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A molecular analysis of African lion (*Panthera leo*) mating structure and extra-group paternity in Etosha National Park

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Abstract

The recent incorporation of molecular methods into analyses of social and mating systems has provided evidence that mating patterns often differ from those predicted by group social organization. Based on field studies and paternity analyses at a limited number of sites, African lions are predicted to exhibit a strict within-pride mating system. Extra-group paternity has not been previously reported in African lions; however, observations of extra-group associations among lions inhabiting Etosha National Park in Namibia suggest deviation from the predicted within-pride mating pattern. We analysed variation in 14 microsatellite loci in a population of 164 African lions in Etosha National Park. Genetic analysis was coupled with demographic and observational data to examine pride structure, relatedness and extra-group paternity (EGP). EGP was found to occur in 57% of prides where paternity was analysed ($n = 7$), and the overall rate of EGP in this population was 41% ($n = 34$). Group sex ratio had a significant effect on the occurrence of EGP ($P < 0.05$), indicating that variation in pride-level social structure may explain intergroup variation in EGP. Prides with a lower male-to-female ratio were significantly more likely to experience EGP in this population. The results of this study challenge the current models of African lion mating systems and provide evidence that social structure may not reflect breeding structure in some social mammals.

Keywords: African lion, extra-group paternity, microsatellites, relatedness, social structure

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Introduction

Over the past several decades, it has become widely accepted that genetic mating systems often differ from social mating systems in many animal species (Griffith *et al.* 2002; Cohas & Allainé 2009). The recent application of molecular methods to analyses of mating systems have revealed that extra-pair or extra-group paternity is much more common than once thought (Griffith *et al.* 2002; Baker *et al.* 2004; Isvaran & Clutton-Brock 2007). Extra-group paternity (EGP) occurs when offspring are sired as the result of copulations with individuals outside of a species' social mating system (Griffith *et al.* 2002; Isvaran & Clutton-Brock 2007). Through a combination of genetic and observational methods, EGP has been reported for many socially monogamous bird species (Griffith *et al.* 2002) and also a number of social mammal species (da Silva *et al.* 1994; Vigilant *et al.* 2001; Ortega *et al.* 2003). Results of recent studies have also shown that EGP occurs in some mammal species previously thought to be social and genetic monogamists, including the white-handed gibbon (*Hylobates lar*; Reichard 2009), the red fox (*Vulpes vulpes*; Baker *et al.* 2004), the alpine marmot (*Marmota marmota*; Goossens *et al.* 1998), and the African wild dog (*Lycaon pictus;* Girman *et al.* 1997). Understanding the extent and context of this variation across mammalian taxa

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will enhance our understanding of the evolution of sociosexual behaviour more generally.

The occurrence of EGP in any population can play an important role in the breeding and social structure and in the dynamics of sexual selection, so knowledge of EGP is critical in gaining a full understanding of the socioecology of a study population (Griffith *et al.* 2002; Isvaran & Clutton-Brock 2007). A number of possible explanations for the occurrence and variability of EGP among and within taxa have been explored (Jennions & Petrie 2000; Griffith *et al.* 2002; Isvaran & Clutton-Brock 2007; Cohas & Allainé 2009). In a recent study of EGP in mammals, Isvaran & Clutton-Brock (2007) found that 46% of species sampled had an excess of 20% EGP that appeared to be correlated with mating season length. Higher levels of EGP were seen in populations where oestrus occurred simultaneously in multiple females making it more difficult for males to defend against extra-group males (Isvaran & Clutton-Brock 2007). Similar correlations have been made for group sex ratio, suggesting that EGP increases as the female-to-male ratio increases, again making it difficult for the males to monopolize the females in their group (Ortega *et al.* 2003; Cohas & Allainé 2009). Cohas & Allainé (2009) have recently argued that social structure influences the occurrence of extra-pair paternity in socially monogamous mammals, providing evidence that individual group social structure may be more important than pair bonding in some species.

The majority of behavioural and social structure studies of natural populations of African lions have focused on those inhabiting the Serengeti Plains ecosystem of eastern Africa (Schaller 1972; Packer & Pusey 1982; Pusey & Packer 1987; Gilbert *et al.* 1991; Packer *et al.* 1991; Scheel 1993). Schaller (1972) described the social structure of Serengeti lions as prides comprised of two or more codominant males with a variable number of kin-linked females and their young. Schaller (1972) and Packer & Pusey (1993) observed that female lions in the Serengeti plains mated exclusively with resident pride males. Gilbert *et al.* (1991) tested these behavioural observations by analysing DNA fingerprints of Serengeti lions and concluded that resident pride males sired all cubs sampled. However, recent studies of African lion populations from other regions have reported variation in behaviour, social structure, phylogenetics, morphology and relatedness, as compared with those of the Serengeti (Funston *et al.* 1998; Kays & Patterson 2002; Spong *et al.* 2002; Dubach *et al.* 2005; Antunes *et al.* 2008).

One aspect of social structure that has been found to differ among regional African lion populations relates to the number of resident pride males. In the Serengeti, all prides are reported to have at least two resident males (Packer & Pusey 1993), while in the Tsavo National Park ecosystem in Kenya, all lion prides only contain one male (Kays & Patterson 2002). In the Etosha population, the number of resident pride males varies from one to three, with several prides sharing males between them. Variation in the numbers of adult males and females affect group sex ratio and can influence mating systems and has been implicated in EGP in some species (Jennions & Petrie 2000; Ortega *et al.* 2003). Lion prides inhabiting the Selous Game Reserve in Eastern Africa have similar variation in numbers of resident males and sex ratio, and while paternity was not directly tested, EGP has been suggested in the Selous population based on relatedness estimates (Spong *et al.* 2002).

Previous paternity studies of individual African lion cubs have been limited to the lions of the Serengeti (Gilbert *et al.* 1991). This study examines both paternity and relatedness in the African lions of Etosha National Park, Namibia, combining genetic, demographic and extensive opportunistic field observational data. Field observations indicated that the Etosha lion social system may be more flexible than other lion groups, with females periodically seen consorting with nonpride males (Lyke 2008). Thus, our primary goal was to examine the mating and social structures of the lions of Etosha, with a particular focus on EGP. We tested three hypotheses: (i) pride males do not sire all cubs within their resident pride during tenure; (ii) females mate with more than one male during oestrus; and (iii) EGP is more likely to occur in groups with high female-to-male sex ratio.

Methods

Study population

The study population included lions inhabiting Etosha National Park (ENP) in Namibia (Fig. 1). The park covers approximately 22 275 km^2 in south-western Africa, 19º 0′ 0″ S latitude and 16º 0′ 0″ E longitude and is considered to be a semi-arid environment, consisting of grassland, forest and desert ecosystems (Stander 1991). A total of eleven lion prides were identified (Fig. 2) and observed during the study period between 1985 and 1998. Three prides were located in the far west, which is savanna woodland, and eight prides located in the eastern plains surrounding the Etosha Pan (Fig. 2). The central region of the park consists primarily of arid desert that did not support established prides during the study period. Pride territories were delineated based on field observations of ranging behaviours.

Observations

Ongoing observations of lions were made three to 5 days per week during daytime and night-time drives

into the bush by one of three personnel: the Chief Control Warden of Etosha National Park, the Research Associate, or the Regional Head Ranger. Each lion was identified visually using several established techniques to ensure accuracy. Most adult pride members were marked with fire brands as part of their ongoing marking exercises or following research immobilizations as part of the Ministry's animal identification policy to identify resident animals within the National Park vs. nomads/transients. Animals that did not have the large numeric or 'pattern' brand were described by scars, whisker patterns and other unique characteristics along with a photograph to assure they were also identified correctly. Only the trained staff, listed above, was involved in the field observations to increase accuracy in the data collection and assure animals were properly identified. Recorded data included individual ID, date, location, pride affiliation, cubs when present and other anecdotal observations.

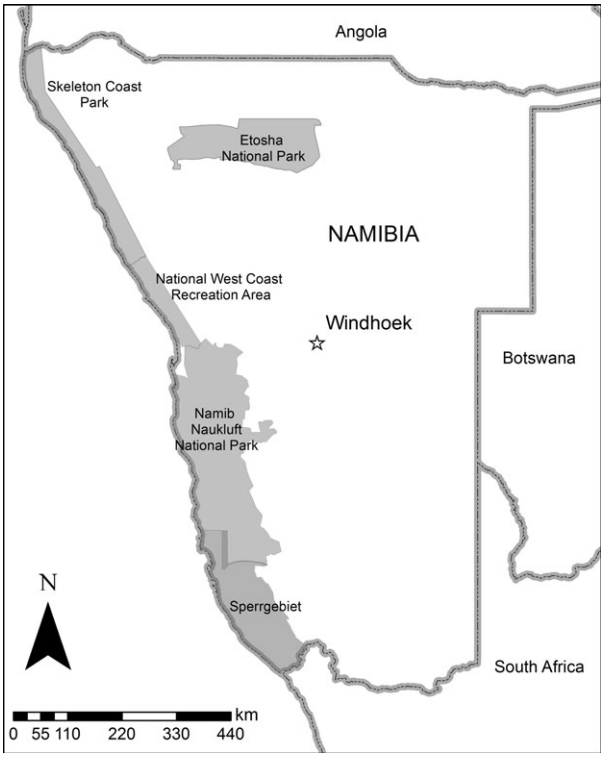

Fig. 1 Etosha National Park, Namibia.

Pride constituency was assigned based on affiliation; males and females that consorted with each other in the same territory were considered to form a pride, along with their offspring. Female lions were not observed to consort with females outside their resident pride. While males did not consort with males outside their prides, males were occasionally observed in close proximity to neighbouring pride males with very few aggressive encounters. Nomadic males were those that did not belong to a pride or associate regularly with any lioness and were generally subadults or older males. Changes in resident male status were generally the result of a conspicuous takeover event by one or more extra-group males.

Sample collection, DNA extraction and amplification

Blood and/or tissue samples were collected from each lion on an opportunistic basis and DNA was extracted as described by Dubach *et al.* (2005). Fourteen

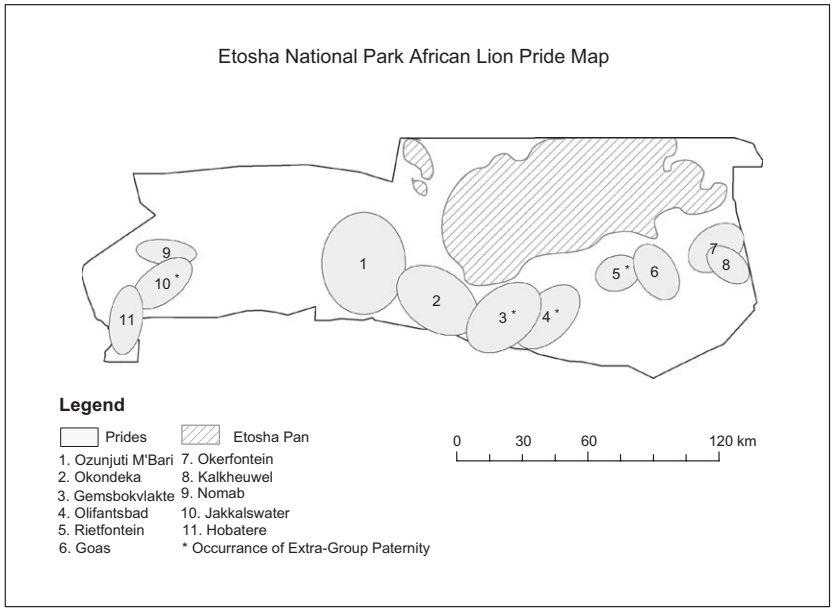

Fig. 2 Etosha National Park lion prides.

microsatellite loci (FCA14, FCA23, FCA26, FCA30, FCA43, FCA45, FCA77, FCA94, FCA96, FCA126, FCA132, FCA187, FCA191 and FCA205) were amplified in each lion (Menotti-Raymond *et al.* 1999). PCR amplification was carried out using 40–90 ng genomic DNA in a 12.5 μL total reaction volume, 0.5U TAQ polymerase (Promega, Madison, WI), 0.2 mM dNTPs, 1 × reaction buffer, 4 pmol of each primer and 1.5 mM MgCl2 (1.1 mM MgCl2 was used for FCA30, FCA191, and FCA205). The following PCR conditions were used for all loci: 94 °C for 3 min, 35 × (94 °C for 30 s, 48.0–65.0 °C for 45 s, 72 °C for 45 s), followed by 10 min at 72 °C in a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA). Annealing temperature was 48 °C, FCA132; 53 °C, FCA26, FCA45; 55 °C, FCA205; 56 °C, FCA23; 58 °C, FCA14, FCA30, FCA43; 65 °C, FCA187; and 50 °C for the remaining five loci. The optimal annealing temperatures and magnesium quantities were determined for each primer by analysis along a gradient. When samples failed to amplify clearly, 1.0 μL of 10 mg/mL bovine serum albumin (BSA) was added to the reaction to improve amplification (Kreader 1996). All forward primers were labelled with Well-Red dyes and the PCR products were sized using the Beckman/Coulter CEQ™ 8000XL DNA Capillary Electrophoresis Genotyping System (Beckman Coulter, Fullerton, CA) and system software. Genotype accuracy was verified by repeating the DNA extraction (from the original sample) and genotyping of 10 lions, from random reamplification of approximately 10% of the population for each locus and sizing the same PCR products before and after each capillary change. In addition, fragment size binning was monitored by hand in an excel spreadsheet for each locus and genotype.

Genetic and statistical analyses

The allele frequency function in the software program Cervus 3.0 (Kalinowski *et al.* 2007) was used to estimate heterozygosity, null allele frequency and deviations from Hardy–Weinberg equilibrium. The inbreeding coefficient (F_{is}) was assessed using FSTAT 2.9 (Goudet 1995). Pairwise relatedness (R) was estimated for all individuals using the relatedness function in the program KINSHIP 1.3 (Queller & Goodnight 1989). All relatedness estimates were based on the allele frequencies within the entire population ($n = 164$), including adults and juveniles, and data were jackknifed across all loci to calculate standard error.

Group sex ratio was calculated for each pride where paternity was analysed to investigate the ability of resident pride males to control mating access to resident females. Sex ratio was calculated based on the pride constituency at the estimated time of conception, approximately 4 months prior to birth. For two prides sharing three males, the male variable was estimated as the average. Association between extra-group paternity and group sex ratio was analysed by logistic regression using JMP 9 (SAS Institute, Inc. 2009). The response variable for the analysis was EGP or no EGP within a pride. We considered $P < 0.05$ significant for all statistical tests.

Paternity analysis

Paternity was examined for all cubs ($n = 43$) born into prides ($n = 7$) with observed dams and established resident males. Prides with individuals lacking genetic data were excluded. For one pride, paternity was analysed during two different time periods with different resident pride males. These two groups were treated as separate prides for all analyses. Two methods of paternity analysis were employed: (i) manual sire-offspring genotype analysis using the principle of exclusion; and (ii) maximum-likelihood analysis using Cervus, version 3.0 (Kalinowski *et al.* 2007). First, all resident pride males in tenure at time of birth and up to 1 year prior were tested for paternity of each resident cub, with genotypes verified manually three times. The sire-offspring pairs were allowed to mismatch at one locus to allow for genotyping errors. Second, Cervus was used to evaluate all adult male lions in the population that were potentially sexually mature (>2 years of age, $n = 44$) at the times the cubs were born. Cervus estimates a ratio of the likelihood of one parent over another, and then calculates the natural logarithm of that ratio, or a Likelihood-of-Difference (LOD) score (Kalinowski *et al.* 2007). The Cervus default parameters of 10 000 offspring and 1% error rate were used for paternity simulations (Kalinowski *et al.* 2007), with 99% loci typed based on the allele frequency analysis. Paternity was assigned at both strict (95%) and relaxed (80%) confidence levels. When a resident pride male met our paternity qualifications using the exclusion principle, that male was assigned paternity over nonpride males regardless of the LOD score assigned by Cervus. This could lead to an underestimation of EGP in this population, as sire-offspring pairs that mismatched at one locus were allowed under the exclusion principle. The Cervus software program assigns parentage based on likelihood calculations that assume Hardy–Weinberg equilibrium and therefore recommends that loci found to deviate be excluded from parentage analysis (Kalinowski *et al.* 2007). Paternity analyses were run with and without these loci and the assignments did not change, and therefore, these loci were not excluded.

Table 1 Genetic analysis of microsatellite genotypes for the Etosha lion population

Locus	No. of alleles	H_e	H_o	F_{is}	Hardy–Weinberg (P-value)	Null allele frequency
Fca014	6	0.57	0.40	0.29†*	<0.001**	+0.170
Fca023	2	0.02	0.03	−0.01	N/A	−0.001
Fca026	5	0.63	0.66	−0.04	0.553	−0.026
Fca030	6	0.75	0.71	0.05	0.142	0.026
Fca043	2	0.33	0.30	0.08	0.380	0.042
Fca045	3	0.46	0.48	−0.04	0.885	−0.017
Fca077	4	0.46	0.46	0.00	0.351	−0.024
Fca094	3	0.63	0.71	−0.12	0.003*	−0.065
Fca096	5	0.74	0.73	0.02	0.686	0.011
Fca126	5	0.56	0.49	0.13	<0.001**	0.051
Fca132	3	0.49	0.45	0.08	0.609	0.035
Fca187	6	0.80	0.76	0.05	0.562	0.025
Fca191	5	0.57	0.56	0.01	0.765	0.007
Fca205	7	0.69	0.78	−0.13	0.019*	−0.070
Mean ± SD	4.4 ± 1.6	0.55 ± 0.20	0.54 ± 0.21	0.03 ± 0.11	N/A	N/A

H_e, expected heterozygosity; H_o, observed heterozygosity; F_{is} is the inbreeding coefficient.
*Significant at the 5% level.
**Significant at the 1% level.
†Indicates significant heterozygote deficit reported.

Results

Genetic and statistical analyses

Genetic data were obtained for a total of 164 lions that included 90 members of 11 prides. The mean number of loci scored was 13.9 (range 12–14), and the average number of alleles was 4.4 (range 2–7; Table 1). To ensure that paternity in this population was not influenced by factors such as inbreeding with subsequent loss of variability, we compared observed heterozygosity levels (H_0) and average number of alleles (A) with findings from Antunes *et al.* (2008). The average observed heterozygosity and number of alleles for the Etosha population in our study ($H_0 = 0.58$; $A = 4.6$) were similar to those reported for Namibian lions by Antunes *et al.* (2008) ($H_0 = 0.577$; $A = 4.4$) and slightly lower than other populations throughout Africa included in that study. This level of variability is considered to be typical of large populations of nonendangered mammals (Frankham *et al.* 2002). In addition, the inbreeding coefficient ($F_{is} = 0.03$) for this population did not suggest that inbreeding was occurring. One locus did show a significant heterozygote deficit (FCA014; $F_{is} = 0.292$; $P < 0.001$; Table 1), and four loci showed evidence of deviation from Hardy–Weinberg (H-W) equilibrium, with three significant after Bonferroni correction (Table 1). While genotyping errors are a common cause of H–W deviations, results can also be influenced by inbreeding, natural selection, population substructure and a high number of related individuals (Marshall 1998). When more than two loci are found to deviate, it is generally indicative of population substructure (Marshall 1998). Results of the logistic regression model indicated a significant association (df = 1, $\chi^2 = 9.56$, $P = 0.002$, $n = 7$) between the occurrence of EGP and the group sex ratio. Groups with a higher adult female-to-adult male ratio (Table 2) were significantly more likely to exhibit EGP.

Table 2 Group composition for lion prides where paternity was analysed

Pride	Adult females	Adult males	Female:Male sex ratio	Cubs	Total animals
Jakkalswater	3	1	3:1	8	12
M'Bari	4	2	2:1	2	8
Okondeka	3	2	3:2	3	8
Gemsbok	3	3*	3:1.5	5	11
Olifantsbad	3	3*	3:1.5	6	12
Rietfontein (pre-1993)	3	1	3:1	4	10
Rietfontein 1993+	3	2	3:2	6	12
Mean	3.1	1.6	-	4.9	10

*Share three males.

Pride structure

The pride compositions recorded from 1991 to 1996 were analysed for this study. Over this time period, eleven prides consisting of a total of 102 lions were

Table 3 Etosha lion paternity assignments

Pride	Resident male(s)	Resident tenure	Cub ID	YOB	Observed dam	Cervus sire	Confidence level%	Assigned sire	Pride male assigned
Jakkalswater	224	1991–1995	213	1992	375	277	80	277	N
			247	1992	370	224	80	224	Y
			248	1992	352	277	95	277	N
			218	1993	370	661	80	661	N
			377	1993	370	87	80	87	N
			220	1993	352	277	-	277	N
			314	1993	352	277	80	277	N
			363	1993	375	271	-	271	N
Gemsbok	96, 97, 261	1990–1995	103	1993	85	74	80	261	Y
			105	1993	85	74	80	74	N
			104	1993	84	631	80	631	N
			107	1993	84	631	-	631	N
			106	1993	95	607	80	607	N
Olifantsbad	96, 97, 261	1992–1995	102	1992	89	96	80	96	Y
			108	1993	75	631	95	631	N
			109	1993	75	86	80	261	Y
			110	1993	89	261	-	261	Y
			111	1993	83	261	-	261	Y
			112	1993	83	261	-	261	Y
M'Bari	62, 65	1989–1995	333	1992	20	65	-	65	Y
			337	1992	20	657	80	65	Y
Okondeka	202, 221	1989–1995	98	1991	73	97	80	202	Y
			99	1991	73	97	80	202	Y
			100	1991	40	97	80	202	Y
Rietfontein 1	633	Pre-1993	709	1992	628	674	95	674	N
			710	1992	628	695	80	695	N
			711	1992	630	633	80	633	Y
			712	1992	687	657	95	633	Y
Rietfontein 2	631, 695	1993–1996	705	1994	628	631	95	631	Y
			707	1994	628	631	95	631	Y
			708	1994	628	631	80	631	Y
			706	1994	630	631	95	631	Y
			714	1994	630	631	95	631	Y
			717	1994	687	695	-	695	Y
Total	-	-	34	-	-	-	-	-	20 Y

Table 4 Etosha lion population average pairwise relatedness (*R*) for known relationships compared with typical values

	Mother-offspring (n = 23)	Sire-offspring (n = 27)	Full-siblings (n = 12)	Second-order (n = 14)
Average *R*-value	0.46	0.44	0.40	0.15
Typical *R*-value*	0.50	0.50	0.50	0.25

n, number of pairs sampled.
*Queller & Goodnight 1989; Glaubitz *et al.* 2003.

observed (Fig. 2). Samples for genetic analysis were obtained for 88% of the lions included in the eleven prides, for a total of 90 lions. For the seven prides where paternity was analysed, the average pride size was 10.0 (range 8–12; Table 2) animals, the average number of adult males was 1.6 (range 1–3), the average number of adult females was 3.1 (range 3–4), and the average number of cubs was 4.9 (range 2–8). The sex ratios for each pride are shown in Table 2.

Paternity analysis

Resident pride male tenure was known during the birth year of 34 of 43 cubs present in the Etosha population

(Table 3). Our genetic data confirmed that a pride male was the sire of 20 of the 34 (59%) assignments. The remaining 14 cubs were sired by a male that was not a resident of the natal pride and were considered to be extra-group paternities (41%). For these fourteen extra-group assignments, all pride males mismatched the candidate cubs at two or more loci. Paternity was analysed in 22 litters in the study population, and EGP occurred in ten (Table 3). Four (18%) of the 22 were mixed paternity litters, where multiple males sired cubs in the same litter, and each mixed litter had at least one extra-group sire (Table 3).

There were 26 Cervus paternity assignments made, including 8 (31%) at the strict (95%) confidence level and 18 (69%) at the relaxed confidence level (80%). Critical and achieved LOD scores for each potential resident pride sire and all assigned sires are given in Table S1 (Supporting information) For seven cubs (ETO103, ETO109, ETO337, ETO98, ETO99, ETO100, ETO712; Table 3), Cervus assigned paternity to extra-group males after a pride male was assigned using the principle of exclusion. Three of the seven cubs (ETO103, ETO337, ETO657) matched both potential sires at all loci. One cub (ETO109) matched the pride male (ETO261) at all loci but mismatched at one locus with the Cervus assigned sire (ETO74). The remaining three cubs (ETO98, ETO99, ETO110) reside in the same pride and mismatched the pride male (ETO202) at one locus but matched the Cervus assigned sire (ETO97) at all loci. All seven cubs were assigned pride male sires over nonpride sires based on the exclusion analysis, which might underestimate the rate of EGP in this population.

Relatedness

Pairwise relatedness (R) was estimated using KINSHIP 1.3 (Queller & Goodnight 1989). The average R-value was first calculated for known mother-offspring (23 pairs, $R = 0.46$), sire-offspring (27 pairs, $R = 0.44$), and full-sibling pairings (12 pairs, $R = 0.40$) as a standard for the assessment of relatedness in this population (Table 4). The average R-values for each were close to the typical values for first-order relatives ($R = 0.50$; Table 4). The average R-value for second-order relatives (e.g. half-siblings; $R = 0.15$; Table 4) was also calculated and was lower than expected ($R = 0.25$; Table 4) and similar to the typical value for third-order relatives (e.g. first cousins; $R = 0.125$; Glaubitz *et al.* 2003; Queller & Goodnight 1989).

Relatedness was then assessed for the population as a whole, for all adult females within each pride, for all adult males within each pride and between adult males and females in each pride (Table 5). The average R-value for females within prides ($R = 0.27$, Table 5) was similar to the typical value of second-order relatives. The average R-value for males within prides ($R = 0.12$, Table 5) was slightly less than that of second-order relatives in this population. The average R-value for males to females was close to zero ($R = 0.03$, Table 5), and the average within pride relatedness for the entire population ($R = 0.21$, Table 5) was slightly less than that typical of second-order relatives.

Table 5 Pairwise relatedness estimates (R) within Etosha lion prides

Pride	Females within pride	Males within pride	Males to females	Entire pride
Nomab	0.49	*	−0.06	0.12
Jakkalswater	0.32	*	−0.08	0.23
Hobatere	0.52	*	*	0.33
M'Bari	0.31	−0.19	−0.02	0.13
Okondeka	0.49	0.22	0.10	0.19
Gemsbok	0.03	0.06	0.03	0.06
Olifantsbad	−0.14	0.06	−0.24	0.03
Rietfontein	0.46	0.21	0.22	0.27
Goas	*	0.31	0.13	0.31
Okerfontein	−0.05	*	0.29	0.42
Kalkheuwel	*	*	−0.10	0.25
Mean ± SD	0.27 ± 0.26	0.12 ± 0.20	0.03 ± 0.16	0.21 ± 0.12

*One or fewer individuals with genetic samples.

Discussion

Our results, presented here, indicate a number of similarities among the Etosha lions and lions inhabiting other regions, with one important difference: extra-group paternity has not been reported in African lion populations. Previous paternity studies of the Serengeti population have shown that EGP does not occur in that region (Gilbert *et al.* 1991; Packer *et al.* 1991). Yet it occurred at a frequency of 41% in the Etosha population and in five of seven prides where paternity was analysed (Table 3). The occurrence of EGP plays an important role in mating and social structure and other life history strategies; therefore, knowledge of EGP is critical for a robust understanding of the overall socioecology of a given population (Griffith *et al.* 2002).

In the Etosha lion population, of the five prides where EGP occurred, two had only one resident male, and two shared three males between them. This suggests that the males may have been unable to monopolize the sexual encounters of all females within their prides. All prides where EGP was not found had more than one resident pride male. While paternity studies of the Serengeti have not demonstrated EGP, all prides reported in the Serengeti have at least two adult males.

The Selous population, where EGP has been suggested based on relatedness estimates, also has prides with only one resident male and prides that share males (Spong *et al.* 2002). These findings provide evidence that pride structure is an important determinant of EGP in some populations.

Additional support for the extra-group paternity assignments in this population come from observational data. Multiple adult male lions in this population were occasionally observed in close proximity without aggressive interactions, and some were frequently encountered outside their home territories (Lyke 2008). Specific to our paternity assignments, the Rietfontein two males (ETO631, ETO695) were observed with some regularity in both the Gemsbok and Olifantsbad pride territories (Fig. 2). One of these adult males (ETO631) sired cubs in both prides, which shared three males between them (Table 2). While no extra-group copulations were witnessed, both males were observed in the presence of the Gemsbok and Olifantsbad females on several occasions, indicating that there may have been mating opportunities. Two males (ETO277 and ETO631) were each assigned as extra-group sires of multiple cubs (Table 3). Observational data indicate that both males were most likely in a nomadic phase during the corresponding breeding events. There were no obvious phenotypic differences noted between resident males that did or did not sire cubs.

Four of the litters where EGP occurred had mixed paternity (Table 3). Mixed paternity litters result from adult female lions mating with multiple males during a typical oestrous period (Packer & Pusey 1983; Gilbert *et al.* 1991). In a paternity study involving the Serengeti lions, where all cubs were sired by resident males, Gilbert *et al.* (1991) reported 1 of the 24 litters examined had mixed paternity. It was also reported that individual resident males in the Serengeti population guarded females for much of the oestrous cycle, providing less opportunity for multiple mating partners (Packer & Pusey 1983). The higher rate of mixed paternity litters in the Etosha population could be correlated with the rate of EGP, as females mating with nonresident males may exhibit less conspicuous mating strategies than those being guarded by resident males. It is also possible that some of the litters reported as mixed paternity here were sired by a nomadic or transient male for which no genetic sample was available, resulting in multiple male paternity assignments in one litter that may actually have a single sire.

We found group sex ratio to be an important variable related to EGP occurrence in this population, indicating that further investigations of African lion and other taxon-specific mating systems should take group demographics into account. It has been argued that the availability of extra-pair males and the ability of males to control access to females are important determinants of EGP in some birds and mammals exhibiting social but not genetic monogamy (Jennions & Petrie 2000; Griffith *et al.* 2002; Cohas *et al.* 2007). Cohas & Allainé (2009) recently investigated a number of possible predictors of EGP in 22 socially monogamous mammal species and reported that social structure was a more useful predictor of EGP than pair-bond strength.

While differences in social structure may offer variable opportunities for females to engage in EG copulations, there are still many questions about the underlying mechanisms driving females to mate with multiple partners (Cohas & Allainé 2009). Several explanations have been proposed, including perceptions of genetic superiority or compatibility of extra-group males over resident pride males (Hughes *et al.* 2003; Cohas *et al.* 2007). Females might also mate outside their groups to increase genetic diversity or to avoid inbreeding (Hughes *et al.* 2003; Cohas *et al.* 2007). However, the mechanisms by which females may be able to assess genetic differences among males are not clear (Cohas *et al.* 2007; Cohas & Allainé 2009). Another possible explanation for some species, including African lions, is that females mate with extra-group males to confuse paternity in an effort to deter infanticide, which has been reported to occur when male lions takeover a new pride (Schaller 1972). Further research relating to ultimate causes of extra-group mating should incorporate investigations of genetic diversity within and among individuals and groups.

The overall within-pride relatedness of this population was close to expected values for second-order relatives, indicating that prides generally do consist of kin-linked individuals. However, the occurrence of EGP may affect the overall within-pride relatedness because 41% of cubs are not related to any resident pride male. In 2004, Baker *et al.* analysed paternity and relatedness in the red fox (*Vulpes vulpes*). Red fox are considered social monogamists; living in kin-linked social groups with only one dominant, breeding pair and within-group relatedness close to that of first-order relatives (Baker *et al.* 2004). The authors reported a 33% extra-pair paternity rate and within-group relatedness estimates approximating those of second-order relatives, and they attributed the lower relatedness estimates to extra-pair paternity (Baker *et al.* 2004).

Recent studies of African lion populations suggest interregional variability in behaviour, social structure and even morphology (Stander 1992; Funston *et al.* 1998; Kays & Patterson 2002; Patterson 2007; Antunes *et al.* 2008). For example, results of observational studies of lions in Tsavo National Park, Kenya, show both morphological and behavioural variation, where all

prides contain only one resident male and most adult males do not have manes (Kays & Patterson 2002; Patterson 2007). In Kruger National Park, male lion dispersal and hunting patterns are different from those reported in the Serengeti (Funston *et al.* 1998, 2003), and in ENP, adult females hunt more cooperatively than in other populations (Stander 1992). These authors have suggested that observed differences in morphology and behaviour may be correlated adaptations to regional differences in ecology, prey and vegetation structure (Stander 1992; Funston *et al.* 1998, 2003; Kays & Patterson 2002). These studies suggest that lions in different parts of their distribution show considerable variation in a number of life history strategies and that conservation management of these populations may benefit from more detailed analyses.

The incorporation of molecular methods into studies of behaviour and social and breeding structure may help to identify patterns not discernible by observation alone. A comparison of genetic paternity analyses with observational paternity predictions for the Etosha lion population indicated that field predictions were incorrect 50% of the time (Lyke 2008). Results of this and other research suggest that observational analyses may not be reliable indicators of animal mating systems (Ortega *et al.* 2003; Baker *et al.* 2004; Lyke 2008). The advance of various types of molecular analyses and noninvasive sampling techniques has made genetic studies much more common, allowing a more robust analysis of population structure. These methods have enabled researchers to uncover hidden genetic mating systems that differ from those predicted by social structure for a number of taxa (Girman *et al.* 1997; Goossens *et al.* 1998; Jennions & Petrie 2000; Baker *et al.* 2004; Reichard 2009). The results of this study give new insight into the reproductive behaviour of at least some African lions, which provides a more accurate understanding of the overall socioecology of the species. Our results also further support the idea that regional variation does exist between populations, which will hopefully lead to additional studies of remaining African lion populations and their respective mating and social systems.

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This research represents part of M.M.L. MS thesis on African lion population structure. M.M.L. is currently a PhD student at the University of Texas at San Antonio, where her focus is primate molecular ecology. The research presented here was completed in the laboratory of J.D. who has conducted genetic research on lions throughout Africa for many years. M.B.B. collected field data, including biological samples and field observations.

Data accessibility

Microsatellite and field observational data: DRYAD entry: doi:10.5061/dryad.47hc8.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 CERVUS generated Likelihood-of-Difference (LOD) scores for each cub-resident male dyad in prides where paternity was analyzed.