Fine-scale population genetic structure in a fission–fusion society

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Abstract

Nonrandom patterns of mating and dispersal create fine-scale genetic structure in natural populations—especially of social mammals—with important evolutionary and conservation genetic consequences. Such structure is well-characterized for typical mammalian societies; that is, societies where social group composition is stable, dispersal is male-biased, and males form permanent breeding associations in just one or a few social groups over the course of their lives. However, genetic structure is not well understood for social mammals that differ from this pattern, including elephants. In elephant societies, social groups fission and fuse, and males never form permanent breeding associations with female groups. Here, we combine 33 years of behavioural observations with genetic information for 545 African elephants (Loxodonta africana), to investigate how mating and dispersal behaviours structure genetic variation between social groups and across age classes. We found that, like most social mammals, female matrilocality in elephants creates co-ancestry within core social groups and significant genetic differentiation between groups ($F_{ST} = 0.058$). However, unlike typical social mammals, male elephants do not bias reproduction towards a limited subset of social groups, and instead breed randomly across the population. As a result, reproductively dominant males mediate gene flow between core groups, which creates cohorts of similar-aged paternal relatives across the population. Because poaching tends to eliminate the oldest elephants from populations, illegal hunting and poaching are likely to erode fine-scale genetic structure. We discuss our results and their evolutionary and conservation genetic implications in the context of other social mammals.

Keywords: African elephant, dispersal, kinship, mating behaviour, microsatellites, population genetic structure

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Introduction

Population genetic models of the distribution and loss of genetic variation in populations classically assume that patterns of mating and dispersal within populations are random and panmictic (Wright 1965). However, this assumption is usually false for social species; instead, sex-biased dispersal and limited reproductive opportunities create fine-scaled genetic structure within populations (Baker & Marler 1980; Chesser 1991a, b; Sugg et al. 1996; Storz 1999). This fine-scale population genetic structure has important potential consequences for both evolutionary processes and conservation genetics: it can structure opportunities for kin selection (e.g. Hägglund & Shorey 2003; Hazlitt et al. 2004; Cutrera et al. 2005; Archie et al. 2006b; Woxvold et al. 2006), influence the rate of inbreeding or outbreeding (Chesser 1991a, b; Sugg 1996), impact processes of local adaptation (Storz 1999; Storz 2005), confound quantitative trait locus (QTL) studies (Ewens & Spielman 1995), and influence the rate at which genetic diversity is lost from natural populations (Melnick 1987; Chesser et al. 1993; Sugg 1994; Sugg 1996; Dobson et al. 2004).
In mammals, fine-scale genetic structure is best understood for one of the most common societies; that is, societies where social group composition is stable, dispersal is male biased, males form relatively permanent associations with female social groups, and mating is polygynous such that matrilocal females all mate with the same relatively few males. Such nonrandom patterns of mating and dispersal create ‘breeding group’ populations where: (i) group members have high co-ancestry, (ii) social groups are genetically differentiated from each other \((F_{ST}\) among groups is significantly greater than zero), and (iii) offspring are unusually heterozygous, given the co-ancestry within groups \((F_{IS}\) is significantly less than zero; reviewed in Dobson et al. 1998; van Staden 1995; Sugg 1996; Storz 1999). Such breeding group species include many nonhuman primates (de Jong et al. 1994; Turner 1981; Dracopoli et al. 1983; Melnick et al. 1984; Melnick & Pearl 1986; Melnick 1987; Pope 1992; de Ruiter & Geffen 1998; Pope 1998), social rodents (Chesser 1983; Schwartz & Armitage 1980; van Staden et al. 1996; Dobson et al. 1997; Dobson et al. 1998; Dobson et al. 2004), some social carnivores (Spong et al. 2002), some bats (Wilkinson 1985), rabbits (Surridge et al. 1999) and rock-wallabies (Hazlitt et al. 2004; Hazlitt et al. 2006).

Less well understood is the fine-scale population genetic structure of mammals that are highly social, but whose societies differ from the breeding group paradigm. There are several such species, and among them are some cetaceans, such as sperm whales, and Asian and African elephants (Douglas-Hamilton 1972; Moss & Poole 1983; Christal & Whitehead 2001; Whitehead 2003). Their genetic structure is important, not only for illuminating underappreciated parameters that influence fine-scale genetic structure, but also because many highly social mammals, including elephants and sperm whales, are charismatic flagship species that are threatened by hunting and habitat destruction.

Elephants and sperm whales share some features in common with breeding group species — for example, males are the dispersing sex, while matrilocal females form predictable social groupings and close and enduring social partnerships (Moss & Poole 1983; Lee 1987; Moss 1988; Whitehead et al. 1991; Whitehead 1996; Christal, Whitehead 2001; Whitehead 2003). However, elephants and sperm whales differ from breeding group species in two important ways. First, they live in fission–fusion societies where social groups are not temporally stable, and instead divide and re-form over the course of hours, days, or weeks (Douglas-Hamilton 1972; Moss & Poole 1983; Whitehead et al. 1991; Whitehead 2003; Wittemyer et al. 2005). Second, and most relevant to population genetic structure, male elephants do not form permanent associations with female social groups. Instead, males move widely within populations, visiting many social groups as they search for sexually receptive females, and siring offspring in multiple core groups (Moss & Poole 1983; Poole 1989b; Poole & Moss 1989; Hollister-Smith et al. 2007); sperm whales appear to have similar behaviour patterns (Whitehead et al. 1991; Whitehead 1996; Christal, Whitehead 2001; Whitehead 2003). As a result, these species do not form ‘breeding groups’ in the strictest sense.

As yet, no data have been available to investigate the impact of these behavioural processes on population genetic structure in nonbreeding group mammalian societies. In this study, we investigate the extent to which such behaviours structure genetic variation in a natural population of wild African elephants. Several alternative outcomes are possible. In elephants, males’ freedom to breed in many social groups, across the population, may reduce co-ancestry within social groups and genetic differentiation between those groups, relative to breeding group species. As a result, genetic structure in elephants may conform more closely to the predictions of panmixia than breeding group species, or it may resemble the relatively weak genetic structure of herd-living ungulates (Petit et al. 1997; Coltman et al. 2003; Nussey et al. 2005).

However, several aspects of elephant social behaviour may reduce panmixia and increase genetic structuring. First, females form predictable, long-term associations with female kin, even though female social groups are not territorial and have broadly overlapping home ranges (Douglas-Hamilton 1972; Moss & Poole 1983; Wittemyer et al. 2005). Specifically, females are matrilocal and form social units called ‘core’ or ‘family’ groups, consisting of 2–30 matrilocal adult females and their immature offspring. Kinship predicts the fission and fusion of elephant groups, and as a result, female elephants spend most of their time with their closest maternal kin (Archie et al. 2006b). This behaviour should result in relatively high co-ancestry within social groups and genetic differentiation between groups. Second, because male elephants reach their peak reproductive success around 40–50 years of age and maintain this peak for 5–10 years (Poole 1989b; Hollister-Smith et al. 2007), this reproductive peak may create cohorts of paternal relatives across the population. That is, individuals may be more closely related to individuals from their own age cohort and to their father’s cohort than to the rest of the population. Third, elephants may engage in nonrandom mating even in the absence of relatively permanent male–female associations. For instance, if female elephants within a single core group all breed with the same set of males, perhaps through female choice or coordinated estrous (e.g. Moss 1983; Rossiter et al. 2005) this will enhance levels of co-ancestry within groups. Alternatively or additionally, male elephants may expend greater mating effort in some core groups than others, and this would also cause elephants to resemble breeding group species.

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We combined 33 years of behavioural observations with genetic information for 545 elephants in a wild population. First, we tested whether nonrandom mating behaviour generated co-ancestry within core groups, and whether paternal relatives occurred in similar-aged cohorts across the population. Then, we tested whether genetic variation in the study population was panmictic or resembled that found in breeding group species. Finally, we simulated the effects of age-biased poaching on the genetic structure of core social groups by excluding the oldest females (with the largest tusks) from our data set. Our results are important, not only for understanding how genetic variation is structured in elephant populations, but also for their conservation. Most elephant populations are isolated by habitat destruction, and are thus threatened by the loss of genetic diversity.

Methods

The study population

Research subjects were the wild, free-ranging African elephants that live in and around Amboseli National Park, Kenya. These elephants have been studied continuously since 1972 by researchers working with the Amboseli Elephant Research Project (AERP) and are among the most natural and intact elephant populations in Africa (Moss 2001). The habitat in and around Amboseli is semi-arid savannah, and the elephant population currently numbers around 1400 individuals. All elephants were individually recognizable from naturally occurring physical features (e.g. tears or holes in the ears, tusk and body shape), which were recorded in a photographic database. All individuals were assigned an age. The ages of elephants born since 1975 were known to within 2 weeks, and the ages of elephants born between 1972 and 1975 were known to within 3 months. Because elephants continue to grow throughout their adult lives, the ages of elephants born before 1972 were estimated based on body size. These estimates were based on well-documented patterns of variance in shoulder height and body shape with increasing age, and were corroborated with tooth eruption data from mortalities for which skulls were recovered; age estimates of the oldest elephants were considered accurate to within 5 years (Haynes 1991; Lindeque & van Jaarsveld 1993; Lee & Moss 1995; Moss 2001; Morrison et al. 2005).

Elephants were categorized as calves or adults depending on their age and/or reproductive status; females were defined as adults when they had given birth at least once (first birth usually occurs between 9 and 17 years of age), and adult males were 21 years of age or older — the youngest age of genetically confirmed paternity in Amboseli (Archie et al. 2007). Between 1976 and 2005, Amboseli’s adult females and calves lived in 54 different core social groups. Almost all of these groups persisted throughout the study; however, two groups went extinct, while another was created by permanent fission of a pre-existing group. Because female elephants are matrilocal, natal core groups were known for all females in the study. Because males disperse from their natal core groups at around 14 years of age, natal core groups were only known for males that dispersed after 1972.

Behavioural data collection

Behavioural observations began in 1972 and were opportunistic. When elephants were sighted, researchers collected several pieces of data, including individual identities, group membership, births, and deaths. Since 1976, researchers also collected observations and focal samples of female oestrus and male sexual behaviour in the presence of oestrous females. Oestrus lasts 4–5 days in female elephants, and researchers identified oestrus with diagnostic behaviours: adult male elephants expressed much greater interest in oestrous females — by smelling their genitals, urine and faeces, and attempting to copulate — and oestrous females exhibited an ‘oestrous walk’ during which they move away from interested males, while glancing back over their shoulder (Moss 1983; Poole 1989b). Non-oestrous females ignored male interest and did not move away from males using the ‘oestrous walk’. Whenever researchers observed a female in oestrus, they recorded the identities of adult males that guarded or successfully copulated with the oestrous female. Guarding occurred when the male that was the closest mature male to the oestrous female maintained this proximity by chasing all other males that approached the oestrous female. Copulation occurred when the male mounted the female from behind, obtained intromission, and was apparently accompanied by ejaculation.

Genetic sampling and genotyping

The analyses described here used genetic samples from 545 individuals, including 256 adult females, 106 adult males, and 183 calves. Genotyping was conducted mainly from noninvasive faecal samples and a few tissue samples. Sample collection and DNA extraction methods are described extensively in Archie et al. (2003) and Archie et al. (2006b). Briefly, faeces were collected from known individuals, almost always within 10 min of defecation, and DNA was extracted using a modified protocol (Archie et al. 2003) for the QIAamp DNA Stool Kit (QIAGEN).

All individuals were genotyped at 11 microsatellite loci, including 10 tetranucleotide loci (LaT05, LaT07, LaT08, LaT13, LaT16, LaT17, LaT18, LaT24, LaT25, LaT26;
Assigning parentage

Maternity was known from direct observation for all calves included in this study, as elephants have a long period of maternal dependence and suckle for 4 years. This enabled repeated sightings of mother–calf pairs, and hence, very accurate mother–offspring relationships. Finally, maternity was confirmed via Mendelian checks for all mother–offspring pairs.

We used CERVUS software (version 3.0; Kalinowski et al. 2007) to assign paternity to 152 of 183 elephant calves for whom we had complete genotypes. All calves for which maternity was assigned were born between 1978 and 2002. This represented approximately 10% of the calves born during this period. We used the following input parameters for all CERVUS simulations: 10 000 cycles, 90 candidate parents, 100% of loci typed, 1% of loci mistyped and confidence levels of 95% strict and 80% relaxed. The proportion of candidate parents sampled from the population varied over the 25-year period. Because CERVUS is sensitive to this proportion (Krutzen et al. 2004), we ran different simulations in CERVUS for periods with different proportions of candidate males sampled: 33% (1977–1980), 45% (1981–1985), 55% (1986–1990), 61% (1991–1995) and 74% (1996–2000) (see Archie et al. 2007; Hollister-Smith et al. 2007; for details).

A father was assigned to a calf when two conditions were met: (i) CERVUS assigned paternity with 95% confidence, and (ii) there were no Mendelian mismatches between the calf and its assigned father. Each of the 152 calves for which fathers were assigned had a unique set of parents (i.e. we found no full siblings); these parents included 42 individual males and 113 individual females.

Statistical analyses

Randomization tests of mating behaviour. In order to test whether nonrandom mating behaviour generated co-ancestry in elephant groups, we ran two Monte Carlo randomization simulations. In the first simulation, we tested whether core groups of female elephants engaged in sexual behaviour and bred with a smaller number of males than expected by chance. To do this, we used Monte Carlo randomization simulations to generate a distribution of the expected number of different males mate guarding, copulating, and siring calves per group, given the number of times we observed these males engaging in these sexual behaviours or siring offspring across the entire population. We then compared these random expectations to the observed number of different males engaging in sexual behaviour or conceiving offspring in each core group. Our hypothesis was supported if the observed number of different males was less than random expectations. Specifically, we counted the number of times any adult male was observed mate guarding or copulating between 1976 and 2004 in each core group (range = 2–60 per group). Then we used POPTOOLS (version 2.7.5, www.cse.csiro.au/poptools) to randomly resample the population-wide observations of mate guarding or copulating (899 observations, involving 147 different males). For each core group, we re-sampled the population-wide data the same number of times we observed mate guarding or copulating in the group (i.e. anywhere from 2 to 60 times), and counted the number of different males that occurred in each re-sample. We replicated this resampling procedure 1000 times and used these data to generate a distribution of the number of different males, expected by random chance, to mate guard or copulate in each core group. In order to evaluate significance, we calculated the mean and 95% confidence limits of each random distribution for each core group, and then compared these distributions to the observed number of different males guarding or copulating in each group. In order to test whether females from the same core group had offspring sired by fewer males than expected by chance, we repeated the same procedure as for mate guarding and copulating, but restricted our analysis to the 29 core groups where we assigned paternity for at least two calves, and we randomly sampled from the list of 42 males who sired 152 offspring.

The second simulation tested whether male elephants were more likely to breed in some core groups than others. To do this we, used Monte Carlo randomization simulations to generate a distribution of the number of different core groups in which we expected each male to mate guard, copulate, or sire offspring, given the number of times those events occurred in each core group over the study period. We then compared these random expectations to the
observed number of different core groups where each male 
guarded, copulated, or sired offspring. Our hypothesis was 
supported if the observed number of different core groups 
was less than random expectations. Specifically, we used 
POPTOOLS to randomly resample the 899 population-wide 
observations of mate guarding or copulating. For each 
male, we re-sampled the population-wide data the same 
number of times we observed him mate guarding or 
copulating (i.e. anywhere from 2 to 56 times), and counted the 
number of different core groups that occurred in each re-sample. We replicated this re-sampling procedure 1000 
times and used these data to generate a distribution of the 
number of different core groups we expected each male to 
be observed mate guarding or copulating. In order to 
evaluate significance, we calculated the mean and 95% 
confidence limits of each random distribution for each 
male, and then compared these distributions to the 
observed number of different core groups we actually 
observed males guarding or copulating. In order to test 
whether males sired offspring with females from fewer 
core groups than expected by chance, we repeated the 
same procedure as for mate guarding and copulating, but 
restricted our analysis to the 29 males who sired at least 
two calves.

Relatedness within age cohorts. In order to test whether 
cohorts of paternal relatives occurred across the population, 
we correlated pairwise genetic relatedness — excluding 
pairs of animals known to come from the same natal core 
group — with difference in age in years (N = 526 individuals 
with known age involved in 149 039 unique pairs). All 
pairwise genetic relatedness values were estimated using 
the program KINSHIP (version 1.3.1, Goodnight & Queller 1999), which uses Queller & Goodnight’s (1989) relatedness 
estimator. We previously determined that this was the best 
kinship estimator for our data (Archie et al. 2007); allele 
frequencies were based on genotypes for all 545 
individuals genotyped from the population. We calculated 
difference in age as an absolute value by subtracting the 
birth years of both animals. Pairs whose age difference was 
zero were born in the same year.

Analyses of population structure. In order to understand 
how genetic variation was distributed within the study 
population, we first used Bayesian assignment techniques 
to test for population structure using the program 
STRUCTURE (version 2.2, Pritchard et al. 2000). This method 
identifies clusters of genetically similar individuals from 
multilocus genotypes without prior knowledge of their 
genetic relationships. The model assumes K genetic 
clusters, each characterized by a set of allele frequencies at 
each locus; the admixture model then probabilistically 
estimates the proportion of individuals with ancestry in 
each cluster. We ran a series of pilot runs to estimate 
Pr(X | K), where X represents the data for K between 1 (the 
extected value if all individuals belong to the same cluster) 
and 53 (the number of social groups in the population in 
2005). From these initial runs, we determined that we only 
had power to detect a maximum of about 17 clusters; Ln 
Pr(X | K) never stabilized even after a Markov chain Monte 
Carlo (MCMC) burn-in period of 1 million. This lack of 
power only allowed us to investigate more broad-scale 
structure, above the level of core social groups. In our final 
runs to determine the most likely K, we assumed that 
populations had correlated allele frequencies, inferred 
alpha from the data, and used a burn-in and MCMC of 
200 000 followed by 1 000 000. Longer burn-in or MCMC 
did not change the results. Because the most likely K was 
not clearly defined (see results section), we used ΔK to 
identify the most likely K, according to the method of 
Evanno et al. (2005).

We also investigated population genetic structure using 
an analysis of molecular variance (AMOVA), as imple-
mented by ARLEQUIN (version 3.01, Excoffier et al. 2005). 
We confined this analysis to the 195 adult females and 142 
calves living in the 21 best genotyped core groups during 
2005 (i.e. the year with most complete genotypes, where we 
genotyped at least three calves and three adult females). 
For three partitions of the data — all adult females, all adult 
females and calves, and all calves alone — we determined 
the degree of correlation among genotypes using hierar-
chical estimates of Φ, which are analogous to Wright’s 
(1931) F-statistics. Specifically, we measured how variation 
was partitioned between core groups in the population 
(ΦST), within individuals relative to the core group (ΦRE), 
and within individuals relative to the population (ΦRT). We 
evaluated the significance of these genetic structures using 
the permutation procedure contained within ARLEQUIN.

We also examined the relationship between average 
pairwise genetic relatedness within the 21 best genotyped 
core groups in 2005 and genetic differentiation between 
core groups in 2005, using linear regression. Average 
pairwise genetic relatedness among adult females was 
estimated using KINSHIP software as described above. We 
estimated genetic differentiation via FST between all pos-
sible pairs of core groups in the population using Weir & 
Cockerham’s (1984) method implemented in GENEPOP 
(version 3.4; Raymond & Rousset 1995).

Finally, poaching in elephants is age biased, and this is 
likely to have strong effects on social and genetic structure 
(Poole 1989a; Ishengoma et al. 2007). In order to simulate 
the effects of age-biased poaching on the genetic structure 
of core social groups, we repeated our AMOVA analysis on 
the same data set described above, but excluded all adult 
females who were over 30 years old and their dependent 
calves (less than 4 years old). While this estimate is a 
relatively conservative measure of the effects of poaching — 
poachers often kill many more than just the oldest
individuals, and it doesn’t include the possible change in reproductive patterns with the loss of large old males (Ishengoma et al. 2007)—we felt this was a simple way to investigate the effects of age-biased poaching. Poachers often remove the oldest individuals first because they have the largest tusks, and 30 was an appropriate age cut-off as animals over 30 are often conspicuously missing from poached populations (Eltringham & Malpas 1980; Hall-Martin 1980; Poole 1989a; Moss 1990; Barnes & Kapela 1991; Aleper & Moe 2006). For instance, intense poaching in Tarangire National Park, Tanzania, in the late 1970s to the mid 1980s eliminated all males over 30 years old (Moss 1990). In Kidepo Valley National Park, only 18% of individuals were over 25 years old (Aleper & Moe 2006).

Results

Mating behaviour does not increase co-ancestry within core groups of elephants

Average pairwise genetic relatedness within core groups of adult female elephants was 0.15 (Archie et al. 2006b). Female matrilocality alone may have been sufficient to generate kinship among female group members. However, elephants may also generate co-ancestry within core social groups if adult females in the same core group breed with a smaller number of males than expected by chance—either through mate choice or coordinated oestrus. In addition, if elephants are like breeding group species, they may also generate co-ancestry within core groups if males sire offspring more often in some core groups than others. However, we found no support for either of these nonrandom patterns of mating behaviour; groups of females were guarded and copulated by the same number of males as expected by random chance, given the number of times each male was observed performing these behaviours across the entire population (Fig. 1). For instance, over the study period, we observed, on average, 16.46 guarding and copulating episodes in each core group (SD = 14.18, median = 12, range = 2–60). On average in each group, 86.86% of these episodes were distributed across different males (SD = 11.82%, median = 86.33%, range = 70% to 100%), and the number of different males almost always fell within the expected confidence limits for random expectations. Open circles that lie outside these confidence limits indicate social groups who engaged in sexual behaviour or conceived offspring with a larger or smaller number of males than expected by random chance; almost all groups fit random expectations.

For instance, over the study period, each male was observed guarding and copulating, on average, 8.92 times (N = 95 males who were observed guarding or copulating at least twice, SD = 9.47, median = 5, range = 2–56). On average paternity to an average of five offspring per core group (SD = 3.44, median = 4, range = 2–15), and on average in each core group, 95.01% of calves were sired by different males (SD = 8.36%, median = 100%, range = 75% to 100%). The number of different males siring offspring in core groups almost always fell within the expected confidence limits, as generated by Monte Carlo simulations (Fig. 1b), and across all social groups, there was no difference between observed and expected number of males siring offspring in groups (chi-squared test, d.f. = 28, \( \chi^2 = 1.67, P > 0.5 \)).

Furthermore, in all 29 social groups where paternity was known for at least two calves, the number of males who sired offspring within each core groups also fit random expectations, given the number of times a given male sired offspring across the population. We assigned paternity to an average of five offspring per core group (SD = 3.44, median = 4, range = 2–15), and on average in each core group, 95.01% of calves were sired by different males (SD = 8.36%, median = 100%, range = 75% to 100%). The number of different males siring offspring in core groups almost always fell within the expected confidence limits, as generated by Monte Carlo simulations (Fig. 1b), and across all social groups, there was no difference between observed and expected number of males siring offspring in groups (chi-squared test, d.f. = 28, \( \chi^2 = 1.67, P > 0.5 \)).

Not only did core groups of females not breed with a smaller number of males than expected by chance, but also, males did not breed with a smaller number of core groups than expected by chance. Instead, males guarded, copulated, and sired offspring in the same number of different core social groups as expected by random chance, given the number of times those groups appeared in the data (Fig. 2). For instance, over the study period, each male was observed guarding and copulating, on average, 8.92 times (N = 95 males who were observed guarding or copulating at least twice, SD = 9.47, median = 5, range = 2–56). On average
for each male, 88.00% of those guards and copulations were distributed across different core groups (SD = 13.92%, median = 92.31%, range = 51.79% to 100%), and this number of different core groups almost always fell within the expected random confidence limits for each male, as generated by Monte Carlo simulations (Fig. 2a). Across all males, there was no difference between observed and expected number of core groups in which males guarded or copulated females (chi-squared test, d.f. = 94, $\chi^2 = 8.21$, $P > 0.5$).

Finally, males were not more likely to sire offspring in some core social groups than others. We assigned paternity to an average of 4.83 offspring per male (N = 29 males who sired at least two offspring, SD = 3.47, median = 4, range = 2–14), and on average, 95.46% of each males’ calves were sired in different core social groups (SD = 9.49%, median = 100%, range = 71.43% to 100%), and this number of different core groups almost always fell within the expected confidence limits, as generated by Monte Carlo simulations (Fig. 2b). Across all males, there was no difference between observed and expected number of core groups where males sired offspring (chi-squared test, d.f. = 28, $\chi^2 = 1.88$, $P > 0.5$).

Male-mediated gene flow creates cohorts of paternal relatives across the population

Male elephants sire offspring across multiple social groups, and tend to remain at their peak reproductive success for about 5–10 years, between about 40 or 50 years of age (Hollister-Smith et al. 2007). Consequently, we hypothesized that male reproductive peaks would create cohorts of similar-aged paternal relatives across all core groups. In support, pairs of elephants (excluding those from the same core group) who were closer in age were more closely related (Fig. 3; N = 148 807 pairs, $r^2 = 0.000042$, $F = 6.3053$, $P = 0.0120$). While elephants were more closely related to animals in their own age cohort than to those in other cohorts, individuals also appeared to be somewhat more closely related to animals from their fathers’ age cohorts (i.e. their fathers and paternal uncles). For instance, the relationships in Fig. 3 suggest the possibility that relatedness was highest among pairs of individuals that were relatively close in age, but was also high among pairs that were 40–50 years apart in age. Indeed, if we limit the regression to pairs of animals that were no more than 41 years apart in age (i.e. the average age difference between fathers and offspring in our data set; N = 138 825 pairs, $r^2 = 0.00015$, $F = 10.5801$, $P < 0.0001$).
function \( (N = 138 \, 825 \, \text{pairs}, \linear\text{regression} \; r^2 = 0.00002, \quad F = 2.7099, \quad P = 0.0997; \) quadratic function \( r^2 = 0.00015, \quad F = 10.5801, \quad P < 0.0001). \)

**Fine-scale genetic structure in elephant populations is age related**

A **structure** analysis of the 526 genotyped elephants in Amboseli did not support panmixia; \( K = 1 \) was the least likely number of distinct genetic clusters; however, **structure** did not reveal striking population genetic differentiation above the level of the core group. The likelihood distribution of \( K \) increased from \( K = 1 \) through 3, and then gradually levelled off and plateaued at around \( K = 13 \). The method of **Evanno** \textit{et al.} (2005) indicated that the most likely \( K \) (i.e. \( K \) with the sharpest change in curvature, or \( \Delta K \)) was 3; however, we could only assign 15\% \( (N = 69) \) of the 526 individuals to any of these three clusters with more than 90\% confidence.

Because we did not have enough statistical power to use **structure** to test genetic variation among elephant core social groups, we conducted an **AMOVA** among the 21 core groups of elephants with the most complete genotypes in 2005 (the year with most complete genotypes). Core social groups of adult females were moderately genetically differentiated; global \( \Phi_{ST} \) indicated that around 5\% or 6\% of the variation in allele frequencies was partitioned between core social groups of adult females (with and without their calves) and these fractions were significantly greater than zero (Table 1). This genetic differentiation was driven by co-ancestry among group members. That is, groups of closer kin had higher average pairwise \( F_{ST} \) values with the other groups in the population, as compared to groups of less closely related females (linear regression, \( r^2 = 0.26, \quad F = 144.35, \quad P < 0.0001; \) Fig. 4). In addition, as is typical of many breeding group populations, fixation indices also reflected unusually high levels of heterozygosity within elephant groups, given their co-ancestry. Global \( \Phi_{TS} \) among adult females, or adult females and their calves, was significantly less than zero (adult females \( \Phi_{TS} = -0.0769, \) adult females and calves \( \Phi_{TS} = -0.0690; \) Table 1).

In breeding group populations, female matrilocality and the fact that males tend to sire more offspring in some social groups than others, create significant genetic differentiation between offspring from different social groups (Pope 1992) — sometimes with more structure between offspring than adults (van Staaden 1995; Dobson \textit{et al}. 1998). In contrast, we did not find significant genetic differentiation among elephant core groups if we only considered the calves living in core social groups in 2005 (age range 0–14 years, average difference in age ± SD = 3.75 ± 2.86); the variation in population allele frequencies among offspring partitioned across social groups was not significantly different than zero (Table 1). This probably occurred because calves that were paternal siblings were distributed across social groups — reflecting substantial male-mediated gene flow between groups — and because most calves living together as immatures were also not maternal siblings; female elephants give birth to a calf once every 4–6 years, and because females mature and male elephants disperse at around age 14, most calves living in the same

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**Table 1** **AMOVA** results describing how genetic differentiation is partitioned among 21 core social groups

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<th>Source of variation</th>
<th>d.f.</th>
<th>Variance components</th>
<th>( \Phi )</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Adult females only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among core groups ( (\Phi_{ST}) )</td>
<td>20</td>
<td>0.2627</td>
<td>0.0583</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Among individuals within core groups ( (\Phi_{TS}) )</td>
<td>174</td>
<td>-0.3264</td>
<td>-0.0769</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Within individuals ( (\Phi_{TT}) )</td>
<td>195</td>
<td>4.5692</td>
<td>-0.0141</td>
<td>0.9539</td>
</tr>
<tr>
<td>Adult females and calves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among core groups ( (\Phi_{ST}) )</td>
<td>20</td>
<td>0.2207</td>
<td>0.0495</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Among individuals within core groups ( (\Phi_{TS}) )</td>
<td>316</td>
<td>-0.2924</td>
<td>-0.0690</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Within individuals ( (\Phi_{TT}) )</td>
<td>337</td>
<td>4.5326</td>
<td>-0.0161</td>
<td>0.9924</td>
</tr>
<tr>
<td>Calves only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among core groups ( (\Phi_{ST}) )</td>
<td>20</td>
<td>0.0967</td>
<td>0.0264</td>
<td>0.9936</td>
</tr>
<tr>
<td>Among individuals within core groups ( (\Phi_{TS}) )</td>
<td>121</td>
<td>-0.1278</td>
<td>-0.0359</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Within individuals ( (\Phi_{TT}) )</td>
<td>142</td>
<td>3.6901</td>
<td>-0.0085</td>
<td>0.7721</td>
</tr>
<tr>
<td>Simulated poaching (no females over 30 years old)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among core groups ( (\Phi_{ST}) )</td>
<td>20</td>
<td>0.1592</td>
<td>0.0359</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Among individuals within core groups ( (\Phi_{TS}) )</td>
<td>230</td>
<td>-0.2317</td>
<td>-0.0541</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Within individuals ( (\Phi_{TT}) )</td>
<td>251</td>
<td>4.5116</td>
<td>-0.0163</td>
<td>0.9775</td>
</tr>
</tbody>
</table>

\( P \) values were obtained by comparisons of observed values with those generated by random permutation in **ARLEQUIN** (version 3.01). d.f. represents the degrees of freedom in each analysis. \( \Phi \)-statistics are analogous to Wright’s (1931) \( F \)-statistics and identify the correlation among alleles at each of the hierarchical levels.
In order to understand how age-biased poaching may impact the distribution of genetic variation within populations, we performed an ANOVA on the 21 core groups of females and their calves with the most complete genotypes, but excluded all social group members (i.e. adult females) over 30 years old. This simulated poaching eliminated, on average, the oldest 26% of group members. As expected, it reduced the average pairwise genetic relatedness within and genetic differentiation between core social groups; average pairwise relatedness between the members of ‘poached’ core groups was 0.0726 (SE ± 0.004, N = 1594 pairs), which was significantly lower than the average pairwise genetic relatedness among the members of intact groups (average $R$ in ‘unpoached’ groups = 0.0937, SE ± 0.0036, N = 2830, $F = 12.5318, P < 0.001$). Poaching also reduced genetic differentiation between core groups; $\Phi_{ST}$ between ‘poached’ core groups was 0.0359, which was significantly greater than zero, but less than in intact core groups of adult females and calves (Table 1).

### Discussion

The fine-scale genetic structure we observed in the Amboseli elephant population was created by patterns of mating and dispersal. Female matrilocality built co-ancestry within core groups and led to significant genetic differentiation between core groups in their entirety, that is, intact lineages of adult female relatives and their calves, whose ages spanned 60 years or more. In addition, gene flow between core groups, mediated most strongly by 40–50 years old males in their reproductive prime, created cohorts of similar-aged paternal relatives in different core groups across the population. This gene flow reduced or erased genetic differences between core groups if we only considered elephants that were around the same age: $\Phi_{ST}$ between similar-aged calves from different core social groups was not significantly different than zero. Finally, the age-dependent nature of the fine-scale genetic structure in our study population, combined with the fact that poaching tends to eliminate the oldest elephants from populations, indicates that illegal hunting and poaching will tend to erode fine-scale genetic differences between female social groups in elephant populations.

**Fine-scale population genetic structure in elephants and other social mammals**

Fine-scale population genetic structure is common in social animals, and among the best-characterized are ‘socially structured’ or ‘breeding group’ mammals where matrilocal females form stable social groups and breed with a subset of males that form permanent or semi-permanent associations with female groups (reviewed in Sugg 1996; Storz 1999). Such social organization creates high co-ancestry within social groups, genetic differentiation between groups, and higher than expected heterozygosity among group members. The most extreme examples are found in black-tailed prairie dogs and red howler monkeys where as much as 23% of the genetic variation in populations occurs between social groups (Chesser 1983; Pope 1992; Dobson et al. 1998; Pope 1998). However, the majority of breeding group societies have more moderate genetic structure where genetic differentiation between social groups ranges from 4% to 11%. Such species include a number of cercopithecine primates, lions, Richardson’s ground squirrels, yellow-bellied marmots, vampire bats, and rabbits (Schwartz et al. 1980; Turner 1981; Kawamoto...
permanent or semi-permanent breeding associations with breeding groups. In breeding group species, paternal... within populations depends partly on demography and population density. For instance, in red deer, a release from hunting led to a more even distribution of mating opportunities across the population, and fine-scale genetic differences between herds consequently declined from 4% to nearly 1% (Nussey et al. 2005). However, in elephants we did not find evidence that nonrandom mating behaviour increased gene correlations within groups and genetic differences between groups—at least on the scale of female groups living in and around Amboseli National Park.

Elephants also appear to differ from breeding group species in that the fine-scale population genetic structure we observed was age dependent. That is, the degree of differentiation across social groups depended upon the age difference between the individuals involved; genetic differentiation among animals more similar in age, from different core groups, was less than between intact social groups. This age-dependent fine-scale genetic structure seems to be a consequence of the fact that, in natural and intact elephant populations, males sire offspring across multiple core groups of females and tend to do so during an extended high-fertility period in their 40s and 50s (Hollister-Smith et al. 2007). This age-dependence at the level of the entire population differs from many breeding group species, where offspring living in the same group are significantly genetically differentiated from those in other groups (Pope 1992; van Staaden 1995; Dobson et al. 1998; Pope 1998). In breeding group species, genetic structure among offspring is partly due to female matrilocality, but also to permanent or semi–permanent associations between males and female social groups, which create gene correlations among offspring from the same group and genetic differentiation between groups. We hypothesize that age-dependent genetic structure may occur in other long-lived species where males wait in a queue to reproduce and breed across the population, including sperm whales, perhaps other cetaceans, or ungulates where males have a discrete period of reproductive dominance and sire multiple offspring across many female ranges.

Evolutionary and conservation genetic implications of elephant genetic structure

Fine-scale genetic structure within elephant populations has at least three potentially important consequences. It
may (i) determine opportunities for kin selection, (ii) intensify founder effects if populations are fragmented, and (iii) influence the rate at which genetic diversity is lost from populations through genetic drift. Of these, the opportunities for kin selection are best understood — especially for adult female elephants (Dublin 1983; Moss & Poole 1983; Lee 1987; Moss 1988; Archie et al. 2006a, b). Kin selection has the potential to influence female relationships, as adult females living in the same core social group are moderately closely related and females spend most of their lives together with their first-order maternal relatives (Archie et al. 2006b). The results we present here confirm our hypotheses about patterns of paternal relatedness in elephant populations. Because males reach their peak reproductive success between 40 and 50 years of age and tend to sire offspring in multiple social groups across the population (Hollister-Smith et al. 2007), an individual’s closest paternal relatives tend to be distributed across social groups in the population, and tend to be similarly aged paternal siblings or paternal aunts and uncles from their father’s age cohort. It is unknown whether elephants form special relationships with their paternal kin; however, at the very least, elephants appear to be able to recognize and avoid inbreeding with their paternal relatives (Archie et al. 2007). One set of relationships where paternal kinship may be most likely to play a role, because these relationships involve interactions between individuals from several social groups, are nonrandom associations between male elephants. Because maternal, and possibly paternal, kinship is an important component of elephant social relationships, conservation strategists and managers should strive to keep natural elephant social organization intact (Slotow et al. 2000; Nyakaana et al. 2001; Bradshaw et al. 2005).

In addition to determining opportunities for kin selection, fine-scale genetic structure — especially the genetic differences between core social groups — has the potential to intensify founder effects (Templeton 1980; Storz 1999). For instance, if a single social group colonizes new habitat, the allele frequencies in that group will probably not accurately reflect allele frequencies in the group’s original population. This genetic structure may intensify the evolutionary change that occurs as a result of the founding event (Templeton 1980; Storz 1999). Emigration of whole core groups of females is probably unusual in natural populations of elephants, as genetic evidence suggests that gene flow between populations is male biased (Nyakaana & Arctander 1999). However, in Amboseli, at least one core social group is thought to be a migrant from a neighbouring population, as the members of this group all share a haplotype that is unique in Amboseli but shared with elephants in northern Kenya (EA Archie, CL Fitzpatrick, CJ Moss, SC Alberts, unpublished). In addition, fine scale genetic structure in elephant populations has important implications for conservation, as translocations of wild elephants risk creating populations with low genetic diversity that do not necessarily reflect the genetic structure of a natural elephant population.

Finally, fine-scale genetic structure in elephant populations may influence the loss of genetic variation due to genetic drift. When population geneticists first considered the genetic structure of social species, many assumed that the division of populations into genetically distinct social groups accelerated the loss of genetic diversity (Bush et al. 1977; Baker et al. 1980; Templeton 1980). This was because social groups were thought to act like small populations where alleles were lost rapidly due to inbreeding and genetic drift. However, this is not the case and instead, inbreeding is usually prevented by sex-biased dispersal, and $F_{st}$ within social groups is almost always negative (Melnick 1987; Sugg 1996; Storz 1999). Hence, an alternative view is that breeding group structure actually slows the loss of genetic diversity from populations (Chesser et al. 1993; Sugg 1994; Sugg 1996). In support, the effective population size of breeding group populations may sometimes be larger than the census size (Chesser et al. 1993; Sugg 1994; Sugg 1996), and if genetic substructure decreases, genetic diversity may be lost from populations (Dobson et al. 2004). For instance, if genetic differences between social groups were lost, then the risk of losing alleles due to genetic drift would increase (Dobson et al. 2004). This result is especially relevant for elephants, as illegal hunting erodes fine-scale genetic structure in elephant populations. In poached populations, older animals are lost and natural social groups are destroyed — both of which lead to smaller, less genetically structured populations. Hence, it is possible for genetic diversity to be lost from poached populations more rapidly than from intact populations — even if those populations have equal census sizes. Perhaps more important is the loss of large, and therefore old, breeding males, which may reduce genetic diversity by increasing the reproductive tenure of younger males (Poole 1989a; Ishengoma et al. 2007). Illegal elephant hunting and limited trade in ivory is increasing in Africa, despite the ivory ban (Stiles & Martin 2001; Martin 2005; Wasser et al. 2007); our results would strongly support conservation efforts that reduce poaching and keep elephant social organization intact.

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References


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