A molecular genetic analysis of kinship and cooperation in African lions

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AFRICAN lions live in complex social groups and show extensive cooperative behaviour[1-10]. Here we describe a new application of DNA fingerprinting that unequivocally demonstrates the kinship structure of lion 'prides': female companions are always closely related, male companions are either closely related or unrelated, and mating partners are usually unrelated. The variability in relatedness among male coalition partners provides an important opportunity to test for the effects of kinship on cooperative behaviour[11]. Paternity analysis reveals that male reproductive success becomes increasingly skewed as coalition size increases, and the tendency to form coalitions with non-relatives drops sharply with increasing coalition size. Thus males only act as non-reproductive 'helpers' in coalitions composed of close relatives.

Lion prides typically contain 2–9 adult females (range, 1–18), their dependent young and a coalition of 2–6 adult males (range, 1–9) that has entered the pride from elsewhere[8]. Incoming males kill or evict the dependent young of the prior coalition[2,7,12,13]. Consequently, females resume sexual receptivity within days, show regular oestrus cycles and mate exclusively with the males of the new coalition by the time they conceive[2,14]. Births are often synchronous within a pride[2] and cubs born less than one year apart comprise a 'cohort'. The resident males typically father only one cohort per pride[4,7]. Two-thirds of all female cohorts are recruited into their mothers' prides, the remainder emigrating together to establish a new pride nearby[7]. By contrast, almost all males leave their natal pride before the age of 4 years and undergo a nomadic phase before gaining residence in a new pride[7]. Large male cohorts usually enter new prides intact, but cohorts of only one or two males often team up with single males from other prides before gaining residence[3-8].

Lions in the Serengeti National Park and Ngorongoro Crater, Tanzania have been studied continuously since the 1960s[1,8,15]. The Serengeti ecosystem covers 25,000 km^2, the total lion population exceeds 3,000 and males disperse over the entire region[7]. More than two-thirds of the 118 males that have bred in our 2,000-km^2 study area originated in other parts of the Serengeti[7,15]. By contrast, the 250-km^2 floor of the crater is a small isolated island of savannah. The current lion population is descended from 15 founders that survived an epizootic in 1962 and includes only 25–45 breeding adults[15]. Compared with the Serengeti population, the crater lions have significantly less allozyme heterozygosity, less restriction-fragment length polymorphism in major histocompatibility complex class I genes, and more sperm abnormalities[15-18].

Hypervariable DNA sequences were identified in the domestic cat and corresponding DNA probes were isolated[19]. In lions, these probes recognized multiple genetic loci with abundant DNA variation and were used in a DNA fingerprinting analysis[20,21] of 193 animals from the Serengeti and 23 from the crater[19]. Data on matrilineal kinship come from long-term records[8]; parentage was also determined for 78 cubs in the Serengeti by DNA band matching. DNA analysis confirmed behavioural estimates of maternity for 77 of 78 cubs; the exception belonged
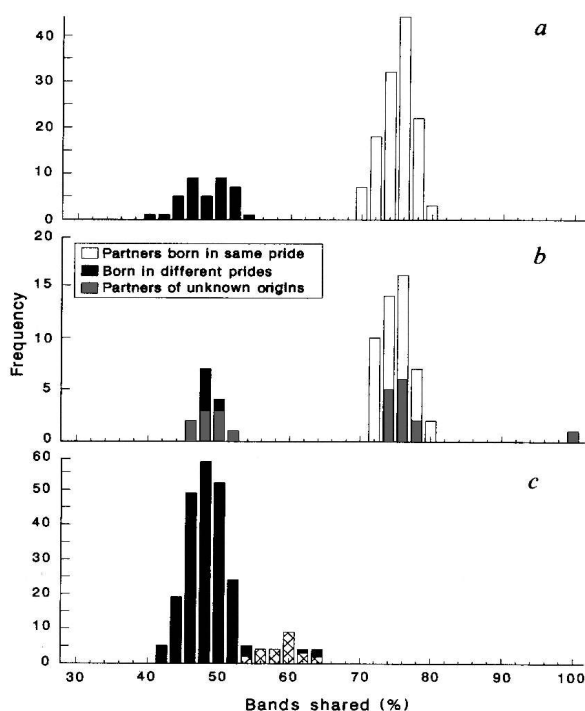
FIG. 1 Frequency distributions of minisatellite band sharing within or between different classes of individuals in the Serengeti. DNA is digested with restriction enzymes and fragments are separated according to relative molecular mass (M_r) by agarose gel electrophoresis. DNA fragments carrying different numbers of the hypervariable minisatellite repeat are seen as bands corresponding to varying M_r after hybridization with a radioactive minisatellite DNA probe. Percentage similarity or 'band sharing' between two individuals is $2F_{ab}/(F_a + F_b) \times 100\%$, where F_{ab} is the number of DNA fragments showing similar M_r and intensity carried by both individuals, F_a is the total number of fragments resolved in individual a and F_b the number resolved in b. All gels were scored by D.A.G., who did not know the geneaological relations of most individuals[19]. For overlapping distributions, the number of pairs showing a particular degree of band sharing is given by the height of the respective hatched region. a, Band sharing between female pridemates (□) compared with band sharing of unrelated individuals born in different parts of the park (■). Data on female pridemates are based on 63 females from 15 prides and include all pairwise combinations of females within each pride (for example, a pride of four females contributes six combinations of females). But each unrelated individual is included in only one combination (76 different individuals in 38 pairs). b, Band sharing between male coalition partners. Data are based on 45 males in 16 coalitions and include all pairwise combinations within each coalition. Data are plotted separately for partners known to have been born in the same pride (□), those known to have been born in different prides (■) and those that had entered the study area from elsewhere (■). One pair of males of unknown origins shared 100% of their bands and are hence presumed to be identical twins. c, Band sharing between resident males and pride females. Data based on 44 males in 18 coalitions and 52 females in 15 prides, including all male–female combinations within each pride. Most males had no known kinship links to the females (■), but one coalition resided in a neighbouring pride that had split off from the males' natal pride 10 years before the males' births (▨).

to a female pridemate of the assumed mother[19]. All 78 cubs were fathered by a member of the resident coalition[19].

The extent of minisatellite band sharing (see legend to Fig. 1) was calibrated against known kinship to provide estimates of relatedness between individuals of uncertain parentage[19]. In the Serengeti population, band sharing is highly correlated with

relatedness but the relationship is not linear: individuals related by a coefficient of relatedness r of 0.5 share 79.6% (s.d. 2.2%) of their bands, animals related by $r = 0.25$ share 74.8% ± 2.2%, whereas those related by $r = 0.02-0.06$ share only 62.0% ± 4.2%. Individuals assumed to be unrelated because they were born in prides with no known kinship links share 49.0% ± 3.3%. The bandsharing data thus distinguish three classes of relationship: kin related by $r \geqslant 0.125$, kin related by $r = 0.02-0.06$ and non-relatives[19]. Band sharing declines more linearly with decreasing relatedness in the crater population and there is a greater variance in band sharing for a given degree of kinship. Crater lions related by $r = 0.5$ share 71.1% ± 11.1% of their bands, and those related by $r = 0.25$ share 59.1 ± 8.4% (note that these estimates of r do not consider the history of inbreeding in this population) and crater lions share 36.9% ± 4.2% with unrelated lions from the ancestral Serengeti population[15,19].

The minisatellite bandsharing data clearly reveal kinship within and between lion prides (Figs 1–3). In the Serengeti population, female pridemates show far greater band sharing than do unrelated individuals (Fig. 1a). Band sharing between male coalition partners from the same natal pride is similar to female pridemates, whereas partners born in different prides show band sharing typical of unrelated individuals (Fig. 1b); band sharing between partners of unknown origins clearly belongs to one distribution or the other (Fig. 1b). Resident males in the Serengeti prides are almost always unrelated to the pride females (Fig. 1c). When a cohort of daughters first splits from

the parental pride, band sharing between the two prides is as close as within each pride (Fig. 3a). But band sharing between prides declines with decreasing kinship as the mothers die (maximum female lifespan is 17 years[8]) and the respective prides produce further cohorts of offspring fathered by different male coalitions (Fig. 3a). Even though the crater population is small and isolated, crater females share more bands with pridemates than with individuals from the most distantly related prides (Fig. 2a); male coalition partners born in the same pride share a proportion of bands similar to female pridemates (Fig. 2b); males resident in non-natal prides share fewer bands with the females than do males resident in their natal pride (Fig. 2c); and band sharing declines with time after prides split (Fig. 3b).

Males cooperate by patrolling the pride's territory and chasing out all male intruders. Because all coalition partners participate in group defence against other coalitions, larger coalitions are better able to gain residence in a pride, maintain residence for longer periods, and gain access to more females over their lifespans[4,8]. A resident coalition is highly effective in preventing extra-pride males from fathering cubs: the paternity analysis demonstrates that the resident males father all cubs conceived during their tenure[19]. Long-term data show that larger coalitions father more surviving offspring per capita than small coali-
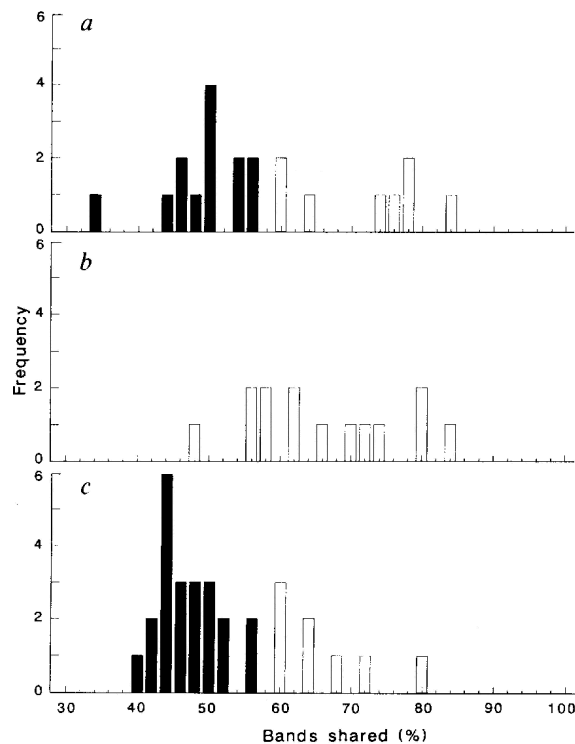

FIG. 2 Frequency distributions of band sharing in Ngorongoro Crater, plotted as in Fig. 1. a, Band sharing between female pridemates (eight females in three prides; (□) compared with band sharing of 22 individuals from crater prides with the fewest kinship links (■). b, Band sharing between male coalition partners (fourteen males in five coalitions). Partners in each coalition were born in the same pride. c, Band sharing between resident males and pride females (eleven males in four coalitions and six females in two prides). All of these males became resident outside their natal pride (■), but members of one coalition later returned to reside in their natal pride (□).
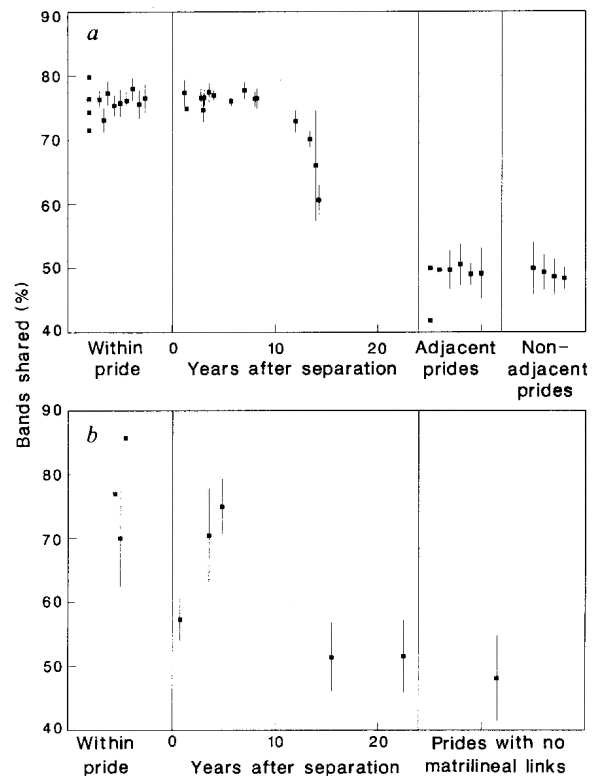

FIG. 3 Degree of band sharing within and between prides. Each point represents comparisons within a pride or between two prides. Where comparisons are made between prides of common female ancestry, the data are plotted against the number of years that have elapsed after the prides separated. The mean and s.d. are plotted across all pairwise combinations for each comparison. a, Serengeti. Data on adjacent and non-adjacent prides only include prides with no known kinship links (either through males or females) since the beginning of the study. All comparisons are of females. b, Ngorongoro Crater. Comparisons between crater prides unavoidably involve numerous kinship links: most contemporary prides are descended from a founding group of four females and there has been frequent exchange of males between all five prides[15]. Between-pride comparisons include both sexes.

tions[4,8]. An increase in coalition size of one additional male results in an additional 0.64 surviving cubs per male[8].

Although a significant proportion of males form coalitions with unrelated companions (Fig. 1b), they do so only under specific circumstances. As coalition size increases, the proportion of coalitions containing non-relatives drops sharply (Fig. 4a). Single males only group to form pairs and trios; all larger coalitions are composed entirely of close relatives. Because per capita reproductive success increases with increasing coalition size, why is it that unrelated males do not continue to join to form larger coalitions? The paternity analysis reveals that it is probably because within-coalition variance in individual reproductive success increases markedly with increasing coalition size (Fig. 4b). Partners had similar reproductive success in all coalitions of only two males, but at least one male failed to breed in each of the larger coalitions.

Cooperation between non-relatives is most likely to evolve when all group members can breed[22–24]. Although each member
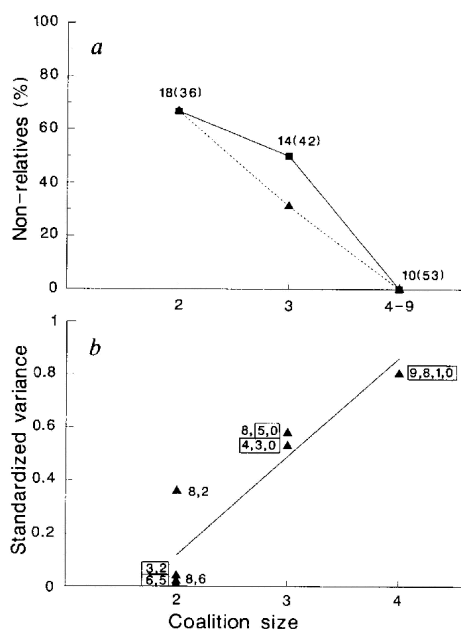

FIG. 4 a, Percentage of coalitions containing non-relatives (■) and of males lacking related partners (▲). Data include all coalitions for which we have demographic or bandsharing information. Coalitions containing unrelated males are significantly smaller than those composed exclusively of relatives ($z = 3.122$, $n_1 = 19$, $n_2 = 23$, $P < 0.002$, two-tailed, Mann–Whitney U-test; if the analysis is restricted to coalitions for which we have demographic data, $z = 3.300$, $n_1 = 14$, $n_2 = 18$, $P < 0.001$). Numbers by each point give the number of coalitions of that size; numbers in parentheses give the number of males. The percentage of males lacking related partners declines even more sharply with increasing coalition size because four of the seven trios containing non-relatives were composed of two relatives and an unrelated third. In these cases, only one of the three males lacked a related partner. b, Standardized variance in individual reproductive success within each coalition. Numbers by each point indicate the number of cubs fathered by each male in that coalition and a box around these points indicates that the males are close relatives. For example, all four males in the coalition of four were relatives; the most successful male in this coalition fathered nine cubs, and the remaining males fathered eight, one and zero cubs respectively. For the regression of standardized variance against coalition size, $r^2 = 0.8370$, $P < 0.001$. Probability for the regression is found by simulation. Simulations were performed by random generation of reproductive success for the 18 males according to the observed distribution of coalition sizes and the observed number of cubs fathered by each of the seven coalitions. The observed regression of standardized variance against coalition size was exceeded in only 2 of 10,000 runs.

of a pair can breed, one or more members of each larger coalition may best be viewed as non-breeding helpers. Their presence increases the reproductive success of their companions (through augmenting coalition size), but they have little personal reproductive success. Such behaviour could only enhance their genetic representation in subsequent generations if it increased the reproductive success of close relatives[11]. A relatively low proportion of males in trios lack any kinship connections with their companions (Fig. 4a). Many trios containing non-relatives consist of a related pair with an unrelated third. The paternity data include one such trio and the non-breeder in this group was closely related to one of the breeders (Fig. 4b). His failure to breed was ameliorated by his relative's success.

Within-coalition variance in male reproductive success results from two components. First, males typically father entire litters. A male will attempt to monopolize a female throughout her 2–4-day oestrus by remaining nearby and preventing his coalition partners from mating with her[5,14]. As in other species[25], this behaviour ensures that the consorting male fathers her entire litter: mixed paternity occurred in only one of 24 litters containing two or more cubs[19]. Second, within-coalition variance in the number of litters fathered by each male increases significantly with increasing coalition size ($P = 0.0084$, $n = 44$ litters; probability determined as described in legend to Fig. 4b). Breeding within each pride is typically synchronous so that several females are often in oestrus at once[2,26]. But larger coalitions do not preferentially reside in larger prides[8], thus the number of males in a large coalition more often exceeds the number of available oestrous females.

Solitary males spend long periods selecting potential companions[7] and behavioural studies indicate that mating success is more disparate within coalitions where partners are less closely matched in age or vigour[5]. The risk of becoming a non-breeder is greater in a trio, and males that form unrelated trios seem to be more sensitive to potential disparities in mating success than males that form pairs: the age difference between unrelated partners is significantly lower in trios (0.75 years) than in pairs (2.5 years, $P < 0.05$).

Previous explanations for cooperation among male lions did not require kinship to maintain the behaviour. Each male was assumed erroneously to father an equal proportion of offspring in all coalition sizes, and therefore to gain equally as per capita reproductive success increased with coalition size[4,5,8]. But it is now clear that this is only true for small coalitions, kinship is essential for the maintenance of larger coalitions where reproduction is highly skewed.

In the Serengeti population, the DNA band-sharing technique allowed us to measure whether companions, neighbours or mating partners were even distantly related. Although these data suggest that similar analyses could be conducted in populations that had not previously been studied in such detail, the relationship between relatedness and band sharing differed between the Serengeti and the nearby Ngorongoro Crater. Thus the degree of band sharing should be calibrated against an independent measure of kinship[19,27]. □

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