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PATERNAL KIN MATTER: THE DISTRIBUTION OF SOCIAL BEHAVIOR AMONG WILD, ADULT FEMALE BABOONS

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CHAPTER 1

Kin selection and kin-biased behavior

Kin-biased behavior has been observed in many taxa, including colonial marine invertebrates, orthopterans, amphibians, fish, and mammals (Kurland 1977; Blaustein and O'Hara 1982; Grau 1982; Holmes and Sherman 1982; Gouzoules 1984; Wilkinson 1984; Grosberg and Quinn 1986; Rabenold 1986; Walters 1987; Dewsbury 1988; Simmons 1989; Keane 1990; Pope 1990; Winberg and Olsen 1992; Brown and Brown 1993; Call *et al.* 1996; Klettenheimer *et al.* 1997; Alberts 1999; Hoglund *et al.* 1999; Pfennig 1999). In most cases listed above, the behaviors chosen to investigate would be strongly selected for under kin selection, either because the behavior was so beneficial to the recipient (cannibal toads avoiding aggregating with kin when hungry (Pfennig 1999)), or because the relatedness of the individuals was so great (female social insects forgoing their own reproduction to help raise sisters ($r = 0.75$) rather than raising their own offspring ($r = 0.5$) (Hamilton 1964).

However, interpreting the results of kin-biasing studies is not always straightforward. Toad tadpoles sometimes do (Blaustein and O'Hara 1982; Cornell *et al.* 1989; Pfennig *et al.* 1993) and other times do not (O'Hara and Blaustein 1981; Fishwild *et al.* 1990; Hall *et al.* 1995) show a preference for aggregating with siblings over non-siblings. Within the same laboratory, pigtail macaques raised in isolation, both do (Wu *et al.* 1980), and do not (Sackett and Frederickson 1987) orient towards half siblings over non-kin. Non-offspring nursing is biased towards close kin in lions (Pusey and Packer 1994) but is not in harbor seals (Perry *et al.* 1998; Schaeff *et al.* 1999). Feeding groups of vultures are composed of kin (Rabenold 1986), while feeding groups of ravens are not (Parker *et al.* 1995). Leks show evidence both for (Hoglund *et al.* 1999) and against (McDonald and Potts 1994) kin-biased composition. Within the same laboratory, male wasps do (Ryan and Gamboa 1986) or do not (Ryan *et al.* 1984) recognize their brothers. Female mice either do or do not show a mating preference for male cousins over brothers or unrelated males (Keane 1990). And finally, cannibalistic spadefoot toad tadpoles avoid eating kin (Pfennig 1999) while willow leaf beetle larvae do not (Breden and Wade 1987).

Part of the confusion in interpreting the results of kin-biased studies comes with failing to distinguish between the evolution, expression, and detection of kin biasing. The first two are biological phenomena and the third is a methodological issue. The expression of a kin-biased behavior may be context dependent, while the evolution of that behavior is not. For example, a kin-biased mate preference for males of an intermediate

degree of relatedness (cousins were preferred over brothers and over unrelated males) has evolved in female mice (*Peromyscus leucopus*), but only females in estrous express the kin-bias (Keane 1990). Further, kin avoidance in aggregating behavior while feeding has evolved in spadefoot toads of the cannibalistic morph, but its expression is correlated with hunger levels (Pfennig 1999). As tadpoles become hungrier, they become less discriminating at avoiding kin. Finally, methodological weaknesses in measuring behavior contribute to confusion in interpreting kin-biasing results. This may have been the cause of the discrepancy between the studies of Wu *et al.* (1980) and Sackett and Frederickson (1987). Wu *et al.* reported that juvenile macaques reared in isolation oriented themselves towards unfamiliar paternal half siblings significantly more often than towards unfamiliar non-kin during forced-choice experiments. These results have not been replicated, despite attempts to do so by researchers part of the original study (Sackett and Frederickson 1987), and by others (Erhart *et al.* 1997). Sackett and Frederickson (1987) suggested that the significant kin biasing observed by Wu *et al.* (1980) was actually a statistical artifact due to small sample size. Although methodological factors are important in interpreting kin-biasing results, the evolution and expression of these kin-biases in the distribution of social behavior are the primary focus of this study.

Cercopithecine primate females (e.g., baboons, vervets, and macaques) bias their social behavior towards maternal kin (members of the same matriline) much more than towards non-maternally related individuals. The proportion of time spent grooming,

grooming 'intensity', grooming reciprocity, maintaining close proximity, and aiding during agonistic bouts, are all biased towards maternal kin (see Chapter 4 for review of maternal kin-biased behavior in cercopithecines).

Despite the clear expression of kin-biased behavior among adult female cercopithecine primates, questions remain about the evolution and proximal mechanisms of this behavior. The behavior of paternal kin can shed light on these issues, first by helping to determine if these behaviors evolved through natural selection (individual) or through kin selection (inclusive) (see below), and then by suggesting which proximal mechanisms underlie the expression of the kin-biased social behaviors.

Kin selection

Hamilton introduced the concept of kin selection (1963, 1964) in an attempt to explain how social behaviors such as cooperation and altruism might evolve. It accounts for the evolution of behaviors that incur a cost to the fitness of the individual performing them, and would therefore not have evolved under natural selection. The theory of natural selection predicts the evolution of heritable social behaviors and physical traits that confer a fitness advantage to the individual possessing the trait, and so, in and of itself, can not explain the evolution of a trait, such as altruism, that lowers the reproductive fitness of its carrier (Hamilton 1963). Hamilton proposed that an individual's fitness included not only their own offspring, gained without the actions of others (direct fitness), but also included the sum of the incremental increases to the

fitness of relatives due to the actions of the individual, weighed by the coefficient of relatedness between the two (Grafen 1978). Hamilton's rule, as it has come to be known, states, that if

$$rb - c > 0$$

where r = the coefficient of relatedness between the actor and the recipient, b = the benefit to recipient's reproductive success, and c = the cost to the actor's reproductive success, then the behavior in question will evolve through kin selection. Therefore, behaviors that exact a high fitness cost from the actor are predicted to be biased nearly exclusively towards an individual's closest relatives (parents, offspring, and full siblings), while behaviors that are beneficial to the recipient, at a relatively low cost to the actor, may be distributed among distant- and non-kin, as well as among close kin.

Two conditions must be met in order for social behaviors to evolve through kin selection. First, populations must be subdivided into kin groups (Wade 1980) and second, a mechanism must exist for behaving differentially to individuals of differing kinship.

Kinship structure of groups

The kinship structures of groups, both the proportion of kin to non-kin, and the degree of relatedness among kin, have important consequences for both the evolution and expression of kin-biased social behavior (Chapter 3). The greater the proportion of kin to non-kin and/or the greater the relatedness among kin, the greater the degree of altruistic

or cooperative behaviors one would expect to evolve through kin selection. The two most extreme cases are groups comprising either only kin or only unrelated individuals. At the one extreme, behaviors can not evolve through kin selection in the absence of kin, (if $r = 0$, then $rb - c$ can never be greater than zero, the necessary condition for evolution by kin selection). At the other extreme, behaviors can evolve through kin selection in the absence of non-kin. Colonies of genetically identical siblings of marine invertebrates (*Botryllus schlosseri*) cooperate to the point of functioning almost as a single organism (Grosberg and Quinn 1986). However, they are not a single organism. Despite being genetic clones of each other, every individual larvae has the potential to disperse from the natal colony and start a new colony by recruiting others (Grosberg and Quinn 1986). Field experiments showed that individuals that disperse aggregate with sibling colonies significantly more than they do with unrelated individuals (Grosberg and Quinn 1986).

However, most individuals do not live in either of these extreme kinship groupings, but rather find themselves in a group or population that is subdivided along lines of kinship, encountering conspecifics of varying degrees of relatedness and non-kin. The expression of behaviors evolved through kin selection often involves a *bias* based on kinship, either towards kin over non-kin, or towards close over distant kin. In either case, a bias assumes variation in the degree of relatedness among potential social partners. Variation in genetic relatedness is required in order to measure the strength of the kin bias.

Both the proportion of kin to non-kin in a group, and the degree of genetic relatedness between kin in the group, are correlated with the degree of cooperative or altruistic social behavior expressed by those individuals. As just mentioned, individuals with a relatedness coefficient of 1 (marine invertebrate clones) behave as a single organism. Among social insects and naked mole rats full sisters have a relatedness coefficient of 0.75 (Alexander *et al.* 1991). While not acting as a single organism as the larvae above, female naked mole rats forgo their own reproduction to help raise younger sisters.

Most mammalian species live in groups in which one sex (usually male) disperses and the other sex is philopatric (usually female). This has two important consequences for the group kinship structure. First, non-kin are introduced into the group through independent dispersal and subsequent immigration. This lowers the average degree of relatedness of the group in comparison to both invertebrate clonal colonies and colonies of social insects. Not surprisingly, cooperative social behavior has not evolved in most mammalian species to the degree that is has in the social insects. Behaviors that have evolved in these mammalian species include grooming, food sharing, aggregating, and forming coalitions to name a few (see above). Although beneficial to the recipient, they may exact a cost to the actor, albeit not as extreme as forgoing one's own sexual reproduction.

The second consequence of sex-specific dispersal and philopatry, to the group kinship structure and therefore kin-biased behavior, is that the average degree of relatedness among adult males and females is different. This difference in relatedness between males and females is expected to result in a difference between the sexes in the evolution and expression of kin-biased social behaviors. For example, as mentioned above, among most mammalian species, females remain in their natal groups and males disperse (Storz 1999). Because of this, the average relatedness among adult females is greater than is relatedness among adult males (Webb *et al.* 1995; Altmann *et al.* 1996; de Ruiter and Geffen 1998). This is true for cercopithecine primates and, as expected, more cooperative and affiliative behavior is expressed among adult females than among adult males. Further, cooperation among females is greater in those species in which groups are made up of a single matriline (related adult females and their offspring) than among females in groups made up of multiple matrilines. Groups of lions and elephants are made up of a single matriline. Female lions nurse each other's young, and female elephants travel in a formation that protects all group young. These behaviors, non-filial nursing and protective formations, are not seen among cercopithecine females, who live in groups composed of multiple matrilines. Finally, Heinsohn *et al.* (1996) show that cooperation among adults of the philopatric sex, begins being expressed among juveniles, when members of both sexes within the group are equally related, at least among lions. Lions are matrilocal, and juvenile females, but not males, become progressively more involved in territorial defense with age. Chimpanzees are an exception. Males are philopatric and females disperse. Therefore the relatedness among the adult males in a

group is greater than among the adult females (Morin *et al.* 1994). Males bond socially, grooming each other, displaying sexual tolerance, cooperating while hunting, and forming alliances during intergroup encounters. This high level of cooperative and affiliative behavior among adult males has been observed in other non-human primates in which males, but not females, are philopatric (reviewed in van Hoof and van Schaik 1994). Furthermore, in these same species, social bonds among females are less obvious than are those among species in which females are matrilocal (Mitchell *et al.* 1991; van Hoof and van Schaik 1994).

Mechanisms

Not only must populations be subdivided into kin groups in order for behaviors to evolve by kin selection, but also a mechanism(s) must exist for distinguishing between individuals based on genetic relatedness. Two proximal mechanisms for kin-biasing have been proposed; familiarity based on learned cues, and phenotype-matching which requires no prior exposure to kin (Hepper 1986). The former mechanism is indirect and therefore prone to error, while the latter is direct and therefore much more accurate. Familiarity to learned cues can be based on environmental factors such as diet (Hall *et al.* 1995), odors (Crosland 1989), or natal habitats such as nest and burrows (Beecher *et al.* 1981; Hepper 1986), or it can be based on familiarity due to exposure early in development (Beecher 1982; Waldman 1987; Haplin 1991). Each of these learned cues estimate genetic relatedness and is only as accurate as the cue is. Unrelated individuals occupying the same natal burrow or nest (i.e., nest parasitism) will be treated as kin if the

kin biasing mechanism is familiarity. Phenotype-matching assumes that some aspect of an individual's phenotype accurately reflects the underlying genotype. Because of this assumption, individuals require no prior experience with kin in order to discriminate between kin and non-kin. An unrelated individual placed in the natal nest, for example, would not be treated as kin. Kin-biasing by phenotype-matching is more accurate than by familiarity, but examples of it are more rare.

Using paternal kin to understand the evolution of kin-biased behavior

Observing the distribution of social behavior among paternal kin may help shed light on the evolution of the maternal kin bias observed among adult female baboons. Consider the implications of failing to observe a kin-bias among paternal kin. One must first rule out that this is not a detection problem on the part of the observer due to a methodological flaw such as small sample size or observing behaviors not evolved through kin selection. In this study, the behavior among maternal sisters was used as a control for detecting kin-biased behaviors evolved through kin selection. Sample sizes were comparable between maternal and paternal sisters in this study, both in numbers of sister pairs, and in number of hours observing those pairs. If a bias could be detected among maternal sisters, then we know that the sample sizes were adequate for detecting a paternal kin bias of similar magnitude.

After ruling out a measurement problem on the part of the observer, failing to observe a paternal kin-bias would cause one to reconsider the evolution of the behavior

through kin selection. The distribution of social behavior among paternal kin tests the assumption that behaviors biased towards maternal kin evolved through kin selection. Stating that behaviors evolved through kin selection hinges on the assumption that degree of relatedness determines the evolution and expression of a social behavior, and not whether the two individuals share genes through their mother or their father. Among baboons, paternal sisters share at least as many, if not slightly more, genes on average than do maternal sisters (see Chapter 4, Discussion for full explanation). Because of this, any behavior evolved through kin selection should be expressed in paternal kin as well as maternal kin. The alternatives are: the behavior did not evolve through kin selection, *r* (the coefficient of relatedness in the kin selection model) should take into account whether relatedness is through the maternal or the paternal line, or maternal siblings have a reliable familiarity cue while paternal siblings do not. Without a mechanism for discriminating kin from non-kin, behaviors will not evolve through kin selection.

Discerning proximal mechanisms through experimental results

In addition to discriminating between natural (individual) and kin (inclusive) selection, the distribution of social behavior among baboon paternal sisters can also illuminate the proximal mechanism(s) underlying kin-biasing. Proximal mechanisms have been worked out for a number of behaviors in several species. Several examples suggest kin-biasing based on familiarity. Bank swallows bias their feeding behavior towards the young chicks in their nest holes. Because nest parasitism does not occur in this species, and because young chicks do not wander off and mix with other young

chicks, biasing behavior towards the young chicks in one's own nest hole would naturally result in biasing behavior towards one's own offspring. However, bank swallows will feed any young chicks experimentally placed into their personal nest holes and will ignore their own chicks if experimentally removed to a nearby nest (Beecher *et al.* 1981). Second, spadefoot tadpoles bias their aggregating behavior towards kin over non-kin, unless close relatives are experimentally raised on different diets. Under these experimental conditions, tadpoles bias their behavior according to diet similarity rather than on genetic relatedness. In their natural habitat, siblings are raised in the same habitat and are raised on similar food, suggesting that the kin-biased behavior expressed is based on familiarity to natal diet (Hall *et al.* 1995).

Examples of kin-biasing through phenotype matching also exist. American toad tadpoles raised in isolation aggregate with siblings over non-kin when given a choice. However, when the nostrils of these tadpoles were experimentally blocked or plugged, they displayed no kin-biasing behavior (Waldman 1985). Waldman (1985) suggests that the tadpoles use a phenotype matching mechanism in which the phenotype being compared is a genetically based chemical signal carried through the water. Mice and rats also display the ability to recognize kin based on a phenotype matching mechanism. Mice (Kareem 1983) and rats (Hepper 1983) removed from their litters at birth, biased their behavior towards siblings over non-siblings when tested as adults.

One thing most mechanism studies have in common is that they take place under unnatural conditions. These experiments have greatly improved our understanding of kin-biased behavior and the proximal mechanisms underlying the expressed bias, but we still know relatively little about the mechanisms underlying kin-biasing in an animal's natural habitat.

Wild baboon paternal half sisters provide a natural experiment for testing the underlying proximal mechanisms of kin-biased behavior

The kinship structure of baboon groups provides a natural experiment for testing the kin-biasing mechanism (see Chapter 4). Although a strong maternal kin-bias has been observed in baboons for decades, it was virtually impossible to tease apart familiarity and genetic relatedness among maternal kin in the wild. One way to do this would be through adoption. However, adoption of neonates has never been reported in Amboseli and is apparently extremely rare among wild baboons.

These two kin-biasing mechanisms can theoretically be teased apart using the distribution of behavior among paternal sisters. Paternal sisters tend to be members of the same age cohort. This is because high-ranking adult males enjoy a 'priority of access' to estrous females, which results in a few high-ranking males siring a disproportionate number of offspring born within a couple of years (Altmann *et al.* 1996). Males maintain high rank for one to two years, on average. Therefore, most of a male's offspring will be of a similar age. However, the priority of access model is not perfect.

Males other than the highest-ranking also sire offspring. For various reasons, (e.g., females may form friendships with lower ranking males and consort with them when in estrous (Smuts 1985), or more than one female may be in estrous at a time, making it impossible for one male to successfully monopolize both simultaneously), age cohorts are made up of both paternal siblings and unrelated individuals. The important point is that within an age cohort, all members are similarly familiar with each other, but the genetic relatedness could theoretically vary from full sibling to non-kin (note that no full siblings were identified in this study, see Chapter 3). If the mechanism for kin-biased behavior is familiarity, one would expect adult females to bias their behavior towards members of their age cohort over females born more than a year before or after them. However, if the mechanism for kin biasing is phenotype-matching, one would predict that adult females would bias their behavior towards related over unrelated individuals regardless of their age cohort.

This study

The adult female savannah baboons (*Papio cynocephalus*) in this study were well suited as subjects for studying the evolution and expression of kin-biased behavior. They resided in three distinct social groups that have been under continual observation for over two decades (Hausfater 1975; Altmann 1980; Muruthi *et al.* 1991; Altmann 1998; Alberts 1999). Studying three groups rather than a single group controlled against a 'group-specific' effect and increased the external validity of the results. Although juvenile age cohorts are larger than age cohorts of adults, adult females were chosen over juveniles as

the subjects of this study because much more was known about kin-biased behavior among adults. Once paternal relatedness was determined (Chapters 2 and 3), both the maternal and paternal kinship structure of the three groups could be compared. Further, two of the three groups resulted from a group fission that occurred the year prior to the study. The group structure, which is predicted to influence the distribution of behavior, could be compared before and after the fission (Chapter 3). Finally, the behavior of the baboons is completely natural, as far as we know. Researchers identify baboons based on individual differences, and avoid interacting with the baboons as much as possible.

Habituated but naturally behaving subjects, availability of long-term data on group kinship structure both before and after fission, known maternal and paternal relatedness among adult females, and availability of long-term background behavioral data on which to base new predictions, all contributed to making the distribution of social behavior among these baboons an excellent resource for studying the evolution and expression of kin-biased behavior.

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CHAPTER 2

Genetic studies in baboon DNA

INTRODUCTION

The goal of this study was to genotype DNA from adult female baboons, make use of the genotypes in identifying paternal half-sisters, and then to analyze the distribution of social behavior among female baboons in light of the genetic relatedness between them and predictions based on kin selection theory. Although many studies have looked at the relationship between maternal kinship and social behavior (see chapter 4), this study used a novel approach to identifying paternal half-sisters. Similar to other genetic studies of nonhuman primates (Constable *et al.* 1995; Gerloff *et al.* 1995; Launhardt *et al.* 1998), genotypes in this study were determined by cross-species amplification of baboon DNA with primers for human simple tandem repeat polymorphism (STRP) loci. However, this study focused exclusively on STRP loci located on the X chromosome. This helped to identify paternal half-sisters among those subjects for whom we had maternal, but not paternal DNA, an approach that was both powerful and novel. Furthermore, the DNA used to genotype the baboons was extracted primarily from feces. Several studies have demonstrated that feces are a non-invasive source of DNA (Hoss *et al.* 1992; Tikel *et al.* 1996; Van der Kuyl *et al.* 1996; Wasser *et*

al. 1997; Frantzen *et al.* 1998) and a few studies have gone further, using feces-derived DNA to identify species and sex (Kohn *et al.* 1995; Taberlet *et al.* 1997; Reed *et al.* 1997). However, only this study and one other (Launhardt *et al.* 1998) have used feces-derived DNA to determine relatedness between individuals. Chapters 3 and 4 discuss the identification of paternal half-sisters and the social behavior among them. In this chapter, the methods used to genotype the baboon DNA are described.

Amplification of baboon DNA

Microsatellite DNA

Microsatellite DNA is composed of tandemly repeated units of 1 to 10 base pairs (bp) of DNA (Bruford *et al.* 1998; Ciofi *et al.* 1998). Often the number of repeated units at specific microsatellite loci varies among individuals. These polymorphic microsatellite loci are referred to as STRPs or SSR (short sequence repeat) polymorphisms. Hamada *et al.* (1982) were the first to call attention to dinucleotide $(TG)_n$ repeats, first in yeast and then in many vertebrate species. Weber and May (1989) were the first to report the suitability of STRPs, in conjunction with the polymerase chain reaction (PCR), as genetic markers in humans, due to the variation in the number of repeats among individuals, the alleles are co-dominantly inherited, and they are ubiquitously distributed throughout eukaryotic genomes.

Cross-species amplification

Cross-species amplification of DNA is extremely useful given the costs associated with identifying and cloning species-specific primers. Microsatellite DNA is conserved between many avian species, between cattle and sheep, and between pilot whales and many other cetacean radiations (see Coote and Bruford 1996 for a review of these studies). Important for this study is that microsatellite DNA and its flanking sequences are highly conserved between humans and many nonhuman primate species (for examples see, apes: Washio 1992; Gerloff *et al.* 1995; Coote and Bruford 1996; Old World monkeys: Morin and Woodruff 1992; Rogers 1992; Constable *et al.* 1995; Altmann *et al.* 1996; Coote and Bruford 1996; Kayser *et al.* 1996; Launhardt *et al.* 1998; von Segesser *et al.* 1999). Therefore , many primers that amplify human microsatellite loci also amplify microsatellite loci in non-human primate species.

Although extremely convenient, cross-species amplification can present problems. In particular, the primer sequence of one primate species often mismatches by as few as a single nucleotide the DNA sequence of a second primate species. This may result in either low amplification rates or in incomplete amplification. The former can result in very weak or absent banding patterns on gels, while the latter can result in banding patterns in which only one homologue amplifies, leading to an excess of homozygotes (see Allelic Dropout section below for discussion). Additionally, levels of heterozygosity in one species may not be indicative of the heterozygosity in the target

species. When selecting primers, most researchers try to use markers that have at least 70% heterozygosity. However, microsatellite markers that are highly polymorphic and heterozygous in humans may fail completely to amplify DNA from nonhuman primates or they may amplify the DNA but contribute little information due to low levels of polymorphism or heterozygosity in the nonhuman primate species (see Screening STRP Primers in Results and Discussion sections).

X-linked STRPs

The purpose of genotyping baboons in this study was to identify females who share a father and are therefore paternal half-sisters. If both the mother's and the father's DNA for each subject were available, identifying paternal half-sisters would have been straightforward. However, the female subjects of this study were adults, making it often impossible to obtain DNA from the parents. In particular, potential fathers for these adult females, especially the older females, had either already died or emigrated to other groups. Maternal DNA was available for approximately 2/3 of the subjects and in these cases, identification of maternally-inherited alleles could be determined.

Markers on the sex chromosomes are more informative for identifying paternal siblings than are markers on the autosomes. Males are haploid on their X and Y chromosomes so all daughters will inherit the same paternal X chromosome from their father, whereas all sons will inherit the same paternal Y chromosome from their father.

Thus, females with the same fathers should have one identical X chromosome haplotype that was inherited from their father.

Microsatellite markers on the X chromosome are useful for identifying paternal half-sisters particularly because of their power to *exclude* pairs as paternal half-sisters. Autosomes are only useful for excluding paternal half-sisters when the paternally-inherited allele is known for both individuals. Whenever the information on two potential paternal half-sisters is incomplete, or whenever two females are each heterozygous for four different alleles, markers on the X chromosome will be more informative for excluding pairs as half-sibs than will autosomal markers, even in the absence of any knowledge of which bands are paternally derived (Table 2.1). X chromosome markers are also useful in cases where no parental DNA is available but DNA from maternal half-brothers is available. At loci where the two maternal half-brothers each inherit different alleles from their mother, it is possible to reconstruct the mother's genotype at that locus. Once the mother's genotype is resolved, the paternal allele in her daughter's (the subject's) genotype can be identified.

Table 2.1 X-linked vs. autosomal STRP loci. Examples of the usefulness of X-linked STRPs in including/excluding two females as potential paternal half-sisters. Known paternal alleles are in bold.

		Exclusion if the marker is on the	**Exclusion if the marker is on the**
F1[a]	F2[b]	X chromosome	Autosome
1, 2	**1**, 3	cannot exclude	cannot exclude
1, 2	2, 3	exclude	cannot exclude
1, 2	3, 4	exclude	cannot exclude

[a] Genotype at one locus of the first female in a pair of potential paternal half-sisters.
[b] Genotype at one locus of the second female in a pair of potential paternal half-sisters.

Feces as a DNA source

Feces compared to other DNA sources

Advances in molecular biology have made it possible to ask questions at the mechanistic level of analysis about the relationship between genetics and behavioral ecology. Blood and other fresh tissue are the best and most commonly used sources of high-quality DNA (see Results below). Protocols for extracting DNA from blood and commercially available kits exist that yield high concentrations of pure (few contaminants), intact DNA that is less susceptible to PCR artifacts (see Results below) than is DNA extracted from lower-quality sources. If these tissues were available, few would use alternative sources of DNA.

However, darting or trapping animals is often necessary to collect blood and other tissues from research animals, and this is inherently risky both to the individual being darted and to the internal validity of behavioral studies if group members behave unnaturally in the presence of the researchers post-darting. Because of these risks, blood and other invasive sources of DNA may not be an option for studies of endangered animals, of animals not yet habituated to humans, or of highly social, habituated animals, such as the baboons in this study, who are responsive to unusual actions of human researchers. A second drawback of collecting invasive sources of DNA is the time-consuming, costly measures that must be taken as precautions against the risks mentioned above. Finally, collecting and transporting blood and other tissues requires permission from, or is even prevented by, various national and international agencies (Kohn and Wayne 1997; Launhardt *et al.* 1998). Because of these considerable constraints on collecting blood and other tissues, it is often necessary to use other, non-invasive sources of DNA.

Hair is one non-invasive source of DNA (Morin and Woodruff 1992; Taberlet *et al.* 1993; Woodruff 1993; Morin *et al.* 1994; Gagneux *et al.* 1997). However, shed hair must usually be collected from sleeping nests (chimpanzees: Morin and Woodruff 1992) or scratching posts (bears: Kohn *et al.* 1995; Taberlet *et al.* 1997) used by multiple animals which renders individual identification of the donor impossible. Freshly plucked hairs with intact roots and sheath cells (the source of hair's DNA) are better than shed hairs for amplifying DNA (Morin and Woodruff 1992), with the advantage of positively

identifying the individual and hence the DNA source. However, plucking hairs may often require darting or trapping, thus having many of the same drawbacks as collecting DNA from an invasive source.

Feces have advantages over both blood and hair as a DNA source in that their collection is non-invasive and the donor's identity is known. Collection and storage are relatively straightforward, convenient, and economically feasible (see Methods below), even for otherwise difficult subjects such as endangered or non-habituated animals. Collecting feces introduces no known risk to either the subject or to the study as a whole, nor does the collection and transportation of feces require permission from as many agencies as does blood or tissue (Gerloff *et al.* 1995; Tikel *et al.* 1996; Kohn and Wayne 1997; Launhardt *et al.* 1998).

Low quantities of DNA in feces

While feces have many advantages as a DNA source, they are equally challenging. All studies that have tried to amplify DNA extracted from feces reported that a portion of amplification attempts failed repeatedly (ranging from 4% in Frantzen *et al.* 1998 to 80% in Taberlet *et al.* 1997). Epithelial cells throughout the feces may be scarce (Gerloff *et al.* 1995) and unevenly distributed (Kohn *et al.* 1995), making it possible to end up with extractions that contain no DNA. While several studies suggested extracting DNA from a homogenized fecal mixture (Deuter *et al.* 1995; Wasser

et al. 1997; Frantzen *et al.* 1998), Flagstad *et al.* (1999) and Kohn *et al.* (1999) found that extracting from the surface of the feces yielded more DNA.

Degradation of DNA from feces

Feces have several things in common with other degraded sources of DNA, and additional drawbacks that are unique to this source. Degraded DNA is often sheared, regardless of the source: hair, feces, or ancient tissue. Sheared DNA is broken in smaller fragments making it difficult to amplify sequences more than 200 – 300 bp long (Launhardt *et al.* 1998), and amplification may fail if the shearing occurs along the stretch of template DNA that binds to the primer sequence or within the sequence flanked by the two primers. This results in a reduced number of copies of template DNA and little or no amplification of the target sequence, and/or non-specific amplification of other regions of the genome. This is especially serious when very little template DNA is available to begin with, as is the case when working with feces.

Inhibitors found in feces

In addition to low quantities of degraded DNA, PCR inhibition may also explain the relatively high failure rate when trying to amplify DNA from feces. Feces contain many compounds such as bilirubin, bile salts, undigested food, mucus, and digestive enzymes that may inhibit amplification by interfering with the *Taq* polymerase during the PCR reaction (Sidransky *et al.* 1992; Deuter *et al.* 1995; Kohn and Wayne 1997). The feces of omnivores, such as the baboons in this study, contain plant polysaccharides that

further inhibit *Taq* (Kohn and Wayne 1997). These various compounds in feces have such strong inhibitory effects that they not only inhibit the amplification of low-quality DNA from feces, but actually prevent the amplification of high-quality DNA when added to blood extracts (see Extraction Protocols below, Wasser *et al.* 1997).

Several extraction techniques are available that reduce the effect of inhibitors found in feces-derived DNA. Paxinos *et al.* (1997) suggested removing visible, polysaccharide-containing plant material at the beginning of the extraction process. However, removing visible plant material may not remove all plant inhibitors, especially those that are released from plants during digestion (S. Alberts, pers. com.). Several studies suggested adding cetyltrimethyammoniumbromide (CTAB) to break down remaining plant inhibitors during the extraction process (Constable *et al.* 1995; Launhardt *et al.* 1998). Deuter *et al.* (1995), using DNA from human stool, found that extractions "using potato flour as an absorption matrix" (p. 3800) had increased DNA yields and were less prone to inhibition during PCR amplification.

Extracting DNA from feces presents a challenge for researchers. Inhibitors can be removed with additional washes during extraction. However, DNA is also lost with each additional wash. Because DNA is scarce in feces, finding the right balance between removing inhibitors and preserving DNA is challenging. Some researchers address this problem by adding BSA during the amplification stage (Gerloff *et al.* 1995; Kohn *et al.* 1995; Van der Kuyl 1996; Paxinos *et al.* 1997; Reed *et al.* 1997; Launhardt *et al.* 1998).

BSA is thought to add another target for the inhibitors, leaving more template DNA free to anneal with the primer DNA during PCR amplification. This also has the benefit of losing less DNA during the extraction.

Allelic dropout in DNA from feces

Preferential amplification of one of the homologues, resulting in an excess of "homozygote" banding patterns, is referred to as allelic dropout. Allelic dropout is thought to be a PCR artifact, probably caused by a mismatch in the primer and template DNA sequences. Allelic dropout is more common in degraded DNA and when using primers from another species.

Several alternatives for reducing the rate of allelic dropout are possible. First, lowering the priming stringency, by either decreasing the annealing temperature or by increasing the magnesium concentration during the PCR, increases the chance of amplifying "difficult" bands by tolerating mismatches between the primer sequence and the template DNA. Second, primers can be redesigned to be species-specific and to avoid mutation sites that may interfere with the amplification of one of the homologues. Finally, performing repeated amplifications may identify a second band, making the final genotype assignment heterozygous despite observation of a single band per amplification (lane).

Literature review of studies using DNA from feces

Feces have long provided ecologists with valuable information about diet, population parameters, and habitat and range use (see Putman 1984 for review), but the importance of feces in genetic and endocrine work has only recently been recognized. This study is concerned only with the growing role of feces in genetic work and will not address feces as a hormone source (see Wasser *et al.* 1988 and 1996 as examples of work being done in that field). In 1992 Albaugh *et al.* reported that DNA could be extracted non-invasively from exfoliated epithelial cells lining the gut. Later that same year Hoss *et al.* reported that DNA could be extracted and amplified from bear feces and many similar studies have followed (Table 2.2).

Testing the results based on feces-derived DNA

The primary purpose of most early studies of feces-derived DNA was to show that DNA can be amplified from feces and to give suggestions for increasing DNA yield and purity (Table 2.2). Because these studies were mostly exploratory, very few utilized internal checks to test the validity of their results. Given the known weaknesses of feces as a DNA source, internal checks testing the reliability of results from studies using feces are essential. All four of the following checks are important for accurate results: 1) use of DNA from known individuals, 2) use of DNA from first degree relatives such as parents or offspring, 3) use of high-quality DNA from blood or another tissue as a positive species-specific control, and 4) multiple independent replications (Table 2.2 'Checks' column for review of these issues).

Only two prior studies reported that the fecal samples came from known individuals (Gerloff *et al.* 1995; Launhardt *et al.* 1998). Without knowing from whom the fecal samples come, it is impossible to test whether different genotypes come from two different individuals, or whether they represent spurious results from two fecal samples originating from the same individual. Although this point seems obvious, many studies (Table 2.2) have used feces-derived DNA from unknown individuals and therefore can not say with certainty whether within sample (or individual) results are repeatable and reliable.

Only Launhardt *et al.* (1998) reported that fecal samples were collected from known relatives. Without known relatives it is impossible to perform Mendelian checks, making sure that mothers and offspring have at least one allele in common at all loci. This check, applicable to all genetic studies regardless of DNA source, is especially important when testing the reliability and efficiency of a new DNA source such as feces.

Although half of the studies reported using species controls from a high-quality DNA source, such as blood or another tissue, for comparison with the fecal results (Gerloff *et al.* 1995; Kohn *et al.* 1995; Van der Kuyl 1996; Paxinos *et al.* 1997; Reed *et al.* 1997; Wasser *et al.* 1997; Launhardt *et al.* 1998), the tissue/fecal comparisons were matched from known individuals in only three studies (Reed *et al.* 1997; Wasser *et al.* 1997; Launhardt *et al.* 1998).

Most studies reported doing multiple replications of fecal amplifications, and some did so under various conditions, but only Taberlet *et al.* (1997) and Flagstad *et al.* (1999) reported that the replications were independent.

Features of this study

The DNA used in the present study comes primarily from feces and was amplified using human STRP primers on the X chromosome. DNA quantities were typically low because (1) the DNA was degraded and sheared, (2) they were derived from feces, (3) the STRP loci were single-copy genes, (4) amplification was hindered by plant inhibitors and, (5) cross-species amplification is less robust.

This study benefited from the work of earlier studies and includes several tests to confirm the results of feces-derived DNA amplifications. The identity of all subjects in this study was known, making it possible to test the consistency of the results from two or more fecal samples from the same individual. Along with Bayes *et al.* (in press) and Launhardt *et al.* (1998), this was one of the few studies of feces that could perform Mendelian checks on the genotypes. Mother-offspring (in this study mother-daughter) pairs were checked to see if they shared at least one allele at each locus. Blood was collected for nearly a quarter of the baboons in this study, enabling me to use the high-quality DNA from blood as a species control group. Results from feces-derived DNA

were compared to results from blood-derived DNA for the following features: allele size, proportion of successful amplifications, and rates of allelic dropout and heterozygosity.

Previous studies have shown that DNA can be amplified from feces and have suggested further applications of those amplifications. However, only one other study (Launhardt *et al.* 1998) set out to determine the genetic relatedness between pairs of individuals in a large group based on genotypes from feces-derived DNA amplifications. Further, this study is unique, in that it makes use of STRP primers located exclusively on the X chromosome in order to identify paternal half-sisters in the absence of paternal DNA.

METHODS

Subjects and site

The subjects of this study are 29 adult female savannah baboons (*Papio cynocephalus*) living in and around Amboseli National Park, Kenya (Altmann 1980; Alberts and Altmann 1995; Altmann *et al.* 1988). The subjects, along with other group members, are habituated to human observers who take precautions to keep human-baboon interactions to a minimum. Each baboon is individually recognized by naturally occurring individual differences. The 29 subjects live in three distinct social groups: Linda's, Weaver's, and Dotty's.

Collection and storage

The DNA used in this study was extracted from feces (n = 22 females) and, when possible, from blood (n = 7 females). Most of the blood samples were collected in 1993; the fecal samples used in this study were collected between 1990 and 1999. Blood was only collected during specific instances when an animal was darted and anesthetized for other reasons (Sapolsky and Altmann 1991; Altmann *et al.* 1996). Approximately 2 – 3 mL of blood were collected, along with hair and tissue, while the animal was unconscious. The blood was collected into vacutainer tubes with EDTA as an anticoagulant. The samples were cooled as soon as possible and then spun down and frozen for shipping within hours of collection (Altmann *et al.* 1996).

Fecal samples were collected almost immediately after defecation and stored in 5 mL Cryogenic tubes that were previously filled with 2.5 mL of 95% ethanol. Approximately 2 g of feces were carefully collected, avoiding any contamination with human DNA. The storage tubes were labeled with the subject's name, the time, and the date and then sealed with parafilm. Samples were stored at ambient temperature in the field for up to six months. Once the fecal samples were in the US, they were frozen at -80° C.

DNA extraction

DNA was extracted from baboon feces and blood. Human DNA from two cell lines was obtained from the Centre d'Etudes du Polymorphisme Humain (CEPH) and used as positive controls in all experiments.

The fecal extraction protocol developed by Gerloff *et al.* (1995) was tested early in this study, but the extractions retained such high levels of inhibitors (most likely plant) that baboon blood and human blood (CEPH) failed to amplify when small amounts of the fecal extractions were added to them. This strong evidence for inhibitors in the feces sampled led to a search for an extraction protocol that addressed the inhibition issue. Paxinos *et al.* (1997) suggested removing visible plant material before extracting and Taberlet *et al.* (1997) suggested freeze-drying the feces before extracting.

DNA was extracted using two different protocols: a slight modification of the method described in Taberlet *et al.* (1997) and a method adapted for feces by Qiagen (Hilden, Germany), using the QIAamp® tissue extraction kit with modified buffers. These two protocols will be referred to throughout this paper as Taberlet and Qiagen extractions respectively.

Several modifications were made to Taberlet's protocol (1997 p.871). First, the dried feces were sifted through a small tea strainer to separate the fecal "powder" from any visible plant material. I then used 0.5 g of dried fecal powder, rather than 50 mg of

dried feces. Second, 10 M guanidine (GuSCN) in the L6 buffer was too concentrated to go into solution and so I used 5 M GuSCN. Third, I washed the samples with L2 buffer twice rather than three times, hoping to lose less DNA during extra washes. Fourth, the pellets usually took more than 10 minutes to dry. The wet parts of the pellet were darker than the dry spots, so I left the samples at 60°C until the pellet was consistently light colored and dry before eluting. The final change was to heat the TE to 60°C to improve the eluting process and increase the DNA yield.

The Qiagen "fecal extraction kit" was actually a modification of the Qiagen tissue extraction kit, and was not yet available to the public in 1998. All DNA extractions were stored at 4°C to avoid the shearing that can occur during repeated freezing and thawing.

PCR amplification and analyses

DNA was amplified using primers for five human STRP loci on the X chromosome: GATA164D10, GATA124B04, GATA144D04, GATA69D06, and AFM240WA9. DNA extracted from the blood of three male baboons was included to ensure that the markers were located on the baboon X chromosome. To minimize linkage (i.e., dependence) between the STRPs, the five primers selected were ≥ 20 cM apart on the human X chromosome.

Various amounts of extracted DNA were pipetted into strip tubes and then allowed to air dry before adding the PCR cocktail mixture. For blood and CEPH DNA,

0.5 µL of extract was added to each tube and for feces-derived DNA, 1 µL of extract was added. Once the DNA was dry, 10 µL of PCR mixture was added. The PCR mixture was made by adding the following reagents in the following order: 246 µL ultra pure H_20, 36 µL of Promega 10 X buffer, 36 µL of 2 mM dNTPs, 36 µL of 25 mM $MgCl_2$ (Promega), 2.9 µL of both forward and reverse primers, 2 µL of alpha ^{32}P-dCTP, and 5 µL of *Taq* polymerase (Promega).

All amplifications were carried out under the following PCR conditions using a Perkin Elmer GeneAmp 9600 thermal cycler: samples were denatured for 5 minutes at 94°C followed by 40 cycles of denaturing for 30 seconds at 94°C, annealing for 75 seconds at 54°C, and extending for 30 seconds at 72°C. Following a final extension period that lasted 10 min at 72°C, the samples were held at 6°C. PCR products were resolved on a denaturing polyacrylamide gel (6.7%) and were visualized on Storm phosphorous screen using Image Quant software.

DNA from two to four females was included on each gel. Between 8 and 12 replicate lanes for each female were run. The replicates were run in groups of four and were often, but not always, from either different fecal samples or from different extractions of the same fecal sample. Baboon blood and CEPH DNA were interspersed between groups of four fecal lanes, both as size ladders and as positive PCR controls. Running multiple replicates for a female on one gel made it possible to identify cases of

allelic dropout and to identify both bands in heterozygous individuals in those cases where only a single allele amplified in each trial.

Independence

Statistical independence between samples was difficult to achieve since the purpose of this study was not methodological, but was to genotype the 29 female baboons, which was often accomplished by using just those fecal samples or extractions that had previously proven successful at other loci. Because of this, the full set of all genotyping attempts is unbalanced and not independent; successful fecal samples and extractions are over-represented and the number of trials per individuals per locus varies with the ease with which the genotype was resolved.

To achieve nominal independence for statistical analyses, each fecal and blood sample was counted only once per locus. Although it took 2,577 amplifications of blood and feces to genotype the 29 females at five loci, to be fairly conservative, only 35 blood samples (7 females x 5 loci) and 163 fecal samples (22 females x 1-5 loci) are considered independent and are therefore included in the methodological results that follow. The single amplification for each fecal sample at each locus was chosen arbitrarily by Microsoft Visual Fox Pro.

Genotype assignments

Allelic dropout was frequent, amplifying only one allele and producing an excess of homozygotes. Therefore, assignment of genotypes was based on a consensus of alleles amplified in the 8 to 48 replications. If, during the first 8 – 12 replications, two alleles were amplified unambiguously at least three times, the individual was considered heterozygous and the genotyping at that locus was considered resolved. The two alleles did not necessarily have to appear in the same amplification product. That is, individuals who appeared 'homozygous' for two different alleles were considered as heterozygotes, even if the two alleles never appeared in the same amplification (Taberlet *et al.* 1996; Bayes *et al.*, in press). If, after the first 8 – 12 replications, less than three replications amplified, or if the individual appeared to be homozygous, an additional 8 – 12 replications were attempted. Individuals were considered to be homozygous if only one allele amplified after 16 replication attempts. Fecal samples from some females consistently yielded very little DNA and therefore as many as 48 replications were carried out in an attempt to genotype these females.

RESULTS

Comparison of extraction protocols

The two extraction methods differed significantly in their ability to amplify baboon feces-derived DNA (Pearson's χ^2, $P < 0.0001$). More than half (53%, 87/163) of the Taberlet extractions but only 12% (6/51) of the Qiagen extractions amplified DNA

that allowed genotype determination. Because the Qiagen extractions yielded so little DNA, I discontinued the use of these kits. There are therefore far fewer independent Qiagen amplifications than Taberlet-extracted amplifications (Qiagen, n = 51, Taberlet, n = 163). The results that follow use independent amplifications of feces-derived DNA, i.e., one amplification / fecal sample / locus, and were extracted using the modified Taberlet method unless otherwise noted.

Screening human STRP primers in baboon DNA

Twenty-nine human microsatellite markers were tested on DNA from baboon blood (Appendix 2.1). Less than half (41%, 12/29) of the primers successfully amplified high-quality baboon DNA. Of these 12 primer pairs, seven amplified only one band, suggesting that all individuals were homozygous for the same allele and that the locus was not polymorphic in baboons. The remaining five of the twenty-nine markers (17%) were suitable for amplification of baboon DNA to be used for genotyping and identifying paternal half-sisters and were therefore used in this study. These results agree closely with those reported by Launhardt *et al.* (1998) who found that 5 of the 32 (16%) human primer pairs amplified DNA extracted from langur feces and were polymorphic.

Marker variation

The five human STRP primers included in this study were trinucleotide (n = 1) or tetranucleotide (n = 4) base pair repeats. These markers were chosen because they were polymorphic in baboons, averaging 6 alleles (Tables 2.3, 2.4) and were heterozygous in

baboons (mean number of animals heterozygous was 86%; range = 79% to 97%). Approximately half of the independent amplifications failed for each marker. These failures were due to problems with feces-derived DNA, as the positive controls and the DNA from baboon blood always amplified in these trials. AFM240WA9 had the highest amplification rate and GATA69D06 had the lowest (67% vs. 44% respectively). However, these differences were not statistically significant (n = 163, Pearson's χ^2, P = 0.40).

The markers tended to differ in their allelic dropout rates (n = 87, Pearson's χ^2, P = 0.06). When considering only those independent trials in which blank lanes were excluded, AFM240WA9 was the least susceptible to allelic dropout, amplifying the complete genotype 77% (17/22) of the time, while GATA164D10 was the most prone to allelic dropout, amplifying the complete genotype only 31% (5/16) of the time. Therefore the latter marker required more amplifications per sample to resolve heterozygous genotypes.

Both the number of alleles and the allele frequencies reported here are comparable to those reported by others (Reed *et al.* 1997; Launhardt *et al.* 1998; Flagstad *et al.* 1999). In this study, markers amplified between 5 and 8 alleles while markers used in other studies amplified between 2 and 10 alleles (Reed *et al.* 1997; Launhardt *et al.* 1998; Flastad *et al.* 1999). The allele frequencies of the markers used here ranged from 2% to

41%, again, within the range reported by others, (Reed *et al.* 1997, 2% to 90%; Launhardt *et al.* 1998, 1% to 65%).

Blood / fecal comparisons

Efficiency and reliability varied between the two DNA sources, blood and feces. In this study, 97% (34/35) of independent amplifications of blood-derived DNA succeeded, while only 53% (87/163) of all independent, Taberlet-extracted, amplification of feces-derived DNA succeeded in producing at least part of the final genotype (n = 198, Pearson's χ^2, $P < 0.0001$). The high quality of the DNA extracted from blood (n = 35) provided no evidence for allelic drop out in this study; DNA extracted from feces (n = 137) exhibited allelic dropout in 48% (66/137) of PCR reactions (total n = 172 Pearson's χ^2, $P < 0.0001$). When allelic dropout occurred it did not affect one allele preferentially so multiple replications eventually amplified both bands, though not necessarily within a single amplification. The final proportion of homozygote genotypes obtained from amplifications of feces-derived DNA was indistinguishable from that of blood DNA (blood, 11% (4/35); feces, 14% (16/113); Pearson's χ^2, $P = 0.67$, Appendix 2.2). This result suggests that running multiple replications alleviates the effects of allelic dropout in amplifications of feces-derived DNA.

Individual variation in DNA quality

The feces-derived DNA from some baboons consistently amplified while the fecal samples from others yielded very little DNA despite using multiple fecal samples and

extractions. Five of 29 individuals (17%) amplified at least one correct allele in each independent amplification, regardless of which marker was used (Asha n = 5, Echo n = 5, Lark n = 7, Laza n = 5, and Wasp n = 5). For other individuals, DNA from feces never amplified in an independent trial (Lassoi n = 5), however their genotypes were eventually resolved through repeated, non-independent trials. Some were extremely difficult to type because of their consistently low DNA yield (Velcro n = 2/12, Wagtail n = 2/9).

Reliability of feces as a DNA source

Three tests confirming the reliability of feces as a source of DNA, and of the genotypes identified in this study, were used. First, the homozygosity rate was compared between DNA extracted from blood and from feces and the two sources were found to be indistinguishable from each other. Second, known mother-offspring pairs were used to perform Mendelian checks. In this study, 60 total mother-daughter checks were made (12 mother-daughter pairs across 5 loci). One mother who had two daughters did not amplify at one locus (Wema at GATA124B04), leaving 58 actual Mendelian checks. All 58 mother-daughter pairs passed the Mendelian check, i.e., they had at least one allele in common. For 48 of the 58 pairs, at least one genotype was derived from feces. Lastly, high-quality, blood-derived DNA from three baboon males was included in all screening trials to make sure that the markers amplified sequences on the baboon X chromosome. In all 15 cases (3 males at 5 loci) males amplified only a single band as expected. This test, although not conclusive as males can be homozygous for autosomal markers, suggests the human STRP primers amplified DNA on the baboon X chromosome, a

condition that must be met in order to assume that paternal-half sisters will share the same paternal allele at each locus.

DISCUSSION

Comparison of extraction protocols

The difference in the amplification rates of the two fecal DNA extraction protocols may reflect an interaction between the extraction and visualization methods. The results reported here suggest that when visualizing by autoradiograph, the Taberlet extractions yield greater amplification rates. However, Bayes *et al.* (in press) visualized DNA by fluorescent labeling on an ABI 377 automated sequencer and reported amplification rates from Qiagen extractions that were similar to the Taberlet amplification rates reported here. The Taberlet extractions were 'dirtier' than were the Qiagen extractions; that is, many Taberlet replications amplified multiple bands in addition to the expected bands. The additional bands were distinguishable from real bands because of differences in thickness, intensity, shape, size, or because of a lack of appropriate stutter bands. These bands never appeared in the amplifications of Qiagen extractions. Most often the amplification attempts from Qiagen extractions failed completely when visualized in ^{32}P-labelled DNA, but when DNA did amplify, the samples were free of any additional bands. The results reported here and by Bayes *et al.* suggest that one should take into account the visualization method when deciding on the method used to extract DNA from feces.

Screening human STRP primers in baboon DNA

The low amplification rate of human primers is probably due to sequence mismatches between humans and baboons (this study) and langurs (Launhardt *et al.* 1998). The difference in the amplification rates among the primers suggest that the DNA sequences flanking the microsatellite loci in humans and the nonhuman target species is conserved at the loci where cross-species amplification is successful. Microsatellites may provide information about the evolutionary distance between two species if the differences in cross-species amplification rates do in fact reflect conservation of sequences between them (Coote and Bruford 1996).

Individual variation in DNA quality

The source of the variation in the ability to amplify DNA from the feces of different females is not clear. Slight variations in diet, digestive physiology, or unknown genetic differences between animals may influence the number of epithelial cells that are sloughed, or the degree of degradation of the DNA in these cells.

In addition to the differences among females, there may also be an interaction between fecal samples and microsatellite markers. DNA from some feces was generally hard to amplify at some loci, but not at others, e.g., 48 replications were necessary to type one female at GATA164D10 but only 8 replications were necessary at AFM240WA9. This was not explained by AFM240WA9 being a robust marker for every female. DNA from another female's feces took 20 replications at AFM240WA9 while genotypes at all

other loci were determined in the first 8 or 12 trials. DNA from a third female's feces required only 12 replications to type her at both AFM240WA9 and GATA69D06, but required 44 replications to type her at GATA164D10. Finally, as in this study, Launhardt *et al.* (1998) report a single individual for whom a final genotype could not be resolved. Here, one individual could not be genotyped at one locus (GATA124B04), despite multiple extractions from multiple fecal samples, and despite amplification of her DNA at all other loci.

These results may be explained by stochastic events. Alternatively, some females may have sequence variation that interferes with primer binding. This would reduce the likelihood of amplification at a specific locus, but would not affect amplification at other loci.

Reliability of feces as a DNA source and importance of validation checks

Ensuring accuracy of genotyping results is especially important when using cross-species amplification and/or low-quality DNA, and is even more critical when using the genotypes to determine relatedness between individuals (Smith *et al.* 2000). This study highlights the importance of multiple replications and of Mendelian checks when using cross-species amplification of feces-derived DNA by demonstrating non-specific amplification of an apparently polymorphic and replicable STRP primer. In this study, STRP primer GATA48H04 passed the traditional checks during the screening process (Appendix 2.1). It amplified baboon DNA extracted from blood and feces of known

individuals, and produced consistent, polymorphic genotypes in repeated replications. However, three of the six mother-daughter pairs tested had no shared allele at this locus. Banding patterns that do not follow Mendelian segregating patterns may result from non-specific amplification of a second locus, or from polymorphisms in the primer binding sites. Some primers such as GATA48H04 may non-specifically amplify two loci in the genome, with the mothers DNA amplified at one locus and the daughters DNA amplified at a second locus.

The ability to perform Mendelian checks was essential despite the fact that fecal samples came from known sources, that blood was used as a species control for allele size, polymorphism, and heterozygosity, and that multiple replications were performed to resolve each genotype.

CONCLUSIONS

In this study, the extraction protocol presented in Taberlet *et al.* (1997) yielded higher quantities of DNA from feces, than did the modified QIAamp® tissue extraction kit. Primers for human STRP loci varied greatly in their ability to amplify baboon DNA. More than half of the primers tested (17/29) failed to amplify baboon DNA. Of those markers that did amplify baboon DNA, more than half were monomorphic in the animals (n = 5) used in the screening tests. Five human microsatellite markers were both polymorphic and highly heterozygous in baboons. The DNA derived from feces was more prone to poor amplification and allelic dropout than DNA derived from blood.

However, with repeated trials, use of blood controls, and known individuals and relatives, feces provided a plentiful, non-invasive source of DNA that was sufficiently reliable to construct genotypes and identify paternal half-sisters, which was the ultimate the goal of this study.

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Table 2.2 Summary of papers publishing results of feces-derived DNA.

Year	Organism	Purpose of study	# Fecal Samples	Checks	Reference
1992	brown bear	amplify DNA	3	2nd species comp.[a]	Hoss *et al.*
1993	fox	identify parasite DNA	29	none	Bretagne *et al.*
1995	olive baboon	amplify DNA	40	2nd species comp.	Constable *et al.*
1995	brown bear	# & sex in group	12	liver[b], 6 replications[c]	Kohn *et al.*
1995	bonobos	amplify DNA	33	blood[b], ID known[d], mult. rep.	Gerloff *et al.*
1995	human	amplify DNA	10	mult. rep.	Deuter *et al.*
1996	dugong	amplify DNA	1	2nd spec. comp.	Tikel *et al.*
1996	green monkey	amplify DNA	1	blood, mult. rep.	vanderKuyl *et al.*
1997	carnivores	ID species	14+	scat from known species	Foran *et al.*
1997	review	NA	NA	NA	Kohn & Wayne*
1997	brown bear	# & sex in group	10+	mult. ind. reps.	Taberlet *et al.*
1997	harbor seal	# & sex in group	20	blood, mult. reps.	Reed *et al.*
1997	kit fox	ID species	10	blood, 2nd species comp.	Paxinos *et al.*
1997	sun &black bear	amplify DNA	15	blood, mult. reps.	Wasser *et al.*
1998	baboon	amplify DNA	22	many rep.under diff. cond.	Frantzen *et al.*
1998	cautionary note	NA	NA	NA	Taberlet, Waits*
1998	extinct ground sloth	amplify DNA	1	46 independent clones	Poinar *et al.*
1998	fossilized feces	NA	NA	NA	Andrews *et al.*
1998	langurs	paternity exclusion	178	Tissue, ID known, Mendelian	Launhardt *et al.*
1999	bonobos	est. pop. size	238	mult. rep., est. Het & HW[e]	Gerloff *et al.*
1999	coyote	?	?	?	Kohn *et al.*
1999	langur	paternity/infanticide	16+	Tissue, ID known, Mendelian[f]	Borries *et al.**
1999	sheep/reindeer	new ext. protocol	52	blood, ind. reps.	Flagstad *et al.*

The Checks column lists the checks that were performed to confirm the reliability of the observed fecal results.

[a] '2nd species comparison' = the sequence of the test feces was compared to that of a closely related species.

[b] 'Liver' and 'blood' = the feces-derived DNA sequence was compared to high-quality DNA derived from either the liver or from blood.

[c] 'Multiple replications' = trials were repeated, sometimes under differing conditions, and twice, the replications were independent.

[d] 'ID known' = subjects are individually recognized, making it possible to perform intra-individuals tests.

[e] Heterozygosity and deviations from Hardy-Weinberg were estimated in Gerloff *et al.* (1999).

[f] Mendelian checks were performed on known mother-offspring pairs by Launhardt *et al.* (1998) and Borries *et al.* (1999).

* Review papers, or studies based on an earlier paper in this table. * These review papers arenot included in this review of methodological studies.

Table 2.3 Allele frequencies.

GATA164D10		GATA124B04		AFM240WA9		GATA144D04		GATA69D06	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
0[a]	.03	1	.21	1	.31	1	.16	0	.03
1	.26	2	.24	2	.14	2	.22	1	.22
2	.28	3	.26	4	.10	3	.03	2	.07
3	.33	5	.07	5	.36	4	.03	3	.41
4	.03	6	.03	6	.03	5	.19	4	.24
5	.07	7	.08	7	.05	6	.09	5	.02
		8	.10			7	.14		
						9	.14		

[a] Allele 0 represents a band, smaller than band 1 that was identified after the numbering scheme I used was already in place.
Data come from Appendix 2. Allele frequencies are pooled across individuals in all three baboon groups, and across both blood and fecal extractions, but individuals are counted only once.

Table 2.4 Marker variation.

Marker	Distance from Pter (cM)*	# Alleles	% Amp. Suc.[a]	% Dropout[b]	%Het[c]
GATA164D10	4.39	5	47	69	97
GATA124B04	8.76	7	55	33	79
AFM240WA9	52.63	6	67	23	87
GATA144D04	71.29	8	52	37	87
GATA69D06	93.17	6	44	47	83

[a] The % of independent amplification attempts in which at least one allele was amplified.
[b] The % of independent amplifications in which allelic dropout was observed.
[c] The % of independent amplifications that were heterozygous.
* Based on human chromosome.

Appendix 2.1 Human microsatellite primer pairs tested on baboon DNA. The primers in the first column were tested on DNA extracted from baboon blood (n = 5 individuals), and on high-quality human DNA (CEPH, n = 2 individuals, 1 male and 1 female) included as a positive PCR control and to insure that the male had only one allele at each X chromosome locus tested. The primers in the next three columns successfully amplified DNA extracted from both baboon blood (n = 5, same individuals used in the initial tests) and feces (n ≤ 24 individuals, depending on whether the primer passed all checks and was used to genotype all individuals in the study). Information on primer sequences, allele sizes, and heterozygosities in humans can be obtained at www.marshmed.org.

Primers that failed to amplify baboon DNA derived from blood, but did amplify human DNA	**Primers that amplified baboon DNA but < 10% of test animals were heterozygous**	**Primer amplified baboon DNA and was polymorphic but failed Mendelian checks**	**Primers that amplified baboon DNA, were ≥ 79% heterozygous in test animals, and passed Mendelian checks**
AFM203YD8	AFM317YE9	GATA48H04	GATA124B04
GATA10C11	DXS1227		GATA144D04
GATA124E07	DXS207		GATA164D10
GATA151F04	DXS6799		GATA69D06
GATA160B08	GATA125B12		AFM240WA9
GATA172D05	AFM248WF9		
GATA186D06			
GATA192D07			
GATA28C05			
GATA31B12			
GATA31F01			
GATA42G01			
GATA72E05			
GATA87A12			
GATA64D08			
GGAT3F08			
XS1059			

Appendix 2.2 Genotypes produced for each adult female baboon in study.

Name	STRP Primers				
	GATA124B04	**AFM240WA9**	**GATA69D06**	**GATA164D10**	**GATA144D04**
Dotty's Group					
Vixen	2, 3	5, 7	1, 3	1, 5	2, 9
Viva[a]	**1**[b], 3	**1**, 5	1, 3	1, **1**	2, **7**
Vortex	3, **4**	**1**, 5	1, **4**	**4**, 5	**6**, 9
Vinyl	3, **7**	5, **6**	**2**, 3	**2**, 5	2, **6**
Velcro	3, **5**	5, **6**	1, **2**	**0**[c], 1	**5**, 9
Dotty	3, 5	1, 5	1, 3	2, 5	5, **5**
Dove	**2**, 3	1, 5	**2**, 3	2, 5	5, **6**
Asha	2, 3	1, **1**	0, 1	1, 2	5, 6
Echo	2, 3	1, 5	1, 4	1, 2	6, 9
Omo	3, 5	1, 7	0, 3	3, 4	5, **5**
Ochre	5, **7**	1, **5**	3, **3**	**1**, 3	5, **9**
Linda's Group					
Nix	1, **2**	**1**, 5	3, **4**	1, 2	2, 3
Nightjar	1, 2	1, 5	3, 4	2, **2**	2, **7**
Linda	2, 6	1, **1**	1, 4	1, 2	2, 5
Kathryn	2, 3	1, 5	3, 4	1, 2	3, 5
Nyota	3, 6	2, 4	3, 4	2, 3	7, 9
Wema		2, 4	1, 3	1, 2	1, 2
Wasp	1, **3**	4, **7**	1, 3	1, **3**	1, 2
Lark	2, 3	2, 4	3, 4	2, 3	7, 9
Mystery	1, 7	1, 4	4, **4**	3, **3**	1, **1**

Appendix 2.2 Genotypes produced for each adult female baboon in study (continued).

Name	GATA124B04	AFM240WA9	GATA69D06	GATA164D10	GATA144D04
Weaver's Group					
Weaver	**1**, 2	**1**, 5	1, 3	**1**, 3	1, 2
Wagtail	2, 3	**2**, 5	3, **3**	**1**, 3	2, **5**
Wendy	1, 2	2, **5**	1, 3	2, **3**	1, **7**
Prudy	**7**, **8**	2, **2**	2, **5**	3, **3**	2, 5
Kelly	2, **8**	2, 5	3, 4	3, **3**	1, 7
Luna	1, 7	1, 5	**4**, **4**	1, 3	**4**, **4**
Limau	**8**, **8**	5, **5**	3, **3**	2, 3	2, 7
Laza					
Lassoi	1, **8**	1, 5	1, 3	2, 3	1, 2

[a] Bolded names are samples extracted from blood.
[b] Bolded alleles are paternal bands.
[c] 0 refers to an allele that is smaller than allele 1 and was first observed after the numbering scheme was already in place.

CHAPTER 3

Group fissions and kinship structure in three wild baboon groups

INTRODUCTION

Animals living in groups are predicted to benefit from early predator detection (Cheney and Wrangham 1987; Wrangham 1987; Rodman 1988), increased foraging efficiency (Wrangham 1987), and increased defensibility of resources (Mitani and Rodman 1979; Cheney 1987; Heinsohn *et al.* 1996). However, group living is also associated with costs such as increased competition for local resources such as food and mates (Hughes 1998), and increased transmission of disease and parasites (Cote and Poulin 1994). Given both the costs and the benefits of group living, with whom animals live, and how they distribute their social behavior among fellow group members become important. This chapter will concentrate on the kinship structure of three wild, natural-feeding baboon groups and how relatedness in two groups was affected by a recent group fission. Chapter 4 will then examine the distribution of social behavior among adult

females within each group and determine whether or not that distribution is biased towards both maternal and paternal kin.

Suites of demographic behaviors (behaviors that alter the composition of groups) such as mating systems and patterns of dispersal have a large effect on the kinship structures of a group, which in turn, influences the distribution of social behavior (Emlen and Oring 1977; Altmann and Altmann 1979; van Schaik and van Hooff 1983). Birds, lions, and chimpanzees provide examples of the relationship between demographic behaviors, the genetic substructuring of a social group, and the distribution of social behavior. Among many bird species, population density and breeding synchrony are both positively correlated with increased occurrences of extra-pair fertilizations (EPFs) (reviewed in Hughes 1998). EPFs, in turn, alter the genetic relatedness of a brood within the nest, which in turn may alter the distribution of parental behavior displayed by putative fathers towards the young within a nest. Male Reed Buntings who sired two broods with the same female within one breeding season contributed more food to the brood to which their paternity was greater (Dixon *et al.* 1994), regardless of brood order. This relatedness-specific investment by adult males was observed in Dunnocks and Barn Swallows as well (reviewed in Hughes 1998).

Several aspects of lion demographic behavior contribute to the genetic structure of the pride. Defense against conspecifics influences much of lion demographic behavior. Young adult males disperse together with their brothers and other cohort mates

in order to defend groups of breeding females against other male lions (Heinsohn and Packer 1995; Heinsohn *et al.* 1996). Related adult females and their offspring live in fission-fusion groups in order to defend the pride both from infanticidal males, and to defend their territory from larger prides (Heinsohn and Packer 1995). The close genetic relatedness among the adult males in the pride may explain why all adult males help defend the pride despite the fact that the young are sired by one, or sometimes by two, males. Close genetic relatedness among the adult females in a pride may explain the unusually high degree of cooperation they display; females hunt, defend their territory, and raise young cooperatively (Heinsohn *et al.* 1996).

Unlike most social mammals, which display female philopatry (Storz 1999), female chimpanzees disperse and males are philopatric. Adult male chimps in the same group are more closely related than are the females (Morin *et al.* 1994) and bond socially, grooming each other, displaying sexual tolerance, cooperating while hunting, and forming alliances during aggressive intergroup encounters. This high level of cooperative and affiliative behavior among adult males has been observed in other non-human primate species in which males, and not females, are philopatric (reviewed in Van Hooff and Van Schaik 1994). Furthermore, in these same species, social bonds among adult females are less obvious than are those observed among species in which females are matrilocal (Mitchell *et al.* 1991; Van Hooff and Van Schaik 1994).

Demographic behaviors contribute to the genetic structure of the multimale-multifemale social groups typical of savannah baboons (*Papio cynocephalus*), the subjects of this study. Females are matrilocal and males disperse independently from the natal group around the time they reach sexual maturity (Alberts and Altmann 1995a). Males continue to disperse throughout their adult lives with their rank in the aggression-submission hierarchy generally declining the longer they remain in a group (Alberts and Altmann 1995b). Finally, the baboons in this study are polygamous i.e., estrous females mate with more than one male and males mate with multiple females. However, matings and conceptions are not distributed randomly among the adult males in the group; the highest-ranking males benefit from priority of access to estrous females on the days when females are most likely to conceive.

Each of the demographic behaviors mentioned above, female philopatry, male dispersal patterns, and the polygamous mating system, have important consequences for the genetic structure of baboon social groups. As with other matrilocal mammalian species (Webb *et al.* 1995), adult female baboons within a group are more closely related to each other than are the adult males (Altmann *et al.* 1996) who are unrelated to other adult group members. The relatedness among breeding females in a matrilocal species leads to 'enhanced' relatedness among paternal siblings (Storz 1999) (see Discussion section for explanation). Patterns of male rank attainment and dispersal, along with a polygamous breeding system also have important genetic consequences. First, paternal siblings tend to be members of the same age cohort (Altmann 1979; Altmann *et al.* 1996).

Second, full siblings are rare (none were identified in this study). And third, inbreeding between fathers and adult daughters is unlikely.

Although cercopithecine social groups are fairly stable over extended periods of time, group fissions in macaques and baboons occur either when the population is expanding (Chepko-Sade 1974; Chepko-Sade and Olivier 1979; Chepko-Sade and Sade 1979) or as a response to increased environmental stresses (Nash 1976; Dittus 1988). Fissioning is the process by which one large socially structured group of animals becomes less cohesive until two or more independent groups are formed. Much of the non-human fission data come from observations of protected, food-provisioned groups in expanding populations (Sugiyama 1964; Furuya 1969; Missakian 1973; Chepko-Sade 1974; Cheverud *et al.* 1978; Chepko-Sade and Olivier 1979; Chepko-Sade and Sade 1979), or from observations of wild, non-provisioned groups in which individuals and their relatedness were not known (Stoltz 1972; Struhsaker and Leland 1984; Malik *et al.* 1985; Ron *et al.* 1994). Only one study of fission has been reported in which the group is wild and non-provisioned, and in which individuals and their maternal kin are known (Nash 1976).

Rank, patterns of affiliative behavior prior to the fission, and maternal relatedness have all been cited as factors determining which individuals eventually become separated during group fissions of cercopithecine primates. Both 'horizontal' and 'Abandon Your immediate Superior' (AYS) describe fissions in which rank in the dominance hierarchy

strongly influences the final composition of the newly formed groups. Horizontal fissions occur when the high-ranking females make up one of the newly formed groups, and low-ranking females make up the second (Chepko-Sade and Olivier 1979; Chepko-Sade and Sade 1979; Dittus 1988; Barton *et al.* 1996). AYS describes fissions in which females with odd ranks, i.e., 1, 3, 5 ..., end up in a different group than the females with even ranks, i.e., 2, 4, 6 ..., (Ron *et al.* 1994). Cords and Rowell (1986) and Nash (1976) found that affiliative social behavior predicted movement during group fissions; individuals that spent disproportionately more time grooming and maintaining close proximity to each other prior to the group fission were more apt to end up in the same group than were others. Finally, maternal genetic relatedness strongly influences which individuals will and will not be separated during a group fission. Rhesus macaque social groups on Cayo Santiago typically fission along matrilineal lines with close maternal relatives ending up together in the same group (Chepko-Sade 1974; Chepko-Sade and Olivier 1979). Larger, higher-ranking matrilines were more likely to remain intact than were smaller, lower-ranking matrilines.

All three of the social groups included in this study had undergone relatively recent group fissions; two of the three study groups were formed by a group fission that occurred only one year before the behavioral data were collected. Group fissions alter the size, and potentially alter the genetic and age structures within the newly formed daughter groups. Both the recent group fission, and the identification of close paternal relatives (e.g., paternal half sisters and aunts and nieces) provided the opportunity to test

several hypotheses about the effects and consequences of relatedness on group fissions and the resulting kinship structures within the newly formed groups.

H1 The average pairwise relatedness within groups will be greater after a group fission than before, when both maternal and paternal kin are considered.

H2 The average pairwise relatedness for each adult female will be greater with the females in the group she 'joined' than it would have been in the group she did not join, when both maternal and paternal kin are considered.

H3 The average pairwise relatedness after the fission will not differ between maternal and paternal kin.

As mentioned above, other studies have reported the strong influence of maternal relatedness on the movements of adult females during the fission process and on the final composition of the newly formed groups. However, the movement of close paternal kin during group fissions is unknown. The behavior of adult females during a group fission will directly affect the kinship and age structures of the newly formed groups (the focus of this chapter), which in turn is predicted to affect the distribution of social behavior among the adult females (the focus of Chapter four).

METHODS

Subjects

The subjects of this study (n = 29) were adult female baboons. They are members of social groups that live in and around Amboseli National Park, Kenya, which are part of

an ongoing, longitudinal study (Hausfater 1975; Altmann 1980; Altmann *et al.* 1988; Muruthi *et al.* 1991; Altmann 1998; Alberts 1999). All individuals are habituated to human observers who take precautions to avoid human-baboon interactions. Each baboon was individually recognized by naturally-occurring individual differences.

Study groups

The female baboons resided in three distinct social groups during the time behavioral data were collected, from July 1996 through February 1997. The three social groups were Dotty's Group (n = 11 adult females), Linda's Group (n = 9 adult females), and Weaver's Group (n = 9 adult females). However, each of these social groups was the product of a recent group fission (Figure 3.1). Dotty's Group was one of three groups formed when Alto's Group completed its fission in 1991; Linda's and Weaver's Groups were formed when Hook's Group fissioned in 1995. Because the adult females in Linda's and Weaver's Groups were all conceived in Hook's Group, it was necessary to consider all pairs of females from Hook's Group when trying to identify paternal sisters, and by extension, paternal aunts and nieces.

Identifying kin

In order to study the kinship structure of the three social groups, it was necessary to identify maternal, paternal, and non-kin. Extrapolating from known mother-offspring pairs identified all maternal kin. Maternity data came from long-term, on-going demographic and reproductive data collected every time the group was visited, several

days a week. Ten mother-daughter pairs, nine maternal half sister pairs, and twelve pairs of maternal aunts-nieces, and cousins were included in this study.

In order to identify paternal half sisters, females were genotyped at five X-linked loci using DNA extracted from blood or feces (see Chapter 2 for details on tissue collection and storage, and DNA extraction and amplification). A 'paternal relatedness score' was calculated for all pairs of females conceived in Hook's or in Dotty's Groups. The score reflected: 1) certainty in the amplification and assignment of specific alleles, 2) confidence that the alleles in question were paternally inherited, and 3) the frequency of those alleles in the group. The scores were then summed across the five loci yielding a 'paternal relatedness score' for every pair of females. Extrapolating from pairs of females identified as paternal half sisters identified paternal aunts and nieces.

Pairs of females were considered paternal half sisters if they met all three of the following criteria, the first a demographic criterion, the second and third, genetic ones. 1). Paternal sisters had to have at least one potential father in common at the time of their conceptions. If the two females did not have at least one adult or subadult male in common in the group when they were conceived, then they could not share a father and therefore could not be paternal sisters. 2). Paternal sisters had to have at least one allele in common at every locus. Because males are haploid on their sex chromosomes, and because all five STRPs were X-linked, paternal sisters must share at least one allele at every locus. 3). Paternal sisters had to have a paternal relatedness score that was at least

two standard deviations above the mean (n = 208 pairs, mean = 5.2, SD ± 1.8, range = 1.09 to 11.8, Table 3.1, Figure 3.2). All three criteria had to be met in order for pairs of females to be considered as paternal half sisters. If pairs of females did not meet one or more of the criteria, they were not considered as paternal half sisters. Meeting these criteria merely estimated paternal relatedness; pairs of females could not be included as paternal half sisters with 100% certainty. However, for the purposes of this study, I will consider paternal relatives to be those that met the criterion described above. Thirteen pairs of paternal half sisters and four pairs of paternal aunts and nieces were identified.

The assignment or exclusion as paternal sisters was inconsistent with the criteria stated above for 3 out of 208 (1%) pairs of females born in the two conception groups (Dotty's and Hook's). In all three cases, assignment (or exclusion) as paternal half sisters depended on the logic that if females A and B shared a father (indicated by high scores), and if females B and C shared a father (also indicated by high scores), then females A and C must also share a father (indicated by a high paternal relatedness score). Using this logic, one pair of females (Nightjar and Kelly) was identified as paternal half sisters despite having a paternal relatedness score that was slightly lower than the 'two standard deviations above the mean' criterion (7.58 vs. 8.8), i.e., Nightjar had high scores with both Limau and Wendy, Kelly's other two paternal sisters. Two other pairs of females were excluded as paternal half sisters despite their high scores (Linda and Weaver = 9.16; Limau and Lassoi = 8.84) for a similar reason. Weaver and Lassoi each shared a high

score with one female (Linda and Limau, respectively), but not with that female's other paternal sisters.

One of the consequences of identifying paternal kin was that individuals who were truly unrelated, rather than just not related maternally, could also be identified. In the literature 'non-kin' often refers to a heterogeneous group consisting of females who are truly unrelated, paternal kin, and individuals of uncertain relatedness (Missakian 1972; Kurland 1977; Massey 1977; Defler 1978; Silk *et al.* 1981; Walters 1981; Bernstein and Ehardt 1986; Cheney and Seyfarth 1990). In this study, 'non-kin' means more specifically, non-maternal and non-paternal kin, i.e., pairs of adult females (n = 94) who were not maternally related, and who were excluded as paternal kin. Of the 58 pairs of females identified as non-kin, half (52%, 30/58) were identified genetically (the pair shared no alleles at one or more X-linked loci), nearly a quarter (22%, 13/58) were identified demographically (the pair had no potential father in common at the time of their conceptions), and the final quarter (26%15/58) were excluded as kin by both genetic and demographic data (Figure 3.3). Pairs of females could be excluded as paternal aunts and nieces by extrapolating from the paternal sister data.

Finally, relatedness could not be determined for 66 pairs of females (32%). These females were not maternally related, but could be neither included nor excluded as paternal kin. In most cases they could not be excluded as paternal sisters because they had at least one male in common at the time of their conceptions, or they had at least one

allele in common at each X-linked locus. However, their paternal relatedness scores were significantly lower than were those of paternal half sisters, and were indistinguishable from those of 'true non-kin' (Tukey-Kramer test, $P < 0.05$, Figure 3.2).

Coefficients of relatedness

After identifying kin, coefficients of relatedness (r) were assigned as follows: half sisters, $r = 0.25$, aunts and nieces, $r = 0.125$, and cousins, $r = 0.0625$. The average pairwise coefficient of relatedness for each female to the other adult females in her group was calculated by summing all rs for her relatives and dividing that value by the number of adult females in the group minus one. These values reflect kinship in the generation immediately under study and they do not include the effects of longer times scales that might inflate (e.g., inbreeding owing to relatedness of paternal pairs) or deflate (e.g., inbreeding avoidance) these values. The number and distribution of loci used in the genotyping study was not appropriate for assignment of relatedness by other techniques such as those of Queller and Goodnight (1989).

RESULTS

Age proximity and kinship as predictors of group composition after the fission

Before we can understand the age and kinship structures of Linda and Weaver's Groups as they existed in 1996, it is necessary first to see how those structures were affected by the just-completed fissioning of Hook's Group. Age and relatedness both

predicted fairly well which females would subsequently reside in the same group and which females would end up in different groups after the fission. A significant percentage of adult females (72%, 13/18, Binomial test, $P = 0.03$) were of more similar ages to the other adult females in the group, on average, after the fission than before, i.e., females tended to end up together with other members of their age cohorts. This was especially true among the older, motherless adult females. All nine females born before 1987 were motherless at the time of Hook's Group fission, and all but one (89%, Binomial test, $P = 0.02$; Prudy was the exception) ended up in the same group as the other members of their age cohort (Table 3.2). As age cohorts tend to be made up of paternal siblings (Altmann *et al.* 1996, results herein), kinship and age are confounded. However, even the three older unrelated females (Wema, Nyota, and Luna), ended up in the same group as the other members of their age cohort.

Among the younger adult females, kinship, better than age, predicted the composition of the groups formed by the fission. Nearly half (44%, 4/9) of the females born from 1987 through 1992 had mothers who were alive at the time of the group fission, and three of those four (75%) ended up in the same group as their mothers. Of the five motherless younger females (females born during or after 1987), four had a sister (either maternal or paternal), and three (75%) ended up in the same group as that sister.

Consequences of the fission for the genetic and age structure

The fissioning of Hook's Group had several important consequences for the subsequent age and kinship structure of Linda's and Weaver's Groups. First, each female's average pairwise relatedness with other adult females was significantly greater after the fission than before (n = 18, Paired t-Test, $P = 0.006$, H1: Table 3.1). Not only was the average pairwise relatedness within groups greater after the fission than before, but each female's average pairwise relatedness to other adult females was greater in the group she 'joined' than it would have been in the group she 'rejected' (n = 18, Paired t-Test, P = 0.01, H2: Table 3.1, Figure 3.4).

The differences between maternal and paternal kin during the fission were interesting. The average pairwise relatedness after the fission did not differ between maternal and paternal kin (n = 18, Paired t-Test $P = 0.50$, H3: Table 3.1). This suggests that when the pairwise r was summed for all females, relatedness within the group was contributed to equally by both among maternal and paternal kin. However there were differences between maternal and paternal kin. First, relatedness among maternally related adult females did not increase after the fission (n = 18, Paired t-Test, P = 0.14, Table 3.1), while relatedness among paternal kin did (n = 18, Paired t-Test, P = 0.03, Table 3.1). The fact that not all kin classes behaved the same way may explain why paternal but not maternal relatedness increased after the fission, despite the fact that the final pairwise relatedness did not differ between the two types of kin. Mothers and

daughters, who have the greatest *r* value of any kin group, tended to end up in the same group, greatly increasing the average pairwise relatedness for females (n = 7) with either a mother or a daughter (Figure 3.5). However, maternal relatives other than mother/daughter pairs (e.g., maternal half sisters and maternal aunts, nieces, and cousins), were less likely to remain together than were paternal relatives (Figure 3.5). Because paternal kin tended to stay together, the average pairwise relatedness among them doubled after the group fissioned (*r* was divided by 17 other adult females before the fission and by 8 other females after the fission). Many of the maternal kin did separate during the fission (so *r* was not greater after fission than before), but because those that did stay together had a large *r* (mothers/daughters, r = 0.5), the average *r* between maternal kin did not differ from the more numerous, but less related, paternal kin.

The kinship / age structure of three groups

A quarter of all pairs of adult females were related (Figure 3.6). Although the proportion of kin to non-kin was consistent across all three groups (n = 127, Pearson χ^2 test, $P = 0.86$), the ratio of maternal to paternal kin varied greatly, ranging from 0.47 in Linda's Group to 6.25 in Dotty's Group (Figure 3.6). In Linda's and Dotty's Groups 67% and 51%, respectively, of female pairs were considered as unrelated using the demographic and genetic methods described above. Relatedness was uncertain for the remaining females (including most of the females in Weaver's Group who were not identified as either maternal or paternal kin).

The mean age of adult females, like the proportion of kin, was consistent across the three groups (n = 29, Tukey-Kramer, $P > 0.05$). However the spread in age varied across groups from 19 years difference between the oldest and youngest females in Dotty's Group, to only 9 years difference in Weaver's Group. Dotty's Group, with the largest age spread, had the lowest proportion of paternal sisters and the highest proportion of maternal sisters. This is not surprising given that maternal sisters tend to be spread out in age while paternal sisters tend to be of similar age (n = 22, mean age difference for paternal sisters = 12.7 months ± 13.3 SD, mean age difference for maternal sisters = 27.6 months ± 12.6 SD, Wilcoxon signed-ranks test, $P = 0.01$).

Variation in the kinship / age environment across females

The kinship / age environment varied greatly across females. The sum of each female's coefficient of relatedness with the other adult females in her group ranged from 0 (Prudy and Luna had no adult female relatives) to 2 (Vixen had four adult female daughters). The proportion of relatives varied among females almost as greatly as did the degree of relatedness. Again, two females were unrelated to any other adult females in their group, while one female (Wendy) was related to some degree, to more than half (63%, 5/8) of the adult females in her group, despite having separated from her mother and two sisters when Hook's Group fissioned.

The ages also varied among females. As the range in age was greatest among the females in Dotty's Group, it is not surprising that those females also exhibited the greatest extremes in the proportion of same vs. differently aged social partners. Five females had no same-aged social partners; i.e., they were at least one year older/younger than all other adult females in their group. At the other extreme, one female in Dotty's Group was born within one year of three other females.

DISCUSSION

Hook's group fission

Relatedness among adult females was greater after Hook's fission than before. Several possible explanations exist for this finding. First, if movement of individuals during a fission is non-random, with individuals tending to end up together with their close relatives, one would predict that relatedness within and genetic variance between groups to be greater after fissions than before (see Slatkin's 1977 propagule-pool model of colonization, reviewed in Storz 1999). Both these predictions are observed among the rhesus macaques on Cayo Santiago where fissions are matrilineal; genetic relatedness within groups and genetic variance between groups increased dramatically after fissions (Chepko-Sade and Olivier 1979; Chepko-Sade and Sade 1979).

While most studies of primate group fissions have concentrated almost exclusively on the behavior of maternal kin (see references above), the results in this study would have been counter-intuitive if only maternal kin had been considered.

Relatedness among maternally related adult females did not increase after the group fissioned, counter to the expectation. It was not until both maternal and *paternal* kin were considered that the final composition of the new groups 'made sense'. Relatedness among paternal kin did increase significantly after the group fissioned suggesting that while the group size decreased, the number of close paternal kin did not; i.e., paternal kin tended to end up together in the same group. This is the first study to demonstrate that groups fission along paternal lines as well as maternal lines.

There are both social and genetic explanations for why paternal half sisters might be more likely to stay together during a group fission than maternal half sisters. Because paternal half sisters are generally of similar ages (see Chapter 4), they have the opportunity to interact socially for a greater proportion of their lives than do maternal half sisters. The degree of overlap in life spans of paternal sisters mean that they will go through similar life history stages synchronously (Altmann 1979); they will be infant and juvenile playmates together, and they will reach menarche, have offspring, and experience old age (assuming they survive) at similar times. Genetic reasons might also help explain why paternal sisters showed a greater tendency to end up together after Hook's Group fissioned than did maternal sisters. Genetically, paternal half sisters share slightly more alleles on average than do maternal half sisters (de Ruiter and Geffen 1998) for two reasons. First, savannah baboons are matrilocal and therefore adult females in the group are more closely related than are the adult males (Altmann *et al.* 1996; de Ruiter and Geffen 1998). As a result, paternal half sisters have 'enhanced' relatedness (Storz

1999), sharing alleles both through their father, and also through their different mothers, who are most likely at least distantly related rather than unrelated. Maternal half sisters, on the other hand, who share alleles through their mother, most likely do not share alleles through their different, unrelated fathers who immigrate independently into the group as adults. Second, paternal siblings of the same sex share identical paternal alleles on the sex chromosome they inherit from their father. While paternal half sisters will share all their X-linked alleles, maternal half sisters will share, on average, only half the alleles on their maternally inherited X chromosome. Although the difference in the proportion of shared alleles between paternal and maternal half sisters is slight, it provides genetic variation between the two by which kin selection can be achieved.

Although most (12/16) females[1] ended up in the newly formed group that would have been predicted by kinship or age similarity (and therefore increased familiarity), 4 of the 16 did not. The cases of two females were particularly interesting. Prudy had no close adult female relatives on either her mother's or her father's side. She was, however, a member of a large age cohort with five other females born within one year of her and a sixth female only 14 months her junior. However, she ended up in a different group than the other members of her age cohort. Rank may partially explain this outcome. Prudy was the second highest-ranking adult female in Hook's Group; Wema, the highest-ranking female, ended up in Linda's Group. Prudy became the highest-ranking female when she 'left' the group that Wema was in and joined what subsequently

became Weaver's Group. Her behavior was similar to that of the adult females described by Ron *et al.* (1994) in which females abandon the female immediately above them in the dominance hierarchy. The second interesting fission case involved Wema's oldest daughter Wendy. Wendy was the only female who ended up in a different group than her mother after Hook's Group fissioned. Before paternal relatives were identified, Wendy's behavior was puzzling as she 'left' her mother and a maternal sister, and she apparently ended up in a group with only two maternal cousins. Maternal relatedness alone did not predict her behavior. However, Wendy had two paternal sisters and a paternal niece in the group she joined, making her average coefficient of relatedness to the other adult females, equal in both groups formed by the fission.

Finally, in this study the proportion of related adult females and the coefficient of relatedness among them were comparable to those reported by others for macaques. Here, a quarter of adult female pairs in all three social groups were related, compared to 23% reported by Call *et al.* (1996) for captive rhesus macaques. Further, de Ruiter and Geffen (1998) reported an average relatedness within a group of long-tailed macaques of 0.068. The average relatedness among adult females in Linda's, Weaver's, and Dotty's Groups were 0.07, 0.06, and 0.09 respectively (the average across all three groups was 0.076).

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[1] For two additional females, both newly formed groups had similar kin/age options and so they did not gain any obvious benefits for joining one group as opposed to the other.

CONCLUSIONS

In this study both genetic and demographic data were used to distinguish between paternal kin and unrelated individuals (pairs unrelated through both their maternal and paternal lines). The age/kinship structure among adult females was compared before and after Hook's Group fissioned. The average pairwise coefficient of relatedness among adult females in a group was greater after the fission than it was before. The majority of females ended up in the newly formed group that made the most genetic 'sense' when both maternal and paternal relatedness were considered, however the tendency to remain with kin was stronger among paternal kin than among maternal kin (except for mother/daughter pairs).

Among the three social groups, the proportion of kin was fairly constant with roughly one quarter of all adult female pairs being related. In Weaver's Group, kin were fairly evenly distributed between maternal and paternal kin; kinship was skewed towards paternal kin in Linda's Group and towards maternal kin in Dotty's Group. The range in the proportion of potential social partners who were adult female relatives varied from zero, for two subjects, to 0.63 for one female. The nearly unique way that each female experiences the kinship and age structures of her social group is predicted to influence the distribution of her social behavior, the subject of Chapter 4.

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Table 3.1 Relatedness before and after Hook's group fission. These results test the hypotheses that the average pairwise relatedness among adult females was greater after a fission than before, and further, that the average pairwise relatedness among females was greater in the group a female 'joined' after the fission than it would have been in the group she 'rejected'. The hypotheses were each tested using a Paired t-Test with n = 18.

Hypotheses	Results	P-value
H1	The average pairwise relatedness was greater after the fission than before.	$P = 0.006$
H2	The average pairwise relatedness was greater in the group 'joined' than in the group 'rejected'.	$P = 0.01$
H3	After the fission, the average pairwise relatedness among maternal and paternal kin did not differ.	$P = 0.5$
H3a	The average pairwise relatedness among maternal kin was *not* greater after the fission than before.	$P = 0.14$
H3b	The average pairwise relatedness among paternal kin was greater after the fission than before.	$P = 0.03$

Table 3.2 Birth years in Linda's and Weaver's Groups for females in this study.

Birth Year	Linda's Group	Weaver's Group
81	Kathryn	
82	Nix, Linda, Wema	Prudy
83	Nyota	
84		
85		Limau
86		Kelly, Luna
87	Lark	Weaver, Wendy
88	Nightjar	
89		Wagtail
90		Lassoi
91	Mystery	Laza
92	Wasp	

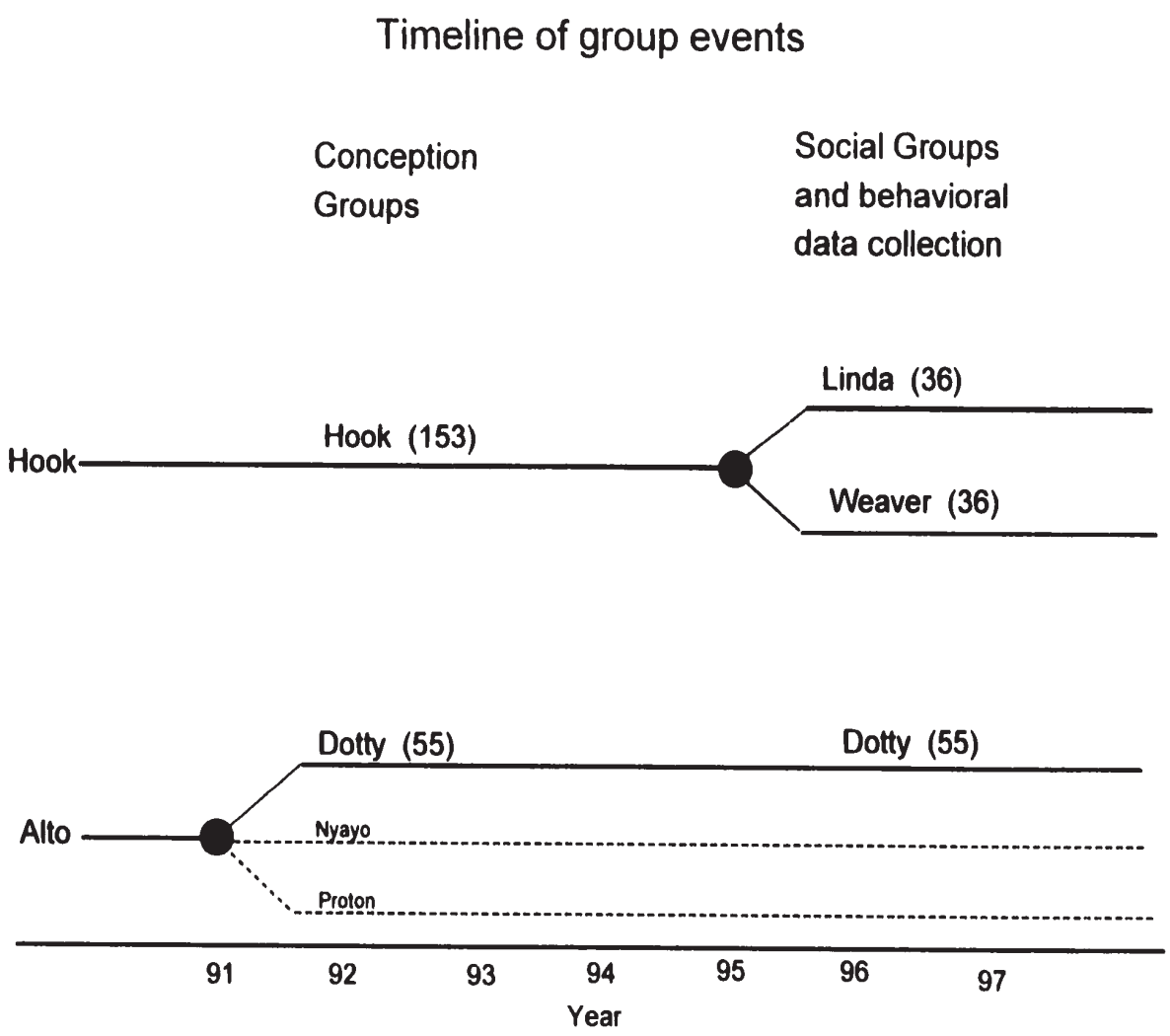

Figure 3.1 Timeline of group events. Numbers in () = number of pairs of adult females. Dots represent years that the fissions were completed.

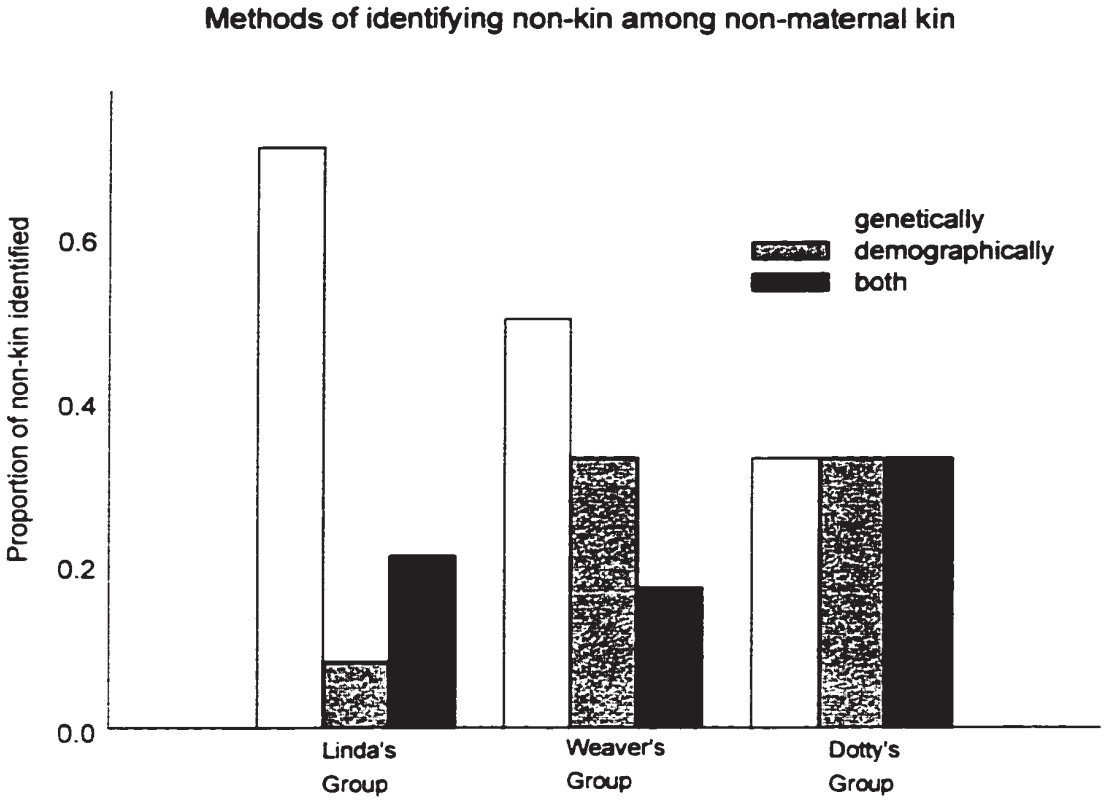

Figure 3.2 Methods of identifying non-kin. Genetically = pairs in which both females were heterozygous and they had no alleles in common at a specific locus. Demographically = pairs of females who were excluded as paternal sisters because they had no potential fathers in common. Both = pairs were excluded as paternal sisters by both genetic and demographic data. All pairs were unrelated maternally.

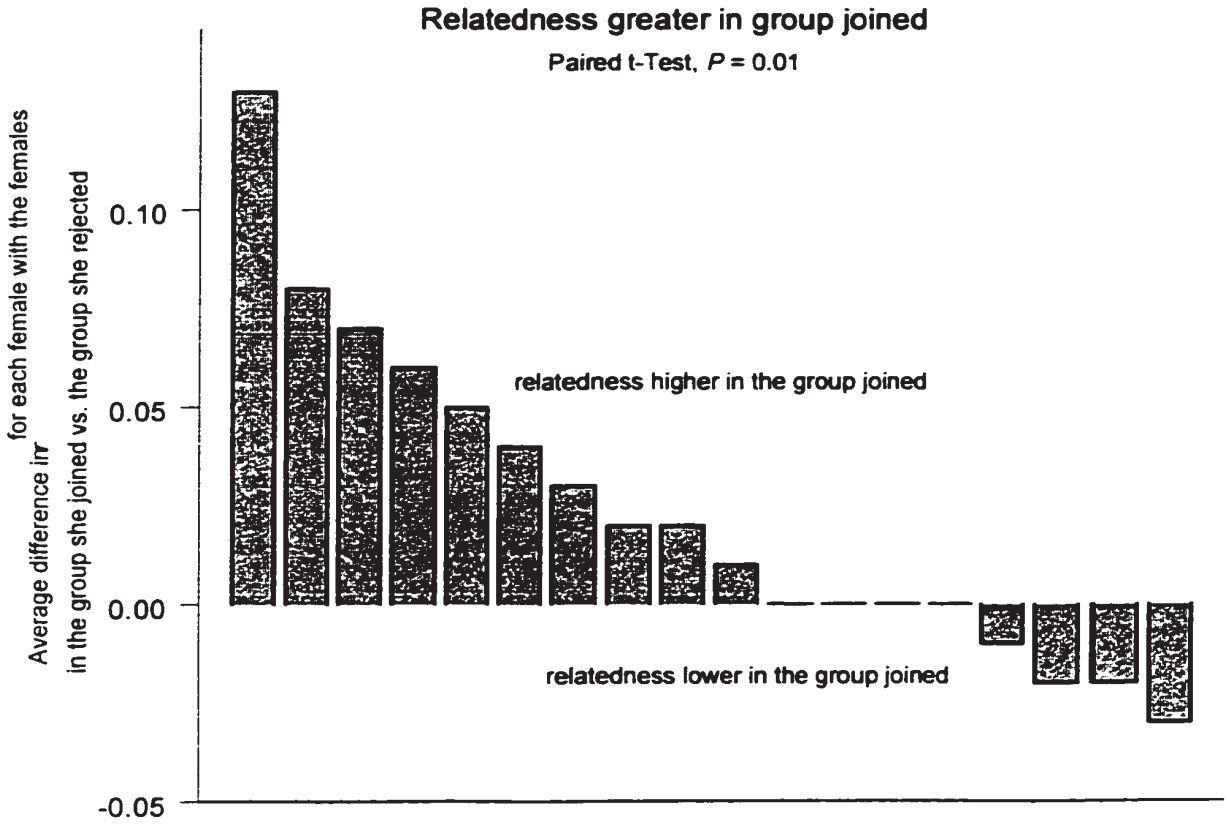

Figure 3.3 Differences in r between 'chosen' and 'rejected' groups formed after Hook's Group fissioned. *r* was averaged for each female with the other females in her newly formed group and then with the females in the new group she did not join. The difference in the average *r* values are shown above for all 18 females. Five females (represented as a dash) were equally related to the adult females in both groups.

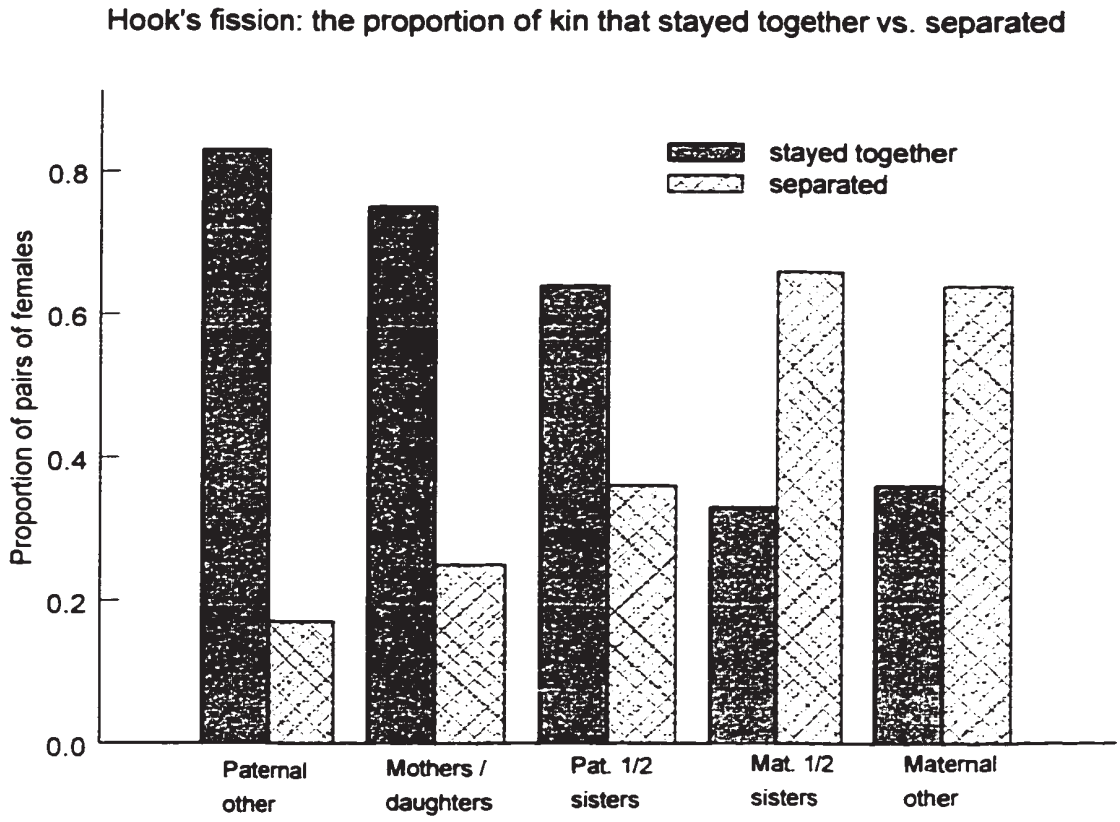

Figure 3.4 Hook's group fission: The proportion of kin that stayed together vs. separating. All kin = maternal and paternal kin. Maternal kin = mother/daughter pairs, half sisters, cousins, and aunt/niece pairs. Paternal kin = half sisters, and aunt/niece pairs.

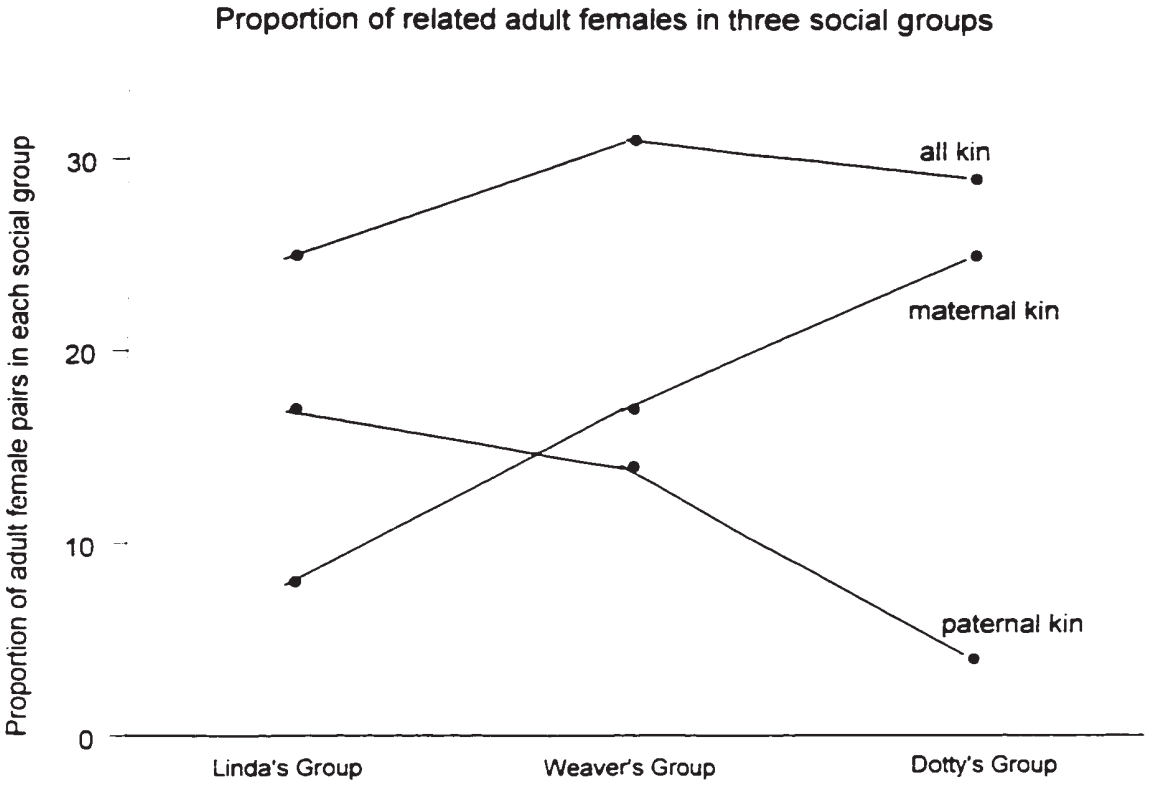

Figure 3.5 The proportion of related adult female pairs in each of the three social groups. All kin = maternal and paternal kin. Maternal kin = mother/daughter pairs, half sisters, cousins, and aunt/niece pairs. Paternal kin = half sisters and aunt/niece pairs.

CHAPTER 4

Paternal-kin-biased behavior and its proximal mechanisms in wild baboons

INTRODUCTION

Maternal kin bias

Field studies show that as adults, cercopithecine primate females (baboons, vervets, and macaques) bias their social behavior towards maternal kin (members of the same matriline) relative to individuals who are not maternally related. Adult females related to each other as mothers, daughters, or maternal half sisters disproportionately bias their grooming behavior towards each other (Gouzoules 1984; Gouzoules and Gouzoules 1987; macaques: Kurland 1977; Defler 1978; Silk *et al.* 1981a; Silk 1982; Chapais 1983; baboons: Seyfarth 1976, 1980; Walters 1981; Saunders 1988; patas monkeys: Rowell and Olson 1983; Loy and Harnois 1988; Watts 1994), spend a greater proportion of their time in close proximity to each other (Kurland 1977; Altmann 1980; Chapais 1983; apes: Watts 1994), and are more likely to aid each other in non-affiliative bouts (Kurland 1977; Massey 1977; de Waal 1977; Walters 1981; Silk 1982; Bernstein

and Ehardt 1985 1986; Gouzoules and Gouzoules 1987; Cheney and Seyfarth 1990; apes: Watts 1994), than are unrelated females.

Although cercopithecine adult females also bias much of their aggressive behavior towards close maternal kin (Kurland 1977; Silk *et al.* 1981a; Bernstein and Ehardt 1985, 1986), this may be due to the close proximity maintained by maternal kin. Further, females reserve severe aggression for non-maternally related females (Silk *et al.* 1981a, 1981b; Walters 1987), and when maternal relatives fight, a higher proportion of those fights are followed by bouts of affiliative behavior than when non-maternally related females fight (Cheney and Seyfarth 1989).

Paternal kin bias

Much less is understood about the distribution of behavior among paternally related cercopithecine females. Many of the maternal kinship studies referred to above made no distinction between paternal kin and non-kin due to the difficulty of identifying fathers (Missakian 1972; Kurland 1977; Massey 1977; Defler 1978; Silk *et al.* 1981b; Walters 1981; Bernstein and Ehardt 1986; Cheney and Seyfarth 1990). Because of the difficulties involved with accessing paternity in wild groups, the few studies of paternal kinship have relied almost exclusively on captive animals (Wu *et al.* 1980; Small and Smith 1981; Kuester *et al.* 1994; Erhart *et al.* 1997). One of the first studies of paternal kinship showed that captive pigtail macaques raised in isolation oriented more often

towards paternal half siblings than towards non-relatives in forced-choice experiments (Wu *et al.* 1980). Although interesting, these results have not been replicated despite attempts to do so by researchers that were part of the original study (Frederickson and Sackett 1984; Sackett and Frederickson 1987). The few studies of paternal kinship among wild monkey populations suggest that in some situations, individuals discriminate between paternal kin and non-kin (Pope 1990; Alberts 1999). Pope (1990) showed that howler monkey coalitions made up of fathers and sons and those among brothers lasted longer and were more stable than were the coalitions made up of unrelated males. Alberts (1999) showed in baboons that the consortships of paternal half brothers and sisters were less sexual than were the consortships of unrelated males and females.

Kin selection

The observed biases of affiliative and cooperative behaviors towards maternal and paternal kin are consistent with the predictions of kin selection theory. The theory states that in order for a behavior to evolve through kin selection,

$$rb - c > 0$$

where r is the coefficient of relatedness between the actor and the recipient of the behavior, B is the fitness benefits to the recipient of the behavior, and C is the fitness costs to the actor (Hamilton 1963, 1964). Individuals are predicted to distribute their social behavior along lines of kinship in a way that maximizes their inclusive fitness, biasing affiliative and cooperative behavior toward, and non-affiliative behavior away from, close kin, all other things being equal.

Important to the present study is that kin selection makes no distinction between maternal and paternal kin. Its reasoning is based solely on the degree of relatedness between individuals. The same predicted selective advantages for biasing affiliative and cooperative social behavior along lines of maternal kinship apply to paternal kin as well. Thus if the observed social bias towards maternal kin among cercopithecines is a result of kin selection, then we expect the same bias to apply to paternal kin.

Proximal mechanisms

The proximal mechanism(s) underlying the observed bias of social behavior towards maternal kin is not known, although two mechanisms are possible. The first is familiarity (ontogenetic association) (Holmes and Sherman 1983). Kin discrimination by familiarity predicts that individuals will treat as kin those conspecifics with whom they have either interacted during critical periods of their development (Halpin 1991) and/or those with whom they have had persistent, long term exposure. Accurate kin discrimination by the familiarity mechanism requires a high correlation between relatedness and early associations.

Baboon infants are extremely dependent on their mothers for a prolonged period of time, relying on their mothers for nutrition, transportation, and protection (Altmann 1980). As a result of the ties between a mother and each of her offspring, maternal

siblings will also develop strong associations that begin at birth. The strong associations between mothers and daughters, and between female maternal siblings persist through adulthood. Thus, ontogenetic association may be a mechanism by which kin selection is achieved among maternally related individuals.

Paternal siblings also have the opportunity to form ontogenetic associations by socializing with similarly aged individuals (members of their age cohort). During their tenure as high-ranking, adult males have priority of access to estrous females, therefore age cohorts are likely to be composed of paternal siblings (Altmann 1979). Older infant and juvenile baboons form early associations, as playmates, with members of their age cohort. Females who bias their social behavior towards similarly aged partners are much more likely to encounter paternal siblings than are individuals who associate partners much older or younger than themselves. Therefore, kin-biased behavior through ontogenetic associations can be achieved among paternal kin as well as among maternal kin. Patterns of male tenure length and schedules of natality and mortality make it unlikely that a pair of adult females will be both maternal and paternal sisters, i.e., full siblings (refs and Results herein).

The second possible mechanism of kin recognition is phenotype matching (Holmes and Sherman 1983). Unlike the familiarity model, kin selection by phenotype matching requires no prior experience or associations with kin. Phenotype matching uses family cues, such as odor, assumed to reliably reflect genetic relatedness (Waldman *et al.*

1988; Halpin 1991). Individuals learn these familial cues from either the phenotypes of family members or from their own phenotype. Once the cues are learned, they are theoretically used as the standard to judge every other individual as either kin or non-kin, and further, to discriminate between close and distant kin. These familial cues are thought to reflect a direct phenotype/genotype correlation (Waldman *et al.* 1988; Halpin 1991; Waldman 1991).

Both mechanisms for kin-biased behavior, familiarity and phenotype matching, make many of the same predictions, making it difficult to tease the two mechanisms apart. Both mechanisms predict that females will bias their affiliative social behavior disproportionately towards maternal and paternal half sisters over unrelated females. Again the ontogenetic associations and the genetic relatedness (and therefore phenotypic familial cues) are stronger among siblings than among unrelated individuals. Finally, both mechanisms predict that maternal and paternal sisters are roughly equally preferable as social partners. Although the mechanisms are different, females have the opportunity to develop strong and early ontogenetic associations with both maternal and paternal sisters. Additionally, maternal and paternal sisters share, on average, similar proportions of genes that are identical by descent.

Using maternal kin to tease apart proximal mechanisms

In theory, the two kin discrimination mechanisms could be teased apart using maternal kin. The two mechanisms lead to different predictions in the case of an infant

adoption; the familiarity mechanism leads to the prediction that the adopted infant will be treated as a genetic relative while the phenotype matching model leads to the opposite prediction. However, infant adoptions among savannah baboons in the wild have never been reported and are probably extremely rare. Comparing the distribution of social behavior among half sisters and among grandmothers and granddaughters provides a second potential test for teasing apart the two mechanisms using maternal kin. These two groups of maternal kin each share roughly the same proportion of the genome by common descent (i.e., the coefficient of relatedness, $r = 0.25$ for both groups), and yet they have ontogenetic associations of varying strengths. However, matrilines in wild, non-provisioned populations, tend to be fairly small, resulting in small sample sizes of grandmothers and adult granddaughters.

The distribution of behavior among maternal half sisters also provides a potential test for teasing apart the proximal mechanism for observed kin biases. Two siblings born sequentially in birth order e.g., the first and second offspring of their mother, have the opportunity to form longer lasting ontogenetic associations than do siblings separated by more than one sibling in the birth order; e.g., a mother's first and fifth offspring. This situation leads to the prediction that if females use familiarity for kin recognition, females will bias their social behavior disproportionately towards maternal half sisters in an adjacent birth order, over maternal half sisters separated by at least one sibling (not predicted by phenotype matching).

Maternal siblings can be used a second way to tease the two mechanisms apart. If a critical period exists for establishing ontogenetic associations, and if this critical period occurs early in life, i.e., soon after birth, females are expected to bias their social behavior disproportionately towards older siblings (to whom they are exposed from birth on) over younger siblings if they are using familiarity as the proximal mechanism. Again, small matrilines with few cases of multiple adult female maternal sisters make it difficult to test these two hypotheses in natural populations, and ultimately, to tease the two mechanisms underlying kin biasing behavior using maternal kin.

Using paternal siblings to tease apart proximal mechanisms

Paternal siblings, and in this study, paternal sisters specifically, provide a natural experiment for distinguishing between familiarity and phenotype matching as the mechanism for observed kin-biased behavior. Age cohorts have two features that make them important as a natural experiment. The first is age similarity, and therefore the assumption of opportunities for similar social familiarity among all cohort members. The second is variability in genetic relatedness. Age cohorts are loosely defined as those individuals born within a narrow range of time, usually between one and two years (Alberts 1999). Here, an age cohort is defined as being composed of those females born within one year of each other.

Being similarly aged has two important consequences in promoting familiarity. First, the more similar the ages between two females, the greater the proportion of their

lives they will have the opportunity to interact with each other (barring death or group fissions). Two females of the same age will have the opportunity to interact their entire lives. This is obviously not the case for the older of two, very differently-aged, females. Second, similarly aged females go through important life history stages, such as infancy, reaching menarche, pregnancy, and motherhood at similar times, while differently-aged females do not (Altmann 1979). Critical to this study, these consequences of age similarity apply to all members of the age cohort, regardless of variation in relatedness among cohort members. While most paternal siblings are members of the same age cohort (Altmann 1979; Chapter 3, results), multiple paternity within an age cohort exists. Although high-ranking males enjoy priority of access to estrous females, the system is not perfect and other group males also consort with estrous females. For example, a single male, even if he is the highest-ranking male, cannot simultaneously monopolize two females in estrous. Because more than one male sires offspring, some members of an age cohort will be paternal siblings while others will be unrelated.

In this natural experimental design, it is inferred that if females make no distinction between paternally related and unrelated members of their age cohorts, familiarity is most likely the proximal mechanism for kin-biased behavior with age being used as estimator of relatedness. In contrast, a biasing of social behavior towards paternally related members of a female's age cohort, over unrelated members of her age cohort suggests phenotype matching as the proximal mechanism.

Kin selection hypotheses

Two sets of hypotheses were tested in this study: kin selection hypotheses and mechanism hypotheses (Table 4.1). Kin-biased social behavior along maternal lines, consistent with the predictions of kin selection theory, have already been the subject of many non-human primate studies (see above). Despite the wealth of information already available about maternal kin biasing, kin selection hypotheses were included in this study for two reasons. The first reason to include kin selection hypotheses was to confirm findings of other studies, i.e., maternal kin bias their social behavior in ways that can easily be measured such as maintaining close proximity towards one another and by grooming each other. Being able to confirm a maternal kin bias demonstrates that the data presented here were adequate, both in terms of sample sizes and in behaviors measured, to detect a paternal kin bias, if one exists. This leads to the second reason for including kin selection hypotheses, which was to test whether adult female baboons show a paternal kin bias similar to the observed maternal kin bias. As stated before, kin selection theory predicts that individuals who can bias social behavior along paternal, as well as along maternal kinship lines, will acquire fitness benefits. This is one of few studies (see above) to examine this possibility. All of the following kin selection hypotheses will be tested.

H1 Adult females will bias their affiliative social behavior disproportionately towards maternal half sisters over unrelated females.

H2 Adult females will bias their affiliative social behavior disproportionately towards their paternal half sisters over unrelated females.

H3 Adult females will not distinguish between maternal and paternal half sisters in the distribution of their affiliative social behavior.

Mechanism hypotheses

Given that females do bias affiliative behavior towards both maternal and paternal kin (see Results), the following hypotheses will be tested.

Maternal sisters

H4 If familiarity is the mechanism of discrimination, adult females will bias their affiliative social behavior towards maternal sisters born next to them in the birth order, over sisters separated by at least one sibling. If phenotype matching is used, these distinctions will not be seen.

H5 If familiarity is the mechanism for discrimination, adult females will bias their affiliative social behavior towards older maternal sisters over younger maternal sisters. This difference is not predicted if phenotype matching is used.

Paternal sisters

H6 If familiarity is the mechanism for discrimination, adult females will bias their affiliative social behavior towards unrelated females born within one year of themselves over unrelated females more than a year older or younger than

themselves. This distinction is not predicted if phenotype matching is the mechanism being used.

H7 If familiarity is the mechanism of discrimination, females will make no distinction between paternal half sisters of the same age and unrelated females of the same age. Further, adult females will make no distinction between differently-aged paternal half sisters and unrelated females when distributing their affiliative social behavior. If phenotype matching is the mechanism of discrimination female will be more affiliative towards paternal half sisters than non-kin, regardless of age.

METHODS

Subjects and site

The subjects of this study were twenty-nine adult (having reached menarche) female savannah baboons (*Papio cynocephalus*) living in and around Amboseli National Park, Kenya (Hausfater 1975; Altmann 1980; Murtuthi *et al.* 1991; Altmann 1998; Alberts 1999) during 1996-7 . The subjects, along with other group members, are habituated to human observers who take precautions to keep human-baboon interactions to a minimum. Each baboon was individually recognized by naturally occurring individual differences. The twenty-nine females lived in three distinct social groups: Dotty's, Weaver's, and Linda's. The groups varied in size and composition. In addition, individuals varied in the number of potential adult female social partners available to them from different kin classes (Table 4.2). All three study groups were products of

relatively recent group fissions. Linda's and Weaver's Groups formed from Hook's Group which completed its fission in January 1995. Dotty's Group was one of three subgroups formed during the fissioning of Alto's Group in 1991.

Behavioral data collection

Both affiliative and non-affiliative behavioral data were collected from July 1996 through February 1997 in order to test the hypothesis that baboons bias their social behavior towards paternal as well as maternal kin, and to identify possible underlying proximal mechanisms. Each of the three groups was observed between two and three days a week, and data were collected between 0600 and 1800 hours. Behavioral data were collected *ad libitum*, and as point and continuous samples within ten-minute focal animal samples (Altmann 1974). The order in which the females were sampled was determined using a random numbers table and was changed each month.

Ad lib data

All observed grooming bouts between two adult females were recorded and used to determine grooming reciprocity i.e., did the two females in each dyad initiate approximately equal numbers of grooming bouts. Grooming reciprocity was then compared among the different kin classes.

Point-sample data

Point-sample data were particularly designed to evaluate interactions between adult females. Every minute during focal animal samples, the focal female's activity (resting, walking, feeding, or grooming) and her nearest adult female neighbor were recorded as point samples. The focal female's activity was scored as grooming only if she was grooming the female who was her nearest adult female neighbor. Two other activities, 'other' and 'being groomed', were recorded but excluded from the analyses. 'Other' activities included grooming an individual other than the nearest adult female neighbor, i.e., an infant, or engaging in an activity other than the four listed above, such as threatening or submitting to another individual. Both excluded activity categories ('other' and 'being groomed') were excluded because they did not represent behavior being directed by the focal female towards her nearest adult female neighbor.

Between 125 and 150 ten-minute focal animal samples were collected for each adult female, resulting in between 1250 and 1500 point samples per female. After the activities 'other' and 'being groomed' were excluded, between 20 and 25 hours (mean = 23.3) of point-sample data were available for each female.

Continuous data

Both affiliative and non-affiliative social behaviors between the focal female and another adult female were recorded each time they occurred within a focal animal sample (see Appendix 4.1 for an ethogram of continuous behaviors). The behaviors recorded as affiliative continuous data included: approaches within one meter, greetings, cohesion grunts, mounts, lipsmacks, following, muzzle-to-muzzle behavior, solicitations of grooming, and the start of grooming bouts. Both aggressive behaviors such as eyelid displays, ground slaps, head bobs, biting, lunging, staring - and submissive behaviors - such as, leaning away, lifting a tail up, presenting, grimacing, and screeching were recorded as non-affiliative continuous data. Leaving a one meter radius and ending a grooming bout were also recorded as non-affiliative behaviors.

Although observations of females ranged from 20 to 25 hours, the number of continuous social behaviors directed by the focal female towards another adult varied greatly, ranging from 9 to 137.

Testing the hypotheses

Identifying kin

To test the hypotheses about the distribution of behavior both within and between kin and age classes, it was necessary to correctly identify members of those classes. The following kin classes were distinguished.

Maternal half sisters

In order to identify maternal siblings, it was necessary to identify mothers. Mothers were identified based on 1). Observations of the external signs of ovulation dates and pregnancy were used to determine expected birth due dates. 2). Observations of females who displayed postpartum signs such as blood on the perineum or hands, along with the presence of an infant who was only hours to days old, or 3). Observations, in rare cases, of the actual birth. Maternity in baboons is unambiguous. Baboons are not seasonal breeders and so females rarely give birth very close in time (the only circumstance in which one female could successfully adopt another's infant). Further, allomaternal care is uncommon, and adoption has never been observed in Amboseli.

Once maternity was established, maternal half sisters and other maternal relatives (aunt/niece pairs, and cousins) were identified by constructing matrilineal genealogies from long-term, on-going maternity demographic data. Seven pairs of adult female,

maternal half sisters were included in this study. It should be noted that 6 of the 7 maternal sister dyads came from the same family (Vixen's daughters in Dotty's Group). Although mother/daughter pairs (n = 9) and maternal relatives more distantly related than half sisters (n = 7 pairs) were not included in this behavioral study, it was necessary to identify them in order to correctly isolate 'true non-kin'.

Paternal half sisters

Paternal half sisters were identified by their high 'paternal relatedness scores' (see Chapter 3), generated through genetic analyses of feces-derived DNA (see Chapter 2). Females were genotyped at five X chromosome loci using human STRP (simple tandem repeat polymorphisms) primers. Paternal relatedness scores were calculated for each pair of females and reflected whether females shared an allele at each locus, whether the shared allele was paternally derived, and the frequency of that allele in the group. Females who shared at least one potential father at the time of their conceptions (from demographic data), and who had high paternal relatedness scores (at least two standard deviations above the mean for all group pairs) were considered paternal half sisters. Nine paternal sister dyads were identified in this way.

Distant kin and pairs of unknown relatedness

In addition to excluding mothers, daughters, and distant maternal kin from the study, paternal aunt/niece pairs (n = 4), and pairs of females of uncertain relatedness (n = 34) were also excluded. Relatedness was considered uncertain when the pair was

unrelated maternally, but could be neither included nor excluded with confidence as paternal half sisters.

True non-kin

True non-kin (n = 57 pairs) were defined as those pairs of females neither maternally nor paternally related.

Kin classes

To test the kin selection hypotheses, comparisons were made between the three kin classes mentioned above: maternal half sisters, paternal half sisters, and true non-kin. The number of pairs in each of these kin classes is presented in Table 4.1.

To test the mechanism hypotheses, it was necessary to make comparisons both within and between kin classes. Two comparisons were made within the class of maternal half sisters: the distribution of behavior among maternal sisters born in adjacent birth order was compared to that of maternal half sisters separated in birth order by at least one sibling (H4), and the behavior directed towards older maternal half sisters was compared to that directed towards younger sisters (H5).

Comparisons were made both among the true non-kin class, and between true non-kin and paternal half sisters. In these comparisons, age, and therefore familiarity, were controlled for. 'Same-aged' refers to pairs of females born within one year of each other,

whether they were related or not. The definition of 'differently-aged' females was context dependent. When making a comparison within the 'true non-kin' class, same-aged vs. differently-aged pairs were mutually exclusive and therefore, differently-aged refers to all pairs of unrelated adult females born more than one year apart (H6). When comparing the distribution of behavior among differently-aged paternal half sisters to that of differently-aged unrelated females, differently-aged refers to females born between 13 and 45 months of each other (H7b). This was the range of age differences observed among paternal half sisters born more than one year apart.

Statistics

The unit of analysis for all of the hypotheses tested was the focal female's behavior directed towards another adult female; it was not the interactions of the pair. Each female's pool of potential partners was distributed differently within and between kin classes, and because of this, A's behavior towards B was not assumed to be indicative of B's behavior towards A. As a result of using each female as the unit of analysis, each pair of females was represented twice as directed pairs, once as AB and again as BA.

The kin selection hypotheses (1-3) were tested using Tukey-Kramer HSD (Honestly significant difference) tests to avoid Type I errors due to multiple comparisons. The Tukey-Kramer HSD test allowed for unequal sample sizes which was appropriate for these comparisons as the number of true non-kin far outnumbered the number of pairs of maternal or paternal half sisters (see Tables 4.1 and 4.2). The mechanism hypotheses (4-

7) were tested using Wilcoxon signed-ranks tests. While the sample sizes of the comparisons used to test the mechanism hypotheses were less disparate than were those used in the kin selection analyses, they were also much smaller (Table 4.1).

Point-sample data: kin selection (Table 4.3) and mechanism (Table 4.5) hypotheses

Point-sample data were used to ask questions about the proportion of time focal females spent in close proximity[2] to, or grooming other adult females, either between kin classes (kin selection hypotheses) or within kin classes (mechanism hypotheses).

To answer the first kin selection and mechanism questions, it was necessary to compare the observed to the expected number of times each female was the focal female's nearest adult female neighbor. An expected value for each recipient was calculated by dividing the focal female's total number of point samples (when her activity was grooming, resting, walking, or feeding) by the number of available adult females in each kin class. A value for each pair was calculated by dividing the observed value by the expected value.

The second and third questions asked essentially whether focal females devoted more or less attention (grooming or maintaining close proximity while resting) to some females than to others. The comparisons were made using values from the following calculation:

$$A_B/A_T,$$

where A_B = the number of point samples in which the focal female A was resting and B was her nearest adult female neighbor (question 2) or in which A was grooming B (question 3)

A_T = the mean of focal female A's maintaining close proximity while resting, or grooming, all adult females.

Values represent the proportion of time the focal female directed towards each recipient, relative to other adult females. Values greater than one represent pairs in which the focal female directed more of her behavior towards the recipient than she did, on average, towards other adult females, and values less than one represent pairs in which the focal female directed less of her behavior towards the recipient.

Continuous data: kin selection (Table 4.4) and mechanism (Table 4.6) hypotheses

Whereas point-sample data were used to ask questions about proximity and the relative proportion of time spent grooming, continuous data were used to ask questions about the relative amount and proportion of affiliative social behavior a focal female directed towards other adult females.

As with point-sample data, the unit of analysis using continuous data was the adult focal female and the social behavior she directed towards other adult females.

[2] The term 'close proximity' is used here and throughout as shorthand for 'nearest adult female neighbor'.

However, information about female A's behavior towards female B came from two sources: 1). A was the focal female and directed behavior towards B and, 2). B was the focal female and was the recipient of behavior directed from A. Therefore, the sum of A's behavior directed towards B was:

$$AB = A \rightarrow B \quad + \quad B \leftarrow A \; .$$

This was true for all analyses using continuous data.

The first questions addressed using continuous data required calculating an expected value. The expected value was derived as above. An expected value for each recipient was calculated by summing the total number of counts of all social behavior (affiliative and non-affiliative) directed by the focal female towards another adult female and dividing that sum by the number of available adult females in each kin class. After summing both sources of data for A's behavior towards B, a value for each pair was calculated by dividing the observed value by the expected value. Values greater than one indicated that the focal female directed more of her social behavior towards the recipient female than was expected due to chance alone.

The second set of analyses using continuous data looked at the proportion of affiliative to non-affiliative social behaviors directed by A towards B. Values for each pair were calculated simply by dividing the total number of affiliative behaviors by the total number of non-affiliative behaviors that A directed towards B.

The final set of analyses using continuous data looked at the number of counts of affiliative behaviors A directed towards B.

Ad lib grooming data

Grooming between adult females was recorded *ad libitum*. These data were used to determine whether the grooming between females A and B was reciprocal. To analyze grooming reciprocity, the total counts of A initiating a grooming bout with B was divided by the total counts of B initiating a grooming bout with A. The greater the deviation from one in either direction, the less reciprocal the grooming between the pair AB.

Power analyses

Statistical power was estimated for the comparisons tested as mechanism hypotheses. Power indicates the probability of committing a type II error, i.e., accepting the null hypothesis when the null hypothesis is false (Sokal and Rohlf 1981). This was particularly critical to many of the mechanism hypotheses in which a 'non-significant' (NS) result was interpreted as being informative about the mechanism(s) underlying the observed behavior. For instance, the hypotheses that tested the distribution of behavior among maternal half sisters were set up in a such a way that failing to reject the null hypotheses suggested the use of a phenotype matching mechanism, while being able to reject the null hypothesis suggested the use of a familiarity mechanism when distributing their behavior. As the sample sizes used to test most of the mechanism hypotheses were

small, it was important to know if failure to reject the null (a non-significant result) was due to small sample size, or because the two 'treatment' groups did not differ. The data in this study did not meet the assumptions of parametric tests and therefore non-parametric statistical tests were used. Because power analyses assume a normal distribution, the power results presented here were used as an estimate only.

RESULTS

Kin Selection hypotheses

Adult female baboons showed a strong preference for directing social behavior towards, and maintaining close proximity to sisters over true non-kin. Further, females rarely distinguished between maternal and paternal sisters. Both of these results are consistent with predictions based on kin selection theory and were consistent between point and continuous data (Tables 4.3 and 4.4 respectively). Point-sample data revealed that a female's nearest adult female neighbor was either a maternal or a paternal sister more often than was expected while unrelated females were the focal female's nearest adult female neighbor less often than was expected. This was true both when the focal female's data were pooled across all activities (n = 146 directed pairs, Tukey-Kramer, $P < 0.05$), and when the focal female was resting (n = 146 directed pairs, Tukey-Kramer, $P < 0.05$). Females also spent significantly more time grooming maternal and paternal sisters than they did true non-kin (n = 146 directed pairs, Tukey-Kramer, $P < 0.05$, Figure 4.1). Finally, maternal and paternal half sisters did not differ, in the proportion of time they spent either in close proximity to each other, or grooming each other (n = 32

directed pairs, Tukey-Kramer, $P < 0.05$, Figure 4.1). Grooming reciprocity did not differ among the three kin groups (n = 126 directed pairs (10 pairs of true non-kin could not be used for the reciprocity analyses as they were never observed grooming, lowering the sample size from 146 directed pairs to 126), Tukey-Kramer, $P > 0.05$, Figure 4.2).

Continuous data were more sparse and therefore the results based on them were less straight forward than those based on point-sample data. Despite fewer data, females directed significantly more of their social behavior towards paternal sisters than towards true non-kin in every measure tested using continuous data (Table 4.4). Females disproportionately biased more (i.e., total number of counts) of their social behavior (both affiliative and non-affiliative), towards paternal sisters than towards true non-kin (n = 45, Tukey-Kramer, $P < 0.05$, Figure 4.3). Females also directed a significantly higher proportion of affiliative to non-affiliative social behaviors towards maternal and paternal sisters, than towards true non-kin (n = 146 directed pairs, Tukey-Kramer, $P < 0.05$). Paternal half sisters directed affiliative behaviors towards each other significantly more (i.e., total number of counts) than did maternal half sisters or true non-kin (n = 146 directed pairs, Tukey-Kramer, $P < 0.05$). Finally, females treated paternal sisters differently from maternal sisters in only one test; as just mentioned they directed affiliative behavior significantly more often towards paternal sisters than towards maternal sisters. However, the distribution of social behavior among maternal and paternal half sisters did not differ in the other measures tested using continuous data, i.e.,

the proportion of affiliative to non-affiliative behaviors, and the observed to expected ratio were similar between the two types of sisters (Table 4.4).

Mechanism hypotheses

Adult female baboons biased much of their behavior towards other adult females of similar ages. Females preferred unrelated partners of the same age to unrelated females of different ages in all measures tested using point-sample data. Females spent significantly more time in close proximity to unrelated members of their age cohort than they spent with unrelated females belonging to different age cohorts when all activities were pooled (n = 114 directed pairs, Wilcoxon signed ranks test, $P = 0.01$). This preference for maintaining close proximity to same-aged, unrelated partners over differently-aged, unrelated partners was even more pronounced when the focal female was resting (n = 114 directed pairs, Wilcoxon signed ranks test, $P = 0.002$, Figure 4.4). Finally, adult females spent significantly more time grooming unrelated members of their own age cohort than they spent grooming unrelated females outside their age cohort (n = 114 directed pairs, Wilcoxon signed ranks test, $P = 0.02$). However, continuous data did not support the notion that females biased the distribution of social behavior towards same-aged, and therefore presumably more familiar, partners (Table 4.6).

In addition to familiarity as a mechanism that adult female baboons used to bias the proportion of time they spent grooming or in close proximity to other adult females, they may also use a phenotype matching mechanism to bias their behavior in some contexts (Tables 4.5 and 4.6). Both point sample and continuous data show that when

age (familiarity) is controlled for, female baboons bias their behavior towards paternal half sisters over non-kin in some contexts. Paternal half sisters of different ages (born between 13 and 45 months apart) spent significantly more time grooming than did differently-aged unrelated females (also born between 13 and 45 months apart) (n = 22 directed pairs, Wilcoxon signed ranks test, $P = 0.05$, Table 4.5, Figure 4.5). In addition, four of the five females (80%) who had both a paternal half sister of the same age, and an unrelated female of the same age spent more time grooming her sister than the unrelated female (Figure 4.6). Further, females directed more social behavior (affiliative and non-affiliative behaviors pooled) towards differently-aged paternal half sisters significantly more often than they did towards differently-aged unrelated females (n = 22 directed pairs. Wilcoxon signed ranks test, $P = 0.05$, Table 4.6).

Two other measures tested with continuous data further suggest that adult female baboons may use a phenotype matching mechanism for distributing social behavior in some contexts. First, females displayed a tendency to bias a higher proportion of affiliative to non-affiliative social behaviors towards paternal half sisters of the same age (born within one year of each other) than towards truly unrelated females of the same age (n = 24 directed pairs, Wilcoxon signed ranks test, $P = 0.08$), although this difference did not reach statistical significance. Second, females biased more counts of affiliative behaviors towards differently-aged paternal half sisters (born 13 to 45 months apart) than towards truly unrelated females of the same age difference range (n = 22 directed pairs, Wilcoxon signed ranks test, $P = 0.08$); again, this test did not reach statistical

significance. Estimates of power suggest that the inability to reject the null hypotheses, i.e., familiarity is the mechanism underlying the observed kin bias, may be due to small sample size in the two results just presented.

Being able to detect significant differences between paternal sisters and non-kin, while controlling for age, is noteworthy given the existence of confounding factors that were not considered (i.e., rank and/or presence of a new infant), and given the small number females available on which to test these hypotheses (n = 11 and 12 pairs for same- vs. differently-aged comparisons). These results testing for genetic variation while controlling for age (familiarity), suggest that while adult female baboons often use familiarity as a general mechanism for distributing behavior, they are capable, in some contexts, of using a more exact phenotype matching mechanism.

Although a single large family contributed disproportionately to the data for maternal sisters, the distribution of behavior among maternal half sisters also suggest that females may use a phenotype matching mechanism in addition to familiarity. Maternal half sisters did not distinguish between those born in an adjacent birth order to themselves, and those separated from them by at least one sibling in any of the six measures tested (n = 14 directed pairs, Wilcoxon signed ranks tests, maintain proximity (obs/exp): $P = 0.56$, Figure 4.7, maintain proximity while resting: $P = 0.2$, proportion of time spent grooming: $P = 0.12$,). Further, females were just as likely to maintain close proximity to, and groom a younger maternal half sister as an older one (n = 14 pairs (not

directed pairs), Wilcoxon signed ranks tests, maintain proximity (obs/exp): $P = 0.94$, Figure 4.7, maintain proximity while resting, $P = 0.57$, proportion of time spent grooming, $P = 0.95$). Although the sample sizes used to test the mechanism hypotheses among maternal half sisters were very small, estimates of power suggest that small sample sizes could account for the non-significant results. Even increasing the theoretical sample size to an extreme size of 300 for power estimates did little to increase the power in these post hoc estimates. To the degree that the females from one family are representative of other maternal half sisters, these results suggest that maternal sisters, much like paternal sisters, do not bias their social behavior based solely on familiarity, but use a phenotype matching mechanism in some contexts.

DISCUSSION

Kin selection hypotheses

One of the important points to come out of this study is that wild, adult female baboons bias their social behavior towards paternal sisters just as they do towards maternal half sisters. Paternal half sisters spend more time grooming and in close proximity to each other than do unrelated females. Although these observations are consistent with the predictions of kin selection theory, they have rarely been tested among wild, non-human primates.

Although female baboons demonstrated an ability to bias their social behavior towards paternal half sisters, they did so in only some contexts. Females spent more time

grooming differently-age paternal sisters than they did differently-age unrelated females, however, they made no distinction between paternal sisters and non-kin as a nearest neighbor while resting.

Others have reported context-dependent kin-biased social behavior similar to those reported here. As mentioned above (Alberts 1999), wild baboon paternal brothers and sisters were just as likely to consort, as were unrelated males and females, however the consortships of paternal siblings were less affiliative and sexual. Keane (1990) showed that female white-footed mice in estrous discriminated between the odors of males of different degrees of relatedness preferring males of intermediate relatedness, while non-estrous females showed no preferences and demonstrated no ability to bias behaviour along lines of kinship. Pfennig *et al.* (1993) showed that spadefoot toad tadpoles, which occur in two morphs, demonstrated kin-biased behavior according to their morph and their hunger level. Given a choice to aggregate with either full sibs or non-kin, the cannibalistic morph avoided kin, while the omnivorous morph preferred aggregating with kin to non-kin. Further, as hunger levels increased, cannibalistic tadpoles became less selective about avoiding kin, showing a hunger dependent kin bias.

The context-dependent results of others, and those reported here (Tables 4.5 and 4.6), highlight the obvious but important necessity of testing hypotheses using multiple measures. This is especially important when sample sizes are small and statistical power is low, as is often the case in studies of wild non-human primates.

Mechanism Hypotheses

The results presented in this chapter suggest that adult female baboons used a two-step model for distributing their social behavior. The first step or rule states, 'treat as close kin, those individuals with whom you have been very familiar since birth (or near birth)'. Individuals who follow this first rule will treat as close kin, mothers, older maternal half sisters, and members of their age cohort. According to the predictions of kin selection theory, individuals who distribute their behavior following this first rule will have a selective fitness advantage over individuals who distribute their behavior randomly among all group members.

However, the female baboons in this study distributed their social behavior in a way that suggests there is a second step or rule in the model that states, 'in some contexts, treat as kin only those individuals with whom you share a reliable familial cue such as odor'. Again, kin selection theory predicts that individuals who can distinguish between kin and non-kin, and between close and distant kin, will acquire fitness benefits over those who use familiarity as an estimator of kinship.

Why do the female baboons in this study seem to use primarily a familiarity mechanism, and use a more accurate phenotype matching mechanism in only some contexts? Several explanations are possible. First, a less exact kin biasing mechanism may promote affiliative social interactions with more group members than would the use,

at all times, of a phenotype matching mechanism. As theoretical advantages exist for group living, such as earlier predator detection, increased foraging efficiency, and increased ability to defend resources (Mitani and Rodman 1979; Cheney 1987; Wrangham 1987; Rodman 1988), distributing affiliative social behaviors among distantly or unrelated group members may confer fitness advantages by promoting group cohesion. Maintaining social bonds towards distantly or unrelated individuals may be especially important among adult females who are the stable core of baboon groups. Second, a phenotype matching mechanism may be more costly, both evolutionarily, and in the day to day execution of it, than a familiarity mechanism, and may therefore be conserved for those situations where it will produce the greatest benefits. Hamilton's rule stresses the importance of the benefit/cost ratio of the behavior to the reproductive fitness of the actor and to the receiver. Perhaps much of baboon social behavior, and specifically those behaviors tested in this study, does not have an extreme enough cost/benefit ratio to justify the constant use of a phenotype matching mechanism, if a phenotype matching mechanism is indeed more costly to implement. In most contexts, a familiarity mechanism may be 'good enough'. Finally, Hamilton's rule also states that individuals should bias their behavior along kinship lines, 'all other things being equal'. However, 'all things' are rarely equal. Many factors, in addition to kinship, influence with whom individuals interact, obscuring the underlying mechanism being used to bias behavior towards kin. Some of these confounding factors include: the proportion of close kin to non-kin an individual has as potential social partners, the reproductive state/value of either the actor or of the recipient, the fitness cost of the behavior to the actor, the fitness

benefit of the behavior to the recipient, the relative ranks in the dominance hierarchy of the actor and recipient, stressful vs. favorable ecological conditions, the process of group fission/fusion, and personal preferences, to name a few.

Several interesting cases suggest that some of these confounding factors may have influenced the behavior of the female baboons in this study. Although females generally biased their behavior disproportionately towards sisters, some of the most extreme cases of biasing (or not), might be partially explained by the relative proportion of kin to non-kin as social partners. Consider the highest data point in Figure 4.1. This point represents the proportion of time Echo groomed her paternal half sister Asha. Echo was the only adult female in her group (Dotty's Group) who had no adult maternal relatives. Furthermore, Asha is the only other female born within a year of Echo. Echo had fewer close relatives to distribute her grooming time among than did the other females in her group. The lowest data point in Figure 4.4 in the 'true non-kin, same age column' provides a second example of how the relative proportion of close kin to non-kin might influence the distribution of social behavior. The data point represents the proportion of time Ochre was Vinyl's nearest adult female neighbor, while Vinyl was resting. Vinyl maintained close proximity to Ochre much less than did any other female towards an unrelated female of the same age. However, Vinyl had more close relatives than did any other female in all three social groups: a mother, three maternal half sisters, and a paternal half sister. It is not surprising then that she spent relatively little time in close proximity to an unrelated member of her age cohort.

Females with new, black infants are very attractive as social partners, and it could be for this reason that Dove and Lark biased so much of their social behavior towards their paternal half sisters (Figure 4.3, two highest data points in the 'paternal half sister' column). Although Dove had a mother and a cousin in her group, she directed three times as much of her social behavior towards Vinyl than was expected. Vinyl had an infant in December 1996, about half way through the field season and Dove was pregnant at the time. Both of these factors, in addition to their genetic relatedness, may have contributed to Dove's disproportional biasing of social behavior towards Vinyl. Lark also biased much more social behavior towards Nyota than was expected, or than was reciprocated (Nyota's behavior towards Lark is the lowest point in the same figure, same column). Nyota had an infant in November 1996 and was the second lowest ranking female in the group, while Lark was one of the highest ranking. Nyota may have been an attractive social partner to Lark not only because of her relatedness, but also because of her new black infant and access to the infant facilitated by Nyota's low rank in the dominance hierarchy.

Rank in the dominance hierarchy might partially explain why Ochre spent much more time in close proximity to Dotty than she spent to other unrelated females on average (Figure 4.4, highest data point in 'true non-kin, different ages' column). Ochre was the lowest ranking female in the group and Dotty was the highest.

Finally, personal preferences for some females may explain the biasing observed by some females, towards other females, that have no other obvious explanation. Wagtail spent a greater proportion of her time grooming than did any other female in the study. Interestingly, she disproportionately biased her grooming behavior towards Wendy, a maternal cousin, two years her senior. Genetic relatedness can not explain this behavior; Wagtail spent less time grooming her maternal half sister Weaver, than she did any other female in the group. The reproductive states of Wagtail and Wendy do not explain Wagtail's preference for Wendy as a grooming partner; both females had older infants and were cycling during most of the data collection time. Nor does rank explain the preference; Wagtail and Wendy held adjacent ranks in the dominance hierarchies (3rd and 4th out of 9 respectively).

CONCLUSIONS

This study of the influence of kinship on the distribution of social behavior is distinctive in several ways: it concentrated on the behavior of adult sisters, it included paternal half sisters in addition to maternal sisters, and it identified and analyzed the behavior of true non-kin, a distinction few kinship studies can make. In every measure tested, adult female baboons preferred paternal half sisters to true non-kin as recipients of their social behavior. In fact, the bias towards paternal sisters over non-kin was stronger in two tests than was the bias towards maternal sisters over true non-kin. Biasing of affiliative behavior towards sisters over non-kin is consistent with the predictions of kin selection theory. Also consistent with kin selection theory is the finding that the

distribution of social behavior among maternal sisters did not differ from that distributed among paternal sisters.

Because unrelated females spend more time in close proximity to, and grooming same-aged social partners than they spend with partners much older or younger than themselves, females most likely use familiarity as a proximal mechanism in the distribution of social behavior. However, their behavior suggests that female baboons can and do use a phenotype matching mechanism in some contexts. Paternal half sisters born more than a year apart spend more time grooming than do unrelated females with the same range of age differences. Adult females also biased significantly more counts of social behavior towards differently-aged paternal half sisters than towards differently-aged unrelated females.

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Table 4.1 Summary of hypotheses.

Comparison	Predicted by: Familiarity	Predicted by: Phenotype matching	Sample sizes (# directed pairs)
Kin selection hypotheses			
Mat 1/2 sisters > true non-kin	yes	yes	14 vs. 114
Pat 1/2 sisters > true non-kin	yes	yes	18 vs. 114
Mat 1/2 sisters ≈ Pat 1/2 sisters	yes	yes	14 vs. 18
Mechanism hypotheses			
Maternal half sisters			
Sequential mat 1/2 sisters > mat 1/2 separated in birth order	yes	no	8 vs. 6 (pairs)
Behavior directed towards older > younger mat _ sisters	yes	no	7 vs. 7 (pairs)

Table 4.1 Summary of hypotheses (continued).

<u>Paternal half sisters and true non-kin</u>			
True non-kin, same-aged >			
True non-kin, differently-aged	yes	no	12 vs. 102
(all true non-kin born more than one year apart)			
Pat _ sisters, same-aged >			
True non-kin, same-aged	yes	no	12 vs. 12
Pat _ sisters, differently-aged >			
True non-kin, differently-aged	yes	no	6 vs. 16
(both pat _ sisters and true non-kin born within one year and 45 months of each other)			

Table 4.2 Number of pairs in each kin category for the three study groups.

Kin Category	Linda's	Weaver's	Dotty's	Total
Close maternal kin (mothers, daughters, and half sisters)	2	2	12	16
Mothers and daughters	2	1	6	9
Maternal half sisters	0	1	6	7
Paternal half sisters	4	3	2	9
Paternal half sisters, same-aged	3	1	2	6
Paternal half sisters, differently-aged (born between 13 and 45 months apart)	1	2	0	3
True non-kin	24	6	27	57
Non-kin, same-aged	3	1	2	6
Non-kin, differently-aged (born more than one year apart)	21	5	25	51
Non-kin, differently-aged (between 13 and 45 months apart, a subset of the preceding category, paralleling 'paternal sister, differently-aged'category).	4	0	4	8

Table 4.3 Kin selection results: Time spent grooming or in close proximity. Hypotheses tested using point sample data and Tukey-Kramer statistics to control for multiple comparisons. Means and standard errors are presented below the results for each comparison. The number of pairs is listed below each comparison. However, directed pairs were used, essentially doubling the sample size. Therefore, the sample size from the first comparison was 128, not 64.

Hypothesis	Comparison	Nearest neighbor Obs/Exp	Proximity while resting	Grooming
1	Mat _ sisters > true non-kin N = 7 pairs vs. 57 pairs	***P*<0.05**, favor kin 1.3 ± 0.1 vs. 0.9 ± 0.03	***P*<0.05**, favor kin 1.2 ± 0.1 vs. 0.9 ± 0.04	***P*<0.05**, favor kin 1.4 ± 0.3 vs. 0.9 ± 0.06
2	Pat _ sisters > true non-kin N = 9 pairs vs. 57 pairs	***P*<0.05**, favor kin 1.2 ± 0.1 vs. 0.9 ± 0.03	***P*<0.05**, favor kin 1.2 ± 0.1 vs. 0.9 ± 0.04	***P*<0.05**, favor kin 1.7 ± 0.2 vs. 0.9 ± 0.06
3	Mat _ sisters ≈ pat _ sisters N = 7 pairs vs. 9 pairs	NS 1.3 ± 0.1 vs. 1.2 ± 0.1	NS 1.2 ± 0.1 vs. 1.2 ± 0.1	NS 1.4 ± 0.3 vs. 1.7 ± 0.2

Table 4.4 Kin selection results: Rates of social behavior. Hypotheses tested using continuous data and Tukey-Kramer statistics to control for multiple comparisons. Means and standard errors are presented below the results in each comparison. Directed pairs were used, doubling the sample sizes.

Hyp.	Comparison	Total count of all behaviors (Obs/Exp)	Prop. AF/N-AF	Total count of affiliative behaviors
1	Mat _ sisters > true non-kin N = 7 pairs vs. 57 pairs	NS, favor kin 1.2 ± 0.2 vs. 0.7 ± 0.1	***P*<0.05**, favor kin 3.0 ± 0.7 vs. 1.7 ± 0.2	NS, favor kin 19 ± 2.6 vs 14 ± 1.5
2	Pat _ sisters > true non-kin N = 9 pairs vs. 57 pairs	***P*<0.05**, favor kin 1.7 ± 0.2 vs. 0.7 ± 0.1	***P*<0.05**, favor kin 3.0 ± 0.6 vs. 1.7 ± 0.2	***P*<0.05**, favor kin 35 ± 5.5 vs 14 ± 1.5
3	Mat _ sisters ≈ Pat _ sisters N = 7 pairs vs. 9 pairs	NS 1.2 ± 0.2 vs. 1.7 ± 0.2	NS 3.0 ± 0.7 vs. 1.7 ± 0.2	NS 19 ± 2.6 vs 35 ± 5.5

Table 4.5 Mechanism results: Time spent grooming or in close proximity. Hypotheses tested using point sample data and Wilcoxon signed ranks statistics. Number in () represents number of pairs tested in each comparison. Note that the number of pairs considered 'true non-kin of different ages' is different in the two analyses. In the first comparison against 'true non-kin, same age' all pairs of non-kin born more than one year apart are included. In the second comparison, non-kin include only the subset of non-kin born between 13 and 45 months apart are included to parallel the age difference range observed among paternal half sisters. Means and standard errors are presented below each result. Directed pairs were used in all analyses except for hypothesis 5.

Hypothesis	Comparison	Nearest neighbor Obs/Exp	Proximity while resting	Grooming
Maternal sisters				
4	Sequential (4) > separated (3) in birth order	NS, P=0.56 1.0 ± 0.06 vs. 1.0 ± 0.03	NS, P=0.2 1.3 ± 0.1 vs. 1.1 ± 0.2	NS, P=0.12 1.1 ± 0.3 vs. 1.8 ± 0.4
5	behavior directed towards older (7) > younger (7) mat. _	NS, P=0.94 1.0 ± 0.04 vs. 1.0 ± 0.04	NS, P=0.57 1.2 ± 0.1 vs. 1.3 ± 0.2	NS, P=0.95 1.3 ± 0.2 vs. 1.5 ± 0.5

Table 4.5 Mechanism results: Time spent grooming or in close proximity (continued).

Hypothesis	Comparison	Nearest neighbor Obs/Exp	Proximity while resting	Grooming
Paternal sisters				
6	true non-kin, same age (6) > true non-kin, diff. ages (51)	***P*=0.01** 1.1 ± 0.1 vs. 0.9 ± 0.03 favor same age	***P*=0.002** 1.3 ± 0.1 vs. 0.8 ± 0.04 favor same age	***P*=0.02** 1.4 ± 0.3 vs. 0.8 ± 0.06 favor same age
7a	Pat _, same age (6) > true non-kin, same age (6)	NS, *P*=1.0 1.2 ± 0.1 vs. 1.1 ± 0.1 favor kin	NS, *P*=0.62 1.2 ± 0.1 vs. 1.3 ± 0.1 favor kin	NS, *P*=0.56 1.7 ± 0.3 vs. 1.4 ± 0.3 favor kin
7b	Pat _, diff. ages (3) > true non-kin, diff. ages (8)	NS, *P*=0.2 1.1 ± 0.1 vs. 0.9 ± 0.1 favor kin	NS, *P*=0.53 1.1 ± 0.1 vs. 1.0 ± 0.1 favor kin	***P*=0.05** 1.6 ± 0.3 vs. 0.9 ± 0.1 favor kin

Table 4.6 Mechanism results: Rates of social behavior. Hypotheses tested using continuous data and Wilcoxon signed ranks statistics. Numbers in () represent number of pairs tested in each comparison. Note that the number of pairs considered 'true non-kin of different ages' is different in the two analyses. In the first comparison against 'true non-kin, same age' all pairs of non-kin born more than one year apart are included. In the second comparison, non-kin include only the subset of non-kin born between 13 and 45 months apart are included to parallel the age difference range observed among paternal half sisters. Means and standard errors are presented below each result. Directed pairs were used in all analyses except for hypothesis 5.

Hyp.	Comparison	Total counts of all behaviors (obs/exp)	Prop. AF/N-AF	Total count of affiliative behaviors
Maternal sisters				
4	sequential (4) > separated (3) in birth order	NS, P=0.25 0.8 ± 0.1 vs. 1.2 ± 0.2	NS, P=0.48 3.2 ± 1.3 vs. 2.8 ± 0.4	NS, P=0.75 16.4 ± 2.1 vs. 21.3 ± 5.4
5	behavior directed towards older (7) > younger (7) mat. 1/2	NS, P=0.9 0.8 ± 0.1 vs. 1.2 ± 0.1	NS, P=0.16 4.1 ± 1.3 vs. 1.9 ± 0.3	NS, P=0.30 20.9 ± 4.1 vs. 16.1 ± 3.2

Table 4.6 Mechanism results: Rates of social behavior (continued).

Hyp.	Comparison	Total counts of all behaviors (obs/exp)	Prop. AF/N-AF	Total count of affiliative behaviors
Paternal sisters				
6	true non-kin, same age (6) > true non-kin, diff. ages (51)	NS, P=0.12 1.0 ± 0.2 vs. 0.7 ± 0.1 favor same age	NS, P=0.95 1.8 ± 0.6 vs. 1.7 ± 0.2 favor same age	NS, P=0.15 23.8 ± 7.8 vs. 12.4 ± 1.4 favor same age
7a	Pat 1/2, same age (6) > true non-kin, same age (6)	NS, P=0.13 1.6 ± 0.2 vs. 1.0 ± 0.2 favor kin	NS, P=0.08 3.1 ± 0.7 vs. 1.8 ± 0.6 favor kin	NS, P=0.2 28.7 ± 5.2 vs. 23.8 ± 7.8 favor kin
7b	Pat 1/2, diff. ages (3) > true non-kin, diff. ages (8)	**P=0.05** 1.9 ± 0.3 vs. 0.9 ± 0.1 favor kin	NS, P=0.91 2.8 ± 1.0 vs. 2.7 ± 0.7 favor kin	NS, P=0.08 48.5 ± 11.9 vs. 24.4 ± 5.5 favor kin

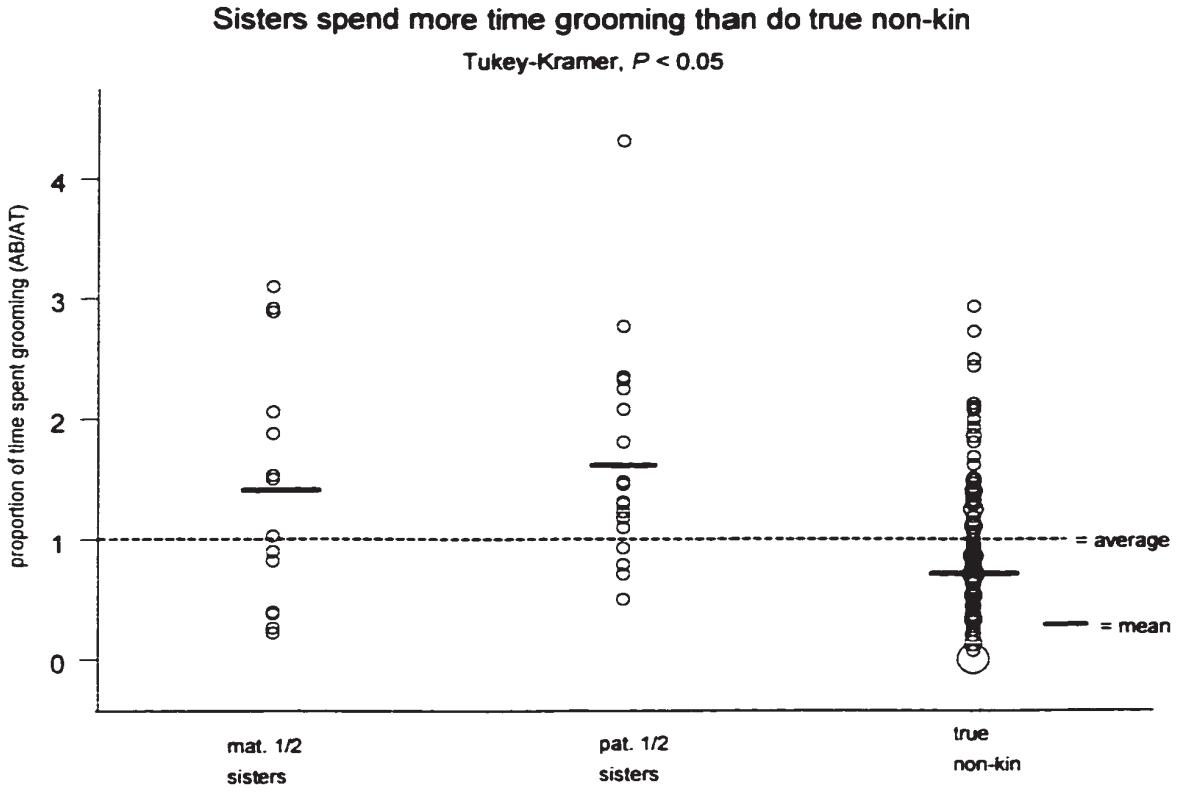

Figure 4.1 Sisters spend more time grooming than do non-kin. Each data point represents the number of times (from point-sample data) female A groomed female B (AB), divided by the mean number of times she groomed all adult females in her group (AT). Points above the dotted line represent pairs in which female A spent more time grooming female B than she spent grooming other adult females on average. Larger circles indicates values that represent more than one pair of females. Bars represent the mean value for each kin class.

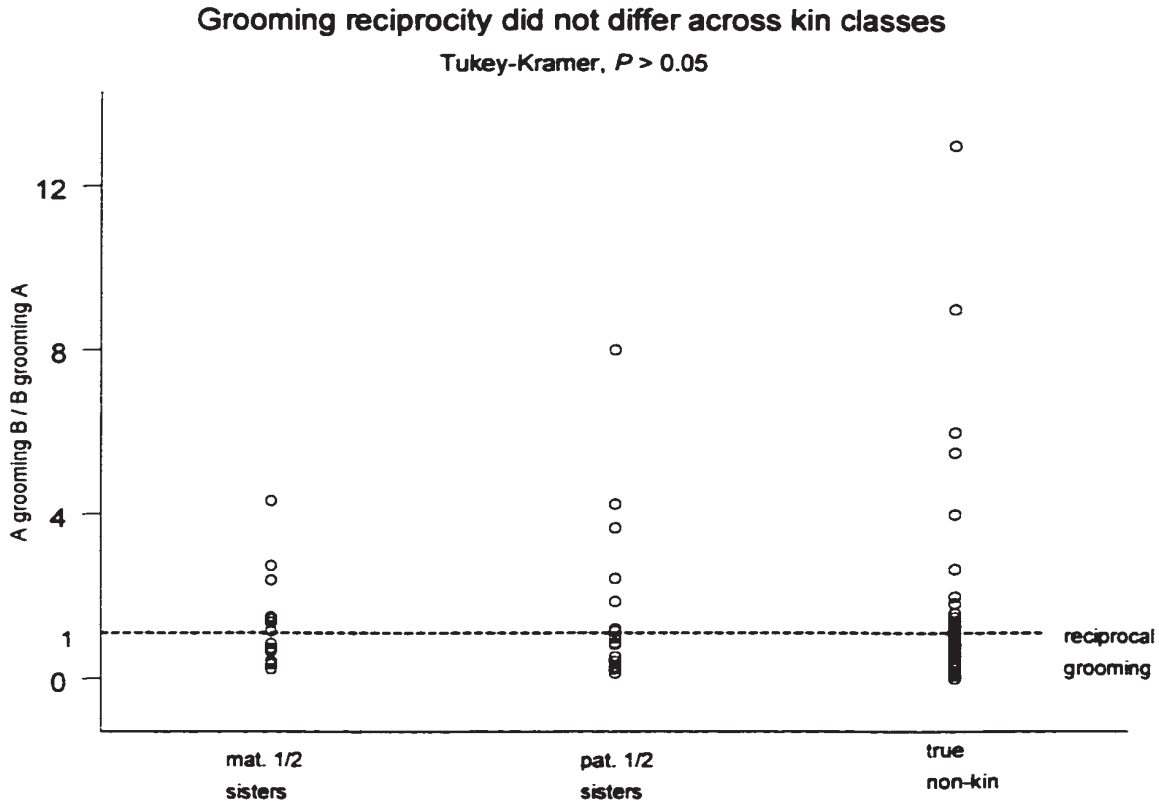

Figure 4.2 Grooming reciprocity did not differ between sisters and non-kin. Each data point represents the initiation of grooming bouts among a pair of adult females where the number of times female A groomed B was divided by the number of times female B groomed A. Data were collected as *ad lib* grooming. Data points close to the dashed line represent pairs in which the grooming was reciprocal, i.e., both females initiated approximately equal numbers of grooming bouts (= 1).

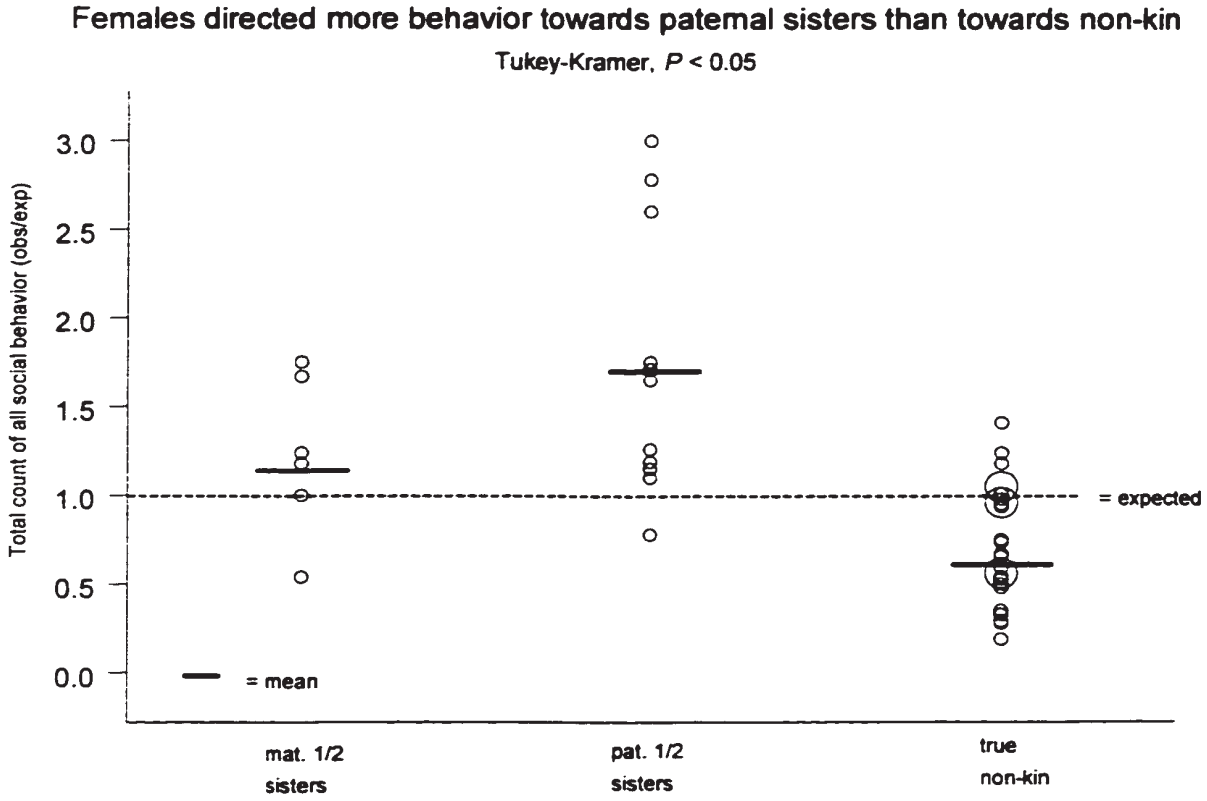

Figure 4.3 Rates of social interactions higher between paternal sisters than between unrelated females. Each data point represents the observed/expected ratio of social behavior (collected as continuous a data) directed by female A towards female B. Data came from two sources within focal animal samples, both when A was the focal female and directed behavior towards B, and also when B was the focal female and received behavior from A. Larger open circles indicate values representing more than one pair of females. Bars represent the mean value of each kin class.

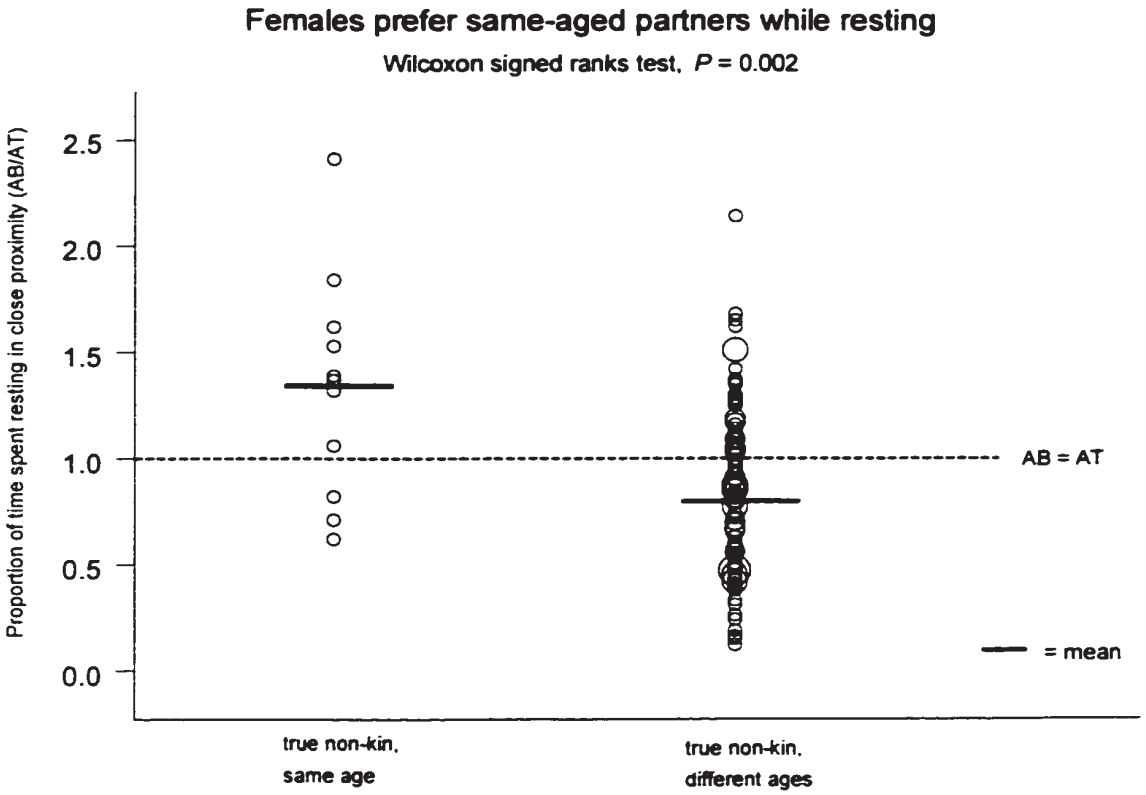

Figure 4.4 Females prefer same-aged partners while resting. Each data point represents the amount of time (number of point samples) that female A was resting and female B was her nearest adult female neighbor (AB), divided by the mean amount of time A was resting with all other females (AT) as her nearest adult female neighbor. Values greater than one indicate that female A spent more time resting in close proximity to B than she spent resting close to all other females, on average. Larger open circles indicate values representing more than one pair of females.

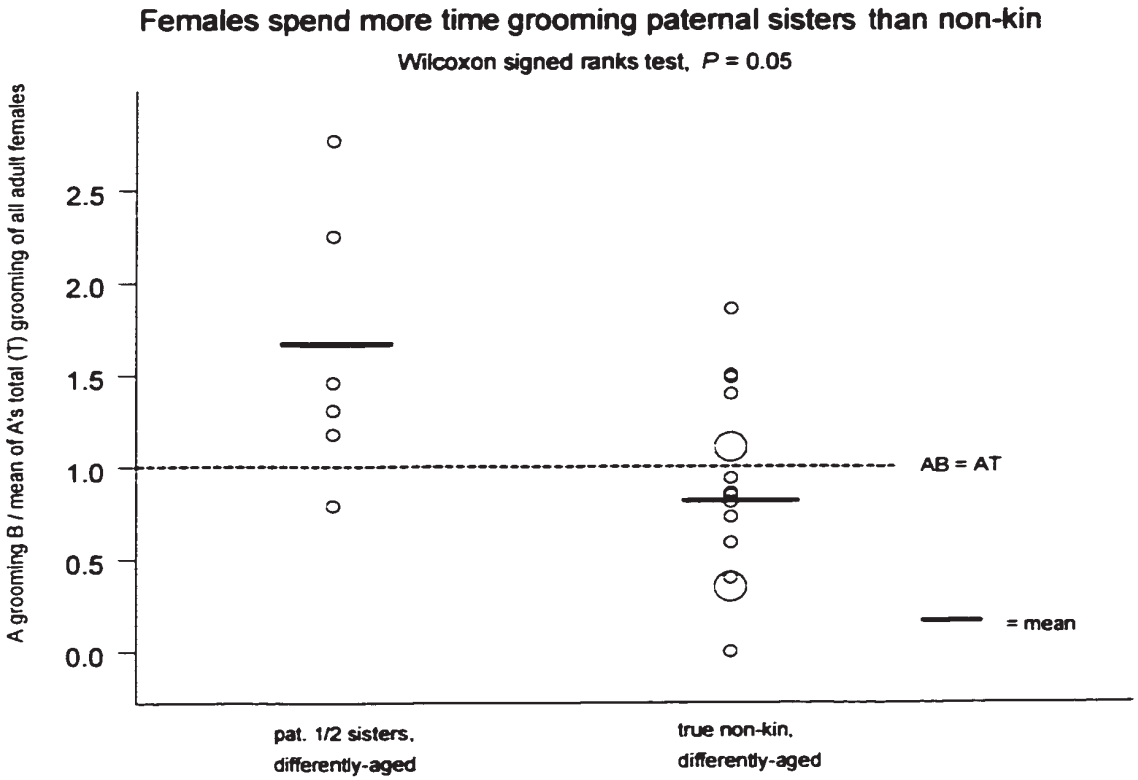

Figure 4.5 Grooming between paternal sisters suggests phenotype matching. Data points represent the amount of time A spent grooming B (AB) (number of point samples), divided by the mean amount of time A spent grooming all females (AT). Values above the dashed line indicate female A spent more time grooming B than she spent grooming other females, on average. Large open circles indicate values representing more than one pair of adult females. Bars represent the mean value of each kin class.

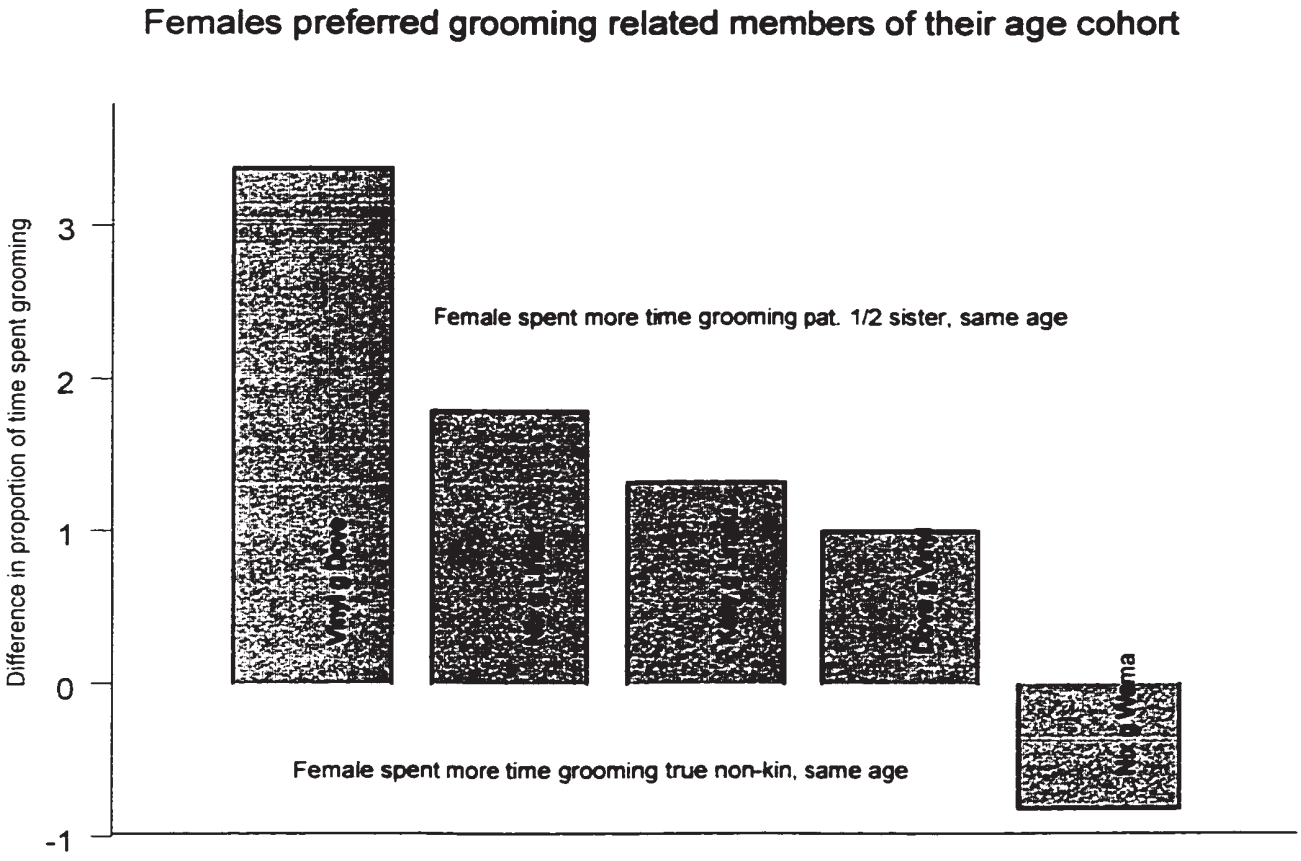

Figure 4.6 Females preferred grooming related members of their age cohort. Four of the five females who had both a related (paternal half sister) and an unrelated member of their age cohort, spent more time grooming their sister than they spent grooming the unrelated female. Data come from point samples. For example, Dove spent three times as much time grooming her paternal sister Vinyl, as she spent grooming Ochre, an unrelated member of her age cohort.

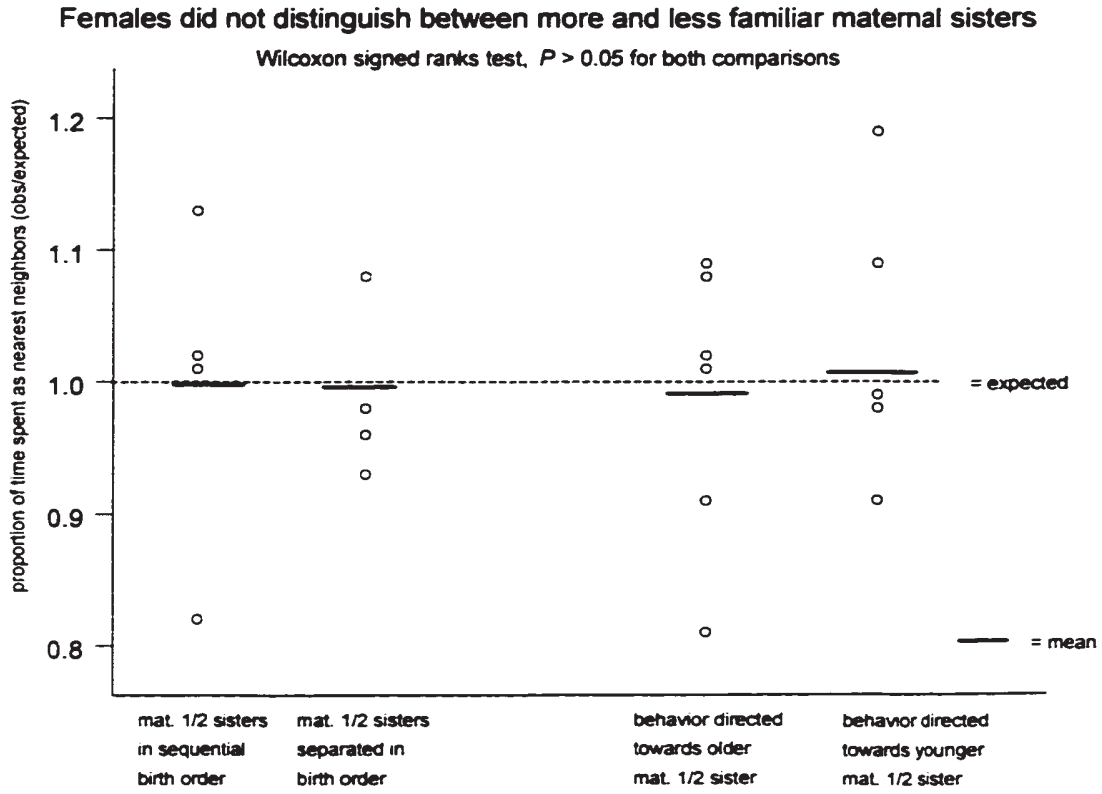

Figure 4.7 Using maternal sisters to test proximal mechanisms. Females did not bias their social behavior among maternal sisters based on familiarity alone in any measure tested. Here, the proportion of time females spent as nearest adult females did not differ among more or less familiar maternal half sisters. Each data point represents the amount of time (number of point samples) A's nearest adult female neighbor was B, divided by the expected value. All means were indistinguishable from each other, and from the expected value.

Appendix 4.1 Ethogram of social behaviors. Behaviors were collected as continuous data. All behaviors were directed by an adult female towards another adult female.

AFFILIATIVE SOCIAL BEHAVIORS

Greet	One female touched another female (Smuts 1995), usually on the torso, when coming in close proximity.
Lipsmack	An adult female moved her lips and tongue in a way that resulted in a smacking noise while looking at another adult female.
Groom	A female had at least one hand on another adult female and moved the hair around. If grooming stopped but was resumed within five seconds, a new grooming bout was not recorded. Grooming was often accompanied by lipsmacking. Lipsmacking that occurred during a grooming bout was not recorded.
Solicit groom	A female conspicuously placed a body part (other than her perineum) into the direct line of vision of another female. Lipsmacking that occurred During a grooming solicitation was recorded.
Mount	A female placed herself behind a second female so that the pelvic regions were aligned. The mounting female also placed at least one hand on the second female's back or hip.
Muzzle to muzzle	A female placed her muzzle in contact (or very close proximity) with a second female's muzzle.
Cohesion grunt	A female emitted a soft grunt, usually while approaching a second female, or while the female was stationary, but in close proximity to and looking at the second female.
Follow	Both females began stationary, but not necessarily in close proximity. As the first female began to move away, the second female responded by moving, within 15 seconds, and in the same direction.
Approach	A female moved within one meter of a second female. Most often approaches occurred when the approached female was stationary. However, it was possible to record an approach when both females were moving if the approaching female was moving faster than the approached female.

Appendix 4.1 Ethogram of social behaviors (continued).

NON-AFFILIATIVE SOCIAL BEHAVIORS

Lean away	A female moved her body, head, or gaze away from another female.
Tail up	A female held her tail upright and away from her body.
Grimace	A female retracted her lips and exposed her teeth which were often clenched.
Screech	A female emitted a high pitched vocalization.
Eyelid display	A female raised her eyebrows to expose the unpigmented skin of her eyelids while looking at another female.
Stare	A female directed a fixed, unblinking, prolonged look at another female.
Head Bob	A female moved her head up and down in a rapid, exaggerated movement.
Bite, nip	Obvious
Ground Slap	A female slapped the ground or some other substrate with her palm while looking at another female.
Lunge	A female leaped or jumped towards another female.
Present	A female backed up towards another female until her perineum was directly in front of the second female's face. The difference between a 'present' and 'soliciting grooming' depended on the body part. Presenting involved the perineum and soliciting grooming involved any other body part (S. Alberts, pers. com).
Leave	A female moved out of a one meter radius of a second female.
End Groom	A female removed her had from the female she was grooming for at least six seconds.

CHAPTER 5

CONCLUSIONS

Historically, studies of kinship and the distribution of social behavior with groups of non-human primates have focused almost exclusively on maternal kin. In fact, kin were often defined as being members of the same matriline. Excluding paternal kin from kinship analyses was largely due to the technical difficulties of assigning paternity and therefore identifying paternal kin. Even when including paternal kin in the same category as non-kin, a strong maternal kin bias has been reported for a wide range of non-human primates involving a wide range of behaviors. Because the maternal kin bias was so strong, it was perhaps natural to question, implicitly if not explicitly, the relative importance of paternal kin to the distribution of social behavior. Perhaps there is something so compelling about the mother-offspring relationship among non-human primates, that biasing social behavior towards maternal kin confers more fitness advantages than biasing behavior towards paternal kin. Perhaps the mother-offspring relationship provides a familiarity mechanism for distinguishing between kin and non-kin that does not exist among paternal kin. Recent developments in molecular techniques allow us now to determine relatedness (both maternal and paternal) between individuals, and to do so non-invasively. With these techniques, it has become possible to ask, "Do paternal kin matter?".

Paternal kin-biased social behavior

The results of this study strongly suggest that yes, paternal half sisters matter. In fact they matter at least as much, if not more, than maternal sisters. Evidence supporting this statement comes from two sources; kin-biased social behavior and the outcome of a group fission.

Females biased their social behavior towards maternal sisters over non-kin in 4 of 6 measures. In contrast, for every measure (6/6), adult females biased their social behavior towards paternal sisters over non-kin. Adult females directed a greater proportion of all social behaviors, both affiliative and non-affiliative, towards paternal sisters than towards non-kin, and the ratio of affiliative to non-affiliative social behaviors directed towards paternal sisters was greater than that directed towards non-kin. Adult females also spent a greater proportion of time grooming with, and maintaining close proximity while resting to, paternal sisters than non-kin.

Paternal sisters were also nearly twice as likely (64% vs. 33%) to end up in the same group after a group fission as were maternal sisters. The two females who did not end up in the same group as their paternal sisters (Nightjar and Laza), were both relatively young adult females who both had mothers alive at the time of the fission, and both females ended up in the same group as their mothers. The tendency of paternal, more so than maternal, sisters to stay together during Hook's Group fission, resulted in all three study

groups containing pairs of adult paternal sisters; only two of the three groups had at least one pair of adult maternal sisters.

Even more recently, the tendency for paternal sisters to stay together during group fissions was observed again in this population. Dotty's Group, one of the three social groups included in this study, completed a group fission in August 1999, two years after the behavioral data for this study were collected. The fission resulted into two new groups, Omo's and Viva's Groups (Altmann, unpublished data). All pairs of sisters, in this case both maternal and paternal, ended up together in the same group. Maternal sisters Viva, Vortex, Velcro, and Vinyl all ended up in Viva's Group, as did Vinyl's paternal sister Dove. Interestingly, Dove and her mother ended up in different groups. Paternal sisters Asha and Echo both ended up in Omo's Group.

Several biological characteristics would predict a greater tendency for the bias towards paternal sisters than towards maternal sisters observed in this study. 'Enhanced' relatedness, age similarity, and/or dominance rank dissimilarity may explain why adult females show a stronger bias towards paternal sisters than towards maternal sisters.

'Enhanced' relatedness

There are genetic reasons why paternal sisters might be important to females as social partners. Paternal sisters share slightly more alleles on average than do

maternal sisters (de Ruiter and Geffen 1998). First, as is true for other matrilocal species (reviewed in Webb *et al.* 1995), the adult female baboons in a group are more closely related to each other than are the adult males (Altmann *et al.* 1996). Therefore the relatedness between paternal siblings is 'enhanced' (Storz 1999) as they share alleles, not only through their common father, but also through their different mothers. On the other hand, maternal siblings are unlikely to share alleles from their different and unrelated fathers, as males immigrate independently into the group and not with brothers or other close relatives, the only way that adult males in the group would be related to each other. Second, as males are haploid for their sex chromosomes, same-sexed paternal siblings will inherit identical sex chromosomes; in the case of paternal sisters, identical X chromosomes are inherited. Because of this, paternal sisters share identical alleles at every X-linked locus while maternal sisters have only a 50% chance of inheriting the same alleles on the maternally-inherited X chromosome. As the proportion of alleles identical-by-descent on the sex chromosomes increases, relative to the proportion of genetic information on the autosomes, a behavioral bias towards siblings sharing those alleles should also increase.

Age similarity

Paternal half sisters tended to be closer in age than were maternal half sisters. The average age difference between paternal sisters was 14.4 months compared to 31 months between adult maternal sisters. This study concentrated exclusively on adult

sisters. However, if siblings are identified from all group members (i.e., siblings of all age/sex categories are considered, and not just adult females), then the difference in age between maternal siblings, but not paternal siblings, will be even greater than when only adult sisters are considered. Because females continue to reproduce their entire adult lives, maternal siblings can be separated in age by a generation or more. The females in this study provide and interesting example. The greatest age difference observed among paternal sisters was 45 months. The largest age difference observed between maternal siblings was 155 months (Viva was born in September 1985 and her younger brother Vibrant was born in August 1998). These age differences have important consequences. First, maternal siblings like Viva and Vibrant, have little in common socially. They are in completely different life history stages and will be until the younger sibling reaches adulthood. Any behaviors that are biased towards individuals of similar ages will not be biased between maternal siblings spread out in age. Second, a relatively small proportion of their life spans will overlap. In this case, Viva was 13 years old, nearing the end of an average life span for females, when Vibrant was born. Decreased overlap in life spans is potentially important to the evolution of familiarity mechanisms. Any familiarity mechanism that requires exposure to kin early in life will not be expressed in Viva's behavior towards Vibrant.

Consider now paternal siblings. They tend to be of similar ages. Therefore a greater proportion of their life spans will overlap, giving them more opportunity both

to develop familiarity mechanisms and to express biased social behavior towards each other. Being of similar ages, they will go through life history stages together and will have more in common socially. They will be infants together, be playmates as juveniles, females will reach menarche and have offspring at similar times and finally paternal sisters will experience old age together, baring death and group fissions. Age similarity may therefore enhance the appeal of paternal siblings over maternal siblings as social partners.

Rank

Rank is a second social reason that females might bias their behavior towards paternal sisters over maternal sisters. While maternal sisters are tightly clustered in rank, paternal sisters are not. The average rank difference among maternal sisters in this study was 1.6 with one representing two females of adjacent ranks in the dominance hierarchy. The average difference in rank for paternal sisters was 3.3 meaning that they had, on average, at least two individuals placed between them in the dominance hierarchy. The range in rank differences was 1 – 3 for maternal sisters and 1 – 6 for paternal sisters. Differences in rank, like differences in age, also have interesting consequences. First, most aggression occurs between individuals of adjacent rank. This would lead one to predict that agonistic behaviors would be biased towards maternal sisters over paternal sisters who are more different in rank. Second, if there are benefits associated with interacting with higher-ranking females,

then these benefits should be more pronounced between paternal sisters than between maternal sisters.

Although the genetic differences between the proportion of alleles shared between maternal and paternal sisters is slight, that genetic variation, along with the greater proportion of life spans that overlap and therefore opportunities for interactions exist, provide necessary conditions for social behaviors to evolve through kin selection.

Evolution of mechanisms for the observed bias

The results presented here suggest that adult female baboons bias their social behavior primarily by familiarity. While females bias their behavior towards paternal sisters over non-kin in every measure tested, the strongest bias observed (the most significant statistical results) was towards same-aged non-kin vs. differently-aged non-kin. Adult females strongly biased their social behavior towards both related and unrelated members of their age cohort. This suggests that females use familiarity, based on age similarity, as a first rule for biasing their behavior towards paternal siblings.

Adult female baboons are most likely using a familiarity mechanism for biasing their behavior along kinship lines. The rule for biasing among maternal sisters may be something along the lines of, "Treat as close kin those individuals who behave in a similar way towards 'mom' ". For paternal sisters the rule may be more like, "Treat as close kin those individuals who are the same age". Although both rules are indirect and

prone to error, both, if followed, will result in female baboons biasing their behavior towards siblings over non-kin, especially in small groups.

However, it is also apparent that these two rules differ. Maternal siblings have one rule and paternal siblings have another. These differences could affect both the evolution of the mechanism, and the strength of the expression of the kin bias. The rule for maternal siblings is actually much more accurate, but also more complicated than is the rule for paternal siblings. The rule to treat as close kin individuals that also have close interactions with mother, similar to those of the young individual learning the rule, is open to much confusion. Assuming a familiarity mechanism develops early in life, what part of an infant's relationship with its mother should he use as a standard for comparing kin to non-kin? Young infants spend much of their time nursing and clinging to their mother's ventrum. Given that no other individual in the group expresses these behaviors towards his mother, these behaviors are not useful as a standard. What if the standard behavior used to learn kin from non-kin is grooming between his mother and others? The grooming between older siblings and their mother may not resemble the grooming relationship between the young, 'learning' individual and his mother. The mother's grooming effort may be biased towards her new infant offspring than towards her older offspring. If so, then her grooming behavior would not provide an accurate cue for kin discrimination. The grooming behavior of older siblings may also not provide a useful cue for learning familiarity. Juvenile males have a less reciprocal grooming relationship with mothers than do juvenile females. Therefore, the grooming behavior of

an older brother towards his mother also may not be helpful for a young individual learning to discriminate between kin and non-kin. Compare all of these issues to the rule, "treat as close kin those individuals who are the same age". This rule has more room for error certainly, however, it is a simpler rule than that used by maternal siblings, suggesting that it might be more likely to evolve in the first place, and more likely to be expressed.

This brings up an interesting point. Can the biasing of social behaviors that evolve through kin selection differ between maternal and paternal kin? Yes, if the mechanisms for distinguishing maternal kin are different from those for distinguishing paternal kin, and if the need/benefit is greater for biasing behavior towards kin of one kind over the other (Altmann 1979). In the case of baboons, groups are subdivided in a way that could have facilitated the evolution of familiarity mechanisms was in place for both maternal and paternal siblings. However, the evolution of a familiarity rule might have been more complicated among maternal than paternal siblings, which might lead to a difference in the strength of the expression of behavior biased towards maternal and paternal siblings. The results presented here suggest that the bias towards paternal sisters was at least as strong, if not stronger, than the bias towards maternal sisters.

Finally, two results suggested that adult female baboons may be capable of distinguishing between kin and non-kin based on a phenotype-matching mechanism. In two of the six measures tested, females biased their behavior significantly more towards

differently-aged paternal sisters over differently-aged non-kin (with comparable age difference between the two categories). The distribution of social behavior between paternal sisters and non-kin was indistinguishable in the other four tests. This suggests that in some contexts, females can distinguish between kin and non-kin even when age similarity (familiarity) is controlled for.

That the phenotype-matching mechanism was observed among differently-aged paternal sisters and not among same-aged paternal sisters may not be surprising. There was no difference in the distribution of behavior between same-aged paternal sisters and same-aged non-kin. Perhaps this is because pairs of females in both categories, related or not, are members of the same age-cohort. As such, they were extremely familiar with each other and had had the opportunity to interact and develop social bonds their entire life, increasing the likelihood that these pairs will interact socially as adults, related or not. However, females did bias their behavior towards differently-aged paternal sisters over differently-aged non-kin. In this case, all pairs are member of different age cohorts and so are less familiar, and therefore the genetic difference (between sisters and non-kin) will be more influential.

Bias observed during ecologically stressful times

The behavioral data used to test whether a bias towards paternal kin existed were all collected during one field season. The eight months I collected behavioral data (between July 1996 and February 1997) were part of a severe drought season (Altmann,

unpublished data). Droughts and other ecologically stressful conditions have interesting implications for this study. First, under stressful conditions, such as droughts, animals have less time to socialize, as they must spend a greater proportion of their time foraging for food and water. Because of this, it is possible that the females in this study interacted socially at lower rates than they would have under better ecological conditions. I may have observed fewer social interactions than I would have during eight months of non-drought conditions. However, it could be precisely during ecologically stressful times when animals have either less time to socialize (increased time spent foraging), or socializing becomes more costly (animals must increase vigilance as predators also become hungrier during droughts), that one would expect to observe the strongest bias towards kin over non-kin.

Implications

Male dispersal and immigration may be influenced by paternal relatedness. Consider a hypothetical situation in which a male is born in one of his father's 'later' groups (i.e., his father sired the greatest proportion of his offspring in a previous group during his tenure as a high-ranking male). This means that there is at least one group in the population that this male should avoid when immigrating into a new group. Take Linda's Group for example. In 1996, three of the nine adult females were paternal sisters. A paternal brother immigrating into Linda's Group would be related to a third of the reproductive females. If there is a cost to reproducing with half siblings, males

should avoid certain groups in the population. The fact that males disperse from their natal groups suggests that there is a cost to reproducing with close maternal kin and Alberts' (1999) study found that familiar paternal siblings also avoided mating with each other, suggesting baboons avoid the cost to mating with paternal siblings. If these costs apply to unfamiliar paternal siblings as well, and if males can discriminate between unfamiliar paternal kin and non-kin, then dispersing males should avoid groups in which he is paternally related to the adult females. Admittedly, this would be hard to test in natural populations. However, the Cayo Santiago colony of rhesus macaques might provide an opportunity for testing this theory. All individual macaques are known in this transplanted, managed colony, both maternal and paternal genealogies are known, and unlike captive groups, males can disperse from group to group. Under these conditions, it is possible to test whether dispersing males avoid immigrating into some groups, and if so, whether they are paternally related to the adult females in those groups.

A second implication of the paternal kin bias observed among baboons is that age and genetic relatedness among adult females in a group should be non-random. The movement of paternal sisters during two group fissions (Hook's and Dotty's) were discussed in this study. In both cases, paternal sisters, who were of similar ages, ended up together after the group fissioned. This results in substructuring both by kinship and by age among adult females in baboon groups (Altmann *et al.* 1996).

Future studies

The results in this study demonstrate that adult female baboons bias their behavior towards paternal sisters. The results also suggest that this bias towards paternal sisters is primarily facilitated by familiarity due to being members of the same age cohort, with a suggestion that under some conditions, adult female baboons can distinguish between kin and non-kin by a phenotype-matching mechanism.

Several interesting questions follow from this study. For instance, it is an assumption of kin selection theory and of this study, that the greater the proportion of alleles identical-by-descent, the greater the bias towards kin, all other things being equal. An interesting test of this theory would be to compare individuals of different species that have sex chromosomes, but that also have varying degrees of genetic material on the autosomes. As paternal siblings of the same sex inherit identical copies of their father's sex chromosome, then the less genetic material they have on the autosomes, then the greater the proportion of alleles identical-by-descent. Is the bias towards paternal siblings greater in species with less autosomal material? The difficulty of this study would be comparing different suites of social behaviors evolved through kin selection in distantly related species.

Several tests to better understand the mechanism underlying the paternal kin bias would be informative. First, how early does the mechanism for biasing between siblings and non-kin develop? If the mechanism is primarily familiarity, then older juveniles and

adults should show a stronger bias than do infants and younger juveniles. It would be very interesting if young juveniles, who spend a large portion of their days interacting with members of their age cohort, showed a bias towards related over unrelated members of their cohort. Further, comparing the bias towards siblings when siblings of all ages and both sexes are considered would be informative. Is the bias towards paternal siblings, who tend to be of similar ages, greater than the bias towards maternal siblings who can be of very different ages?

A second test of mechanism that would be interesting to do in baboons and/or other species, would be to test if the cost of the behavior alters the strength of the kin bias that is expressed. Several studies suggest that this is the case. Pfennig (1999) shows that spadefoot toad tadpoles that are the greatest threat to their relatives show the strongest kin-bias. Alberts (1999) showed, in the same population of baboons studied here, that while paternal siblings did not avoid each other as consort partners, their consortships were less sexual than were the consortships between unrelated individuals. Both these studies do suggest that both 'costlier' behaviors (e.g., aiding during an agonistic bout, nursing non-filial young), and behaviors with costlier consequences (e.g., cannibalism or mating) should be strongly biased along kinship lines.

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