Behavioural inbreeding avoidance in wild African elephants

ELIZABETH A. ARCHIE,* JULIE A. HOLLISTER-SMITH,† JOYCE H. POOLE,‡ PHYLLIS C. LEE,§ CYNTHIA J. MOSS,‡ JÉSUS E. MALDONADO,* ROBERT C. FLEISCHER* and SUSAN C. ALBERTS¶
*Center for Conservation and Evolutionary Genetics, NZP/NMNH, Smithsonian Institution, Washington, D.C., USA, †Oregon Health & Science University, Portland, OR, USA, ‡Amboseli Trust for Elephants, Nairobi, Kenya, §Department of Psychology, University of Stirling, UK, ¶Department of Biology, Duke University, Durham, NC, USA

Abstract

The costs of inbreeding depression, as well as the opportunity costs of inbreeding avoidance, determine whether and which mechanisms of inbreeding avoidance evolve. In African elephants, sex-biased dispersal does not lead to the complete separation of male and female relatives, and so individuals may experience selection to recognize kin and avoid inbreeding. However, because estrous females are rare and male–male competition for mates is intense, the opportunity costs of inbreeding avoidance may be high, particularly for males. Here we combine 28 years of behavioural and demographic data on wild elephants with genotypes from 545 adult females, adult males, and calves in Amboseli National Park, Kenya, to test the hypothesis that elephants engage in sexual behaviour and reproduction with relatives less often than expected by chance. We found support for this hypothesis: males engaged in proportionally fewer sexual behaviours and sired proportionally fewer offspring with females that were natal family members or close genetic relatives (both maternal and paternal) than they did with nonkin. We discuss the relevance of these results for understanding the evolution of inbreeding avoidance and for elephant conservation.

Keywords: African elephant, inbreeding, kin recognition, mate choice, microsatellites, relatedness

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Introduction

Mating with close relatives often leads to inbreeding depression; in many wild and captive populations, inbred offspring have lower survival or fertility and consequently lower fitness (reviewed in Pusey & Wolf 1996; Crnokrak & Roff 1999; Keller & Waller 2002; Edmands 2006). As a result, behavioural mechanisms of inbreeding avoidance have evolved in many species, and in mammals at least three such mechanisms may reduce the costs of inbreeding depression. First, individuals may delay maturation and/ or suppress their own reproduction when kin are available as mates (Pusey *et al.* 1996; O'Riain *et al.* 2000). Second, dispersal may be sex-biased; female mammals are usually matrilocal, while males tend to disperse and do not return to breed in their natal group or territory (Greenwood 1980; Pusey 1987). Third, individuals may use kin recognition — either by familiarity or phenotype matching — to avoid

Correspondence: E.A. Archie, Smithsonian Genetics Program, 3001 Connecticut Ave. NW, Washington, D.C. 20008, USA. Fax: (202) 673-4648, E-mail: archiee@si.edu

inbreeding (Porter *et al.* 1978; Holmes & Sherman 1982; Halpin 1991; Pusey *et al.* 1996; Alberts 1999; Mateo & Johnston 2000).

Although inbreeding depression may be costly, studies that demonstrate behavioural inbreeding avoidance in long-lived mammals are rare (e.g. Keane *et al.* 1996). One reason is that such studies require detailed information about the life histories and mating behaviour of many individuals, and these data are difficult to collect. However, in some cases, inbreeding avoidance may be more costly than inbreeding depression (Waser *et al.* 1986; Kokko & Ots 2006). In particular when mating opportunities are rare, reproductive success is highly skewed, or kin are difficult to identify, individuals may miss valuable chances to reproduce if they attempt to avoid mating with kin (Waser *et al.* 1986). Hence, the balance between the costs of inbreeding depression and the opportunity costs of inbreeding avoidance will determine whether and which mechanisms of inbreeding avoidance evolve.

Here we use this conceptual framework to investigate behavioural inbreeding avoidance in wild African elephants (*Loxodonta africana*), a long-lived mammal in which

male and female kin seem to encounter each other often, and where males must compete intensely for mates. Female elephants live in a fission–fusion social system, but for the most part remain with their natal social group, which is composed of around two to 20 female maternal kin (Douglas-Hamilton 1972; Moss & Poole 1983; Moss 1988; Archie *et al.* 2006). While females are matrilocal, males leave their natal family group at adolescence (Douglas-Hamilton 1972; Moss *et al.* 1983; Moss 1988). However, males do not join other social groups as permanent members. Instead, they travel freely and widely within and often between local populations in search of estrous females for breeding opportunities (Poole & Moss 1989). Males are attracted to the acoustic and olfactory signals of estrous females over great distances, and they search widely for estrous females, but they spend only brief periods — a few minutes to a few days — with any one social group of females (Poole 1989a, b; Poole & Moss 1989).

Although males leave their natal family at about 14 years of age, kin appear to be commonly available as mates in natural populations of elephants. Males are regularly re-sighted in their natal population — often for years or decades after they leave their natal group (Lee *et al.* in press; Moss 1988; Sukumar 2003; Moss and Poole, unpublished data) — and this creates opportunities for inbreeding. For instance, males may have the opportunity to return and breed with their mother, sisters, or other females in their natal family, although the extent to which they do this is as yet unknown. Moreover, paternal kin may be even more commonly available as mates than maternal kin. For instance, males will often still be reproductively active when their daughters mature (between 9 and 17 years of age) because males are reproductively active for several decades (if they survive that long), and male reproductive success increases with age (Poole 1989b; Moss 2001; Hollister-Smith *et al.* 2007). In addition, because males breed across several social groups, cohorts of paternal half-siblings occur across the population (Hollister-Smith *et al.* 2007). When those paternal kin mature, they presum-ably regularly encounter each other as potential mates. However, as with natal family members, it is unknown whether paternal kin from across the population are able to recognize each other and avoid inbreeding.

Although the availability of kin may result in selection for inbreeding avoidance, the costs of inbreeding depression may not be strong enough in elephants to overcome the opportunity costs of inbreeding avoidance. This is unlikely to be the case for female elephants, who invest heavily in each offspring, but it may be especially true for males, who provide no parental care and for whom reproductive skew is strong and breeding opportunities are rare (Douglas-Hamilton 1972; Moss *et al.* 1983; Poole 1989b; Lee & Moss 1999; Hollister-Smith *et al.* 2007). A given female enters estrus only about once every 4–5 years,

females can enter estrus throughout the year, and when there is an estrous female in the population she may be the only one available (Moss *et al.* 1983; Poole 1989b; Poole & Moss 1989). Estrous females are usually followed by five or more males, and in most cases only the largest and oldest males are able to mate at peak estrus (Poole & Moss 1989). Male elephants wait several years to reach their maximum reproductive success, which occurs around age 45 or 50 (Poole 1989b; Hollister-Smith *et al.* 2007). If males strongly avoid mating with maternal or paternal relatives, they may reduce their mating opportunities even further.

Here we measured patterns of inbreeding avoidance in a wild population of African elephants. Specifically, we tested whether males engaged in sexual behaviours and sired offspring with a smaller proportion of female kin than nonkin, and we did tests for three overlapping categories of female kin: (i) females who were the males' natal family members; (ii) female 'close kin' who had high pair-wise genetic relatedness with the males (R significantly > 0.25 giving rise to offspring with inbreeding coefficient (F) greater than or equal to 0.125); (iii) and a subset of these 'close kin' — females who could only be paternally related to the males and were not obviously familiar through social cues. Our results contribute to a general understanding of kin recognition and the evolution of inbreeding avoidance, and they also have consequences for the conservation of natural elephant populations. African elephant habitat is increasingly fragmented and isolated, and the degree to which elephants avoid inbreeding will partly determine the rate at which genetic diversity is lost from wild populations.

Methods

The study population

Research subjects were the elephants that live in and around Amboseli National Park, Kenya. This population has been studied continuously since 1972 by the researchers of the Amboseli Elephant Research Project (AERP; Moss 2001). Currently, the population numbers around 1400 elephants; all are individually recognizable from their physical features, which are recorded in a photographic database.

Although detailed information on dispersal are not yet available for elephant populations other than Amboseli, the Amboseli elephants represent a natural, intact population that is continuous with other elephant populations — especially to the south, southwest, and east (Western & Lindsay 1984; Grimshaw & Foley 1990; Moss 2001; Kioko *et al.* 2006). Elephants range freely within the protected area (390 km²) and well beyond its borders. Males from other populations are regularly seen in Amboseli, indicating that there are no barriers to movement between

Amboseli and its neighbouring populations, and that Amboseli is not in any sense isolated. Male elephants are not territorial and have larger home ranges than females; in this study, male home ranges encompassed the home ranges of all female social groups.

Our study focused on the adult males and females who were alive and sexually mature between 1977 and 2005 ($N = 677$, including 483 females and 194 males). We defined sexually mature females as those who had given birth (first birth occurs between 9 and 17 years of age). We defined sexually mature males as males that were 21 years of age or older, as this is the youngest age a male has been known to sire an offspring in Amboseli (see paternity analysis conducted in this study). Because female elephants are matrilocal, natal families were known for all females in the study; these females live with their immature offspring in approximately 53 family groups, which remained fairly constant across the study period. Because males disperse at around age 12, natal families were only known for males that dispersed after 1972 ($N = 96$ males).

All individuals were assigned an age. The ages of elephants born since 1975 ($N = 24$ males and 237 females in this study) were known to within 2 weeks, and the ages of elephants born between 1972 and 1975 ($N = 14$ males and 16 females in this study) were known to within 3 months. Because elephants continue to grow throughout their adult lives, the ages of elephants born before 1972 ($N = 156$ males and 230 females in this study) were estimated using a variety of techniques (see Moss 2001). These estimates were mainly based on well-documented patterns of variance in body size with increasing age; 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). Animals whose ages were estimated in 1972 are reassessed at death when jaws are found (from tooth ages), by using early photographs, and by constant reference to the maturational and growth changes among the known-aged sample.

Behavioural data collection

Researchers observed the elephants opportunistically and collected several different types of behavioural and demographic information with each sighting, including spatial association patterns, births, and deaths. Especially relevant to this study, since 1976, researchers have collected records of estrus and mating behaviour. Estrus lasts 4–5 days in female elephants, and researchers identify estrus with diagnostic behaviours: adult male elephants express much greater interest in estrous females — by smelling their genitals, urine and faeces, and attempting to mount — and estrous females exhibit an 'estrous walk' during which they move away from interested males, while glancing back over their shoulder (Moss *et al.* 1983). In contrast, nonestrous females ignore male interest and do not move away from males using the 'estrous walk'.

Whenever researchers observed a female in estrus, they recorded the identities of independent males (i.e. males that had permanently dispersed from their natal group), which were following her. In these 'estrus records', researchers also recorded whether any of the males guarded or successfully copulated with the estrous female. Guarding occurred when the male that was the closest mature male to the estrous female maintained this proximity by threatening or chasing all other males that approached the estrous female. Copulations occurred when the male covered the female from behind, and was often accompanied by ejaculation. We extracted all instances of these sexual behaviours from the AERP long-term records.

Genetic sampling and genotyping

The analyses described here used genetic samples from 545 individuals, including 256 adult females, 106 adult males, and 183 calves. These samples represent approximately 40% of the extant population (approximately 50% of adult females and males, and 20% of calves). Genotyping was conducted mainly from noninvasively collected faecal samples and from a few tissue samples. Sample collection and DNA extraction methods are described extensively in Archie *et al.* (2003, 2006). 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 QIAmp 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; Archie *et al.* 2003) and one dinucelotide locus (LaFMS02; Nyakaana & Arctander 1998). Polymerase chain reaction (PCR) amplification protocols are in Archie *et al.* (2003, 2006). In addition, we sequenced 672 bp of the mitochondrial control region for all adult males and females (primers were MDL3 and MDL5; Fernando & Lande 2000). PCR products were separated using either an ABI 3700 or ABI 3100 DNA Analyser. Sequences were analysed using SEQUENCHER software (version 4.1.2 Gene Codes) and microsatellite alleles were analysed using GENOTYPER 2.0 software (version 2.5, PE–Applied Biosystems).

To minimize genotyping errors, we conducted microsatellite genotyping according to the protocol described in Archie *et al.* (2006), which was a modified version of the multiple tubes approach (Taberlet *et al.* 1996). Whenever possible, individuals were genotyped from two or more faecal samples collected from independent defecations (89% of individuals). All heterozygote genotypes were replicated at least twice and all homozygote genotypes were replicated at least seven times. A given allele was assigned

to an individual only if it amplified at least twice during all replicates. Finally, Mendelian checks were conducted for all mother–offspring pairs, and all loci were in Hardy–Weinberg equilibrium.

Assigning parentage

Maternity was known from direct observation for all calves included in this study, as all were born after the onset of demographic data collection in 1972. In addition, elephants have a long period (e.g. 4 years) of maternal dependence, which enabled repeated sightings of mother–calf pairs over a number of years, and hence very accurate mother–offspring designations. Finally, maternity was confirmed via Mendelian checks for all mother–offspring pairs.

Of the 183 elephant calves for which we had complete genotypes, we were able to assign paternity to 152 elephant calves with known mothers, born between 1978 and 2003, using CERVUS software (version 3.0; Kalinowski *et al.* 2007). This sample 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 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 was 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.

Calculating pairwise genetic relatedness and identifying 'close kin'

The literature reports several estimators of pairwise genetic relatedness (Queller & Goodnight 1989; Li *et al.* 1993; Lynch & Ritland 1999; Wang 2002). These estimators vary in their assumptions, and their accuracy varies across data sets (Van De Casteele *et al.* 2001). In order to choose the most accurate estimator for our data, we compared four of the most common estimators (Queller *et al.* 1989; Li *et al.* 1993; Lynch *et al.* 1999; Wang 2002) using SPAGEDI software (version 1.2b; Hardy & Vekemans 2002). We calculated average pairwise genetic relatedness for 402 unique pairs of individuals from six known relationship categories and compared these estimates to their expected value (Table 1).

We found that there was no single best estimator. All estimators were relatively good at reproducing the expected pairwise genetic relatedness values, although Lynch & Ritland (1999) had the largest deviation between estimated and expected for four of the six relationship categories (Table 1). The Lynch & Ritland (1999) method also tended to have the highest standard deviation (smallest in zero of six cases), followed by Li *et al.* (1993; smallest in one of six cases), Wang (2002; smallest in two of six cases), and Queller & Goodnight (1989; smallest in three of six cases).

For our analyses, we chose to use Queller & Goodnight's (1989) estimator for two reasons: first, it was one of the best performers and was approximately equivalent to the other good performers, Li *et al.*'s and Wang's estimators, and second, the program KINSHIP (version 1.3.1; Goodnight & Queller 1999) allowed us to test the significance of pairwise genetic relatedness values among individuals. This latter quality is important because all relatedness estimators have wide confidence intervals — especially with only 11 microsatellite loci — and thus will result in erroneously high pairwise genetic relatedness values to some pairs of nonkin (and erroneously low values to some pairs of kin). The program KINSHIP allowed us to eliminate some of these erroneous assignments by conducting maximum-likelihood hypothesis tests that identify pairs with sufficient power to estimate genetic relatedness and evaluate the significance of a hypothesized relationship.

To estimate relatedness, allele frequencies were based on genotypes for all 545 individuals genotyped from the population. We defined '*close kin*' as any pair of individuals from across the population whose pairwise genetic relatedness was significantly ($P < 0.05$) greater than 0.25, and '*nonkin*' as any pair of elephants whose pairwise genetic relatedness was not significantly greater than 0.25. Our relationship category, *close kin*, should include most parents and offspring, some (but not all) siblings, and relatively few less-closely related pairs (e.g. excluding most with erroneously assigned high relatedness values), making it a conservative criterion.

Testing inbreeding avoidance

We tested inbreeding avoidance using two data sets. First, the sexual interactions data set included all observations of male sexual behaviours with females, collected between 1977 and 2005 (1976 was excluded because we had very little genetic information for these few records). These data consisted of records of males following, guarding, or copulating with estrous females. Based on these records, we assigned each instance of sexual behaviour to a given male–female pair. If a given male was seen following, guarding or copulating with a given female multiple times during the same estrous period (i.e. during the same 7 days), we recorded only one instance during the estrous

Table 1 Comparisons between the observed average and expected mean pairwise genetic relatedness (*R*) among six known relationship categories for four common estimators of genetic relatedness

Known relationship category	N (number of pairs)	Expected mean R	Estimation method	Observed mean R	SD	Difference in expected and observed mean R
Father/offspring	40	0.5	Li	0.526	0.096	0.026
			Lynch & Ritland	0.550	0.172	0.050
			Queller & Goodnight	0.522	0.094	0.022
			Wang	0.524	0.090	0.024
Mother/offspring	79	0.5	Li	0.481	0.085	0.019
			Lynch & Ritland	0.485	0.152	0.015
			Queller & Goodnight	0.475	0.097	0.025
			Wang	0.483	0.079	0.017
Paternal sibling	131	0.25	Li	0.252	0.142	0.002
			Lynch & Ritland	0.250	0.167	0.000
			Queller & Goodnight	0.237	0.137	0.013
			Wang	0.254	0.142	0.004
Maternal sibling	68	0.25	Li	0.218	0.126	0.032
			Lynch & Ritland	0.204	0.142	0.046
			Queller & Goodnight	0.210	0.124	0.040
			Wang	0.220	0.126	0.030
Grandmother	26	0.25	Li	0.250	0.111	0.000
			Lynch & Ritland	0.286	0.182	0.036
			Queller & Goodnight	0.249	0.133	0.001
			Wang	0.253	0.113	0.003
Aunt or uncle/niece or nephew	58	0.125	Li	0.123	0.139	0.002
			Lynch & Ritland	0.097	0.139	0.028
			Queller & Goodnight	0.111	0.129	0.014
			Wang	0.123	0.139	0.002

SD, standard deviation.

period. Second, the genetic paternity data set identified the mothers and fathers of 152 pairs of assigned parents.

We used each of these data sets, to test three hypotheses. First, we tested whether males avoided sexual interactions or siring offspring with adult female members of their natal family, where '*natal family members*' were defined as individuals that were born into the same social group as the male in question; '*non-natal family members*' were known to have been born into different social groups. Average pairwise genetic relatedness among *natal family members* in Amboseli is 0.14, but families contain a range of genetic relatives, from distantly related cousins to parents and offspring (Archie *et al*. 2006). Second, we tested whether males avoided sexual interactions or siring offspring with *close kin*, where *close kin* were defined as described above. Third, we tested whether males avoided inbreeding with '*close paternal kin*'. To do this, we tested for inbreeding avoidance in a subset of *close kin* that excluded all male–female pairs who could possibly be maternal relatives (i.e. pairs known to come from the same natal family, or when natal families were unknown, pairs that shared the same mitochondrial DNA haplotype). Thus, our definition of *close paternal kin* was conservative, only including animals with pairwise genetic relatedness significantly higher than 0.25 and no possible maternal relationship.

We tested these hypotheses using Wilcoxon signed rank tests. As an example, we describe one test of the first hypothesis in detail; all other tests follow a similar form. This first test asked whether males were less likely to follow estrous females from their own natal family as compared to non-natal family members. To do this, we calculated — for each male — the proportion of *non-natal family members* the male was observed following; that is, the number of times the male followed *non-natal family members*, divided by the male's total reproductive opportunities with *non-natal family members*. A male's total reproductive opportunities were defined as the total number of offspring born in the population while he was alive and sexually mature (i.e. from at least 21 years old). We then calculated the proportion of *natal family members* the male was observed following as the number of times the male followed a *natal family member*, divided by the male's total reproductive opportunities with *natal family members*. We then calculated the difference between these proportions for each male, and then used Wilcoxon signed rank tests to determine whether this proportion was significantly different from zero across all males (i.e. positive differences support inbreeding avoidance; males should follow a significantly higher proportion of his *non-natal family members* as compared to *natal family members*). In the text, we use the

Table 2 Tests of the hypotheses that males avoid sexual behaviours or siring offspring with different categories of relatives (i.e. *natal family members*, *close kin*, and *close paternal kin*). Wilcoxon signed rank tests were performed on the difference in proportion of sexual behaviours or offspring sired by males with different categories of kin vs. nonkin; inbreeding avoidance was supported by values that were significantly greater than zero

Testing the hypothesis that males avoid ...	Type of interaction	Number of males included in test	Observed % of male's sexual interactions that were with relatives	Median difference in the percentage of interactions with kin and nonkin (positive values indicate larger % with nonkin)	Wilcoxon signed rank W	P
natal family members	Following	63	3.91% (11 of 281)	0.36	479	< 0.0001
	Guarding	34	2.00% (2 of 100)	0.15	230	< 0.0001
	Copulating	26	0.00% (0 of 49)	0.19	175.5	< 0.0001
	Siring offspring	10	9.09% (2 of 22)	0.12	8.5	0.2160
close kin	Following	80	4.44% (32 of 720)	0.54	458	0.0140
	Guarding	60	5.60% (21 of 375)	0.03	239	0.0390
	Copulating	50	5.15% (7 of 136)	0.22	308.5	< 0.0010
	Siring offspring	41	3.29% (5 of 152)	0.25	244.5	< 0.0010
close paternal kin	Following	79	3.68% (19 of 516)	0.58	633	< 0.0100
	Guarding	54	4.65% (12 of 258)	0.50	352	< 0.0100
	Copulating	44	4.72% (5 of 106)	0.29	290	< 0.0001
	Siring offspring	36	3.85% (4 of 104)	0.32	195	< 0.0100

median to describe the difference in proportions because males always had many more *non-natal family members* and *nonkin* than *natal family members*, *close kin*, or *close paternal kin* and so the distribution of proportions was skewed.

We used a similar procedure to test the remaining hypotheses (e.g. differences in the proportion of guarding, copulating with or siring offspring with kin vs. nonkin). The sample sizes varied for each test because some behaviours were more common than others. For instance, copulations were less common than guarding or following. Sample sizes also varied depending on the category of kin we chose to test (*natal family members* vs. *close kin* vs. *close paternal kin*). This variability occurred because these categories of kin were naturally more or less common in the population (e.g. *close paternal kin* were a subset of all *close kin*) and because we did not have perfect knowledge of the natal families, or complete genotypes, for all elephants.

Results

Elephants avoid sexual behaviour with natal family group members

In support of the hypothesis that male elephants avoid sexual interactions with members of their natal families (i.e. maternal kin), males engaged in proportionally fewer sexual interactions with *natal family members* than *non-natal family members* (Wilcoxon signed rank tests; Table 2; Fig. 1). For example, during the study period, we observed 63 males with known natal families following estrous females. Each of these 63 males was observed following an estrous female at least once, and the largest number of
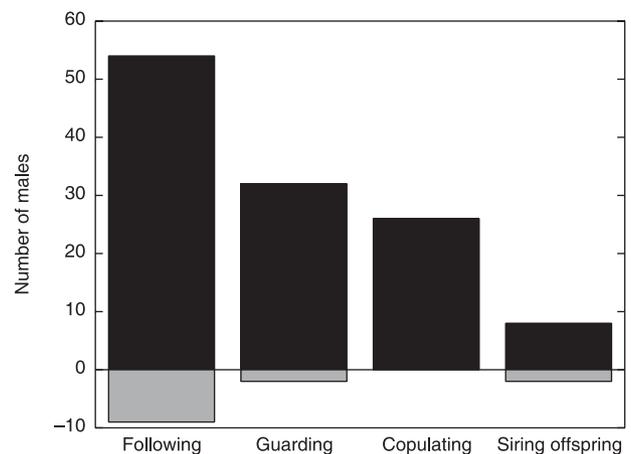

Fig. 1 The number of males who biased sexual behaviours towards *non-natal family members* (black bars, above the line), compared with the number of males who biased sexual behaviour towards *natal family members* (grey bars, below the line). Data for each category of sexual behaviour (following, guarding, copulating, and siring offspring) are depicted. In each case, a larger black bar than grey bar is evidence for inbreeding avoidance.

records of a male following different estrous females was 29 (mean number of follows per male = 4.46 ± 5.24 SD). Males followed proportionally fewer *natal family members* as compared to *non-natal family members*, and this was also the case for mate-guarding and copulations; males mate-guarded proportionally fewer *natal family members* than *non-natal family members*, and copulated with proportionally fewer *natal family members* than *non-natal family members* (Table 2, Fig. 1).

In further support, as sexual interactions were more likely to lead to reproduction, males tended to avoid natal family members more strongly — from following behaviour (least avoidance), through mate-guarding (intermediate avoidance), and copulating (highest avoidance). That is, 14.29% (nine of 63) of males with known natal families followed females from their own natal family, but only 5.88% (two of 34) of the males who mate-guarded, guarded an estrous female from their own natal family, and none of the males who copulated with estrous females were observed copulating with a *natal family member*.

While males appeared to avoid sexual interactions with *natal family members*, it was not clear whether males sired proportionally fewer offspring with *natal family members* than *non-natal family members*. We only knew the natal family for 10 assigned fathers, and two of these males fathered offspring with a member of their natal family. A Wilcoxon signed rank test indicated that there was no significant difference in the probability that males sired offspring with females from their natal vs. non-natal families (Table 2; Fig. 1); Although the power of this test is low because of small sample size, the median difference in offspring sired with *natal family members* vs. *non-natal family members* was in the expected direction.

Elephants avoid sexual behaviour and inbreeding with close genetic relatives

In support of the hypothesis that males avoid inbreeding by recognizing and avoiding their genetic relatives, males engaged in proportionally fewer sexual interactions with *close kin* than *nonkin* (Wilcoxon signed rank tests; Table 2; Fig. 2), where *close kin* were defined as pairs of animals whose pairwise genetic relatedness was significantly greater than 0.25 according to the program KINSHIP. For instance, 80 males with known genotypes were observed following estrous females. Each male followed an estrous female at least once and up to 49 times (mean number of follows per male ± SD = 9.01 ± 8.82). These 80 males followed *close kin* during proportionally fewer reproductive opportunities as compared to *nonkin*, and this was also the case for mate-guarding and copulating; males guarded proportionally fewer *close kin* than *nonkin*, and copulated with proportionally fewer *close kin* than *nonkin* (Table 2, Fig. 2). Furthermore, males' tendencies to engage in sexual behaviours with *close kin* decreased as behaviours were more likely to lead to reproduction. That is, 30% (24 of 80) of males who followed estrous females, followed *close kin*, and 26.67% (16 of 60) of males who ever guarded estrous females, guarded *close kin*, while only 14% (seven of 50) of males observed copulating ever copulated with *close kin*.

The results presented above demonstrate that male elephants avoided sexual interactions with close genetic
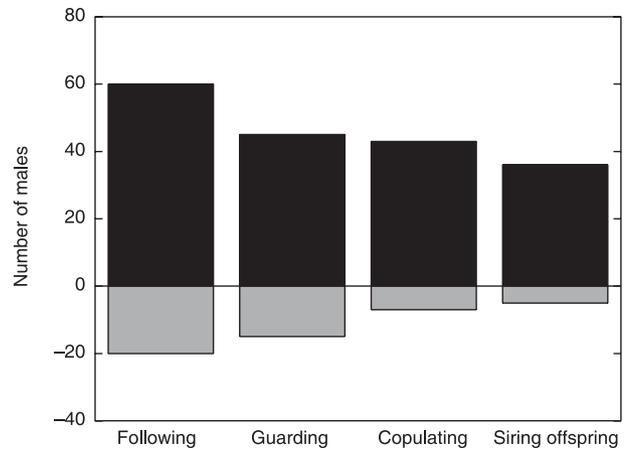

Fig. 2 The number of males who biased sexual behaviours towards *nonkin* (black bars, above the line), compared with the number of males who biased sexual behaviour towards *close kin* (grey bars, below the line). Data for each category of sexual behaviour (following, guarding, copulating, and siring offspring) are depicted. In each case, a larger black bar than grey bar is evidence for inbreeding avoidance.

relatives, and paternity data confirm that inbreeding avoidance behaviour meant that males sired proportionately fewer offspring with *close kin* than *nonkin* (Wilcoxon signed rank test; Table 2; Fig. 2). Only 3.29% (five out of 152) pairs of parents were *close kin*. Of these five pairs, one pair was a known father–daughter pair, and the relationship between the remaining pairs is unknown.

Elephants avoid inbreeding with paternal kin

We tested the hypothesis that males avoid inbreeding with their paternal kin by determining whether we could detect inbreeding avoidance in a test sample that excluded all pairs of animals that could be maternal kin (i.e. those from the same natal family and/or with matched mitochondrial DNA haplotypes). In support of the hypothesis that males recognize and avoid inbreeding with paternal kin, we found that males engaged in proportionally fewer sexual interactions with *close paternal kin* (Wilcoxon signed rank tests; Table 2; Fig. 3). For instance, 79 males followed estrous females who were *close kin* but could not be maternally related to the male. These males followed this subset of estrous females at least once and up to 49 times (mean number of follows per male ± SD = 6.53 ± 6.95). In support of paternal kin recognition, these 79 males followed *close paternal kin* during proportionally fewer reproductive opportunities as compared to *nonkin*, and this was also the case for mate-guarding and copulating behaviours; males guarded proportionally fewer *close paternal kin* than *nonkin*, and copulated with proportionally fewer *close paternal kin* than *nonkin* (Table 2, Fig. 3).
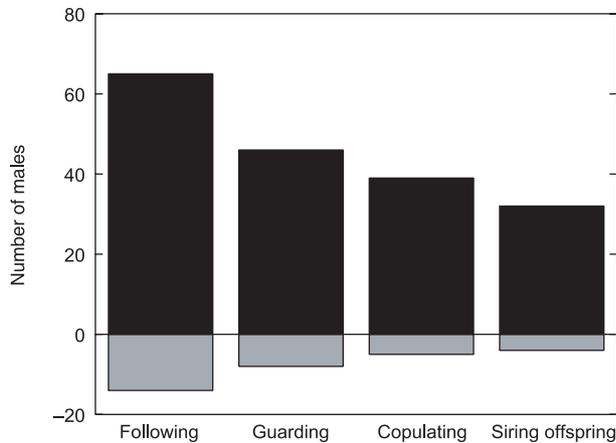
Fig. 3 The number of males who biased sexual behaviours towards *nonkin* (black bars, above the line), compared with the number of males who biased sexual behaviour towards *close paternal kin* (grey bars, below the line). Data for each category of sexual behaviour (following, guarding, copulating, and siring offspring) are depicted. In each case, a larger black bar than grey bar is evidence for inbreeding avoidance.

Furthermore, males' tendency to engage in sexual behaviours with *close paternal kin* decreased as behaviours were more likely to lead to reproduction. That is, 24.05% (19 of 79) of males ever observed following, followed *close paternal kin*, and 22.22% (12 of 54) of males ever observed mate-guarding, guarded *close paternal kin*, but only 11.36% (five of 44) of males we observed copulating, copulated with *close paternal kin*.

The behavioural results presented above suggest that elephants recognize and avoid inbreeding with their paternal relatives, and indeed only 3.85% (four of 104) pairs of parents were *close paternal kin*, and males sired proportionally fewer offspring with *close paternal kin* than *nonkin* (Wilcoxon signed rank test; Table 2; Fig. 3).

Discussion

In elephants, sex-biased dispersal does not lead to the complete separation of male and female kin, and as a result opposite-sexed relatives have the opportunity to interact, mate, and sire offspring. Because kin are available as mates, and because mating with kin may lead to inbreeding depression, elephants may experience selection for inbreeding avoidance. In support of this hypothesis, our results show that male elephants avoided engaging in sexual behaviours and siring offspring with their close genetic relatives. This was true for several categories of kin, including members of the males' natal families, close kin pairs (pairs whose pairwise genetic relatedness was significantly greater than 0.25), and close kin pairs who were related paternally.

Inbreeding depression and opportunity costs for male and female elephants

The result that male elephants avoided inbreeding is interesting in light of the fact that males compete intensely for mates and male reproductive success is strongly skewed (Poole 1989a, b; Poole & Moss 1989; Hollister-Smith *et al.* 2007). These factors predict high opportunity costs for males who strongly avoid inbreeding; yet our data indicate that males avoided inbreeding in spite of the fact that they may miss mating opportunities. One explanation for our results is that females may exert considerable control over mating outcomes. Across mammals, and especially in elephants, females are thought to experience lower opportunity costs of inbreeding avoidance and much higher fitness costs of inbreeding depression than males (Clutton-Brock & Harvey 1976; Waser *et al.* 1986). Females invest heavily in each offspring and the reproductive success of females does not appear to be strongly skewed. Female elephants might avoid inbreeding by resisting males and failing to cooperate with male kin who attempt to mate-guard and copulate; females run from undesirable males who attempt to follow, guard and mount them, and females must stand stationary for males to successfully copulate (Moss *et al.* 1983; Poole 1989b). However, although females are able to exert some mate choice, it is unknown exactly how much control females have. Male reproductive dominance is determined by age, size, and musth; musth males are able to dominate all other non-musth males in the population, large and old males stay in musth the longest, and dominance among musth males is determined by age and size (Moss 1988; Poole 1989a, b; Poole & Moss 1989; Hollister-Smith *et al.* 2007). The most reproductively successful males can be twice the mass of a female, and females can be harassed and worn down by persistent males (Moss *et al.* 1983). Hence, sexual dimorphism, and intense male–male competition make it difficult to know exactly how much control females have over whom they mate with.

Another explanation for our results is that the costs of inbreeding are higher for males than previously thought. In support, males appeared to avoid inbreeding, even during following behaviour where females probably have the least control. Males may avoid inbreeding because the opportunity costs of inbreeding avoidance may be outweighed by the costs of competing for access to reproductive females. Competition among males for mates is intense. Males occasionally kill each other over mating contests, and certainly risk injury when they compete for females in estrus (Moss 1988; Poole 1989a). Consequently, each offspring that a male sires may represent a real risk to his survival or health, and the fitness payoff of producing an inbred offspring may not be sufficient to overcome these costs.

Mechanisms of kin recognition in elephants

In our study, male elephants avoided sexual interactions with natal family members and especially with close kin in those families. To achieve this avoidance, they may follow simple mate choice rules based on familiarity: that is, 'avoid members of your own natal family' and 'avoid your mother and her offspring even more.' However, we also found that males avoided paternal kin, which appear to have very few, if any, social cues to genetic relatedness; hence it would be much more difficult for males to avoid paternal kin by following simple social rules. For instance, males might avoid mating with their daughters if they followed a rule that states, 'avoid the daughters of females you mated with.' Since males are likely to contact their daughters' social groups at a similar frequency to all other social groups, this rule necessitates that males remember all the females they mated with, and those females' daughters. Males who followed this rule might exclude many nonkin because females mate with multiple males during an estrous period so a male's copulations are not necessarily good predictors of his paternity (Poole 1989b; Hollister-Smith *et al*. 2007). Similarly, males might avoid paternal siblings by following an age-proximity rule that states: 'avoid all females that were born in my same age cohort.' However, this cohort is fairly broad (e.g. 10–15 years) because successful males are reproductively active for many years (Hollister-Smith *et al*. 2007; Poole 1989b). In addition, because many males are breeding in the population at the same time (and hence most members of the same age cohort have different fathers), males who followed such a rule would probably exclude many nonkin as potential mates.

Because males avoid inbreeding with paternal kin, and simple social rules would be very crude, error-prone measures of paternal kinship, our results raise the possibility that males may also use phenotype matching to refine their ability to recognise and avoid their relatives. Such phenotype matching has been demonstrated in several other vertebrate species, and can occur through spatial, vocal, visual, or olfactory cues (Holmes *et al*. 1982; Blaustein *et al*. 1987; Price 1999; Mateo & Johnston 2000; Busquet & Baudoin 2005; Gerlach & Lysiak 2006). A growing number of studies implicate the major histocompatibility complex (MHC) in kin recognition (Manning *et al*. 1992; Brown & Eklund 1994; Hurst *et al*. 2001; Zelano & Edwards 2002; Rajakaruna *et al*. 2006). In elephants, olfactory cues in urine, including the MHC, as well as vocal cues might all contain information about genetic relatedness (Buss *et al*. 1976; Moss 1988; Rasmussen 1998; McComb *et al*. 2000, 2003; Rasmussen & Krishnamurthy 2000; Sukumar 2003); however, it is currently unknown whether either of these signals have sufficient variability and are closely correlated enough with relatedness to be sensitive cues for phenotype matching.

Implications for elephant conservation

Inbreeding and outbreeding can have important consequences for the conservation of natural populations (reviewed in Crnokrak *et al*. 1999; Keller & Waller 2002; Edmands 2006). The expansion of human populations, the subsequent loss of elephant habitat, and increasing rates of conflict between humans and elephants all contribute to a current trend in African and Asian elephant population management, which is to contain elephant populations in fragmented and isolated populations (Armbruster & Lande 1993; Hoare & Du Toit 1999; Hoare 2000; Sukumar 2003; Bradshaw *et al*. 2005). Such isolation can lead to inbreeding depression, especially when populations are small, reproductive rates are low, and populations are contained in very small areas.

Our results suggest that elephants have behavioural mechanisms for inbreeding avoidance. However, in our study, 3.29% of elephant parents were close kin. This rate of inbreeding is similar to other wild populations of mammals; in black-tailed prairie dogs, for instance, 4.68% (36 of 770) copulations involved pairs with $R > 0.25$ (Hoogland 1992). Both Ralls *et al*. (1986) and Marshall *et al*. (2002) report rates of inbreeding between 0% and 5.5% for several natural mammal populations. However, even with low rates of inbreeding, genetic diversity will decline in small, isolated elephant populations due to genetic drift. Furthermore, elephants may increase their rate of inbreeding when faced with a higher proportion of potential mates that are kin. Consequently, we recommend that for this large, long-lived vertebrate, which is especially vulnerable to habitat fragmentation and dwindling populations, conservation efforts strive to maintain robust population sizes and connectivity between elephant habitats.

Acknowledgements

References

Alberts SC (1999) Paternal kin discrimination in wild baboons. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 1501–1506.

Archie EA, Moss CJ, Alberts SC (2003) Characterization of tetra-nucleotide microsatellite loci in the African savannah elephant (*Loxodonta africana africana*). *Molecular Ecology Notes*, **3**, 244–246.

Archie EA, Moss CJ, Alberts SC (2006) The ties that bind: genetic relatedness predicts the fission and fusion of groups in wild African elephants (*Loxodonta africana*). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **273**, 513–522.

Armbruster P, Lande R (1993) A population viability analysis for African elephant (*Loxodonta africana*): How big should reserves be? *Conservation Biology*, **7**, 602–610.

Blaustein AR, Bekoff M, Daniels TJ (1987) Kin recognition in vertebrates (excluding primates): empirical evidence. In: *Kin Recognition in Animals* (eds Fletcher DJC, Michener CD), pp. 287–332. Wiley, Chichester, UK.

Bradshaw GA, Schore AN, Brown JL, Poole JH, Moss CJ (2005) Elephant breakdown. *Nature*, **433**, 807.

Brown JL, Eklund A (1994) Kin recognition and the major histo-compatibility complex: an integrative review. *American Naturalist*, **143**, 435–461.

Busquet N, Baudoin C (2005) Odour similarities as a basis for discriminating degrees of kinship in rodents: evidence from *Mus spicilegus*. *Animal Behaviour*, **70**, 997–1002.

Buss IO, Rasmussen LE, Smuts GL (1976) The role of stress and individual recognition in the function of the African elephant's temporal gland. *Mammalia*, **40**, 437–451.

Clutton-Brock TH, Harvey PH (1976) Evolutionary rules and primate societies. In: *Growing Points in Ethology* (eds Bateson PPG, Hinde RA), pp. 195–237. Cambridge University Press, New York.

Crnokrak P, Roff DA (1999) Inbreeding depression in the wild. *Heredity*, **83**, 260–270.

Douglas-Hamilton I (1972) *On the ecology and behaviour of the African elephant: the elephants of Manyara*. PhD thesis, Oxford University, UK.

Edmands S (2006) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology*, **16**, 463–475.

Fernando P, Lande R (2000) Molecular genetic and behavioral analysis of social organization in the Asian elephant (*Elephas maximus*). *Behavioral Ecology and Sociobiology*, **48**, 84–91.

Gerlach G, Lysiak N (2006) Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching. *Animal Behaviour*, **71**, 1371–1377.

Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, **8**, 1231–1234.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behavior*, **28**, 1140–1162.

Grimshaw JM, Foley CAH (1990) *Kilimanjaro Elephant Project: Final Report*. Friends of Conservation, Nairobi, Kenya.

Halpin ZT (1991) Kin recognition cues of vertebrates. In: *Kin Recognition* (ed. Hepper PG), pp. 220–258. Cambridge University Press, Cambridge.

Hardy OJ, Vekemans X (2002) SPAGEDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Haynes G (1991) *Mammoths, Mastodons, and Elephants: Biology, Behavior, and the Fossil Record*. Cambridge University Press, New York.

Hoare R (2000) African elephants and humans in conflict: the outlook for co-existence. *Oryx*, **34**, 34–38.

Hoare R, Du Toit J (1999) Coexistence between people and elephants in African Savannas. *Conservation Biology*, **13**, 633–639.

Hollister-Smith JA, Poole JH, Archie EA *et al.* (2007) Age, musth and paternity in wild male African elephants, *Loxodonta africana*. *Animal Behaviour*, **74**, 287–296.

Holmes WG, Sherman PW (1982) The ontogeny of kin recognition in two species of ground squirrels. *American Zoologist*, **22**, 491–517.

Hoogland JL (1992) Levels of inbreeding among prairie dogs. *American Naturalist*, **139**, 591–602.

Hurst JL, Payne CE, Nevison CM *et al.* (2001) Individual recognition in mice mediated by major urinary proteins. *Nature*, **414**, 631–634.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1006.

Keane B, Creel SR, Waser PM (1996) No evidence of inbreeding avoidance or inbreeding depression in a social carnivore. *Behavioral Ecology*, **7**, 480–489.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 130–241.

Kioko J, Okello M, Muruthi PM (2006) Elephant numbers and distribution in the Tsavo-Amboseli ecosystem, south-western Kenya. *Pachyderm*, **40**, 61–68.

Kokko H, Ots I (2006) When not to avoid inbreeding. *Evolution*, **60**, 467–475.

Krutzen M, Barre LM, Connor RC, Mann J, Sherwin WB (2004) 'O father: where art thou?' — Paternity assessment in an open fission-fusion society of wild bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. *Molecular Ecology*, **13**, 1975–1990.

Lee PC, Moss CJ (1995) Statural growth in known-age African elephants (*Loxodonta africana*). *Journal of Zoology*, **236**, 29–41.

Lee PC, Moss CJ (1999) The social context for learning and behavioural development among wild African elephants. In: *Mammalian Social Learning: Comparative Ecological Perspectives* (eds Box HO, Gibson KR), pp. 102–125. Cambridge University Press, Cambridge, UK.

Lee PC, Poole JH, Moss CJ (in press) Male elephant social dynamics: independence and beyond. In: *The Amboseli Elephants: A Long-Term Perspective on a Long-Lived Mammal* (eds Moss CJ, Croze H). University of Chicago Press, Chicago.

Li CC, Weeks DE, Chakravarti A (1993) Similarity of DNA fingerprints due to chance and relatedness. *Human Heredity*, **43**, 45–52.

Lindeque M, van Jaarsveld AS (1993) Post-natal growth of elephants *Loxodonta africana* in Etosha National Park, Namibia. *Journal of Zoology*, **229**, 319–330.

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. *Genetics*, **152**, 1753–1766.

Manning CJ, Wakeland EK, Potts WK (1992) Communal nesting patterns in mice implicate MHC genes in kin recognition. *Nature*, **360**, 581–583.

Marshall TC, Coltman DW, Pemberton JM *et al.* (2002) Estimating the prevalence of inbreeding from incomplete pedigrees. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 1533–1539.

Mateo JM, Johnston RE (2000) Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 695–700.

McComb K, Moss CJ, Sayialel S, Baker L (2000) Unusually extensive networks of vocal recognition in African elephants. *Animal Behaviour*, **59**, 1103–1109.

McComb K, Reby D, Baker L, Moss C, Sayialel S (2003) Long-distance communication of acoustic cues to social identity in African elephants. *Animal Behaviour*, **65**, 317–329.

Morrison TA, Chiyo PI, Moss CJ, Alberts SC (2005) Measures of dung bolus size for known-age African elephants (*Loxodonta africana*): implications for age estimation. *Journal of Zoology*, **266**, 89–94.

Moss CJ (1988) *Elephant Memories*. University of Chicago Press, Chicago.

Moss CJ (2001) The demography of an African elephant (*Loxodonta africana*) population in Amboseli, Kenya. *Journal of Zoology*, **255**, 145–156.

Moss CJ, Poole JH (1983) Relationships and social structure of African elephants. In: *Primate Social Relationships* (ed. Hinde RA), pp. 315–325. Sinauer Associates, Sunderland, Massachusetts.

Nyakaana S, Arctander P (1998) Isolation and characterization of microsatellite loci in the African elephant, *Loxodonta africana*. *Molecular Ecology*, **7**, 1431–1439.

O'Riain MJ, Bennett NC, Brotherton PNM, McIlrath GM, Clutton-Brock TH (2000) Reproductive suppression and inbreeding avoidance in wild populations of co-operatively breeding meerkats (*Suricata suricata*). *Behavioral Ecology and Sociobiology*, **48**, 471–477.

Poole JH (1989a) Announcing intent: the aggressive state of musth in African elephants. *Animal Behaviour*, **37**, 140–152.

Poole JH (1989b) Mate guarding, reproductive success and female choice in African elephants. *Animal Behaviour*, **37**, 842–849.

Poole JH, Moss CJ (1989) Elephant mate searching: group dynamics and vocal and olfactory communication. In: *The Biology of Large African Mammals in Their Environment* (eds Jewell PA, Maloiy GMO), pp. 111–125. Clarendon Press, Oxford, UK.

Porter RH, Wyrick M, Pankey J (1978) Sibling recognition in spiny mice (*Acomys cahirinus*). *Behavioral Ecology and Sociobiology*, **3**, 61–68.

Price JJ (1999) Recognition of family-specific calls in stripe-backed wrens. *Animal Behaviour*, **57**, 483–492.

Pusey AE (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology & Evolution*, **2**, 295–299.

Pusey A, Wolf M (1996) Inbreeding avoidance in animals. *Trends in Ecology & Evolution*, **11**, 201–206.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Rajakaruna RS, Brown A, Kaukinen KH, Miller KM (2006) Major histocompatibility complex and kin discrimination in Atlantic salmon and brook trout. *Molecular Ecology*, **15**, 4569–4575.

Ralls K, Harvey PH, Lyles AM (1986) Inbreeding in natural populations of birds and mammals. In: *Conservation Biology: The Science of Scarcity and Diversity* (ed. Soule ME), pp. 35–56. Sinauer Associates, Sunderland, Massachusetts.

Rasmussen L (1998) Chemical communication: an integral part of functional Asian elephant (*Elephas maximus*) society. *Ecoscience*, **5**, 410–426.

Rasmussen LEL, Krishnamurthy V (2000) How chemical signals integrate Asian elephant society: the known and the unknown. *Zoo Biology*, **19**, 405–423.

Sukumar R (2003) *The Living Elephants*. Oxford University Press, Oxford, UK.

Taberlet P, Griffin S, Goossens B *et al.* (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, **24**, 3189–3194.

Van De Casteele T, Galbusera P, Matthysen E (2001) A comparison of microsatellite-based pairwise relatedness estimators. *Molecular Ecology*, **10**, 1539–1549.

Wang J (2002) An estimator for pairwise relatedness using molecular markers. *Genetics*, **160**, 1203–1215.

Waser PM, Austad SN, Keane B (1986) When should animals tolerate inbreeding? *American Naturalist*, **128**, 529–537.

Western D, Lindsay WK (1984) Seasonal herd dynamics of a savanna elephant population. *African Journal of Ecology*, **22**, 229–244.

Zelano B, Edwards SV (2002) An MHC component to kin recognition and mate choice in birds: predictions, progress, and prospects. *American Naturalist*, **160**, S225–S237.

Elizabeth Archie studies the evolution of social behaviour and its genetic causes and consequences. Julie Hollister-Smith is interested in reproductive behaviour and chemical communication. Joyce Poole studies elephant social and sexual behaviour and communication. Phyllis Lee is interested in mammalian growth and social development, reproductive strategies, longevity and life history evolution and biodiversity conservation. Cynthia Moss has spent more than 30 years in Africa studying elephants and working for their conservation. Jesús Maldonado is interested in using molecular genetic tools to answer basic and applied questions dealing with conservation genetics, systematics, behavioural ecology and the evolution and genetic diversity of a variety of mammals. Rob Fleischer is head of the Center for Conservation and Evolutionary Genetics at the Smithsonian's National Zoo and has research interests in evolutionary biology and in the application of genetic markers to conservation, population and systematic studies of birds and mammals. Susan C. Alberts studies demographic, environmental and genetic correlates of and sources of variance in fitness components in natural population of large mammals, particularly African elephants and baboons.