent in our

study population and also enable us to ascertain specific relationships (e.g., parent-offspring, full-sibing, etc.) among individuals that share home ranges.

In addition to informing population dynamics, pedigrees are key to studying the heritability of quantitative traits (Kruuk & Hill 2008). For example, DiBattista et al. (2009) established a molecular marker-based pedigree to estimate the quantitative genetic parameters of body size metrics in lemon sharks (*Negaprion brevirostris*). Over the past three decades, global shark harvesting has grown sharply both directly and indirectly as a result of by-catch (Barker & Schluessel 2005). Such harvesting tends to target larger individuals, which may lead to selection for smaller body sizes and eventually evolutionary change in exploited populations (Fenberg & Roy 2008). Using their pedigree, DiBattista et al. (2009) found that body mass and length—both predictors of survival—were moderately heritable among juvenile sharks. Consequently, harvesting that targets large individuals may ultimately lead to smaller-bodied populations that are less fit and thus less viable.

Other selection pressures, such as severe environmental change, can also influence life-history traits such as body size. In fact, researchers have found that body size and/or growth rate decrease during drought years in myriad organisms from tropical trees to mammals (Sheridan & Bickford 2011). In recent years, California has experienced a severe drought as a result of high temperatures and El Niño conditions (Hanak et al. 2015). Whether the body size of bobcats in the Simi Hills has changed during this time period is currently unknown although the NPS has collected data on various bobcat body size metrics since 1996. We plan to take advantage of this rich dataset to estimate the heritability of body size using the pedigree reconstructed herein and determine whether body size has been influenced by shifting environmental conditions such as the drought.

Molecular marker-based pedigrees are also essential to investigating mating behavior and reproduction patterns, especially in species where the direct observation of mating events is rare or not feasible. The pedigree results of this study have already revealed some aspects of bobcat reproductive behavior, but the thoroughly and continuously studied Simi Hills population presents a unique opportunity to discover more about this elusive mesocarnivore by comparing mating patterns before, during, and after the severe genetic bottleneck. As described above (see Introduction), Simi Hills bobcats suffered an extreme population decline due to a notoedric mange epizootic concurrent with high levels of anticoagulant exposure from 2002–2005. Serieys et al. (2015b) sought to advance our understanding of the profound genetic impact disease imposed on this population by genotyping individuals in the Simi Hills at nine neutral and seven immune-linked microsatellite loci. Remarkably, they found that a genetic bottleneck occurred over the three-year period, causing greater genetic differentiation between the pre- and post-mange Simi Hills population than that between populations that have been separated by major freeways for more than 60 years. Although there was local extinction of bobcats in some habitat patches throughout the Simi Hills region during the population decline (Riley et al. 2007), the population appears to be in strong recovery in recent years (NPS, unpublished data). However, as our pedigree study suggests, multiple lines of genetic evidence based on neutral markers points to the repopulation occurring as a result of few founder individuals originating in the Simi Hills region. These results indicate that strong selection occurred on the neutral loci over a period of one to two bobcat generations; yet there is also evidence of balancing selection that maintained variation at immune-linked loci (Serieys et al. 2015b). Taken together, these intriguing observations raise questions concerning how mating patterns might have contributed to the observed maintenance of variation at immune-linked loci across the population bottleneck.

Understanding the mechanisms underpinning the production and maintenance of genetic variation in natural populations is a fundamental goal in evolutionary biology. From a conservation perspective, a better grasp of these processes is of importance for the successful management of the ever-growing number of threatened wildlife populations. Both directional selection and drift can act to reduce genetic variation over time whereas balancing selection can maintain or even enhance variation. In recent years, immune-related genic regions, such as the major histocompatibility complex (MHC), have emerged as important models for the study of adaptive genetic diversity across vertebrate taxa (Piertney & Oliver 2006). In fact, the role that MHC variation plays in mate choice has been the subject of a growing number of studies over the past four decades, but such investigations have yielded mixed results (Spurgin & Richardson 2010; Sutton et al. 2011; Kamiya et al. 2014). Moreover, research on these questions in non-model, natural populations has been rare, especially among long-lived, elusive species. Addressing the factors contributing to the maintenance of adaptive variation in a natural population undergoing demographic change, such as the Simi Hills bobcats, would help fill this current knowledge gap (Oliver & Piertney 2012).

Using the pedigree established in this study along with the immune-linked microsatellite data, we will investigate whether there is evidence of non-random mating patterns in Simi Hills bobcats that might explain the observed maintenance of immune-linked genetic variation in this population. Since we found that many of the immune markers were in LD (see Results), we plan to generate haplotypes with the immune markers. Next, we will run demographic simulations to determine whether mated pairs are less similar at immune-related loci than would be expected under random mating and to test for disassortative mating at the neutral loci, as well as evaluate

other mating scenarios as in previous work (Schwensow et al. 2008; Juola & Dearborn 2012; Moore et al. 2015).

Conclusion

In this study, we reconstructed a multigenerational pedigree for a unique population of urban bobcats that has undergone rapid demographic change related to both natural environmental challenges and human-induced interference. Our results have improved our understanding of the mating system and social dynamics in this elusive solitary carnivore by shedding light on individual life histories and lifetime reproductive fitness and will provide an invaluable tool for future research into ecological and evolutionary questions in this population. From a broader conservation perspective, mammalian carnivores are particularly susceptible to local extinction in fragmented areas due to their small population sizes, wide home ranges, and direct persecution by humans (Woodroffe & Ginsberg 1998); yet, they play a key role in the maintenance of biodiversity and ecosystem health by keeping herbivores in check (Estes et al. 2011). In a survey of nine mammalian carnivore species in southern California, Crooks (2002) found that because bobcat populations require sufficient connectivity among habitat patches to persist, they have the potential to serve as a fundamental indicator species for the health of southern California's fragmented ecosystems. Thus, by improving our knowledge of bobcat reproductive behavior and social dynamics, our study has the potential to inform conservation management decisions in numerous species threatened by urbanization.

APPENDIX A: TABLES

Table 1. Summary of 28 microsatellite loci characteristics in 196 bobcats sampled in the Simi Hills from 1996–2015. Number of genotypes analyzed (*n*), number of alleles (*k*), polymorphic information content (PIC), the significance level for Hardy-Weinberg Equilibrium (HWE), observed (H_O) and expected heterozygosity (H_E), and probability of identity (PID) and PID among siblings (PIDsibs) were calculated in CERVUS 3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007). Locus name, species developed from, chromosome (chr.), and repeat motif (di, dinucleotide; tri, trinucleotide; tetra, tetranucleotide) were obtained from the cited reference: [a] Menotti-Raymond et al. (1999), [b] Menotti-Raymond et al. (2005), [c] Faircloth et al. (2005), [d] Carmichael et al. (2000), and [e] Serieys et al. (2015).

Locus	Species	Repeat	Chr.	Multiplex	*n*	Size range (bp)	*k*	PIC	H_O	H_E	HWE	PID	PID_{sibs}
FCA008[a]	Domestic cat	Di	A1	N/A	196	142–154	6	0.68	0.68	0.73	NS	0.12	0.42
FCA023[a]	Domestic cat	Di	B1	N/A	195	149–159	6	0.71	0.82	0.76	NS	0.10	0.40
FCA026[a]	Domestic cat	Di	D3	N/A	196	140–168	12	0.80	0.75	0.83	NS	0.05	0.35
FCA031[a]	Domestic cat	Di	E3	1	195	238–254	6	0.70	0.72	0.75	NS	0.11	0.40
FCA043[a]	Domestic cat	Di	C2	N/A	196	131–139	5	0.68	0.66	0.72	NS	0.12	0.42
FCA045[a]	Domestic cat	Di	D4	N/A	192	148–174	8	0.72	0.65	0.76	<0.01	0.10	0.40
FCA077[a]	Domestic cat	Di	C2	N/A	196	145–167	9	0.75	0.79	0.78	NS	0.08	0.38
FCA082[a]	Domestic cat	Di	E1	2	193	250–264	7	0.75	0.79	0.78	NS	0.08	0.38
FCA090[a]	Domestic cat	Di	A1	N/A	195	115–125	6	0.51	0.49	0.56	NS	0.24	0.53
FCA096[a]	Domestic cat	Di	E2	N/A	196	186–210	8	0.66	0.65	0.71	NS	0.14	0.43
FCA132[a]	Domestic cat	Di	D3	N/A	195	181–195	7	0.66	0.70	0.71	NS	0.13	0.43
FCA149[a]	Domestic cat	Di	B1	1	195	133–159	9	0.75	0.77	0.78	NS	0.08	0.38
FCA559[a]	Domestic cat	Tetra	B1	2	196	119–135	5	0.54	0.67	0.62	NS	0.22	0.50
FCA742[b]	Domestic cat	Tetra	D4	3	193	120–136	5	0.55	0.63	0.61	NS	0.21	0.50
BC1AT[c]	Bobcat	Tetra	Unknown	2	194	298–326	8	0.76	0.78	0.79	NS	0.08	0.38
BCD1T[c]	Bobcat	Tri	Unknown	2	194	279–285	3	0.27	0.33	0.31	NS	0.52	0.73
BCE5T[c]	Bobcat	Tetra	Unknown	3	194	255–275	6	0.60	0.60	0.63	NS	0.17	0.48
BCH6T[c]	Bobcat	Di	Unknown	2	193	170–184	8	0.62	0.58	0.66	NS	0.16	0.46

LC109[d]	Canada lynx	Di	Unknown	1	188	180–188	5	0.49	0.38	0.58	<0.001	0.26	0.53
LC110[d]	Canada lynx	Di	Unknown	1	196	90–100	4	0.60	0.63	0.66	NS	0.18	0.47
LC111[d]	Canada lynx	Di	Unknown	3	189	154–212	7	0.68	0.68	0.73	NS	0.12	0.42
DRA1[e]	Bobcat	Di	B2	N/A	196	216–240	10	0.70	0.74	0.73	NS	0.11	0.41
DRB1[e]	Bobcat	Di	B2	N/A	196	287–303	4	0.63	0.59	0.68	NS	0.16	0.45
DRB3[e]	Bobcat	Di	B2	N/A	196	245–263	8	0.82	0.80	0.84	NS	0.04	0.34
DRB4[e]	Bobcat	Di	B2	N/A	196	275–297	8	0.80	0.75	0.82	NS	0.06	0.36
FLA1[e]	Bobcat	Di	B2	N/A	183	198–210	7	0.70	0.68	0.75	NS	0.11	0.41
TLR3[e]	Bobcat	Di	B1	N/A	196	283–303	8	0.55	0.51	0.58	NS	0.21	0.52
TLR4[e]	Bobcat	Tetra	D4	N/A	196	263–271	3	0.29	0.33	0.33	NS	0.50	0.71
Avg.							*6.71*	*0.64*	*0.65*	*0.68*			
Avg. (ped)							*6.38*	*0.62*	*0.64*	*0.66*			
Comb.												*$2.11x10^{-25}$*	*$1.07x10^{-10}$*
Comb. (ped)												*$3.56x10^{-18}$*	*$6.00x10^{-8}$*

Table 2. Characteristics of seven microsatellite loci tested in seven 1996–2015 Simi Hills bobcats that were excluded from the study after some loci amplified poorly in the multiplex assays. Locus name, species developed from, chromosome, and repeat motif (di, dinucleotide; tri, trinucleotide; tetra, tetranucleotide) were obtained from the cited reference: [a] Menotti-Raymond et al. (1999), [b] Menotti-Raymond et al. (2005), and [c] Faircloth et al. (2005).

Locus	Species	Repeat	Chromosome	Multiplex	*n*	No. of alleles	Size range (bp)
FCA035[a]	Domestic cat	Di	D2	4	7	5	143–167
FCA126[a]	Domestic cat	Di	B1	5	7	6	144–154
FCA391[a]	Domestic cat	Tetra	B3	5	7	4	215–227
FCA740[b]	Domestic cat	Tetra	C1	4	7	6	218–244
FCA741[b]	Domestic cat	Tri	D1	4	7	5	174–186
BCB12D[c]	Bobcat	Di	Unknown	5	7	4	278–314
BCG3D[c]	Bobcat	Di	Unknown	4	7	6	214–240

Table 3a. Null allele frequency estimates for the 196 individuals in the 1996–2015 Simi Hills population using four methods performed by MICRO-CHECKER (Van Oosterhout et al. 2004).

Population	Locus	Evidence of null alleles?	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
Full	FCA008	no	0.029	0.030	0.024	0.024
Full	FCA023	no	-0.041	-0.039	-0.035	0.016
Full	FCA026	yes	0.046	0.046	0.040	0.040
Full	FCA031	no	0.014	0.016	0.013	0.040
Full	FCA043	yes	0.045	0.042	0.034	0.034
Full	FCA045	yes	0.074	0.075	0.060	0.115
Full	FCA077	no	-0.012	-0.010	-0.009	0.000
Full	FCA082	no	-0.010	-0.012	-0.010	0.051
Full	FCA090	yes	0.065	0.072	0.049	0.074
Full	FCA096	no	0.046	0.042	0.034	0.034
Full	FCA132	no	0.005	0.007	0.005	0.037
Full	FCA149	no	0.011	0.006	0.005	0.033
Full	FCA559	no	-0.048	-0.043	-0.035	0.000
Full	FCA742	no	-0.014	-0.017	-0.013	0.065
Full	BC1AT	no	0.004	0.003	0.002	0.045
Full	BCD1T	no	-0.032	-0.026	-0.013	0.072
Full	BCE5T	no	0.039	0.026	0.019	0.068
Full	BCH6T	yes	0.057	0.060	0.045	0.098
Full	LC109	yes	0.153	0.204	0.124	0.219
Full	LC110	no	0.027	0.024	0.019	0.019
Full	LC111	no	0.038	0.037	0.030	0.124
Full	FLA1	yes	0.043	0.042	0.034	0.176
Full	DRA1	no	-0.004	-0.006	-0.005	0.000
Full	DRB1	yes	0.068	0.070	0.053	0.053
Full	DRB3	no	0.027	0.028	0.025	0.025
Full	DRB4	yes	0.043	0.043	0.037	0.037
Full	TLR3	yes	0.054	0.064	0.044	0.044
Full	TLR4	no	-0.002	-0.005	-0.002	0.000

Table 3b. Null allele frequency estimates for the 127 individuals sampled before and during the disease-induced Simi Hills population decline (1996–2005; before/during mange, BDM) using four methods performed by MICRO-CHECKER (Van Oosterhout et al. 2004).

Population	Locus	Evidence of null alleles?	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
BDM	FCA008	no	-0.006	-0.006	-0.006	0.000
BDM	FCA023	no	-0.060	-0.056	-0.052	0.000
BDM	FCA026	yes	0.051	0.050	0.042	0.042
BDM	FCA031	no	0.007	0.005	0.004	0.004
BDM	FCA043	no	0.032	0.031	0.025	0.025
BDM	FCA045	no	0.005	0.004	0.004	0.004
BDM	FCA077	no	-0.023	-0.024	-0.021	0.000
BDM	FCA082	no	-0.021	-0.021	-0.019	0.042
BDM	FCA090	no	0.034	0.042	0.028	0.028
BDM	FCA096	no	0.027	0.020	0.016	0.016
BDM	FCA132	no	-0.039	-0.033	-0.029	0.000
BDM	FCA149	no	-0.017	-0.018	-0.016	0.000
BDM	FCA559	no	-0.034	-0.027	-0.021	0.000
BDM	FCA742	no	-0.019	-0.018	-0.014	0.000
BDM	BC1AT	no	0.023	0.021	0.018	0.050
BDM	BCD1T	no	-0.046	-0.036	-0.019	0.000
BDM	BCE5T	no	0.023	0.015	0.012	0.012
BDM	BCH6T	yes	0.104	0.117	0.083	0.129
BDM	LC109	yes	0.115	0.144	0.090	0.090
BDM	LC110	no	0.012	0.009	0.007	0.007
BDM	LC111	yes	0.060	0.061	0.048	0.077
BDM	FLA1	no	0.042	0.042	0.034	0.117
BDM	DRA1	no	-0.016	-0.017	-0.014	0.000
BDM	DRB1	yes	0.069	0.074	0.057	0.057
BDM	DRB3	no	0.029	0.029	0.026	0.026
BDM	DRB4	no	0.023	0.021	0.018	0.018
BDM	TLR3	yes	0.076	0.092	0.062	0.062
BDM	TLR4	no	0.010	0.005	0.003	0.003

Table 3c. Null allele frequency estimates for the 69 individuals sampled after the disease-induced population decline (2006-2015; post-mange, PM) using four methods performed by MICRO-CHECKER (Van Oosterhout et al. 2004).

Population	Locus	Evidence of null alleles?	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
PM	FCA008	yes	0.072	0.076	0.055	0.055
PM	FCA023	no	-0.041	-0.035	-0.029	0.049
PM	FCA026	no	-0.012	-0.009	-0.008	0.000
PM	FCA031	no	-0.038	-0.026	-0.022	0.050
PM	FCA043	no	0.050	0.041	0.032	0.032
PM	FCA045	yes	0.122	0.147	0.096	0.226
PM	FCA077	no	-0.006	0.002	0.002	0.002
PM	FCA082	no	-0.020	-0.023	-0.019	0.055
PM	FCA090	yes	0.103	0.116	0.078	0.125
PM	FCA096	no	0.050	0.045	0.036	0.036
PM	FCA132	no	0.066	0.065	0.048	0.099
PM	FCA149	no	0.042	0.033	0.027	0.080
PM	FCA559	no	-0.122	-0.112	-0.088	0.000
PM	FCA742	no	-0.018	-0.025	-0.020	0.118
PM	BC1AT	no	-0.047	-0.043	-0.040	0.034
PM	BCD1T	no	-0.014	-0.013	-0.005	0.141
PM	BCE5T	no	0.005	-0.004	-0.003	0.122
PM	BCH6T	no	-0.038	-0.035	-0.029	0.052
PM	LC109	yes	0.225	0.327	0.187	0.374
PM	LC110	no	0.031	0.028	0.022	0.022
PM	LC111	no	-0.030	-0.028	-0.024	0.171
PM	FLA1	no	0.040	0.035	0.028	0.265
PM	DRA1	no	0.004	0.003	0.002	0.002
PM	DRB1	no	0.012	0.006	0.004	0.004
PM	DRB3	no	0.002	0.000	0.000	0.000
PM	DRB4	no	0.054	0.056	0.048	0.048
PM	TLR3	no	0.011	0.011	0.007	0.007
PM	TLR4	no	-0.138	-0.062	-0.025	0.000

Table 4. Twelve microsatellite locus pairs that were found to be in linkage disequilibrium in 196 Simi Hills bobcats sampled from 1996–2015. Only BC1AT and BCH6T were included in relatedness and pedigree analyses.

BC1AT	DRB4
BCH6T	FCA026
DRA1	DRB1
DRA1	DRB3
DRA1	DRB4
DRA1	FLA1
DRB1	DRB3
DRB1	DRB4
DRB1	FLA1
DRB3	DRB4
DRB3	FLA1
DRB4	FLA1

Table 5. Pedigree results for 196 bobcats sampled in the Simi Hills from 1996–2015. Maternal and paternal assignments are based on a consensus of outputs from ML-RELATE, CERVUS, and COLONY2 along with field observations. Ten bobcats that were sampled post-mortem are labeled BM before their respective ID numbers. Predicted but unsampled individuals are labeled with UF for unsampled females and UM for males. Both sample year and age class refer to the individual's first sampling event.

ID	Sex	Sample Year	Age Class	Mother	Father
B001	Female	1996	Adult	Unresolved	Unresolved
B002	Male	1996	Adult	Unresolved	Unresolved
B003	Female	1996	Adult	Unresolved	Unresolved
B004	Male	1996	Adult	Unresolved	Unresolved
B005	Male	1996	Adult	Unresolved	Unresolved
B006	Female	1996	Adult	Unresolved	Unresolved
B007	Male	1996	Yearling	Unresolved	Unresolved
B011	Female	1996	Adult	B053	B005
B016	Male	1996	Adult	Unresolved	Unresolved
B018	Female	1997	Adult	Unresolved	Unresolved
B022	Male	1997	Adult	Unresolved	Unresolved
B023	Male	1997	Adult	Unresolved	Unresolved
B030	Female	1997	Adult	B006	B023
B031	Female	1997	Adult	Unresolved	Unresolved
B032	Female	1997	Adult	Unresolved	Unresolved
B033	Female	1997	Adult	Unresolved	Unresolved
B034	Female	1997	Yearling	B033	Unresolved
B035	Male	1997	Adult	Unresolved	Unresolved
B042	Male	1998	Yearling	Unresolved	Unresolved
B044	Female	1998	Yearling	B079	B064
B051	Female	1998	Adult	Unresolved	Unresolved
B052	Male	1998	Adult	Unresolved	Unresolved
B053	Female	1998	Adult	Unresolved	Unresolved
B054	Female	1998	Adult	Unresolved	B115
B055	Male	1998	Adult	Unresolved	Unresolved
B057	Female	1998	Yearling	B051	B052
B058	Male	1998	Adult	B051	B052
B059	Female	1998	Adult	B078	B115
B060	Female	1998	Adult	Unresolved	B115
B061	Male	1998	Adult	Unresolved	Unresolved
B063	Female	1998	Adult	Unresolved	Unresolved
B064	Male	1998	Adult	Unresolved	Unresolved
B065	Male	2000	Adult	Unresolved	Unresolved
B066	Male	2000	Yearling	Unresolved	Unresolved
B067	Female	2000	Yearling	B069	B052
B068	Female	2000	Yearling	B067	B066
B069	Female	2000	Adult	UF01	UM01
B070	Male	2001	Yearling	B078	B115
B071	Female	2001	Yearling	B075	Unresolved
B072	Female	2001	Yearling	B003	B077
B073	Female	2001	Yearling	Unresolved	Unresolved

B074	Male	2001	Yearling	Unresolved	Unresolved
B075	Female	2001	Adult	B001	Unresolved
B076	Male	2001	Adult	B051	Unresolved
B077	Male	2001	Adult	B018	Unresolved
B078	Female	2001	Adult	UF02	UM02
B079	Female	2001	Adult	UF02	UM02
B080	Female	2001	Yearling	B069	B066
B081	Male	2001	Kitten	B075	B007
B082	Female	2001	Yearling	Unresolved	Unresolved
B085	Male	2001	Unknown	Unresolved	Unresolved
B086	Male	2001	Adult	Unresolved	Unresolved
B087	Female	2001	Yearling	UF03	UM03
B088	Female	2001	Yearling	B079	B064
B091	Male	2001	Adult	Unresolved	Unresolved
B092	Female	2002	Adult	UF03	UM03
B093	Male	2002	Adult	UF03	UM03
B094	Male	2002	Adult	Unresolved	Unresolved
B095	Male	2002	Yearling	Unresolved	B077
B096	Female	2002	Yearling	Unresolved	Unresolved
B097	Male	2002	Adult	Unresolved	Unresolved
B098	Female	2002	Yearling	Unresolved	Unresolved
B099	Female	2002	Adult	B078	Unresolved
B100	Male	2002	Kitten	B078	B058
B101	Female	2002	Kitten	B078	B058
B102	Female	2002	Kitten	B060	B058
B103	Male	2002	Kitten	B060	B058
B104	Female	2002	Kitten	B060	B058
B105	Female	2002	Kitten	B069	B066
B106	Female	2002	Kitten	B071	B114
B107	Male	2002	Kitten	B071	B114
B108	Female	2002	Kitten	B075	B070
B109	Female	2002	Kitten	B075	B070
B110	Male	2002	Kitten	B075	B070
B111	Male	2002	Kitten	B078	B058
B112	Female	2002	Adult	B069	B076
B113	Female	2002	Adult	Unresolved	Unresolved
B114	Male	2003	Adult	Unresolved	Unresolved
B115	Male	2003	Adult	UF01	UM01
B116	Male	2003	Yearling	UF04	B115
B117	Female	2003	Yearling	UF04	B115
B118	Male	2003	Adult	UF04	B115
B119	Male	2003	Adult	Unresolved	Unresolved
B120	Male	2003	Kitten	B071	B114
B121	Male	2003	Kitten	B071	B114
B122	Male	2003	Kitten	B075	Unresolved
B123	Male	2003	Kitten	B069	B064
B124	Male	2003	Kitten	B069	B064
B125	Female	2003	Kitten	B069	B064
B126	Female	2003	Kitten	B117	B058

B127	Male	2003	Kitten	B117	B058
B128	Male	2003	Kitten	B117	B058
B129	Male	2003	Kitten	B088	B022
B130	Female	2003	Kitten	B113	B064
B131	Female	2003	Kitten	B113	B064
B132	Female	2003	Adult	Unresolved	Unresolved
B133	Female	2003	Yearling	B132	B118
B134	Female	2003	Yearling	B096	B094
B137	Male	2003	Yearling	Unresolved	B119
B139	Male	2003	Adult	Unresolved	Unresolved
B140	Female	2003	Yearling	UF05	B119
B143	Female	2004	Adult	B078	B058
B144	Male	2004	Yearling	Unresolved	Unresolved
B145	Male	2004	Kitten	B117	B144
B146	Female	2004	Kitten	B117	B144
B147	Female	2004	Kitten	B117	B144
B148	Female	2004	Kitten	B132	B094
B149	Male	2004	Kitten	B132	B094
B150	Female	2004	Kitten	B108	UM04
B151	Male	2004	Kitten	B108	UM04
B152	Male	2004	Kitten	B108	UM04
B153	Female	2004	Kitten	B108	UM04
B154	Male	2004	Adult	Unresolved	Unresolved
B155	Male	2004	Adult	Unresolved	Unresolved
B156	Female	2004	Adult	UF05	B119
B157	Male	2005	Yearling	Unresolved	B119
B158	Male	2005	Yearling	UF06	B119
B159	Male	2005	Adult	Unresolved	Unresolved
B161	Male	2005	Kitten	B132	B094
B162	Male	2005	Kitten	B132	B094
B163	Male	2005	Kitten	B132	B094
B164	Male	2005	Kitten	B132	B094
B165	Male	2005	Kitten	B132	B094
B166	Male	2006	Adult	Unresolved	Unresolved
B167	Male	2006	Adult	B168	B154
B168	Female	2006	Adult	UF06	B119
B169	Male	2006	Yearling	B168	B154
B171	Female	2006	Kitten	B133	UM05
B172	Male	2006	Kitten	B133	UM05
B173	Female	2006	Kitten	B133	UM05
B174	Male	2006	Unknown	Unresolved	Unresolved
B175	Male	2007	Adult	Unresolved	Unresolved
B176	Female	2007	Adult	Unresolved	B155
B177	Male	2007	Yearling	Unresolved	Unresolved
B178	Male	2007	Yearling	Unresolved	Unresolved
B179	Male	2007	Yearling	Unresolved	Unresolved
B180	Male	2007	Adult	Unresolved	Unresolved
B181	Male	2008	Yearling	B132	B094
B182	Female	2008	Kitten	B176	UM06

B183	Male	2008	Kitten	B176	UM06
B184	Male	2008	Kitten	B146	Unresolved
B185	Male	2008	Kitten	B171	B181
B195	Male	2009	Kitten	B171	B181
B196	Female	2009	Kitten	B146	B179
B197	Male	2009	Kitten	B146	B179
B211	Female	2009	Unknown	Unresolved	Unresolved
B225	Male	2010	Kitten	B171	B179
B226	Male	2010	Kitten	B171	B179
B227	Male	2010	Kitten	B171	B179
B228	Female	2010	Kitten	B171	B179
B229	Female	2010	Kitten	B171	B179
B255	Female	2010	Adult	B176	UM06
B258	Female	2010	Kitten	B255	B178
B259	Female	2010	Kitten	B255	B178
B260	Male	2010	Yearling	UF07	B183
B261	Female	2010	Yearling	UF07	B183
B263	Male	2010	Yearling	UF07	B183
B279	Female	2011	Kitten	B255	B178
B280	Female	2011	Kitten	B255	B178
B281	Male	2011	Kitten	B196	B181
B291	Male	2011	Kitten	B293	B181
B292	Female	2011	Kitten	B293	B181
B293	Female	2011	Adult	Unresolved	B177
B294	Female	2011	Unknown	B171	B179
B295	Female	2012	Adult	B171	B179
B296	Female	2012	Adult	Unresolved	B179
B297	Male	2012	Adult	B293	B181
B300	Female	2012	Adult	B293	B181
B301	Male	2012	Adult	Unresolved	Unresolved
B302	Female	2012	Adult	B296	B181
B303	Female	2012	Adult	B261	B177
B304	Female	2013	Unknown	B255	Unresolved
B305	Male	2013	Kitten	Unresolved	Unresolved
B306	Female	2013	Adult	Unresolved	B263
B308	Male	2013	Kitten	B306	UM06
B309	Female	2013	Kitten	B306	UM06
B311	Female	2013	Yearling	B255	B297
B312	Male	2013	Yearling	B258	B297
B313	Male	2013	Yearling	B258	B297
B314	Female	2013	Yearling	B255	B297
B315	Female	2013	Yearling	B255	B297
B326	Female	2015	Kitten	B255	B226
B327	Female	2015	Kitten	B255	B226
B328	Female	2015	Kitten	B295	B181
B329	Female	2015	Kitten	B295	B181
B330	Male	2015	Kitten	B295	B181
BM003	Male	1998	Unknown	B079	B064
BM004	Male	2001	Fetus	B073	UM07

BM005	Male	2001	Fetus	B073	UM07
BM007	Male	2003	Unknown	Unresolved	Unresolved
BM016	Male	2007	Unknown	Unresolved	Unresolved
BM064	Unknown	2012	Unknown	Unresolved	Unresolved
BM071	Unknown	2012	Unknown	Unresolved	Unresolved
BM076	Unknown	2012	Unknown	Unresolved	Unresolved
BM083	Unknown	2012	Unknown	Unresolved	Unresolved
BM092	Unknown	2014	Unknown	Unresolved	Unresolved

APPENDIX B: FIGURES

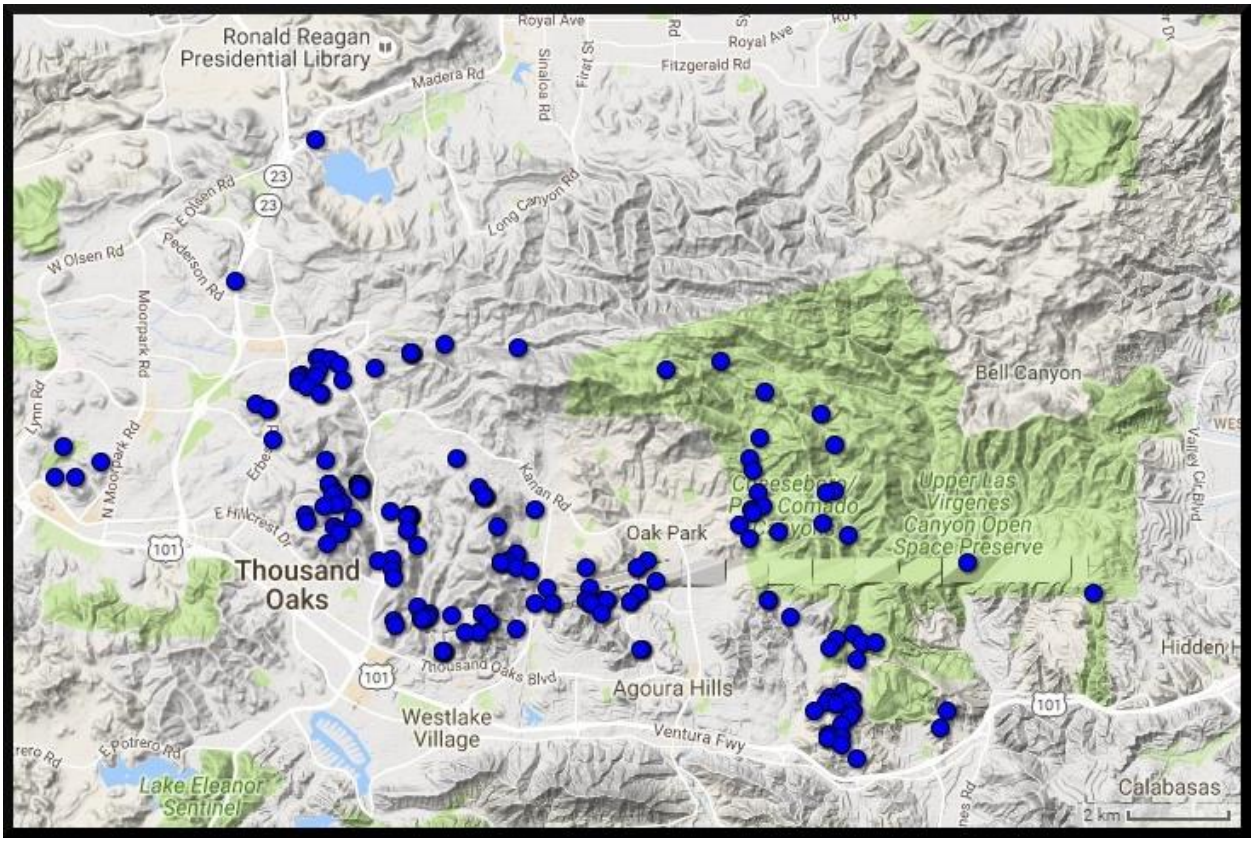

Figure 1. Map of Simi Hills and surrounding area in southern California where 196 bobcats were sampled from 1995–2015. Blue circles represent individual bobcat capture and mortality sampling locations. Map was created using data from Google at GPSVisualizer.com.

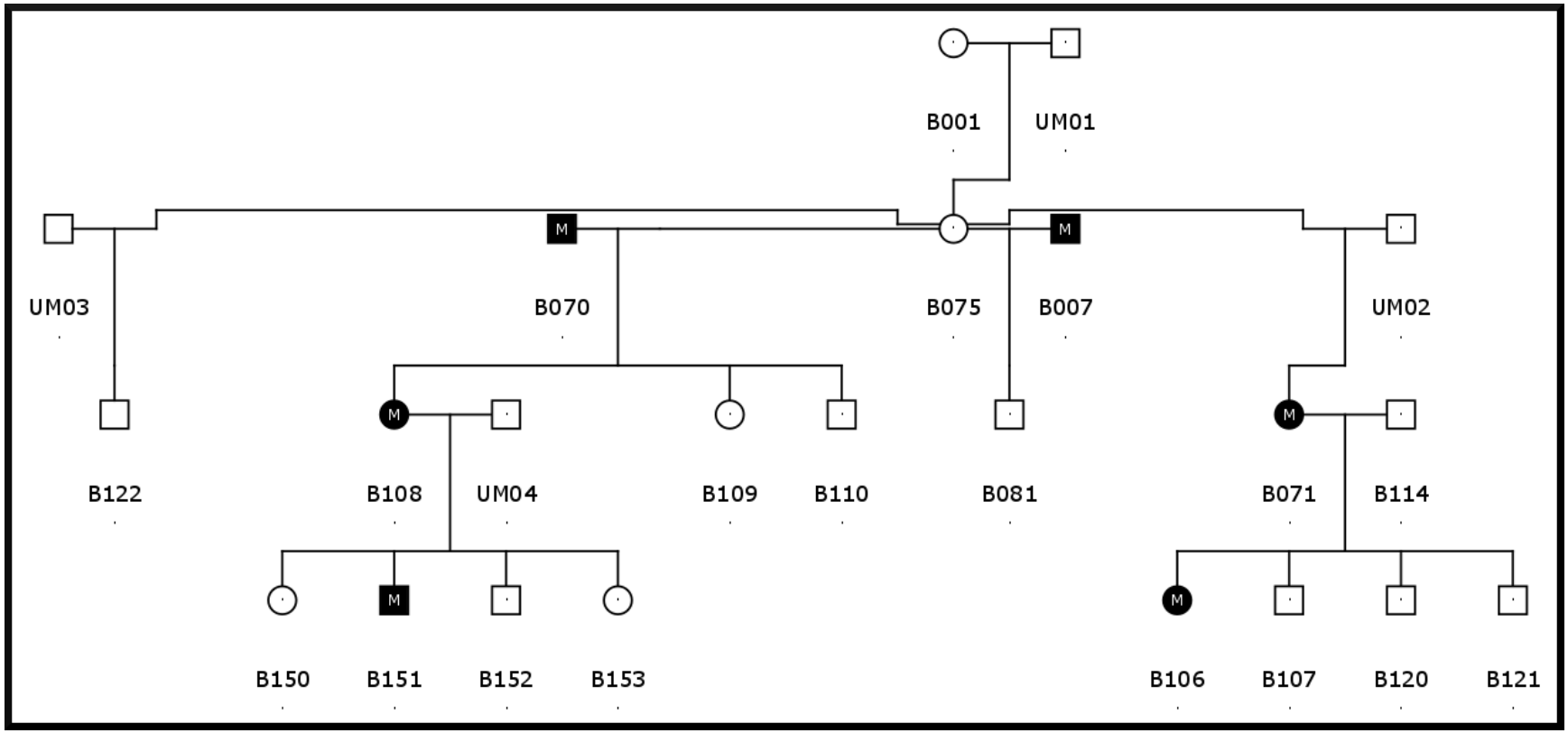

Figure 2. Pedigree of the B001 family lineage from the 1996–2015 Simi Hills bobcat population. ID labels are below the circles and squares, which represent females and males, respectively. UF and UM IDs indicate unsampled females and males, respectively. Note that unsampled ID numbers here may not correspond to unsampled IDs in the complete pedigree (Table 5). Dark symbols marked "M" denote individual bobcats who died of notoedric mange. Members of this family line seem to have either dispersed out of the area and/or met their demise in part because of the mange epizootic among other sources of mortality.

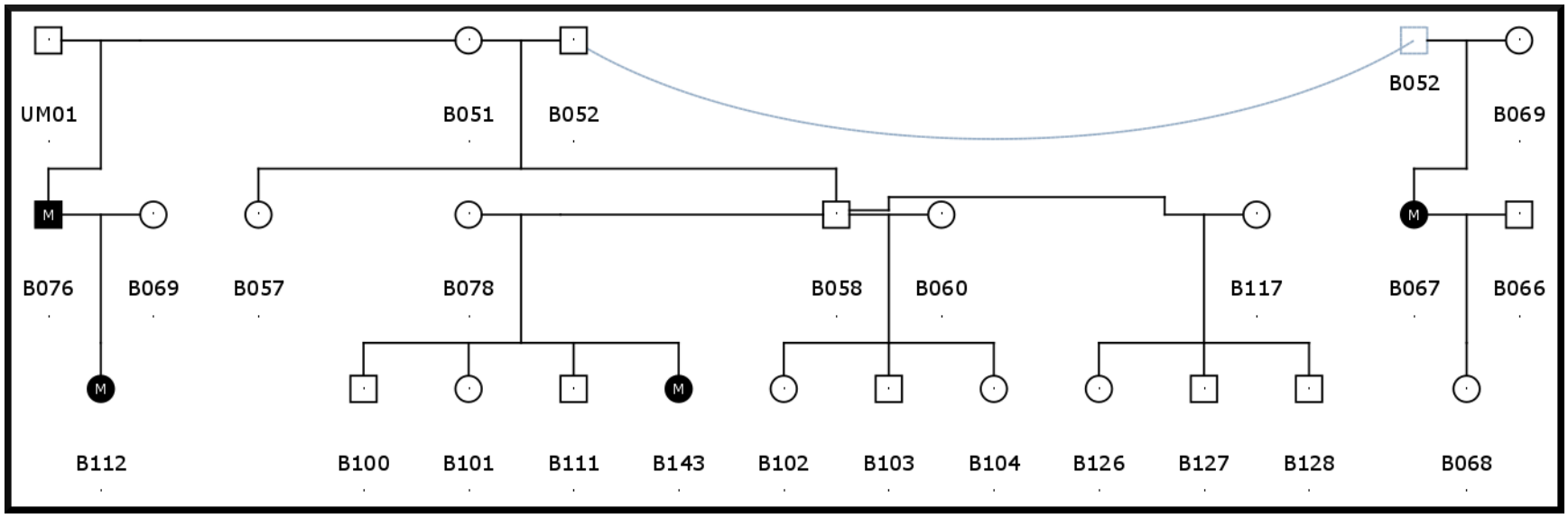

Figure 3. Pedigree of the B051/B052 family lineage from the 1996–2015 Simi Hills bobcat population. ID labels are below the circles and squares, which represent females and males, respectively. UF and UM IDs indicate unsampled females and males, respectively. Note that unsampled ID numbers here may not correspond to unsampled IDs in the complete pedigree (Table 5). Dark symbols marked "M" denote individual bobcats who died of notoedric mange. Members of this family line seem to have either dispersed out of the area and/or met their demise in part because of the mange epizootic among other sources of mortality.

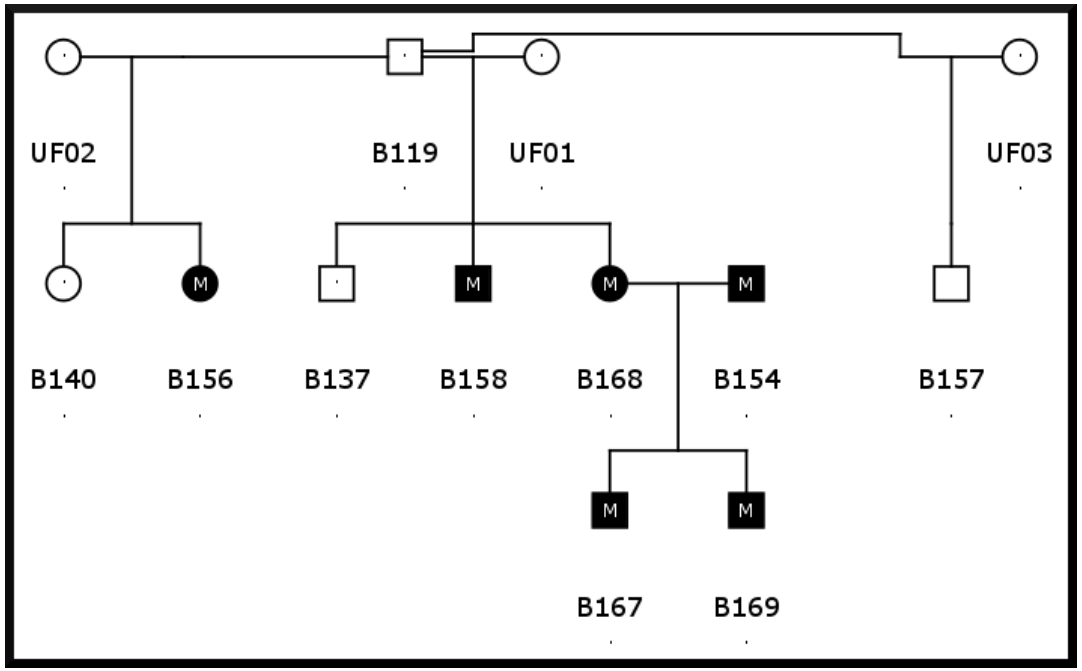

Figure 4. Pedigree of the B119 family lineage from the 1996–2015 Simi Hills bobcat population. ID labels are below the circles and squares, which represent females and males, respectively. UF and UM IDs indicate unsampled females and males, respectively. Note that unsampled ID numbers here may not correspond to unsampled IDs in the complete pedigree (Table 5). Dark symbols marked "M" denote individual bobcats who died of notoedric mange. Members of this family line seem to have either dispersed out of the area or met their demise largely because of the mange epizootic among other sources of mortality.

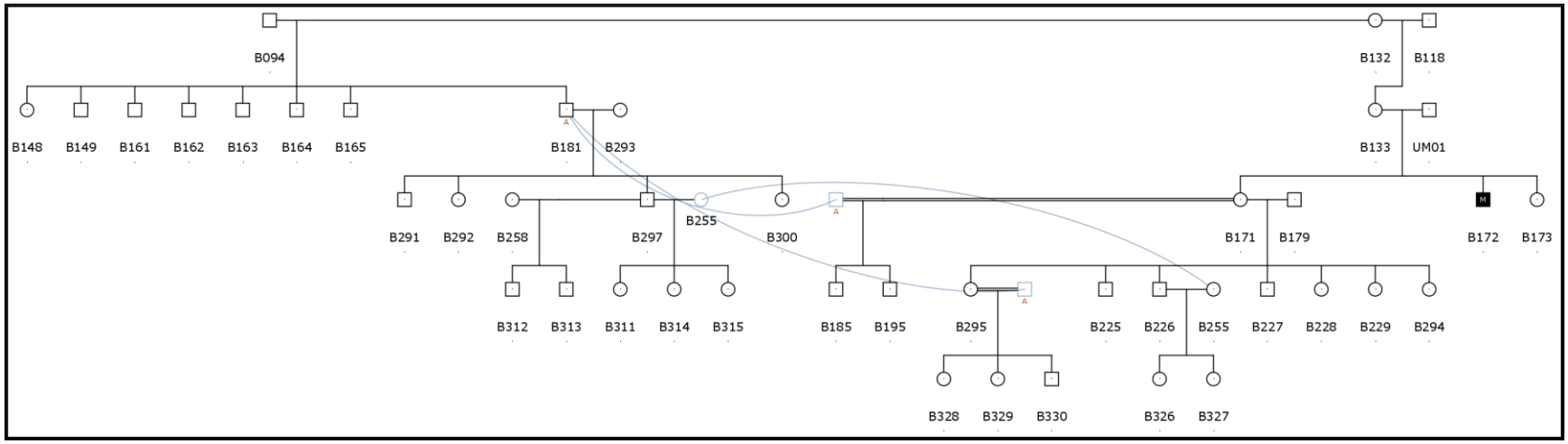

Figure 5. Compete pedigree of the 1996–2015 Simi Hills B132 family lineage, which illustrates its remarkable proliferation in the post-mange epizootic years. It also depicts B181's reproduction with both his half-niece, B171, and his great-half-niece, B295. ID labels are below the circles and squares, which represent females and males, respectively. UF and UM IDs indicate unsampled females and males, respectively. Note that unsampled ID numbers here may not correspond to unsampled IDs in the complete pedigree (Table 5). The dark symbol marked "M" denotes one bobcat who died of notoedric mange in this family line.

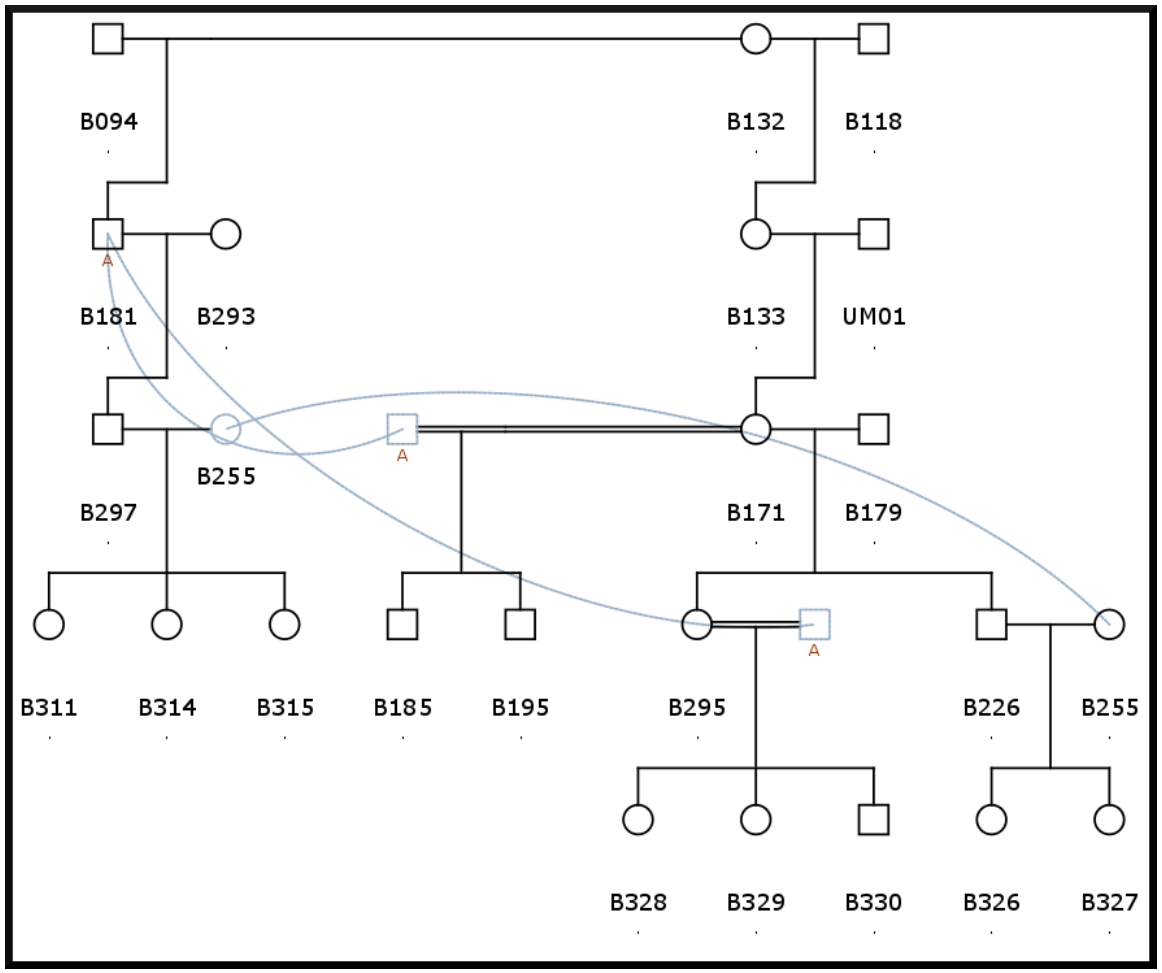

Figure 6. Partial pedigree of the B132 family lineage, which highlights its most successful members from the 1996–2015 Simi Hills bobcat population. It also depicts B181's reproduction with both his half-niece, B171, and his great-half-niece, B295. ID labels are below the circles and squares, which represent females and males, respectively. UF and UM IDs indicate unsampled females and males, respectively. Note that unsampled ID numbers here may not correspond to unsampled IDs in the complete pedigree (Table 5). Along with B155's descendants, members of this family line dominated the post-mange epizootic years.

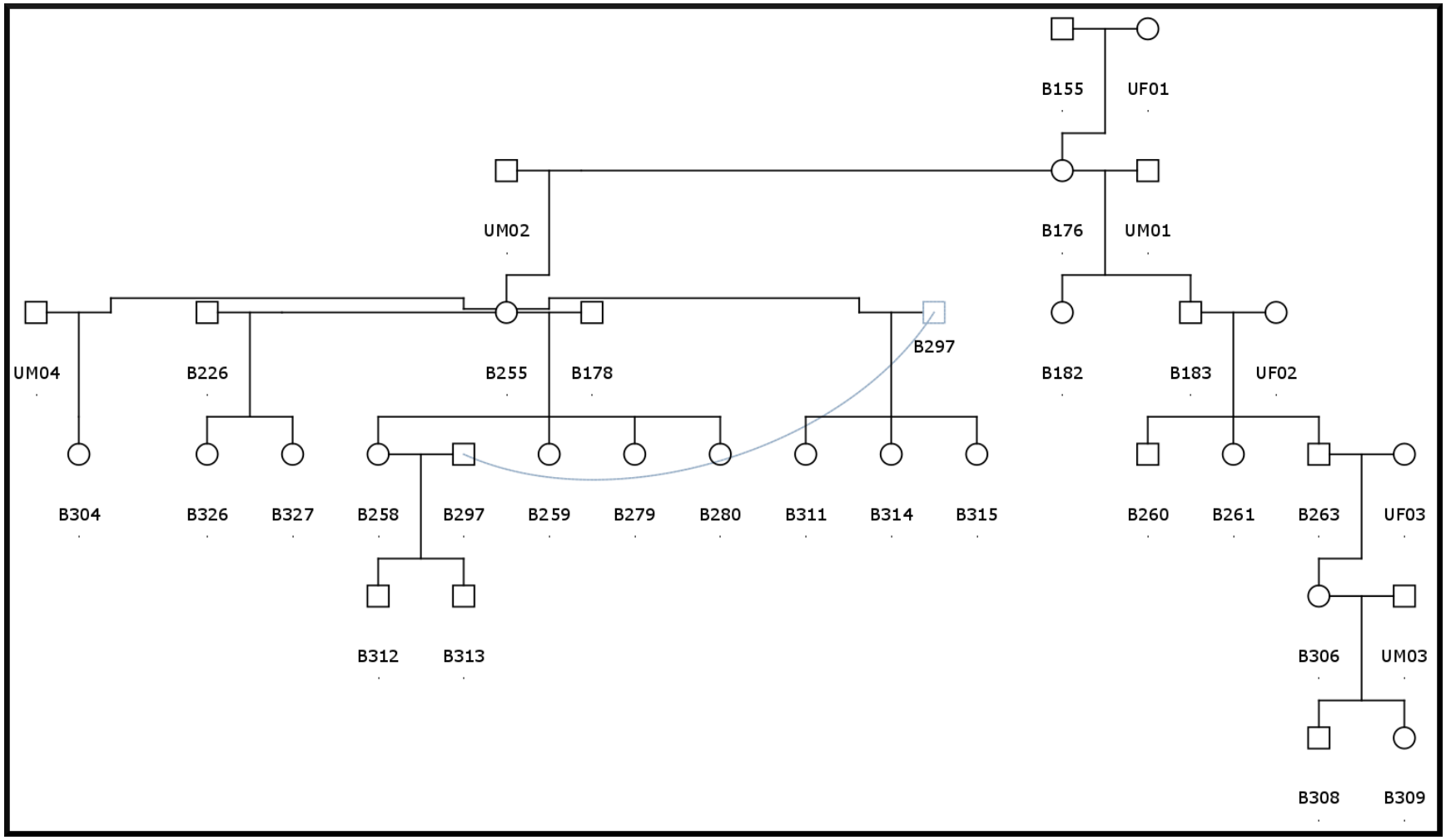

Figure 7. Pedigree of the B155 family lineage from the 1996–2015 Simi Hills bobcat population. ID labels are below the circles and squares, which represent females and males, respectively. UF and UM IDs indicate unsampled females and males, respectively. Note that unsampled ID numbers here may not correspond to unsampled IDs in the complete pedigree (Table 5). Along with B132's descendants, members of this family line dominated the post-mange epizootic years.

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