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Genetic relatedness and disrupted social structure in a poached population of African elephants

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Abstract

We use genetic measures of relatedness and observations of female bonding to examine the demographic signature of historically heavy poaching of a population of free-ranging African elephants. We collected dung samples to obtain DNA and observed behaviour from 102 elephant families over a 25-month period in 2003–2005 in Mikumi National Park, Tanzania. Poaching reduced the population by 75% in the decade prior to the 1989 ivory trade ban; park records indicate that poaching dropped significantly in Mikumi following the ban. Using 10 microsatellite loci, DNA was genotyped in 203 elephants and pair-wise relatedness was calculated among adult females within and between groups. The Mikumi population is characterized by small group size, considerable variation in group relatedness, females with no first-order adult relatives and females that form only weak social bonds. We used gene-drop analysis and a model of a genetically intact pedigree to compare our observed Mikumi group relatedness to a simulated genetically intact unpoached expectation. The majority of groups in Mikumi contain 2 to 3 adults; of these, 45% were classified as genetically disrupted. Bonding, quantified with a pair-wise association index, was significantly correlated with relatedness; however only half of the females formed strong bonds with other females, and relatedness was substantially lower for a given bond strength as compared to an unpoached population. Female African elephants without kin demonstrated considerable behavioural plasticity in this disturbed environment, grouping with other females lacking kin, with established groups, or remaining alone, unable to form any stable adult female-bonds. We interpret these findings as the remaining effect of poaching disturbance in Mikumi, despite a drop in the level of poaching since the commercial trade in ivory was banned 15 years ago.

Keywords: gene-drop analysis, *Loxodonta africana*, microsatellite loci, poaching, relatedness, social structure

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Introduction

Studies of heavily poached African elephant (*Loxodonta africana*) populations reveal considerable variation in group structure that differs from that of historically protected populations (Poole 1989; Barnes & Kapela 1991; Abe 1994; Ereckson 2001; Nyakaana 2001; Foley 2002). In the heavily poached Queen Elizabeth Park, Uganda, genetic analysis on mitochondrial DNA (mtDNA) extracted from tissue samples showed that some female social groups contain multiple haplotypes and therefore, females from different matrilines (three groups out of nine contained more than one mtDNA haplotype) (Nyakaana *et al*. 2001). By contrast, the highly protected population in Amboseli, Kenya, consists primarily of maternally related female groups (37 groups out of 39 had complete mtDNA haplotype uniformity) (Archie *et al*. 2006b). Secondary bonding also exists in this population: related matriarchs from two different core groups frequently and preferentially associate to form a large 'bond group' (Archie *et al*. 2006b).

Poaching peaked across Africa in the 1970–1980s, then lessened in many areas with the listing of African elephants as an Appendix I species by the Convention for the

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International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1989. A demographic signature of this extreme disturbance, which caused the swift removal of the majority of male and female adult elephants in some populations, is likely to still be evident today. In this long-lived, slow-reproducing, highly social species, several decades may be required to repopulate the adult cohort. Female African elephants typically mature around the age of 12 years old, have a generation time of 17.4 years, produce four offspring that survive to adulthood (at a 1:1 sex ratio) on average, and may survive past the age of 60 years old (Moss 2001). They are known for their highly developed social system in which stable, cooperative female-bonded groups are a hallmark (Dublin 1983; Moss & Poole 1984). Led by an old matriarch (≥ 30 years), related adult females cooperatively forage and care for their offspring (Dublin 1983; Moss & Poole 1984). Their society is characterized by a fission–fusion association pattern where groups will join together into larger aggregations during the wet season when food and water are abundant, but divide into the smallest social unit possible, the family or core group, during the dry season or drought (Western & Lindsay 1984). In this way, female life-history characteristics and behaviour stabilize core groups. This model of elephant social organization is primarily based on a few long-term studies of relatively unpoached populations in which groups were characterized via observed births and genetic analysis (Douglas-Hamilton 1972; Moss 2001). A different reality may exist in heavily poached elephant populations where close adult kin have been exterminated and the opportunities for cooperation and its attendant benefits altered.

Preferential bonding and grouping with kin is standard in matrilineal social species (Alexander 1974). Individuals cooperate with close relatives over unrelated individuals, thereby increasing their inclusive fitness (Hamilton 1964). However, kin selection requires that individuals who interact are closely related; benefits of this social behaviour are more likely to accrue when bonds are maintained over time. Loss of a large number of related individuals from a population through severe over-exploitation, a disease epidemic or prolonged drought may have marked effects on its social structure beyond the time period of the original disturbance, particularly for long-lived species with long generation times. Under these circumstances, lack of kin, typical social structure and strong bonds may produce novel, alternative social tactics. The demographic disruption associated with poaching for ivory of the African elephant provides such a circumstance.

We used contemporary genetic data from a heavily poached elephant population in Mikumi National Park, Tanzania, to investigate whether a demographic signature of poaching is evident in terms of group size, relatedness and behavioural associations between adult females. The elephant population of Mikumi was reduced by nearly 75% in the 1980s due to poaching. However, increased anti-poaching efforts since the 1989 ivory ban have since resulted in only a few adult female mortalities per year (Balozi 1989; Idhe 1991; TWCM 1998; J. Shemkunde, personal communication). Current patterns were compared with those of this population in 1989 reported by J. Poole. Simulated data and published data on unpoached populations were also used to examine whether some Mikumi groups still display altered structures despite a 15-year drop in the level of poaching. Ecological variables that may influence grouping behaviour logically cannot be held constant when comparing extant elephant populations. However, differences in size, relatedness and behaviour of the primary elephant social unit of a historically protected population to one that was heavily poached is the best available comparison to make, outside a simulation. We predict that a loss of adult female kin in a poached population will produce high variance in group relatedness, with group size patterns described just prior to the ivory ban persisting, as adult female kin are only added slowly in an elephant population. In contrast to present–day unpoached populations, we expect Mikumi to currently have small group sizes, disrupted genetic patterns and lower relatedness for a given bond strength due to a lack of adult female kin.

We also compared association behaviour along the continuum of group relatedness to investigate the versatility and plasticity of female-bonding tactics in this disturbed setting. Relatedness and association behaviour (i.e. female bonding) are observed to be highly correlated in an unpoached population (Archie *et al*. 2006b). However, when kin are absent, adult female elephants may also lack close female relationships, cohesive groups and extended social ties with other groups. Alternatively, female elephants that lack kin may group with others who lack kin, integrate themselves into established groups permanently at the group level, or at least associate occasionally with established groups at a secondary level, as a 'bond group'.

Methods

Identifying and aging elephants

Mikumi National Park, Tanzania (6.9 –7.7 S, 36.9 –37.4 E, area: 3230 km^2) supports a population of approximately 1140–3100 free-roaming elephants (lower bound: African Elephant Database, 2002; upper bound TWCM, 1999). Its growth rate is uncertain because survey reliability and migration of elephants to and from the neighbouring Selous Game Reserve (area: 43 000 km^2) confounds population estimates (Mpanduji *et al*. 2002). Mikumi consists of a diverse mosaic of open woodland (primarily *Brachystegia* and *Combretum* species) and long-grass savanna habitat of the Mkata floodplain; annual rainfall averages 750 mm, typically beginning in December and peaking in March–May

Fig. 1 Map of Mikumi National Park, Tanzania, with the Selous Game Reserve partially shown to the southeast and Udzungwa National Park partially shown to the southwest. Tourist transects within park boundaries demarcated by solid lines; locations of sampled elephant groups demarcated by filled circles.

(Norton *et al*. 1987; Wasser & Norton 1993). We identified individual elephants based on unique physical characteristics and a photo identification file, built over repeated sightings of 102 known groups. We considered female elephants to be mature if they were ≥ 10 years of age, consistent with Poole (1989). We aged adult females as young (10–19 years old), middle-aged (20–29 years old), and old (≥ 30 years) based on anatomical cues including shoulder height, back length, circumference of tusks (if present), ear position, shoulder protrusion, breast development and abdomen depth with known-age elephants (Laws *et al*. 1975; Kangwana 1996; Foley 2002), following in the field training by C. Foley.

Behavioural data collection

To observe and sample this population, we conducted repeat vehicle surveys of six navigable tourist track transects (totalling 110 km) across all habitat types in the northern third of Mikumi (1000 km^2 out of a total area of 3230 km^2) over 15 months across a 25-month period from July 2003 to August 2005 (Fig. 1). When a group was sighted, we recorded its latitude and longitude on a Global Positioning System device. We performed an initial scan from a maximum distance of 100 m, recording the number of individuals, their sex and age class (Laws *et al*. 1975; Kangwana 1996). Observation sessions continued off-road if necessary, concluding when the group left our field of view.

We defined a group as one or more adult female elephants and her immature offspring moving and behaving in a coordinated manner with no single individual at a distance greater than the width of the main body of the group based on Moss (2001). Each elephant was assigned membership to the group of female elephants that she was behaving in this manner with for the majority (> 50%) of her sightings. Accordingly, a group's size was determined by its majority membership during sightings across days, seasons and years. Based on these definitions, the number of adult females observed per group averaged 2.2 (SE 0.11, range 1–6, n = 102 out of 109 groups). We were unable to define an absolute group size for seven highly fluid 'groups'. In these 'groups', each female changed associates frequently among a cluster of females such that her association with any individual was considerably less than the threshold of 0.5 used to define group membership. This behaviour pattern differs from a fissioning of groups into smaller core units because no core unit was ever recognizable across days, seasons or years for up to 22 sightings for some females.

A 'bond group' was evident when females from two or more previously known groups repeatedly affiliated (≥ 2 times, but less than 50% of their sightings indicating they are two distinguishable groups) (Moss 1988, 2001; Archie *et al*. 2006b). We detected six bond groups in Mikumi; this is likely an underestimate of the occurrence of intergroup bonding given our sampling scheme because one-time casual associations observed between two groups may have actually represented a true bond group. Therefore, our analysis of bond groups reflects only the most salient bonding at this secondary level in Mikumi. Nonetheless,

an examination of this conservative subset of bond groups should reveal a correlation between relatedness and intergroup bonding if it exists in the population.

Each known elephant that was sighted at least two times was assigned a mean association index (AI) based on her interactions with all other adult females and a maximum AI reflecting the strongest social bond she developed with another adult female elephant. These metrics assume that the frequency that two individuals are in close proximity reflects their social bond (Hinde 1976). AI is defined as the number of times two individuals were sighted in close proximity behaving as a group, divided by the total number of times each elephant was sighted (ranges from 0–1, with 1 indicating that the two elephants were together every time each was sighted) (Cairns & Schwager 1987; Ginsberg & Young 1992; Whitehead 1997). The index was derived and analysed using SocProg version 1.2 (Whitehead 1997). A sampling period of 1 day was used to ensure that behavioural observations were not auto-correlated. Individuals were sighted an average of six times across days, seasons and years. Maximum AI was not correlated with the number of sightings ($R^2 = 0.01$, $F_{1,195} = 1.2$, $P = 0.28$).

We compare female elephant bonding between 102 known Mikumi groups to that of 10 groups from the Amboseli National Park, Kenya, quantified in a similar manner across 3 years of observation (average number of sightings per group not specified) by Archie *et al*. (2006a). These two East African elephant populations vary in their ecological resource availability; for example, Amboseli experiences less annual rainfall but has more year-round standing water (swamps) than Mikumi. However, the most prominent difference between the two populations arguably is their poaching histories, with Amboseli representing the only unpoached population in the region with comparable data to ours (Idhe 1989; Poole 1989; Ereckson 2001; Moss 2001).

Genetic methods

We attempted to collect faeces from all observed defecations by known adult females as soon as a group departed. We successfully sampled 203 adult female elephants from 94 known groups (86% of all known groups). Samples were collected by pinching off the outside of several boli and placing the faeces in a 40-mL vial with 25 mL of 20% dimethyl sulfoxide buffer (Wasser *et al*. 2004). Samples were stored at room temperature until shipment to the USA, where they were then stored at –20 C until subsequent DNA analysis.

Faecal DNA (approximately 0.5 g) was extracted from duplicate sub-samples of thoroughly mixed dung using a QIAamp Stool Kit (QIAGEN Inc.) and purified using QBio GeneClean III kit (Wasser *et al*. 2004). We amplified the DNA at 10 dinucleotide microsatellite loci. Polymerase chain reaction (PCR) volumes contained 2 L of total genomic DNA, 0.2 L of 20 M 5′ end labelled forward primer, 0.2 L of 20 M unlabelled reverse primer (Integrated DNA Technologies), 0.4 L 10 mM dNTPs, 12.1 L distilled water, 0.8 L 10× PCR buffer, 0.5 L 25 mM $MgCl_2$, 0.4 L 10 mg/mL BSA, 1.2 L antibody buffer, 0.3 L *Taq* antibody (1:1 g/mL) (Promega), and 0.3 L *Taq* DNA polymerase (5 U/ L) (Promega) for a total volume 20 L. We used a cycling regime comprised of one cycle of initial denaturation at 94 C for 4 min, followed by 40 cycles of denaturing at 94 C for 1 min, annealing at 58 C for 1 min, extending at 72 C for 1 min, followed by a final extension step at 72 C for 2 min in a 9600 ABI thermocycler. We subjected 1 L of PCR product to fragment analysis using GeneScan mode on Model 3100 ABI Capillary Array Genetic Analyzer. Allelic frequency and size were scored with Genotyper version 3.7 software; categories were defined by the weighted average of histogram plots for each allele size bin with tolerance of 0.5 base pairs (Comstock *et al*. 2000; Wasser *et al*. 2004).

We successfully genotyped all 203 known elephants at the following 10 microsatellite loci: FH067, FH129, FH048, FH102, FH103, FH126, FH127, FH153, LAfMSO3, and LAfMSO4 (Nyakaana & Arctander 1998; Comstock *et al*. 2000). These loci had the greatest amplification success, observed heterozygosity and allelic diversity of 16 loci initially tested on a subset of the data (10 random samples). The number and selection of loci was also based on the additional probable statistical power provided by each locus in a rarefaction analysis. Allele frequencies, observed and expected heterozygosities were calculated in Cervus version 2.0 (Marshall *et al*. 1998). The average observed heterozygosity across loci was 0.66, ranging from 0.39 to 0.83 per locus, with our most polymorphic locus having 22 alleles (see Appendix). Tests for linkage and Hardy-Weinberg equilibrium were performed on GenePop version 3.1, applying the sequential Bonferroni test a *posteriori* (Rice 1989; Raymond & Rousset 1995). All loci were unlinked and met Hardy-Weinberg expectations. We calculated the unbiased probability of identity (P_{id}) (with sample size correction) as < 0.0001 and that between siblings (P_{id} sib) as 0.0004 with Match-Maker, version 1.0 (Rudnick *et al*. unpublished). These values indicate that the combination of microsatellite markers in our data set have sufficient discriminatory power that is consistent with similar studies of genotyped free-ranging wildlife (Waits *et al*. 2001).

All alleles were scored twice by the primary researcher, 10% of the scored alleles were randomly selected and scored a third time by a second researcher. There was 98.1% agreement of scores between the two researchers. To guard against incorrect genotyping due to allelic drop-out, we repeatedly amplified samples until heterozygote alleles were observed at least two times and homozygote alleles at least three times consistent with the multiple tubes approach (Taberlet *et al*. 1996). Thirty-seven per cent of the

individuals sampled were genotyped twice from two different dung samples collected on different days to confirm their genotypes. We screened our data set for all identical or near-identical genotypes with an identity check allowing up to one mismatched locus out of 10 loci on Cervus (Marshall *et al*. 1998). All samples from the same individual were in 100% agreement and no matches or near-matches were found among different elephants. All loci tested negative for null alleles ($P > 0.33$) with 10 000 randomizations on ML-Relate version 1.0 (Kalinowski *et al*. 2006) (see amplification success, number of detected null and false alleles per locus in Appendix).

Genotypes were used to generate a pairwise coefficient of relatedness between all adult female pairs using Kinship, with an *r*-value of zero representing the population average (Queller & Goodnight 1989). The program uses genotypic information for single-locus, codominant genetic markers (e.g. microsatellite DNA loci) and derives an *r*-value (range –1 to 1; negative values indicate relatedness below that of the population average) between any two individuals based on the ratio of the number of alleles they share over the alleles' combined frequency in the entire data set. Values approximate the theoretical *r*-values derived from known pedigree analysis. In the algorithm, the denominator (the population allele frequencies) is corrected by excluding the pairs' genotypes and any of their defined group members (likely relatives). We calibrated our relatedness values by computing the relatedness between seven known Mikumi mother–infant pairs. Their average pairwise relatedness was 0.41 (SE 0.05) (no Mendelian mismatches), approaching the theoretical *r*-value of 0.5 for first-order relationships. Based on this calibration, elephant pairs were defined as close relatives (i.e. first-order) if their $r \geq 0.37$. We substantiated close relative designation by determining the most likely relationship of all within-group pairs of females (e.g. first-order $r = 0.5$, second order $r = 0.25$ or unrelated $r = 0$) using maximum-likelihood methods via ML-Relate version 1.0 (Kalinowski *et al*. 2006). In consideration of the resolution of our data set, we then definitively distinguished first-order relationships from unrelated females at the 0.05 significance level. Close relative assignment was in agreement for 95% (144 out of 152) of within-group pairs tested with both methods.

Simulated intact genotypic data set

We examined the deviation between observed and expected group relatedness, with expected values derived from a simulated intact female elephant population unaffected by heavy adult mortality due to poaching, disease, drought or starvation. Expected genotypic data were created through a gene-drop analysis program written in C++ (version 6.0), based on the observed allele frequencies of the Mikumi population. This program is based on MORGAN (version 2.7, Thompson 2005), only simplified for use with our data set. Gene-drop analysis uses a Markov chain Monte Carlo simulation technique that successively drops alleles through a pedigree from founders to descendants. To accomplish this, the analysis randomly assigned genotypes to the pedigree's founders, the matriarch and all fathers, on the basis of allele frequencies derived from the Mikumi matriarchs [i.e. the eldest female per group born prior to peak poaching (1973) from 94 observed families, $n = 50$ elder females]. We assumed that the Mikumi founders were an unrelated, random sample of the population; any alleles they shared would represent the background relatedness level of the population [relatedness across these elder females was 0.008 (0.1 SE)]. Descendants' genotypes were then produced by simulating meiosis, recombination (genes were unlinked) and mating in chronological order.

The use of 50 extant elder females' genotypes for the simulation does not completely represent the true Mikumi 'founder generation' because no poached elder females are included. However, the observed heterozygosity and average alleles per locus of the simulated and observed data sets are similar (Table 1). Four rare alleles (frequency of ≤ 0.006) are absent in the simulation; therefore, the four observed elephants (of 86) with these alleles that formed a total of six within-pairs (of 120) are not accurately represented in the simulation and their *r*-values are likely underestimated. Two of these elephants belonged to groups subsequently defined as intact and two from groups defined as disrupted.

We modelled the intact pedigree for gene-drop analysis on matrilineal elephant group structure as described in Douglas-Hamilton (1972) and Moss (1983, 1988, 2001). This group structure is based on the premise of female philopatry, a 1:1 sex ratio at birth, and the recruitment of two female offspring into adulthood per mother. These trends were recorded for the Lake Manyara and Amboseli elephant populations during times when poaching and drought were virtually absent (Douglas-Hamilton 1972; Moss 2001).

Our model included the expectation that all connections were parsimonious with no missing 'nodes' (i.e. female relatives). We assumed that if a group had not kin lost to poaching, disease or drought, the connections between any two adult females should be the shortest branch lengths possible. We assumed that natural mortality would not disrupt intact pedigrees because it is typically concentrated on the youngest and eldest individuals in the population. Furthermore, only 10% of all known deaths in an unpoached population over a 27-year span were attributed to the loss of an adult female to a natural cause ($n = 20$ deaths) (Moss 2001). We also assumed that no natal transfers of adult females into the group or permanent separation of females from the group occur. Both of these events are rare in unpoached populations (Douglas-Hamilton 1972; Moss 2001; Archie *et al*. 2006b). Our model included 15 distinct

Table 1 Summary of characteristics of simulated intact groups and observed Mikumi groups that were compared in this study

	Simulated intact groups	Observed Mikumi groups
No. of adult females per group	2–3	2–3
No. of female founders per group	1	1
No. of male founders per group	1–2	1–2
No. of unique pedigrees	3 for two-adult groups 8 for three-adult groups	Not applicable
No. of groups	1000 iterations per pedigree	n = 24 with 2 adults n = 13 with 3 adults
Allele frequency data	Genotypes of 50 Mikumi elder females born before 1973 from 50 different groups	Genotypes of 203 Mikumi females born in 1993 or prior from 94 different groups
Assumptions	Female philopatry, 1:1 sex ratio at birth, minimal adult female mortality, no natal group transfers	Not applicable
Observed heterozygosity	0.65	0.67
Average no. of alleles per locus	8.3	8.5
Range in average group relatedness	Two-adult groups: (0.26–0.53) Three-adult groups: (0.26–0.50)	Two-adult groups: (–0.21–0.66) Three-adult groups: (–0.08–0.40)

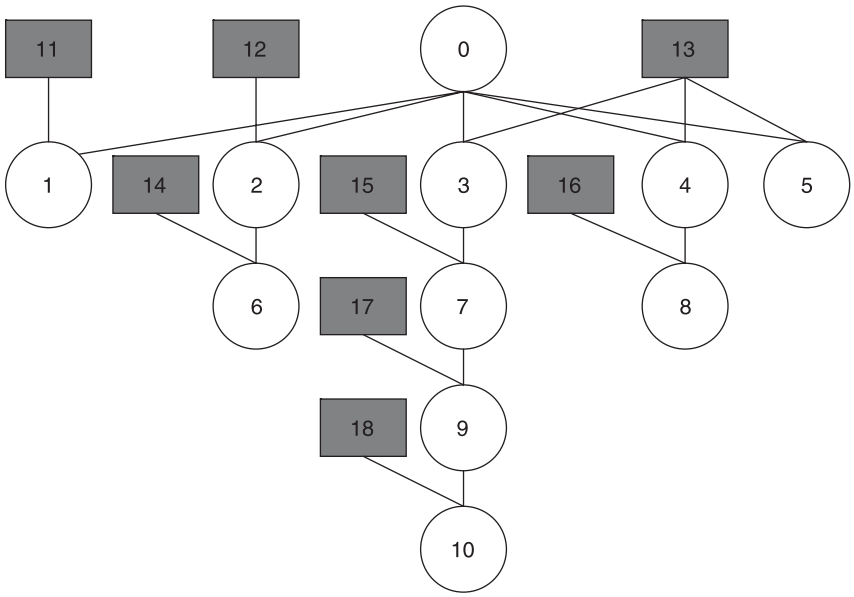

Fig. 2 Model pedigree of an intact group spanning multiple overlapping generations (circles are females, numbered 0–10; rectangles are males, numbered 11–18).

female relationships, from first-order (e.g. mother:daughter) to fourth-order (e.g. great grand-aunt: niece) relatives, representing a group with multiple females of different, overlapping generations (Fig. 2).

We assumed a new male mated with each female, as would be expected in a polygynously mating, long-lived species (Poole 1987). The only exception to this was to simulate full-sisters (the founder had two daughters with the same father). Male African elephants come into musth periodically at which time they dominate a large percentage of matings with estrus females (Poole 1987). However, musth males mate with estrus females at random, not concentrating their efforts on any one group at any one time (Archie *et al*. 2007). Thus, in our modelled intact group, it seemed reasonable for each sire to have a different identity. This resulted in a total of eight different males included as founders in our gene-drop analysis. However, if males were to mate with multiple females in one group, any two females in our model would be more closely related than calculated. Therefore, including the maximum number of fathers rendered the model conservative.

Our intact group model included a total of 11 adult females, approaching the maximum number observed in groups in an unpoached population (Moss 2001; Archie *et al*. 2006b). The assumptions inherent in our model render it an ideal but realistic portrayal of elephant group structure

when female survivorship and reproduction are minimally disrupted. However, it was necessary to compare a Mikumi group's average *r*-value to that of a simulated group of the same size because the average pairwise relatedness becomes diluted as group size increases. For example, a lower proportion of pairings in a group is likely to be first-order relatives as the total number of pairs increase. Naturally, each adult female has a limited number of close adult female relatives (i.e. first-order) in her group: her mother and her adult daughters (the latter slowly increases as she ages because the sex ratio for elephants at birth is 1:1 and females mature around 12 years of age).

Eighty-six per cent of multi-adult groups sighted in Mikumi have two to three adult females; therefore, we focused our observed vs. simulated relatedness comparisons on these group sizes. We independently simulated all possible parsimonious pedigrees of size 2–3. We defined parsimonious pedigrees as those with the shortest branch lengths possible between females; 3 unique pedigrees exist for groups of size 2 and 8 for groups of size 3. For example, a group of 3 with no missing adult females could be a mother and 2 adult daughters (full sisters) (simulated average $r = 0.50$) or three half-sisters (simulated average $r = 0.26$). We repeated the gene-drop 1000 times for each pedigree to derive its average group *r*. All pedigrees were embedded in our full model; this design allowed us to easily repeat the procedure for each pedigree, simply averaging across the appropriate number females of the appropriate positions to arrive at an average *r*.

The minimum simulated average *r* for parsimonious pedigrees with 2–3 adult females was 0.26 (i.e. simulated groups of all maternal half-sisters). Observed groups were classified as disrupted if their observed average *r* was ≤ 1 SD below the expected average (simulated average $r = 0.26$ (0.18 SD); groups with $r < 0.08$ were designated as disrupted). Otherwise, groups were considered intact (i.e. likely missing no adult females for their group size). Comparing the average pairwise relatedness of each observed Mikumi group with 2–3 adult females to this minimum simulated average determined the percentage of these Mikumi groups that differed from an intact expectation in which adult female mortality to any cause other than old age is minimal. Characteristics of the simulated population and observed Mikumi population are summarized in Table 1.

Statistical analysis

We conducted statistical analyses in SPSS (version 11.5) and JMP SAS (version 6.0). We performed Kolmogorov–Smirnov (K–S) tests, one-way analysis of variance (ANOVA), chi-squared analysis to compare distributions and variance in group size, relatedness and AIs of the Mikumi population and the unpoached Amboseli population or simulated data. We tested for correlations between AI and relatedness with Mantel tests at two levels: across the whole sampled Mikumi population and within Mikumi groups. Groups of size 1 to 2 adults and those with no variance in AI were excluded from the analysis because a minimum of three pairs was required per group to compare matrices and to compute the tests correctly.

Results

Minimal change in group structures

The demography of the Mikumi population today ($n = 102$ groups) compared to Poole's 1989 report ($n = 69$ groups, 466 elephants) demonstrates that poaching has declined over the last 15 years in the park; however, group structure has remained disrupted. If poaching has declined in Mikumi, then we expect more families to have old, tusked matriarchs. Indeed, since 1989 the proportion of old matriarchs with tusks has increased by 14.2%. Little change in the number of solitary adult females and group size would indicate static group structures; the percentage of solitary adult females has decreased from 33.1 to 30.3% presently. The median group size of 6 (all cows and calves) reported in 1989 remains the same today (average number of adult females per group was not reported in 1989).

Mikumi demography and relatedness vs. intact, unpoached groups

We expected that high female mortality due to poaching would render surviving groups in Mikumi small, many with disrupted genetic relatedness patterns. If poaching has had these long-term effects on group structure, significant differences in group size and relatedness between Mikumi and unpoached populations should be apparent. In support of our hypothesis, the unpoached Amboseli and poached Mikumi populations differ significantly in their group size distribution, with the Mikumi population heavily skewed to the smaller sizes (K–S test $Z_{102,45} = 3.813$, $P < 0.0001$) (Archie *et al*. 2006b). Also, no solitary groups exist in Amboseli whereas a third of groups in Mikumi had only one adult female.

Mikumi group size averaged 2.2 (SE 1.1, $n = 102$ groups) and pairwise relatedness averaged 0.13 (SE 0.01; range: –0.22 to 0.67, $n = 55$ completely genotyped groups; solitary females omitted). Average group relatedness varied greatly in Mikumi; 10 established groups and all seven highly fluid groups had average group *r*-values < 0 (Fig. 3). Amboseli group size averaged 6.5 (SE not reported, $n = 34$ genotyped groups) and pairwise relatedness averaged only slightly higher at 0.15 (SE 0.02) than for Mikumi (average group relatedness was not available). However, differences in the group size distributions between the two populations would make direct statistical comparisons of average

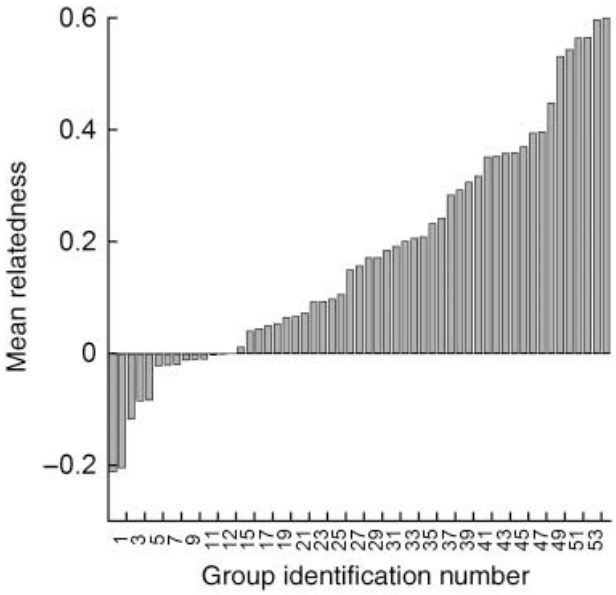

Fig. 3 Distribution of average group relatedness for 55 known groups with 1–6 adult females each in Mikumi National Park Tanzania, averages below zero are likely groups of unrelated females.

group relatedness spurious due to the dilution effect (described in Methods). Therefore, we compared *r*-values of observed groups to those from a simulated intact population, with group sizes similar to those found in Mikumi created through gene-drop analysis.

If poaching had a long-term effect on genetic patterns, we expected that a large percentage of the observed Mikumi groups would be classified as disrupted based on comparisons to our simulated intact expectation. Average group *r* of 37 observed groups (84 females) was compared to the minimum expected group *r* for a simulated intact group of the same size. If an observed group *r* was at least 1 SD below this minimum, the group was likely missing adult female relatives and therefore classified as disrupted. Overall, 45.2% of these adult females (38 out of 84) came from disrupted groups (Table 2).

Groups with unrelated females

To test the hypothesis that unrelated females ($r \leq 0$) primarily come together when they lack adult matrilineal kin, we examined the occurrence of first-order relatives and variance in relatedness across group size. Females in large groups (3–6 adults) were predicted to have at least one first-order relative solidifying her inclusion in the group; non-relatives would not be welcome (i.e. no natal transfers). If relatedness is the means by which group membership is typically maintained, then non-relatives will most commonly group when kin are wholly absent. Thus, if bonding between non-relatives occurs, we expect it to be between two solitary females. Preferential bonds with kin are not an option for such females, thus opening an opportunity for strong bonds between non-related females. Accordingly, the range in average relatedness in small groups is expected to be extended due to the two extremes: pairs of close relatives and pairs of non-relatives. We expected large groups to be more consistently related (i.e. lower variance in average group relatedness) than small groups.

Table 2 Per cent disrupted and intact Mikumi elephant groups (size 2 to 3 adult females); disrupted groups were those with an average group relatedness 1 SD below the mean of simulated intact groups of the same size

Group size (N = groups)	Intact (range in average group *r*-values)	Disrupted (range in average group *r*-values)
2 (24)	72% (0.15–0.66)	28% (–0.21–0.04)
3 (13)	44% (0.09–0.40)	56% (–0.08–0.07)

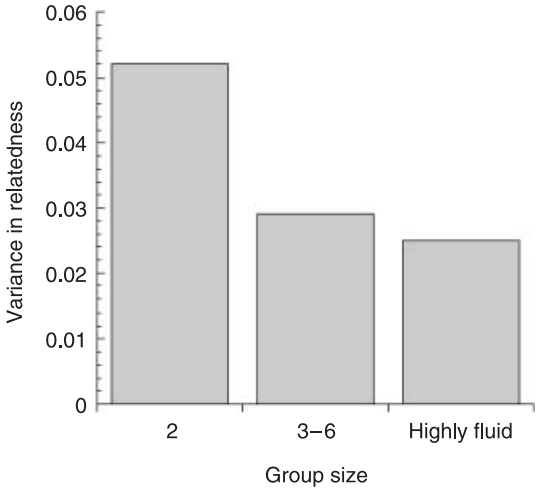

Fig. 4 Variance in mean pairwise relatedness was significantly greater in small groups (2 adult females) than highly fluid and large groups (3–6 adult females).

Females in the largest groups (n = 47 females) were most likely to have a first-order relative in their group [likelihood ratio test R^2 (U) = 0.07, n = 137 d.f. = 2, χ^2 = 13.832, P = 0.001]. Variance in average pairwise relatedness was significantly greater in small groups compared to large groups as predicted (K–S test $Z_{24,26}$ = 1.52, P = 0.02) (Fig. 4). We did not include females in highly fluid groups (n = 20 females) in the variance comparison because they displayed weak bonding and invariantly low relatedness. Average pairwise relatedness of all highly fluid groups was < 0, which was significantly lower than that of established groups (highly fluid groups: n = 5, mean r = –0.007 (SE 0.06); established groups: n = 50, mean r = 0.19 (SE 0.03), t test$_{1,54}$ P < 0.0001).

These findings also support our idea that non-relatives will most commonly group when kin are wholly absent. However, having a first-order relative was not a strict requirement for inclusion in a large group as evidenced by the classification of 56% of three-adult groups as genetically disrupted (Table 2). Their lopsided genetic pattern is consistent with our original hypothesis if they represent two unrelated females who grouped, with one subsequently having a daughter who recently matured.

Correlation of genetic and behaviour patterns

In a poached population, we predicted the loss of kin and the resulting genetic disruption would negatively affect bond strength in the population. If poaching had this long-term effect, then we expected bond strength (maximum AI) to be variable in Mikumi, correlating with relatedness. Accordingly, females in larger groups should have higher average bond strength because larger groups were shown to be more consistently related (i.e. lower variance in average relatedness). We also expected that Mikumi elephants would have a lower relatedness on average than elephants in an unpoached population for a given bond strength because of a lack of available kin in Mikumi. Only half of the adult females in the Mikumi population form an extremely strong bond with at least one other adult that they are always with ($n = 218$ females in 109 groups) (Fig. 4). Across the population, a positive correlation between maximum AI and relatedness was significant as predicted (Mantel $R = 0.09$, $n = 201$, $P = 0.001$). However, exceptions were noteworthy on an individual group basis. Of the groups examined, 56% had an association-relatedness correlation below the population mean; genetically disrupted groups had significantly higher Mantel *R*-values [mean Mantel *R*: 0.02 (SE 0.11)] than intact groups [mean Mantel *R*: –0.06 (SE 0.08)]. Overall, a female's maximum AI increased with her group size as expected (one-way ANOVA, $F_{4,174} = 2.85$, $P < 0.02$). As group members were added, bonds became stronger as opposed to more dilute (Fig. 5).

In comparison with the unpoached Amboseli population, within-group average pairwise AI in Mikumi was unexpectedly higher [Mikumi average AI: 0.72 (SE 0.03) for 152 dyads in 55 groups; range: 0.5–1.0; Amboseli average AI: 0.64 (SE 0.01) for 317 dyads in 10 groups; range: 0.2–1.0]. We defined individuals with AI ≥ 0.5 as group members, whereas behavioural data collected across 30 years was used to determine group membership in Amboseli. Therefore, it seemed more appropriate to compare relatedness for a given range of AIs between the two populations. Relatedness was substantially lower in Mikumi for the same bond strength (maximum AI of 0.9 to 1.0) as compared to Amboseli [Mikumi: $r = 0.23$ (SE 0.03) $n = 46$ dyads in 26 groups; Amboseli: $r = 0.42$ (SE not reported), $n = 10$ groups] (Archie *et al*. 2006b). This difference was driven by 22 Mikumi females with maximum AI of 0.9 to 1 and no first-order relatives in their group.

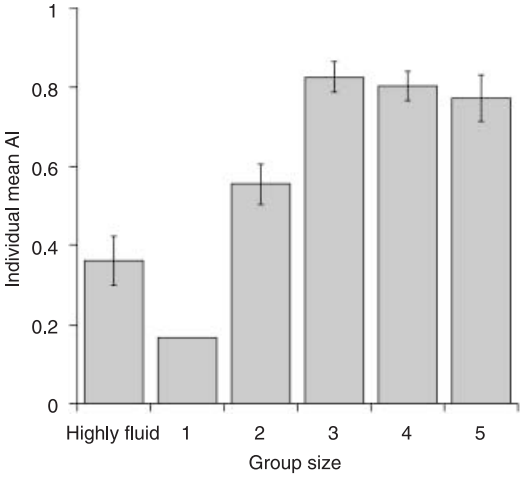

Fig. 5 Individual mean AI was significantly greater in large groups (3–6 adult females) than small groups (2 adult females) and solitary females.

Secondary bonding

Bond groups have been described in the literature as resulting from the temporary re-fusion of previously fissioned groups. To test whether the availability of 'extra' relatives drives this behaviour, we examined the relatedness within and between the constituent groups of each bond group. We predicted that each constituent group of a bond group would be genetically intact, indicating high internal relatedness (i.e. not classified as disrupted via gene-drop analysis). Accordingly, we also predicted that solitary females and groups classified as disrupted, indicating a lack of first-order relatives for their given group size, would not participate in bond groups.

We identified six bond groups in the Mikumi population, representing 14 groups (25 adult females). Constituent groups participating in bond groups ranged in size from 1–5 females; total bond group size varied from 3–6 females. Internal relatedness varied in the constituent groups; only five groups out of 14 contained first-order relatives [i.e. pairs with $r \geq 0.37$ which were significantly more likely to be first-order relatives ($r = 0.5$) vs. non-relatives ($r = 0$) with ML-Relate]. Contrary to expectation, four solitary females and two disrupted groups participated in bond groups. However, the majority of bond groups (four of six) were characterized as one related group (i.e. contained first-order pairs) bonding with another group with had a female that was a relative of the old matriarch of the former (intergroup pair mean $r = 0.29$ (SE 0.5), and all were significantly more likely to be to be second-order relatives ($r \geq 0.25$) vs. non-relatives ($r = 0$) with ML-Relate).

Discussion

Our results demonstrate that a demographic signature of poaching persists in the Mikumi elephant population on the basis of group size, relatedness and social bonding among adult female groups. Descriptions of the Mikumi population in 1989, preceding the international ban on the sale of ivory, still persist today. We also observed striking differences in the ways female elephants in Mikumi form groups as compared to an unpoached population. Poaching rather than any other agent is known to have precipitated their vastly divergent rates of adult female mortality since 1980 (Balozi 1989; Idhe 1989; Poole 1989; Ereckson 2001; Moss 2001; Archie *et al*. 2006b). These differences appear to have persisted despite a 15-year drop in the occurrence of poaching in Mikumi (Seige & Baldus 2000). Considerable variation in grouping behaviour exists in this population that has not been reported for unpoached populations. This variation demonstrates flexibility in elephant behaviour: a behavioural resilience to the loss of kin for some female elephants, and an apparent social deficiency for others.

Disrupted demographic patterns persist

The close match between demographic patterns among the elephants of Mikumi observed in this study and those reported by Poole in 1989 attest to the slow recovery of this long-lived species to an extreme disturbance that occurred at least 15 years prior. Overall, group size was small with just two adult females on average. Six percent of the groups were highly fluid in Mikumi, displaying unusually weak bonds. Thirty percent of the females had no other adult females in their group. Solitaries have been observed in other poached populations, such as those in Tsavo, Kenya, and in the Selous and Tarangire of Tanzania; thus, this extremely small group size is not unique to Mikumi (Poole 1989; Foley *et al*. 2001). All of these findings differ from what has been observed in unpoached populations, typified by large groups, with no solitaries or highly fluid groups reported.

Several grouping options exist for elephants lacking kin

In Mikumi, females across the genetic relatedness spectrum were observed to form groups. Group relatedness varied greatly across groups, with highly fluid groups having the lowest relatedness. Pre-ban poaching in Mikumi did not uniformly affect the population. Therefore, we expected a proportion of the groups in the Mikumi population to have a relatedness pattern similar to what is observed in unpoached populations. The majority of groups in Mikumi have two to three adult females; only 54.8% of these groups were related in a way consistent with an unpoached model. Small groups with just two adults had the highest variance in relatedness, suggesting that some small groups consisted of females who lost kin and form only weak bonds with the elephants most available to them — other individuals who also lost kin. Larger groups were more consistently related, but many included non-relatives. In combination, our findings suggest that females without close kin use a full range of options: live alone (33%), form a small group with another female lacking kin (24%), join a large group unrelated to them (20%), or form a loose connection with a number of kinless females (e.g. highly fluid group; 22%).

Poaching leads to increased variation in bonding

Elephants have been characterized as having fission–fusion societies; however, the number of female elephants in the Mikumi population that only occasionally associate with other females and form only very weak bonds is inconsistent with that described for unpoached elephant populations (Douglas-Hamilton 1972; Moss & Poole 1983; Whitehouse & Hall-Martin 2000; Moss 2001; Archie *et al*. 2006b). Only half of the adult females in Mikumi form an extremely strong bond with at least one adult that they are always with (maximum AI: 0.9–1); some associated with no other adults (maximum AI: 0). Females in large groups (3–6 adult females) formed the greatest number of bonds with the highest average bond strength.

Overall, relatedness drives bonding in Mikumi on both the individual group and bond group level, consistent with unpoached populations. Relatedness correlated with strength of association population-wide, and the majority of bond groups consisted of two related constituent groups. However, the quality and quantity of social bonds among adult female elephants varied greatly in Mikumi. Although some adult female elephants were prevented from forming stable groups because they lacked kin, others somehow overcame this obstacle and formed very tight bonds with non-relatives (11 pairs of unrelated females had a maximum AI of 0.9 to 1). Thus, the relatedness among female elephants that formed strong bonds (AI ≥ 0.9) was considerably lower than that for similar female elephant pairs in an unpoached population. This suggests that when kin are unavailable, female elephants still seek out strong female relationships, presumably moving down their pedigree to the next available, often distant kin eventually choosing non-relatives. Within a group, the context of relatedness appears to be important in dictating the correlation of relatedness and association. Our Mantel test results suggest that if average group relatedness is low (disrupted group *r*-value range: –0.22–0.07), bonding preferentially occurs among distant kin vs. non-relatives. When average group relatedness is relatively high (intact group *r*-value range: 0.09–0.54), differentiating among elephants who are all likely kin becomes less important. An abundance of kin is not a prerequisite for creating a second-tier association in Mikumi, as evidenced by the

participation of solitaries and groups with low relatedness in bond groups. However, the majority of bond groups contained groups of high relatedness and an intergroup pair that was also highly related. This pattern suggests that having close kin outside the natal group increases the chances of a bond group being maintained.

Long-term effects of poaching persist

Our results suggest that it can take upwards of 20 years or 1–2 elephant generations for a group to recover from destruction of its social network of kin. An enormous number of elephants were illegally killed in Tanzania in the late 1970s through the 1980s. The passing of the ivory ban by CITES in 1989 provided a respite for many of the hard-hit elephant populations. Yet, the lack of kin manifests itself in the lives of many female elephants, as reflected by their lack of strong bonds they have with other elephants. Thus, a sizable proportion of the Mikumi adult female population currently lacks kin and the associated strong, cohesive groups. Adverse fitness consequences are also apparent from this disturbed social structure (Gobush *et al*. 2008). Some females are able to overcome this loss by grouping with non-kin, but many remain alone. The extent of possible loss in fitness of these two alternatives may vary for females depending on their age, reproductive status, habitat context or protection milieu, but overall these multiple strategies represent plasticity in female elephant social structure.

Conserving a species means conserving its unique qualities, including its social complexity and the way it manipulates the environment to fulfil its resource needs. Disrupting the social fabric of Mikumi elephant population has resulted in some adult females unable to bond and group, more than a decade after the threat of poaching in the area subsided. Continued and strengthened protections must become a priority if these female elephants are to successfully raise daughters to maturity and rebuild their adult–kin network.

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Appendix

Unlinked microsatellite loci used in this study on 203 known adult female African elephants; no significant deviations from Hardy–Weinberg expectations were detected for any loci using the Bonferroni correction for multiple comparisons

Locus	No. of alleles	Allele size range (bp)	H_O	Total attempted amplifications	% failed to amplify	% with null alleles	% with false alleles
FH067	9	87–105	0.718	1238	0.89	0.40	1.45
FH129	6	152–162	0.759	1157	1.99	0.69	0.43
FH048	9	166–184	0.830	1266	1.03	0.95	1.50
FH102	6	171–183	0.658	1256	0.72	0.40	2.23
FH103	5	145–153	0.602	1108	0.99	0.30	1.79
FH126	10	94–122	0.733	1106	0.27	0.18	1.18
FH127	22	143–289	0.885	1285	0.39	1.25	1.71
FH153	12	155–179	0.794	1287	0.62	0.47	0.62
LAfMSO3	5	139–147	0.470	1200	0.88	1.00	2.00
LAfMSO4	7	150–162	0.396	1210	1.74	0.27	1.34

H_O, observed heterozygosity.