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Molecular Genetic Insights on Cheetah (*Acinonyx jubatus*) Ecology and Conservation in Namibia

Laurie L. Marker, Alison J. Pearks Wilkerson, Ronald J. Sarno, Janice Martenson, Christian Breitenmoser-Würsten, Stephen J. O'Brien, and Warren E. Johnson

From the Cheetah Conservation Fund, PO Box 1755, Otjiwarongo, Namibia (Marker and Wilkerson); the Wildlife Conservation Research Unit, Department of Zoology, Oxford University, South Parks Road, Oxford OX1 3PS, UK (Marker); the Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD 21702-1201 (Sarno, Martenson, O'Brien, and Johnson); and the KORA, Thunstrasse 31, CH 3074 Muri b. Bern, Switzerland (Breitenmoser-Würsten).

Address correspondence to W. E. Johnson at the address above, or e-mail: johnsonw@ncifcrf.gov.

Abstract

The extent and geographic patterns of molecular genetic diversity of the largest remaining free-ranging cheetah population were described in a survey of 313 individuals from throughout Namibia. Levels of relatedness, including paternity/maternity (parentage), were assessed across all individuals using 19 polymorphic microsatellite loci, and unrelated cheetahs ($n = 89$) from 7 regions were genotyped at 38 loci to document broad geographical patterns. There was limited differentiation among regions, evidence that this is a generally panmictic population. Measures of genetic variation were similar among all regions and were comparable with Eastern African cheetah populations. Parentage analyses confirmed several observations based on field studies, including 21 of 23 previously hypothesized family groups, 40 probable parent/offspring pairs, and 8 sibling groups. These results also verified the successful integration and reproduction of several cheetahs following natural dispersal or translocation. Animals within social groups (family groups, male coalitions, or sibling groups) were generally related. Within the main study area, radio-collared female cheetahs were more closely interrelated than similarly compared males, a pattern consistent with greater male dispersal. The long-term maintenance of current patterns of genetic variation in Namibia depends on retaining habitat characteristics that promote natural dispersal and gene flow of cheetahs.

In the early 1900s, cheetahs (*Acinonyx jubatus*) were found in areas of suitable habitats throughout the Sahel area of Africa and from the Middle East and the Arabian Peninsula to India and the southern provinces of the former Soviet Union (Myers 1975; Nowell and Jackson 1996). Today, cheetahs have been extirpated from a large portion of this area. Asian populations are nearly extinct, with the largest confirmed population (of less than 100 animals) inhabiting central Iran. In Africa, an estimated 15 000 cheetahs remain, with the largest populations existing in Namibia, Botswana, Zimbabwe, Kenya, and Tanzania (Marker and Schumann 1998). The majority of these populations are threatened by competition from other large carnivores, habitat loss, poaching, and widespread killing to protect livestock outside protected areas (Nowell and Jackson 1996; Marker and Schumann 1998).

One of the largest remaining cheetah populations inhabits large portions of north-central Namibia (Marker-Kraus et al. 1996; Nowell and Jackson 1996). As in other parts of Africa, these populations have historically been persecuted, and the Namibian cheetah population decreased 50% in the 1980s as over 6700 individuals were trapped and killed as vermin (CITES 1992). However, over the last decade, management practices have been gradually changing (Marker and Schumann 1998; Marker, Mills, and Macdonald 2003) and landowner perceptions of cheetahs have allowed increasing levels of coexistence (Marker, Mills, and Macdonald 2003). However, many farmers still trap cheetahs as a precautionary measure to reduce livestock loss (Marker, Dickman, Mills, and Macdonald 2003). In the past, these cheetahs were mostly killed, but many trapped cheetahs today are instead released or translocated to areas where farmers are more tolerant of cheetahs (Marker, Dickman, Mills, and Macdonald 2003). Over the past decade, the Namibian cheetah population has increased to around 3000 animals, over 90% of which inhabit unprotected areas on privately owned commercial livestock or game farms (Hanson and Stander 2004). These farms generally have no lions or spotted

hyenas and support over 70% of the country's game animals (Richardson 1998).

Namibian cheetahs form a variety of social groups, including coalitions of adult males, single adult males, single adult females, and family groups (females accompanied by dependent cubs or groups of siblings that have recently reached independence) (Marker-Kraus et al. 1996). Male coalitions of 2–4 animals have an average yearly home range minimum convex polygon of 1665 km^2 compared with 1836 km^2 for females (Marker 2000; Marker LL, Dickman AJ, Mills MGL, Jeo RM, MacDonald DW, submitted). Cheetahs in Namibia have also been recorded as occasionally forming unusually large social groups (McVittie 1979; Marker-Kraus et al. 1996) of unknown interrelatedness.

Various aspects of cheetah evolutionary history have been well characterized and discussed (O'Brien et al. 1983; May 1995; Driscoll et al. 2002). The cheetah evolved from a common ancestor with the puma (*Puma concolor*) and jaguarundi (*Puma yaguaroundi*), presumably in North America, in the late Miocene (4.92 million years before the present) (van Valkenburgh et al. 1990; Johnson and O'Brien 1997; Johnson et al. 2006), and predecessors of modern day cheetahs were once distributed across North America and Europe (Adams 1979). However, by the end of the last glacial period, the cheetah had disappeared from most of its prior distribution, and the few surviving cheetahs experienced at least one severe demographic bottleneck that significantly reduced levels of molecular genetic variation as measured by several methods, including mitochondrial DNA DNA sequence variation (Menotti-Raymond and O'Brien 1993), allozyme size variation (O'Brien et al. 1983), variation in the major histocompatibility complex (O'Brien et al. 1985; Yuhki and O'Brien 1990), and minisatellite variation (Gilbert et al. 1991; Menotti-Raymond and O'Brien 1993). The bottleneck and associated loss of genetic variation have also been linked to several important life history characteristics of cheetahs, including increased fluctuating asymmetry in metric skull measurement (Wayne et al. 1986), relatively low levels of normal spermatozoa in males (Wildt et al. 1983), immunologically accepted reciprocal skin graphs between unrelated individuals (O'Brien et al. 1985), dental anomalies and palatal erosion (Marker and Dickman 2004), and an increased susceptibility to infectious disease agents (O'Brien et al. 1985; Evermann et al. 1988; Heeney et al. 1990; Brown et al. 1993; Munson 1993). Approximate molecular dating places this bottleneck at the end of the last ice age. Since that time, cheetah populations have been reconstituting genetic variation, and current levels of cheetah microsatellite variation approach those of several other outbred populations of felids (Culver et al. 2000; Uphyrkina et al. 2001; Driscoll et al. 2002).

Here, we use variation at 38 microsatellite loci to 1) characterize patterns of molecular genetic variation across the Namibian cheetah population to determine if there were any major barriers to gene flow or recognizable substructure, 2) compare levels of molecular genetic variation among geographic regions in the country, 3) utilize microsatellite size variation to describe aspects of the social behavior of Namibian cheetahs by assessing relatedness within known social groups including male coalitions, females with young, and sibling groups, and 4) assess the efficacy of several important conservation management practices in Namibia.

Methods

Sample Collection and DNA Extraction

Samples were collected from wild-caught cheetahs live-trapped in cages (Marker, Dickman, Jeo, et al. 2003; Marker, Dickman, Mills, and Macdonald 2003) from 1991 to 2000 from throughout the range of the cheetah within Namibia (Supplementary Appendix I, Figure 1). The largest number of samples are from the core study area encompassing portions of the Otjiwarongo (Otj), Okahandja (Oka), and Omaruru (Oma) districts. In this core area, 41 unrelated adults (26 males and 15 females) were fitted with radio telemetry collars, and their activities and movements were monitored for an average 19.4 (± 13) months (Marker 2000; Marker LL, Dickman AJ, Mills MGL, Jeo RM, MacDonald DW, submitted). Overall home range size was estimated for 27 cheetahs with at least 30 fixes per year using the 95% kernel method (Marker 2000; Marker LL, Dickman AJ, Mills MGL, Jeo RM, MacDonald DW, submitted). Cheetahs were classified according to the age and sex composition of the group with which they were caught into 1 of 5 social groups: single males, male coalitions, single females, mother with cubs, and independent siblings without an adult female (dam) (as in Marker et al. 2002b and 2002c) (Supplementary Appendix I).

Total genomic DNA from 313 individual cheetahs was extracted from frozen leukocytes and blood stored in a concentrated salt solution (100 mM Tris, 100 mM ethylenediaminetetraacetic acid, 2% sodium dodecyl sulfate) following 1 of 2 standard extraction techniques: phenol–chloroform (Sambrook et al. 1989) or salt precipitation (Montgomery and Sise 1990).

Microsatellite Markers

Nineteen microsatellite loci derived from the domestic cat (FCA8, FCA26, FCA51, FCA85, FCA96, FCA97, FCA117, FCA126, FCA133, FCA169, FCA187, FCA212, FCA214, FCA224, FCA247, FCA290, FCA298, FCA310, and FCA344) were characterized in 313 cheetahs to address questions of behavioral ecology. An additional 19 microsatellites (FCA14, FCA42, FCA69, FCA75, FCA78, FCA80, FCA88, FCA94, FCA105, FCA113, FCA161, FCA166, FCA171, FCA192, FCA208, FCA225, FCA230, FCA327, and FCA559) were characterized in a representative subset of 89 unrelated cheetahs from throughout Namibia (Supplementary Appendix I). Unrelated cheetahs were selected based on behavioral observations, parentage analyses, and estimates of genetic relatedness as described below.

Microsatellites were amplified following previously described polymerase chain reaction (PCR) amplification conditions (Menotti-Raymond et al. 1997, Menotti-Raymond

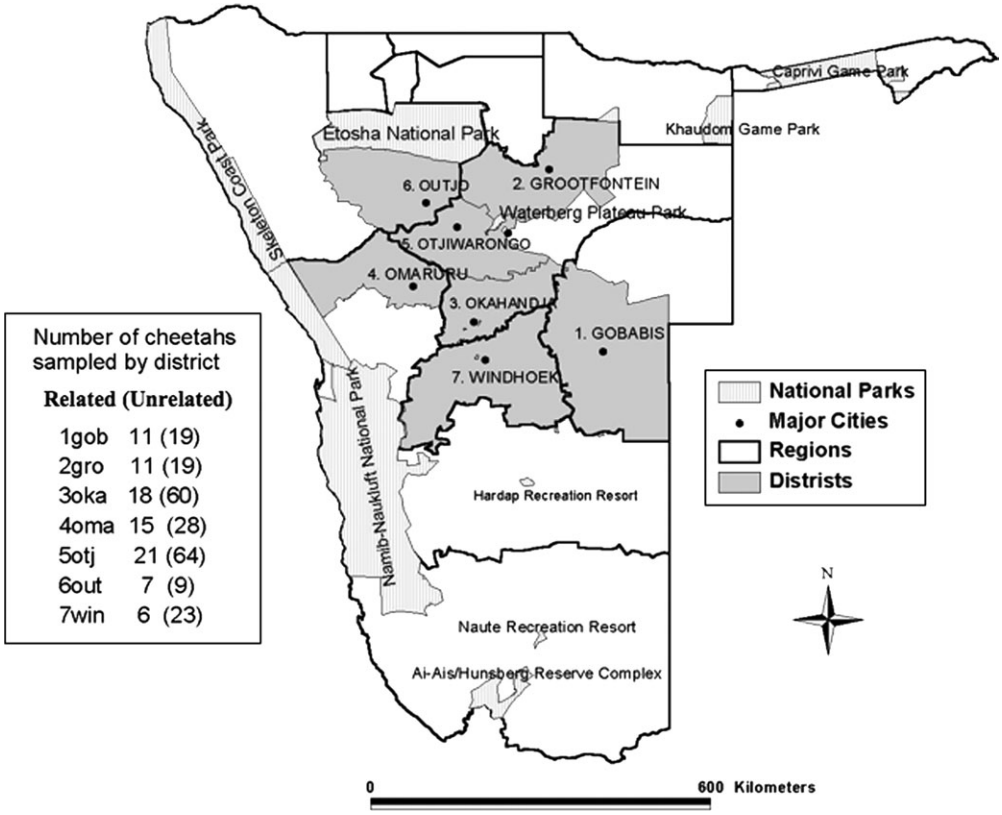

Figure 1. Map of Namibia with the 7 geopolitical regions and the number of cheetahs sampled from each area. The number of animals utilized in analyses requiring unrelated individuals is listed in parenthesis.

1999). All microsatellites were dinucleotide repeats except Fca559 and Fca42, which had tetra-nucleotide repeats. These 38 microsatellites have been mapped to 11 of the 19 domestic cat chromosomes. Of the 38 loci, 23 were unlinked or at least 20 cM (centimorgams, a unit of recombination distance between genes on chromosomes) apart in the domestic cat and are therefore assumed to be unlinked in cheetah (Menotti-Raymond et al. 1999; Driscoll et al. 2002). Five pairs of loci were separated by an estimated distance of 12 cM (Fca85/Fca96), 9 cM (Fca75 and Fca96), 6 cM (Fca212/Fca126), 4 cM (Fca224 and Fca161), and 1 cM (Fca171/Fca161). The dye-labeled PCR products of the microsatellite primer sets were pooled and diluted together based on size range and fluorescent dye so that 3–6 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, Foster City, CA) 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), which is known in the domestic cat but not for the cheetah.

Analysis of Genetic Variation

Cheetah samples were initially classified into 7 geographic regions that in general lacked recognizable physical borders but coincided with magisterial districts that defined the perimeters of large farms (of around 8000 ha each) within the districts (Figure 1). Estimates of microsatellite size variation such as average expected heterozygosity, average variance, number of unique alleles, and average number of repeats were derived for the 89 unrelated cheetahs from the program MICROSAT (version 1.5) (Minch et al. 1996) for the 7 geographic regions. These estimates of cheetah microsatellite diversity could be biased upward relative to estimates from other felid populations as only polymorphic loci were used in the present study. Fisher exact test for deviations from Hardy–Weinberg equilibrium (Guo and Thompson 1992), genotypic linkage disequilibria between pairs of loci (Garnier-Gere and Dillman 1992), and pairwise genotypic and genic differentiation between populations (Goudet et al. 1996) were calculated using GENEPOP version 3.2 (Raymond and Rousset 1995). The *F*-statistic was calculated according to Weir and Cockerham (1984) using FSTAT, version 1.2 (Goudet 1995) and ARLEQUIN (Schneider et al. 2000) to assess possible geographic structure and levels of gene flow among the geographic regions. Standard errors of F_{st} and F_{is} estimates were obtained by jackknifing over all loci as implemented in FSTAT. When appropriate, we corrected type 1 error levels for multiple tests following the sequential Bonferroni procedure (Rice 1989). All computed *P* values were 2 tailed.

Phylogeographic Cluster Analyses

The extent of geographic structure among Namibian cheetahs was assessed using 3 approaches. The first approach was to estimate pairwise genetic distances among the set of 89 unrelated individuals and among unrelated individuals from the same area from composite microsatellite genotypes using the proportion of shared alleles (Dps) algorithm with a $(1 - M)$ correction as implemented in MICROSAT (version 1.5) (Minch et al. 1996). Three loci (Fca 169, Fca 559, and Fca 42) were excluded from phylogeographic analyses because of insufficient data. A phylogenetic tree was constructed with bootstrap values (from 1000 iterations) from a Dps distance matrix using PHYLIP (version 3.572) (Felsenstein 1993) and was drawn using the program TREEVIEW (version 1.5) (Page 1996). The second approach was to perform a principal component analysis (PCA) and produce a population cluster matrix using the program GENETIX (4.01) (Belkhir 2000). The third approach was a Bayesian procedure, implemented in the program STRUCTURE (Pritchard et al. 2000), to identify populations or genetic clusters and to assign individuals to one of these groups. All the unrelated samples were pooled and were assumed to belong to an unknown number of genetically unique clusters (K), and the posterior probability (log likelihood, lnL) was estimated assigning priors from 1 to 7 (number of populations) to determine the number of clusters that maximized the lnL of the data. Structure was run for 5–10 repetitions of 100 000 iterations with a burn-in period of 100 000 iterations, assuming a model of admixture with correlated genotypes among clusters. The estimated proportion of membership (q_i), or the average proportion of genotypes in each predefined group, was inferred, and based on previous studies with similar data sets, individuals with $q_i > 0.80$ were assigned to one cluster or jointly to more than one cluster if $q_i < 0.80$ (individuals with evidence of admixture).

Parentage, Relatedness, and Dispersal

The probability that putative mothers were the correct biological mothers was estimated using the program CERVUS (version 2.0) (Marshall et al. 1998) using both the "one-parent known" and the "neither-parent known" options. In addition, all individuals were compared against all others, as potential parents, to identify possible parents or situations where the individual had inadvertently been sampled more than once or when females were captured with cubs that were not their own offspring.

A standard curve depicting the estimated degree of relatedness (R) among known mothers and offspring, siblings, and unrelated animals was established using the program RELATEDNESS 5.0.5 (Queller and Goodnight 1989). Based on the standardized curve, we categorized cheetah pairs of unknown kinship as related if R was >0.2. The difference in relatedness of between 24 male and 13 female radio-collared cheetahs and between 65 male and 24 female unmarked cheetahs in our study area was assessed (using actual R values) using a Mann–Whitney U-test.

To analyze genetic relatedness and dispersal patterns, the distance in kilometers between the capture site of the related individuals was compared. Social groups of known related individuals, including dam and daughter, dam and son, sire and daughter, sire and son, and siblings were compared using a Mann–Whitney U-test.

Results

Genetic Variability of Microsatellite Loci

In the 89 unrelated animals, 248 alleles were observed for the 38 microsatellites. After correcting for multiple testing, none of the loci deviated from the Hardy–Weinberg equilibrium for any of the regions. There were 3–10 alleles per locus (Table 1), and mean expected heterozygosity (H_e) ranged from 0.640 to 0.708 in the 7 geographic regions (Table 1) compared with 0.599 in Serengeti cheetah. The mean number of alleles per locus was higher in Otj ($n = 4.6$), in the center of the Namibian cheetah's distribution area, than in Outjo (Out) ($n = 3.7$), which is the westernmost area of their Namibian range (Figure 1). By comparison, Serengeti cheetahs had an estimated 4.1 alleles per locus.

Genetic Relationship between Regional Groups

Both the neighbor-joining tree, constructed from a distance matrix based on the proportion of shared alleles (Dps) of animals within each region (Figure 2) and the population cluster graph (Figure 3), showed modest substructure that was related to the relative geographic locations of the 7 regions in Namibia. Both methods grouped the east central regions of Oka, Oma, and Otj, the southeast regions of Gobabis (Gob) and Windhoek (Win) and the northern regions of Grootfontein (Gro) and Out.

However, when the 89 unrelated cheetahs were treated individually, a phylogenetic analysis of their composite microsatellites using the proportion of shared allele distances revealed no apparent structure, with individuals from different regions being intermixed (Figure 4). The multivariate approach using a PCA corroborated the general lack of differentiation among individuals from the 7 regions, with only the group from Out showing a clear difference from the other populations (Figure 3). Bayesian analyses provided some support for geographic subdivisions, partitioning these unrelated cheetahs into either 2 or 3 clusters (average lnL for x repetitions $\pm$ 1 standard error $= -7900.4 \pm 46.9$ for $K = 2$ and -7829 ± 15.2 for $K = 3$; Table 2). In the 2 cluster scenario, 15 of 18 cheetahs from Out and Gro were assigned to cluster 1, with the 3 others showing affinity with both 1 and 2 ($q_1 < 0.80$ and $q_2 < 0.80$) (Table 3). All except one individual from Gob, Win, and Oka were in cluster 2 or in both 1 and 2, whereas individuals from Oma and Otj were more equally split between cluster 1 and 2 or were of mixed origin. In the 3-cluster scenario, the 7 cheetahs from Out formed a cluster distinct from individuals from Gro, animals from Win, Gob, and Oka remained grouped, and Oma and Otj again appeared to be of

Table 1. Expected heterozygosity (H_e) and number of alleles (A) by district (as defined in Figure 1) and total number of alleles (AT) for each microsatellite locus

	GOB (n = 11)		GRO (n = 11)		OKA (n = 18)		OMA (n = 15)		OTJ (n = 21)		OUT (n = 7)		WIN (n = 6)		(n = 89)
Locus	H_e	A	H_e	A	H_e	A	H_e	A	H_e	A	H_e	A	H_e	A	AT
Fca 8	0.718	4	0.764	5	0.667	4	0.65*	5	0.763	6	0.643	4	0.8	4	6
Fca 26	0.727	6	0.611	3	0.577	4	0.544	3	0.707	6	0.762	4	0.767	4	6
Fca 51	0.573	4	0.364	2	0.343	4	0.295	3	0.138	3	0.433	3	0.533	3	5
Fca 85	0.714	6	0.805	6	0.387	4	0.66	5	0.518	7	0.69	4	0.375	3	7
Fca 96	0.836	7	0.791	6	0.813	7	0.764	6	0.785	7	0.75	5	0.833	5	10
Fca 97	0.833	4	0.875	5	0.726	5	0.8	5	0.695	4	1	3	0.75	4	9
Fca 117	0.75	4	0.727	6	0.783	6	0.85	7	0.862	7	0.821	6	0.817	5	7
Fca 126	0.709	5	0.695	4	0.644	5	0.702**	5	0.55	3	0.607	3	0.583	3	7
Fca 133	0.659	5	0.65*	5	0.715	5	0.703	5	0.64	4	0.595	4	0.917	7	10
Fca 169[a]	0.667	3	NA	2	0.75	3	0.5	2	0.683	4	NA	NA	0.917	4	4
Fca 187	0.445	3	0.632	3	0.556	3	0.59**	3	0.53	3	0.536	3	0.533	3	3
Fca 212	0.783	5	0.689	4	0.557	4	0.777	5	0.662*	7	0.786*	5	0.7	3	8
Fca 214	0.745	6	0.701	5	0.835**	7	0.786*	6	0.758	7	0.381	3	0.733	4	8
Fca 224	0.686	4	0.736	5	0.733	5	0.667	4	0.721*	5	0.619	3	0.767	4	6
Fca 247	0.677	3	0.732	4	0.699	4	0.673	5	0.765	7	0.65	5	0.825	4	7
Fca 290	0.618	4	0.741	5	0.668	6	0.558	4	0.651	4	0.774	5	0.7	4	7
Fca 298	0.255	2	0.464	2	0.488	2	0.514	2	0.42	3	0.143	2	0.55	3	3
Fca 310	0.709	4	0.786	6	0.651	4	0.721	4	0.638	4	0.702	4	0.75	4	6
Fca 344	0.491	2	0.509	2	0.472	3	0.51	2	0.498	2	0.548	2	0.4	2	3
Fca 14	0.764	5	0.755*	5	0.771	5	0.66	5	0.669	5	0.702	4	0.8	5	6
Fca 69	0.732	4	0.778	4	0.703	4	0.743*	4	0.741	4	0.738	5	0.733	3	5
Fca 75	0.691	5	0.814*	7	0.639*	5	0.84	7	0.627	5	0.762*	4	0.733	4	8
Fca 78	0.823	6	0.756	5	0.608***	6	0.75	6	0.779	7	0.675	3	0.75	5	9
Fca 80	0.6	4	0.573	4	0.619	3	0.47	3	0.621	4	0.607	3	0.6	3	4
Fca 88	0.727	5	0.7	6	0.694	4	0.705	5	0.623	4	0.631	4	0.817	5	6
Fca 94	0.636	3	0.723	5	0.678*	3	0.733	4	0.684**	3	0.762	4	0.683	3	6
Fca 105	0.741*	4	0.782	5	0.667	6	0.757	5	0.699	5	0.702	4	0.717	4	6
Fca 113	0.572	3	0.556	3	0.572	4	0.561	4	0.591	4	0.262	2	0.717	3	6
Fca 161	0.795	5	0.709	4	0.7	4	0.686	5	0.631	5	0.69	4	0.65	3	5
Fca 166	0.75	5	0.705	5	0.714	5	0.423	4	0.569	5	0.81**	4	0.7	4	5
Fca 171	0.85	8	0.8	5	0.741	6	0.821	6	0.787	5	0.464	3	0.833	5	8
Fca 192	0.673	5	0.644*	4	0.629	4	0.654	5	0.574	4	0.6	3	0.5	3	5
Fca 208	0.783	5	0.709	5	0.544	4	0.724*	5	0.751	5	0.683	4	0.7	3	5
Fca 225	0.736	5	0.655	3	0.709	5	0.712	4	0.641	4	0.488	3	0.767	4	5
Fca 230	0.823	6	0.768	4	0.765	5	0.827	6	0.764*	5	0.75	3	0.85	5	7
Fca 327	0.6	4	0.7	4	0.75	4	0.753	4	0.725	5	0.583	3	0.783	4	5
Fca 559[a]	0.75	3	0.75	3	0.833	4	0.833	4	0.8	4	NA	NA	NA	NA	4
Fca 042[a]	1	4	1	3	NA	1	0.75	3	NA	1	NA	NA	NA	2	5
Average	0.700	4.5	0.701	4.3	0.655	4.4	0.671	4.5	0.642	4.6	0.640	3.7	0.708	3.8	6.1
Standard Error	0.130	1.3	0.121	1.3	0.118	1.3	0.136	1.3	0.137	1.5	0.144	1.0	0.130	1.0	1.8
F_{is} (mean)	−0.045		0.040		−0.029		−0.003		0.099		−0.040		0.038		
F_{is} (standard deviation)	0.255		0.244		0.181		0.272		0.187		0.187		0.235		

[a] Loci excluded from future analysis due to insufficient data, as noted by NA (insufficient data for calculation).

* Significant at < 0.05. ** Significant at < 0.01. *** Significant at < 0.001 (Hardy–Wienberg probability test).

mixed geographic heritage and were assigned more randomly to 1 of the 3 groups or to a combination of groups (Table 3).

Population differentiation among the 7 regions was tested using an analysis of molecular variance approach with 10 000 permutations as implemented in Arlequin. Overall, F_{st} and R_{st} values were low (0.02 and 0.03, respectively) and not significant ($P > 0.05$). Pairwise values showed very little evidence of genetic differentiation between most of the subpopulations of cheetahs (Table 4). Only animals from the districts of Oka and Out showed moderate differentiation. F_{st} and R_{st} pairwise values were significantly correlated (Spearman's correlation $r_s = 0.853$, $n = 21$, $P < 0.001$, Pearson $r^2 = 0.7273$).

Relatedness and Dispersal

Estimates of relatedness (R) were obtained for the Namibian cheetah population based on comparisons between individuals of known genetic relationships. Average relatedness among 89 presumed unrelated cheetah was −0.0067 (±0.177, n =

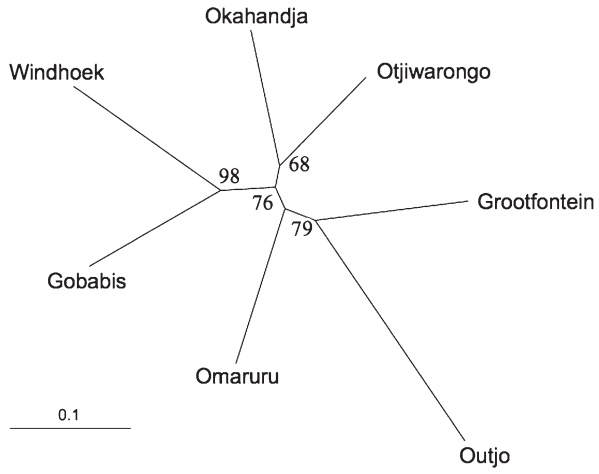

Figure 2. Depiction of relationships among 7 subpopulations in Namibia, constructed using the proportion of shared alleles distances among the 7 geographic groups and the neighbor-joining algorithm. Nodes are labeled with percent bootstrap support from 1000 replicates.

7470 pairwise comparisons, Supplementary Appendix I) (Figure 5). First-order relatives (e.g., mother–offspring) had a mean R value close to the expected value of 0.5 (0.481 ± 0.141, $n = 57$) (Supplementary Appendix I). Average relatedness among siblings (136 pairwise comparisons) was $R = 0.391$ (±0.167) (Supplementary Appendix I, Figure 5), slightly lower than the theoretical expectation of 0.5 and perhaps indicative of some misidentification of siblings or of multiple paternity. Estimates of relatedness and parentage analyses using CERVUS provided strong support that 21 of 23 females in identified family groups were correctly classified as the biological mothers of the proposed offspring with logarithm of the odds (LOD) scores ranging from 3.6 to 8.56, suggesting that the probability the proposed relationship might be incorrect was 1 in 1000 to 1 in 10 000 000. The probability of nonexclusion of mothers ranged from 1.4×10^{-3} to 5.9×10^{-11}. A probable dam (AJU986) and sire (AJU881) was identified for only one cheetah, AJU985 (Table 5).

In the analysis of the sibling groups with no dam, 80.8% ($n = 21$) of the groups were composed of related individuals. Similarly, among cheetahs in male coalition groups, 88.5% ($n = 26$) of the groups had R scores suggesting that they were siblings. The 3 groups that appeared to be unrelated all were of uncertain origin (see Discussion). An analysis of the degree of relatedness (R) among all individuals also identified several closely related pairs of individuals that were captured separately and had therefore been assumed to be unrelated. Combined with knowledge of the age of the individuals and their capture location, 14 dam/offspring groups, 26 sire/offspring relationships, and 8 sibling groups were inferred that had previously not been suspected (Table 5) and one animal that had been captured twice and assigned 2 identification numbers was proven to be the same individual.

Overall, there was no significant difference in the amount of home-range overlaps between unrelated cheetahs ($R < 0.2$) and more related cheetahs ($R > 0.2$) (Mann–Whitney $U = -1.033$, $P = 0.302$). There was also no significant difference in the amount of home-range overlap and the degree of relatedness among cheetahs in social groups (Kruskal–Wallis $\chi^2 = 4.182$, degree of freedom = 3,

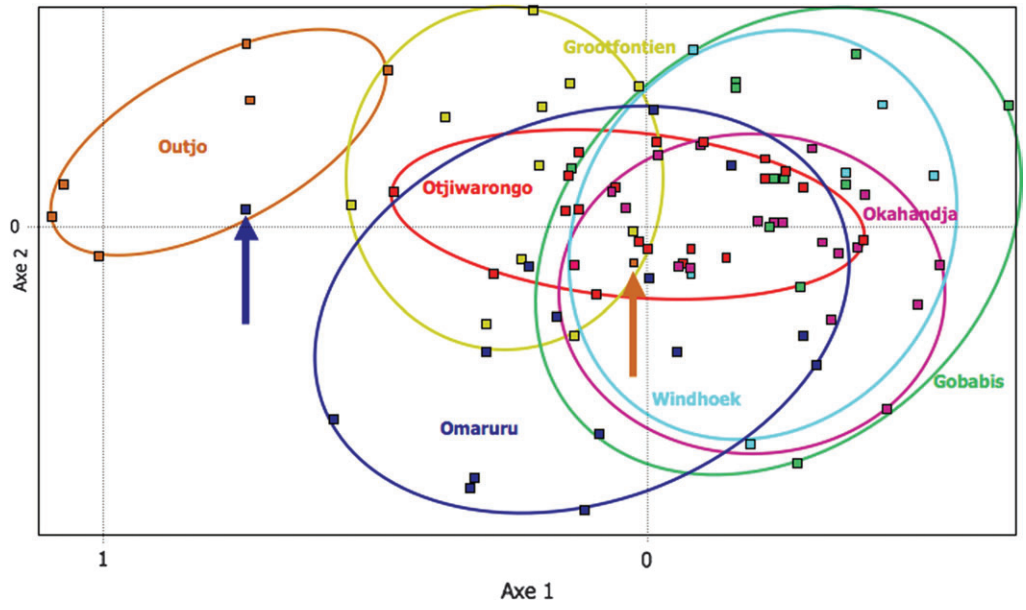

Figure 3. Population cluster graph from PCA of 7 subpopulations in Namibia.

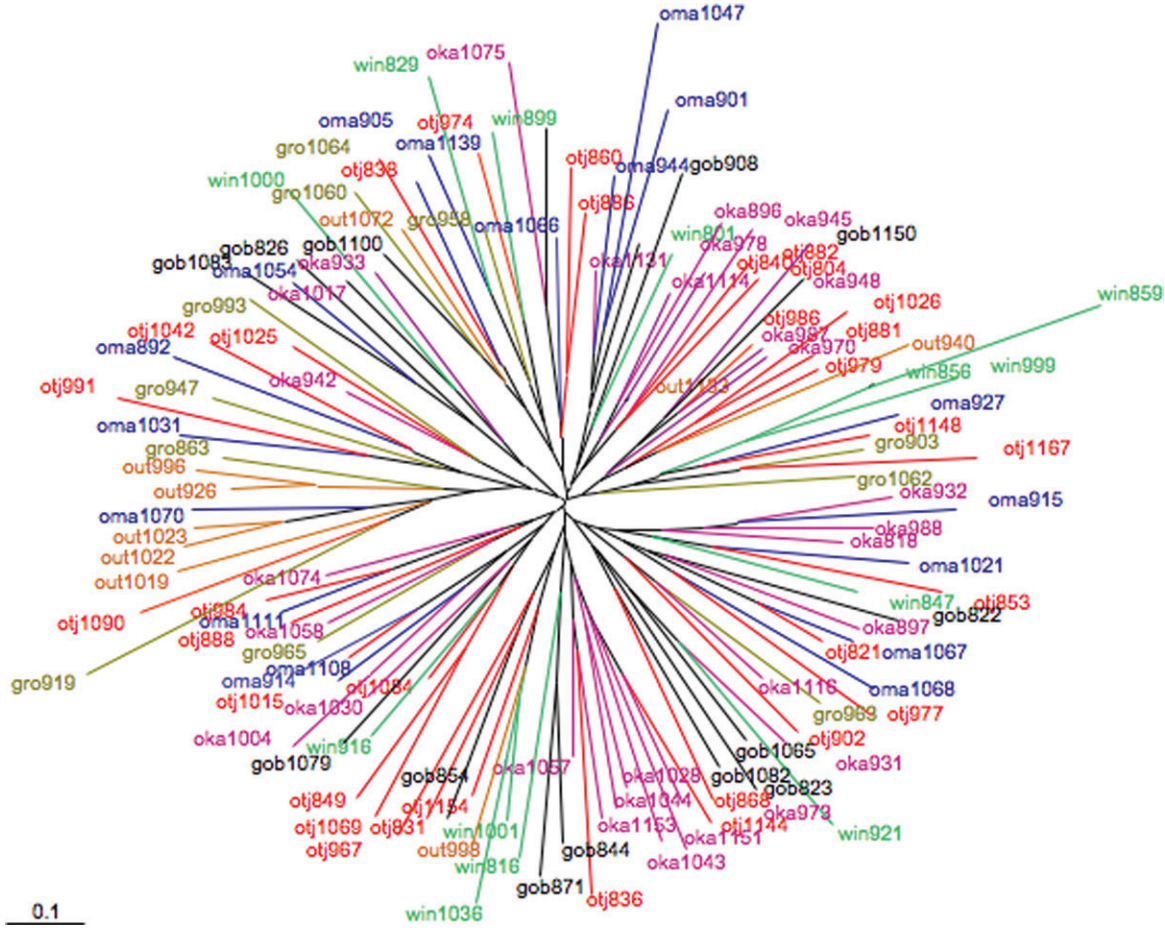

Figure 4. Phylogenetic depiction of relationships among individual cheetahs. Colors indicate the region from which the individual was sampled.

$P = 0.242$) (Figure S1). However, home-range overlap among related females was significantly greater than among nonrelated females ($U = -2.315$, $P = 0.021$).

The mean distance between social groups, including groups made up of related and unrelated animals, was 90.7 km (standard deviation = 80.66 km) (Table 6). The mean distance between dams and daughters was significantly less than between dams and sons ($U = -2.08$, $P = 0.0048$), and the mean difference between dams and daughters was significantly less than between sires and daughters ($U = -2.86$, $P = 0.004$).

Discussion

Regional Patterns among Cheetah Populations

Phylogeographic, multivariate, and Bayesian approaches provided consistent evidence that there are only weak subdivisions among the 7 regions. Cheetahs from the northern regions of Out and Gro are somewhat isolated from the other Namibian regions, as is illustrated in the phylogram and PCA of the 7 populations (Figure 2) and in the Bayesian population structure analyses. Similarly, animals from the southern regions of Oka, Win, and Gob are somewhat genetically distinct. Cheetahs from Oma and Otj, which are located geographically between the northern and southern groups, have genetic affinity with animals

Table 2. The estimated probability of the number of population (K) for prior values of $K = 1$ to 6 using a Bayesian clustering analysis of 89 unrelated cheetah samples as implemented in the program STRUCTURE (Pritchard et al. 2000). The highest likelihood was found for the 3 populations model ($k = 3$)

K	LnL	Standard deviation
1	−7949.4	6.19
2	−7900.4	46.81
3	−7829.9	15.24
4	−7856.1	34.23
5	−7912.5	90.27
6	−8088.2	243.02

Table 3. The number of cheetahs from seven geographical regions in Namibia assigned to a single clusterwith a qi > 0.80 or to a combination of clusters under a two population scenario ($k = 2$) and a three population scenario ($k = 3$) using a Bayesian clustering analysis of 89 unrelated cheetah samples as implemented in the program STRUCTURE (Pritchard et al. 2000)

	1	1 and 2	2
Out	6	1	0
Gro	9	2	0
Oma	7	4	3
Otj	8	6	7
Oka	0	2	4
Win	1	0	10
Gob	0	4	13

	1	1 and 2	2	2 and 3	3	1 and 2 and 3
Out	5	1	0	1	0	0
Gro	1	4	5	1	0	0
Oma	1	5	2	3	2	1
Otj	0	6	2	9	2	1
Oka	0	0	0	8	9	0
Win	0	0	1	2	3	0
Gob	0	1	0	6	4	0

from both the north and the south. In the structure analyses, individual cheetahs from Oma and Otj are either assigned to the northern cluster or the southern cluster or are assigned to both (have a mixed heritage). This pattern could result from the higher removal rates of cheetahs in the middle part of the country, leading to increased immigration into this region. Immigration of cheetahs into Out from the north is perhaps more limited as Out borders Etosha National Park where cheetah populations are less dense due to endemic anthrax and predation by a comparatively high density of lions and hyenas (Berry et al. 1996). Immigration into Gro is probably restricted by limited habitat, as 65% of the Namibians inhabit this area and prey species have been almost completely extirpated.

Cheetahs from Oka and Otj in central Namibia had the highest average number of alleles per locus (Table 1), perhaps due to gene flow from both the more northern and more southern regions of the country. The F_{st} values, a measure of the overall level of genetic divergence among groups, were relatively low. Recently, animal movements and subsequent gene flow in Namibia may have increased as the extensive removal (killing and trapping) of cheetahs by farmers over the past 30 years or more (CITES 1992; Marker-Kraus et al. 1996; Marker et al. 2002b) may have precipitated increased dispersal (Johnson et al. 2001) into unoccupied ranges. Additionally, translocation of animals has been increasingly used for management and conservation purposes.

Inference on Behavioral Ecology

These genetic findings, many from cheetahs that were opportunistically tagged, present valuable behavioral information, which would not have been available through tag-and-release or radio-tracking studies. The relatedness estimates among individuals from different social groups and the identification of potential family groups (parent/offspring and siblings) provided important insights into aspects of cheetah social and behavioral ecology that have been poorly understood or for which plausible explanations have been poorly documented or controversial. These include the confirmation that most social groups are composed of related individuals, evidence of differences in dispersal patterns between males and females, and documentation of successful social integration of translocated animals into new regions.

From a behavioral ecology perspective, one of the most important findings is that most groups of cheetahs in Namibia, whether they were family groups, sibling groups, or male coalitions, consisted of related animals. In almost every case when a group consisted of unrelated animals, there were extenuating conditions that could explain the finding. For example, of the groups preclassified as sibling groups, 81% of these were composed of related animals. The 5 presumed sibling groups that had low relatedness scores were sampled at captive holding facilities with inadequate identification systems, and the initial classification of the group was probably the result of incorrect assignment of individuals into a group while in captivity. Similarly, of the 26 coalition male groups caught together on farms, only 3 groups consisted of apparently unrelated animals based on their low relatedness scores. Subsequent field observations of these groups and radio telemetry data supported the inferences resulting from the molecular genetic data. These were not intact male groups, but instead

Table 4. Population pairwise F_{st} (about diagonal) and R_{st} (below diagonal) estimates and standard errors (in parentheses). Significant values are noted with an asterisk (*)

	1gob	2gro	3oka	4oma	5otj	6out	7win
1gob		0.012 (0.089)	−0.002 (0.062)	0.017 (0.090)	0.016 (0.073)	0.020 (0.013)	−0.014 (0.099)
2gro	0.022 (0.054)		0.025 (0.071)	0.016 (0.090)	0.012 (0.063)	0.017 (0.127)	0.016 (0.132)
3oka	0.023 (0.047)	0.025 (0.049)		0.027 (0.082)	0.015 (0.040)	0.086*(0.142)	0.003 (0.094)
4oma	0.039 (0.059)	0.005 (0.041)	0.029 (0.051)		0.025 (0.081)	0.032 (0.113)	0.022 (0.102)
5otj	0.027 (0.048)	0.004 (0.036)	0.011 (0.030)	0.024 (0.045)		0.066 (0.130)	−0.003 (0.092)
6out	0.022 (0.054)	0.023 (0.073)	0.074*(0.096)	0.044 (0.098)	0.052 (0.088)		0.056 (0.160)
7win	−0.011 (0.049)	0.012 (0.079)	0.008 (0.064)	0.027 (0.055)	0.003 (0.058)	0.060 (0.102)	

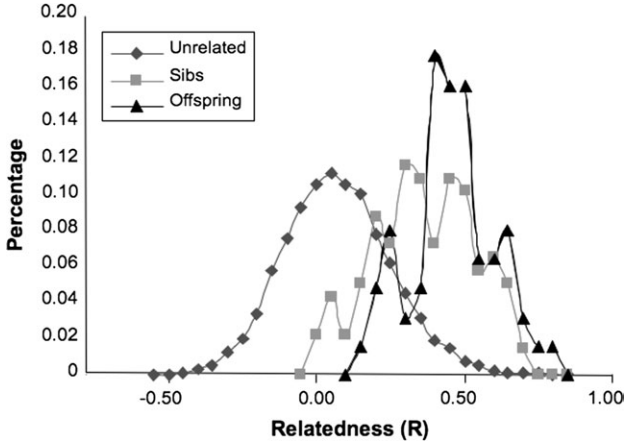

Figure 5. Relatedness (*R*) curves depicting relatedness (percentage of individuals in each category) for pairs of parent/offspring, siblings, and unrelated animals.

one of the captured males had been defending his territory when captured. It is a fairly common practice in Namibia for captured cheetahs to be held at the capture site as a lure to capture other cheetahs. These results suggest that, in contrast with male coalition groups in the Serengeti (Caro 1994), in Namibia, male coalition groups generally consist of related individuals. Finally, based on analyses of parentage and relatedness, there was strong support that most of the groups (21 of 24) classified as family groups a priori by observation or proximity of capture were composed of related mothers and their offspring.

Our analyses also provided insights on patterns of dispersal and long-distance movements of cheetahs, generally supporting previous tag-and-release and radio-tracking studies that showed that cheetahs (especially males) migrate into different regions of the country, have large home ranges, and that young male cheetahs during dispersal can move long distances from their natal home range into another region in a few days (Morsbach 1985, 1986; Marker-Kraus et al. 1996; Marker 2000; Marker LL, Dickman AJ, Mills MGL, Jeo RM, MacDonald DW, submitted). From our genetic analysis, we found a number of related animals that were previously not known to be related (Table 5). From a comparison of the distance between the capture locations of these individuals (the presumed distance the offspring had dispersed), we confirmed that male offspring dispersed significantly farther than female offspring and that female offspring dispersed farther from their sire than from their dam (Table 6). This is

Table 5. Inferred parent/offspring relationships among pairs of cheetahs based on CERVUS analyses. LOD scores were derived using the program CERVUS (version 2.0) (Marshall et al. 1998) using the "neither-parent known" option

LOD score	AJU #	Suggested relationship	Parental AJU #	Suggested relationship	LOD score	AJU #	Suggested relationship	Parental AJU #	Suggested relationship
5	846	Daughter	871	Dam	3.15	1085[a]	Son	868[a]	Sire
7.8	844	Daughter	871	Dam	2.19	1086[a]	Son	868[a]	Sire
4.3	1084	Daughter	1002	Dam	2.97	1088[a]	Son	868[a]	Sire
5	846	Daughter	871	Dam	4.2	1142	Son	1128	Sire
8.8	837	Son	1067	Dam	3.9	977	Son	842	Sire
3.6	1167	Son	1025	Dam	4	988[b]	Son	932[b]	Sire
5.7	1072	Son	820	Dam	4	1003[b]	Son	932[b]	Sire
10.2	863	Son	926	Dam	4.9	1139	Son	934	Sire
4.69	985[c]	Son	986[c]	Dam	7.5	985	Son	881	Sire
4.6	1095	Son	901	Dam	4	1018	Son	1123	Sire
4	1163	Son	1006	Dam	4.6	1059	Son	946	Sire
5	1075	Son	1168	Dam	6.1	1096	Son	947	Sire
10	863	Son	926	Dam	6.1	1097	Son	947	Sire
4	990	Son	892	Dam	4.2	1142	Son	1128	Sire
5.9	895	Daughter	882	Sire	4.6	1076	Son	826	Sire
11.8	1026	Daughter	881	Sire	5.5	902	Sibs	1170	Sibs
7.55	1029	Daughter	989/988	Sire	5.5	1040	Sibs	1103	Sibs
4.8	1157	Daughter	1071	Sire	6.8	1076	Sibs	1054	Sibs
6.5	1144	Daughter	868	Sire	7.1	919[d]	Sibs	866[d]	Sibs
5.2	1092	Daughter	832	Sire	6	861	Sibs	860	Sibs
4.6	902	Daughter	890	Sire	5.9	1139	Sibs	863	Sibs
6.5	1014[b]	Daughter	932[b]	Sire	6.5	1119	Sibs	988/989	Sibs
3.6	1057	Daughter	842	Sire	4.2	1123	Sibs	864	Sibs
6.5	1144	Daughter	858	Sire	6.7	1162	Same animal	878	Same animal

[a] Identification of sire when dam was known.

[b] Offspring of relocated cheetahs.

[c] Cheetahs caught together at play tree.

[d] Dispersal of male from natal home range.

Table 6. Mean distance between capture for identified related social groups

	No. animals	Mean distance between capture (km)	Standard deviation
Dam and daughter	4	13.00	0.00
Dam and son	8	116.38	69.39
Sire and daughter	10	93.50	85.74
Sire and son	16	99.06	89.09
Sibs	6	121.00	68.97
Overall	46	90.66	80.66

consistent with the finding that female cheetahs within our study area were more closely related than were males and that home range overlap was greater among related versus unrelated cheetahs.

We also gained insights from the unusual behavior of specific individuals. For example, although it has been well established that male cheetahs often visit and mark "play trees" with urine and feces as a part of their territorial display (Marker-Kraus and Kraus 1995; Marker-Kraus et al. 1996), little is known or understood about the behavior of females at these marking trees. One example from our study suggests that play trees, much like other prominent geographic features like water holes or prominent overlooks, might be important for a wide variety of social interactions, but in ways that might be complex. In this case, an adult pair of cheetahs (AJU985 and AJU986; Table 5) that was caught together at a play tree was presumed to be a mating pair. However, parentage analyses suggested that these animals were instead a dam and son, and unexpectedly, based on the general pattern that males disperse from their natal area, subsequent radio telemetry data showed that the presumed son occupied a home range in the area of the play tree and that his dam, which occupied a completely nonoverlapping home range 100 km away, most likely had made a long-distance foray to the tree.

Long-distance movements of greater than 100 km have been regularly documented in cheetahs using both radio telemetry and tag-and-release data (Morsbach 1986; Marker-Kraus et al. 1996). Our individual relatedness scores were insightful for understanding the dispersal of young male cheetahs and to demonstrate that long-distance movements are probably common. For example, one of our tagged males, AJU866, who was trapped and sampled in Otj, was found from genetic evidence to be a sibling to female AJU919, who was captured in Gro when still with their dam (Table 5) over 100 km south.

Finally, our results also provided evidence of both social stability in Namibian cheetahs (e.g., AJU868 sired a litter of cubs AJU 1085, AJU1086, and AJU1088 in 1997, 2 years after siring AJU1144 in the same region) and also that the decade-long program of relocating cheetahs from farms where they are viewed as pests to other parts of the country can be successful. For example, male AJU932 sired AJU988 in Oka prior to being translocated to Otj, a distance of over 250 km where he sired siblings AJU1003 and AJU1014.

Conservation Implications

This study and the relatively stable and panmictic Namibian cheetah populations provide a baseline model for future studies in other range countries, and a benchmark for the amount of area and type of landscape connectivity might be necessary to maintain a sustainable, unfragmented population. The absence of strong genetic divisions suggests that cheetahs from the different regions in Namibia do not need to be managed separately. However, the persistence of current patterns of genetic variation in Namibia is likely to depend on maintaining gene flow throughout the country, especially between the northern and central regions. Therefore, efforts to insure connectivity of remaining habitat throughout the country should continue.

In addition, because cheetahs in Namibia appear to fit the pattern expected from a large panmictic population, our data imply that animals can be translocated within Namibia without significantly altering historic patterns of gene flow, and we have shown that these translocations can lead to successful integration and reproduction. Genetic information provided here, accompanied with ecological and ecosystem approaches, will be useful in developing management strategies and setting priorities for cheetah conservation in Namibia. Therefore, as we use this model for other countries, not only is land use an important issue but also that of the social dynamics of the population.

Supplementary Material

Appendix Table I can be found at http://www.jhered.oxfordjournals.org/.

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Corresponding Editor: Scott Baker