Genetic relatedness and socioecology of wild Hanuman langur monkeys

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Katherine Alexandra Little

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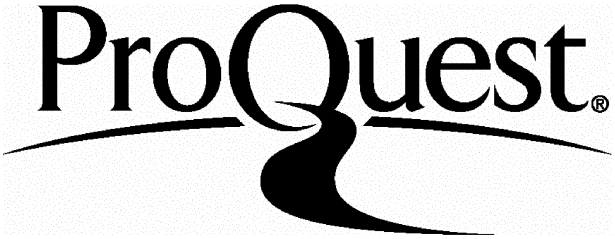

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Abstract

This genetic study is based on a geographically isolated population of Hanuman langurs that live around the city of Jodhpur, north-west Rajasthan. Here, the majority of langurs live in harem troops (with a single resident male for 95% of the troop's history) and bachelor bands. Behavioural studies of these langurs suggest that the troops are matrilineal, with males being the dispersing sex. It has therefore been hypothesised that females of a troop are closely related, both through their mothers and through cohorts sharing the same father. This would explain the high levels of cooperation seen between females, such as allogrooming and home range defence. Conversely, members of all male bands, particularly the young adults who control the bands' movements, are unlikely to be related, because of the constantly changing membership and the high mortality rate suffered by the nomadic males.

This study has tested these hypotheses using non-invasive techniques to obtain DNA samples from troops and bands in the population. 89 individuals of five troops and one band have been genotyped at eight polymorphic microsatellite loci. Analysis of the microsatellite data using Queller and Goodnight's RELATEDNESS and KINSHIP programs has shown that on average, troops are related by 0.17 ± 0.04, troop females by 0.14 ± 0.07, and non-adult troop members by 0.27 ± 0.07. Conversely, the relatedness of the band was only 0.05 ± 0.08. In three troops the resident male could not be excluded as the father of any non-adult, suggesting that these residents had had long term mating monopoly in these troops, whereas in the remaining two troops where takeover had recently occurred, the new residents could be excluded as fathers in all but 2/12 cases. Additionally, the population proved to be highly structured, and troops appeared outbred, an indication of female philopatry combined with polygyny. These results provide genetic evidence in support of the social organisation suggested from long-term behavioural data.

GENETIC RELATEDNESS AND SOCIOECOLOGY OF WILD HANUMAN LANGUR MONKEYS

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1. THE INTERPLAY BETWEEN RELATEDNESS AND COOPERATION

1.1 ALTRUISM OR GENETIC SELFISHNESS?

Examples of cooperative behaviours are seen across a variety of animal taxa, including allomaternal behaviour in elephants (Lee, 1987), food sharing in vampire bats (Wilkinson, 1984), helpers at the nest in many species of birds (e.g. the Seychelles warbler, Komdeur, 1992), and individuals sacrificing their own reproduction to help another reproduce, as seen, for example, in wild dogs (Girman *et al.*, 1997), naked mole rats (Sherman *et al.*, 1992) and eusocial insects (Bourke and Franks, 1995). Until the 1960s, cooperative behaviours were explained as being 'good for the species', an argument that proposed that selection operated at the level of social groups. It was the publication of Wynne-Edwards' (1962) review of social behaviour, paradoxically championing the group selectionist theory, that led others (e.g. Lack, 1966) to the realisation of the flaw of this idea - that such a system would be open to invasion by selfish 'cheats' acting for their own benefit. Group selectionist reasoning, it was highlighted, contradicts Darwin's evolutionary theory (1859), which emphasises the struggle of the individual to outcompete other members of the population, in order that it may leave the greatest number of surviving offspring. Natural selection thus operates at the level of the individual, not at that of the group, and can easily account for observations of competition and conflict between individuals. However, it was not immediately apparent how such a framework could accommodate cooperative behaviours, in which animals spend time and resources investing in the survival of others, often to the detriment of themselves.

A convincing explanation for the evolution of cooperative behaviours was given by Hamilton (1964), who recognised that if an individual preferentially directed helping behaviours toward its kin, it would be benefiting genes that it shared with the recipient by descent. The most obvious example of this 'kin selection' is parental care. It is not questioned that parents, especially mothers, should invest in their young, for offspring carry their parents' genes into future generations. In a diploid organism, offspring inherit half of their genes from their mother and half from their father; the parent–offspring coefficient of relatedness, r, in an outbred mating is 0.5. Parental care, though phenotypically altruistic, is therefore genotypically selfish behaviour, as investment in the offspring ensures the survival of the parent's genes and benefits the parent's direct fitness (Brown, 1980). However, there is genetically nothing unique about the

relatedness of parent to offspring. Full sibs share 50% of their genes on average, being related through both parents; their coefficient of relatedness is also 0.5. Similarly, grandparents are related to grandchildren by 0.25, aunts to nieces by 0.25, and cousins by 0.125. The advantages of helping closely related kin are greater than helping less related individuals; nevertheless, to help any of these relatives in surviving to reproduce will be beneficial to the genes that the actor and recipient have in common by descent, and will benefit an individual's indirect fitness. Direct and indirect fitness combine to give an individual's inclusive fitness, an overall measure of reproductive success.

There are, however, costs to helping behaviours; by aiding another individual, a helper may be reducing its own reproductive chances. Behaviours such as sharing food with another individual, rather than selfishly defending it, may increase the recipient's fitness whilst decreasing that of the donor. The relative gains in terms of inclusive fitness (directly and indirectly related offspring) need to be taken into account before it can be estimated whether the helping behaviour is ultimately beneficial to the altruist in terms of increasing its overall genetic contribution to future generations.

Situations in which altruistic behaviours will spread by kin selection are quantified by Hamilton's rule (1964), in which

$$rB > C$$

where r is the relatedness of the actor to the recipient, B is the benefit to the recipient, and C is the cost to the actor. Costs and benefits may be measured in terms of survival chances, or in terms of number of offspring gained or lost through the act. Hence it can be seen that if the relatedness of the actor and recipient is high (as in the case of close relatives) the benefit of the act to the recipient need not be many times greater than the cost of performing the act to the actor, whereas if they are distantly related, the benefit to the recipient must be very much greater than the cost to the actor. The latter situation of costs and benefits is less likely to occur; therefore altruistic acts between distantly related individuals will be very unlikely to spread through a population.

Extreme altruistic and cooperative behaviours between close kin are observed in social insects; worker castes can forgo reproduction completely, or even sacrifice their lives to enable a close relative, usually their mother, to reproduce (Bourke and Franks, 1995). In mammals, there is not usually such a division of reproduction, although this is seen in, for example, naked mole rats (Sherman *et al.*, 1992) and wild dogs (Girman *et al.*, 1997). More often altruism and cooperation in mammals is manifested in behaviours

such as alarm calling in Belding's ground squirrels (Sherman, 1977), allomothering in many primates (Nicolson, 1987) and the formation of large coalitions amongst male lions (Packer *et al.*, 1991a). The effect of the degree of relatedness is illustrated in the case of Belding's ground squirrels. These rodents give alarm calls when predators approach. Giving alarm calls is costly, as it makes the actor more prone to attack. The beneficiaries of the alarm call (other individuals in the vicinity) gain by being made aware of the presence of the predator. Individuals are more likely to give alarm calls when close relatives are near, whether these relatives are offspring or non-descendant kin. Males are less likely to give alarm calls; being the dispersing sex, they are not often in the company of close kin, therefore there is less benefit to offset the cost of making the alarm call. A comparison in primates comes from a study of co-feeding in Japanese macaques (Belisle and Chapais, 2001); aggression over a limited supply of desirable food is lessened with increasing kinship, suggesting that primates are able to differentiate between differing degrees of relatedness. The role of kin selection in primate groups is reviewed by Silk (2002).

An alternative mechanism by which cooperative acts may be perpetuated is reciprocal altruism, or reciprocity. Interacting individuals can balance the cost and benefit of altruistic acts over time; on one occasion one individual will benefit from the altruism of another, and will repay this act – reciprocate - at some time in the future (Trivers, 1971). This tit-for–tat scenario necessitates individuals encountering each other over a period of time, so they have the opportunity to engage in a series of interactions. They must also have the capacity to recognise one another, and so identify individuals who will reciprocate and those who may be inclined to cheat on an altruistic act (Axelrod and Hamilton, 1981). Thus, as noted by Silk (2002), both reciprocity and kin selection rely on altruists being able to interact selectively with other altruists. An example of reciprocity is found in vampire bats (Wilkinson, 1984). Individual bats that fail to feed during the course of a night will beg blood meals from others in the communal roost. Regurgitation of blood meals occurs only either between relatives, or between roost-mates, who are familiar with one another and can be depended upon to reciprocate this donation in the future. Reciprocity is invoked as the driving force behind cooperative behaviours in many species of mammals and birds (see Ligon, 1991, for review).

For mammals living in groups, it is difficult to determine what drives them to cooperate. Philopatric individuals are likely to be in the company of relatives – females are often the philopatric sex, so matrilineal relatedness can be determined. However, often the

genetic relationships between individuals are not known; this is especially true of animals which are long lived, so long term field data is not always available, individuals may disperse over large distances, and paternity is often uncertain. In these situations, cooperative behaviours cannot unambiguously be attributed to kin selection without the support of genetic measures of relatedness, which has only recently become possible to investigate on a large scale for wild primates because of limitations of DNA sampling. If cooperating individuals within a group are closely related then the evolution and maintenance of the cooperative behaviours can be attributed to kin selection; this is the most parsimonious explanation for their occurrence. To infer reciprocity in such a situation is a plausible explanation but is unnecessarily complex if kin selection can be invoked.

If, however, individuals are found to be unrelated, then the occurrence of cooperative behaviours cannot be explained by kin selection. Individuals should act to maximise their own direct fitness, as they cannot gain indirect fitness benefits in a group where they have no relatives. Members of alliances of many mammals, particularly males who have dispersed from their natal groups, are unlikely to be in the company of relatives. Any apparently cooperative behaviours must here be attributed to alternative mechanisms, such as reciprocity (Ligon, 1991), manipulation of some group members by others (Hrdy, 1977), or even a revised group selection theory (Sober and Wilson, 1998). There are several explanations currently proposed for the occurrence of altruistic behaviour, compounded by the continued redefining of terms such as 'altruism' and 'reciprocity' by theorists (Ligon, 1991). The present study is therefore confined to exploring the potential effects of kin selection on both cooperative and competitive behaviours.

1.2 AIMS OF THESIS

The Hanuman langur, *Presbytis entellus*, is a primate of great behavioural flexibility. Across its range, it exhibits a variety of social organisations, from one-male multi-female troops and all male bands, to multi-male, multi-female troops. (Terms used to describe the langurs' social organisation are given in TABLE 1.1.) These different social groups are likely to contain differing proportions of related and unrelated individuals, providing an ideal species to test predictions of how relatedness may affect the occurrence of cooperative and competitive behaviours. Langur behaviours raise a

number of questions; for example, why do females cooperate in defence of their home ranges? Why do bands of males take over troops together, when only one male will ultimately remain as resident? Why do incoming males often attack unweaned infants? By measuring the genetic relatedness of various groups and subgroups of a population of langurs at Jodhpur, Northwest India, it is possible to quantify the role that kin selection may have in the occurrence of some of these behaviours.

TABLE 1.1 Definitions of terms used to describe langur social organisation in this study.

Term	**Definition**
Group	A discrete social unit of langurs living and moving in the same home range and interacting socially with one another
Troop	A group of females and their offspring accompanied by one or more adult males
All male band (AMB)	A group of males ranging from juveniles to old adults
Subgroup	A subset of individuals within a group, defined by age class and sex
Adult females	Female members of a troop who have given birth, or are pregnant
Offspring	Male and female juvenile and white coat members of a troop, assumed to be the offspring of adult females in the troop

Langurs have been studied most intensively at Jodhpur, Rajasthan, for over 30 years, leading to an unrivalled database from which many hypotheses regarding the social behaviours of the monkeys have been formulated (e.g. Sommer, 1994; Sommer and Rajpurohit, 1989; Borries *et al.*, 1994; Rajpurohit *et al.*, 1995). These hypotheses highlight the interplay between the ecology and behaviour of the langurs, demonstrating the profound effect the habitat has on their social organisation. Socioecology now has a new tool available in non-invasive genotyping, which means that unique genetic identities of many individuals can be obtained, allowing the test of hypotheses pertaining to the relatedness of members of different groups. This thesis addresses issues of relatedness at various levels within the Jodhpur population, and compares the results with the previously studied population at Ramnagar, Nepal (Launhardt, 1998), which has a contrasting social organisation.

1.3 STRUCTURE OF THESIS

Six further chapters are presented here. Beginning with Chapter 2, the sociobiology of the Hanuman langur, with particular reference to the Jodhpur population, is outlined. A popular science article addresses the main assumptions about their behaviour, followed by an overview of the derivations of these theories and exploration of how genetic data can be used to test them. The chapter concludes with a review of the molecular genetic techniques that have been applied to this and similar primate studies.

Chapter 3 introduces the study site and langur groups sampled, and the materials and methods used. It includes the results of the population microsatellite genotyping, and discusses the problems encountered when working with these non-invasive samples.

In Chapter 4, genetic data is analysed at the level of dyadic relatedness scores and relatedness of groups of langurs, testing hypotheses about the cooperative behaviours seen within troops and all male bands. Chapter 5 explores different methods of assigning parentage in the sample in an attempt to assess the breeding monopoly of resident males. An analysis of population structure is provided in Chapter 6, giving further insights to the breeding structure and implications this may have for the evolution of social behaviour.

Finally, Chapter 7 reiterates the main findings and outlines the major problems encountered in this study, and suggests improvements that should be considered for future similar work. Further investigations that could be undertaken, particularly in the comparison of contrasting social organisations, are also proposed.

2. LANGUR SOCIOECOLOGY AND GENETICS

2.1 THE LANGURS OF JODHPUR

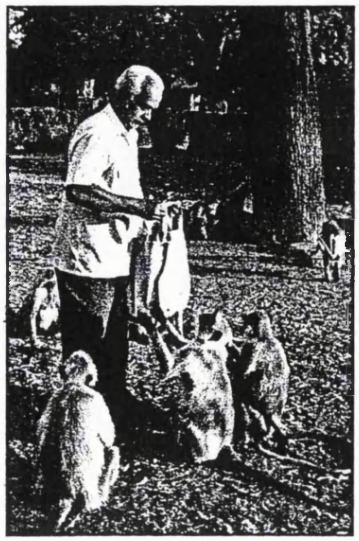
The Hanuman langur, *Presbytis entellus*, is found across the Indian subcontinent. Its long association with humans is revealed by its name; Hanuman is a Hindu god worshipped by millions for many hundreds of years. Combined with its terrestrial nature, this has made the langur a popular subject for naturalists for over 150 years; an early account published in an 1836 issue of the Bengal Sporting Magazine tells of how 'one male more valorous than the rest will be found in possession of the flock [of females]' (quoted in Hughes, 1884). Though the attribution of virtues such as valour are now less common in behavioural literature, the langur has not lost its appeal to primatologists. Many studies of this colobine monkey have been undertaken across its range over the last 40 years, the most long term of these being at Jodhpur, Rajasthan. There is a wealth of literature, both scientific and popular, relating to its behaviour and social organisation. The popular article reprinted here (Sommer, 2002) gives an overview of the current knowledge of langur researchers, and some of the prevalent hypotheses invoked to explain the observed behaviour. It is evident that many of these hypotheses make assumptions about the genetic relatedness of the monkeys. These claims, reasonable and plausible as they may be, have nevertheless not been tested directly for Jodhpur – a gap in our knowledge that this thesis aims to address.

The holy Hanuman

by Volker Sommer

(Modified from an article first published in BBC Wildlife magazine, January 2002; pictures by K. Little)

"Au! Au!" The man pants heavily, dragging a sizeable bag uphill. His calls, however, do not express pain but devotion. *"Au!"* means "Come!" in Hindi. And come they do: Dozens of greyhound-sized creatures in silvery coats emerge from shady crevices in the sandstone cliffs, appear from behind bushes of spiky *Euphorbia*, climb out of the canopy of *Acacia* trees, jump from the roof of a temple. "*Le! Le!* - Take! Take!" The man opens his bag and places potatoes and aubergines in the open palms that surround him.



A troop is provisioned by a devotee of Hanuman

The scene plays in Jodhpur, a town in North India, but is re-enacted all over the subcontinent where devoted Hindus serve a favourite deity: Hanuman. Biologists call the God's incarnations variously grey langurs, Hanuman langurs or Indian langurs and place them as either *Semnopithecus entellus* or *Presbytis entellus* in a genus of Colobine primates, a subunit of Old World Monkeys.

Hindu believers call them the incarnations of Hanuman, the monkey god who is a key actor in India's national epic. The "*Ramayana*" praises God Rama and his beautiful wife Sita. Alas, the demon king abducts Sita to the island of Lanka. Hanuman masterminds Sita's rescue by commanding a victorious army of monkeys. Halfway through the saga, demon guards capture

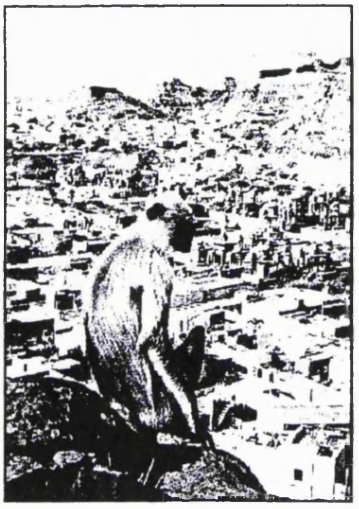
Hanuman and wrap his tail with oil-soaked cloths before setting it on fire. The noble monkey escapes and drags his long torched tail across the roofs of Lanka, burning the villain's residence to ashes. The charcoal black feet, hands and faces of India's temple monkeys are testimony to their heroic ancestor Hanuman, who suffered burns while setting the demon city ablaze. This tale explains why langurs are favourites amongst the pantheon of holy animals roaming India.

Flexible Ecology and Sociality

Like good Hindus, langurs follow a rather strict vegetarian diet. There is the occasional munch of a locust or larvae, but the monkeys are really specialised to exploit hundreds of different plants. A chambered stomach, similar to ruminants, teems with bacteria that can process cellulose. This allows the digestion of large quantities of leaves, though langurs prefer shoots, blossoms, fruit and occasional snacks of gum that seeps from the bark of trees. Almost nothing will put off langurs; they can even tackle strychnoid plants which instantly kill similar-sized animals such as hardy goats.

Thanks to their unfussy dietary requirements, langurs can be found in many different environments, allowing them the largest geographical distribution of any of the 230 or so non-human primates. They dwell from the Himalayan mountains through the semi-arid zones of Rajasthan to the cultivated plains along the mighty rivers; they are as at home in the urban environments of countless cities in the Indian peninsula as in the tropical forests of Sri Lanka. Only one other primate surpasses such tremendous ecological adaptability: *Homo sapiens*.

Hanuman langurs share another feature with humans which also reflects their ubiquitous status in the primate world: a considerable social flexibility. In some areas, langurs form troops with multiple adult males and females. Elsewhere, a single male lives with multiple females in a so-called harem while surplus males join bachelor bands. At yet again other places, varying proportions of one-male versus multi-male troops are found simultaneously.



A langur overlooks the city of Jodhpur

The semi-arid habitat around Jodhpur is home to a geographically isolated langur population with a particularly rigid one-male structure. In the mid 1990s, researchers counted 1300 monkeys. Bachelors were found in 14 bands with an average of 12 members. In the 29 bisexual troops, harem holders enjoyed average tenures of 27 months amongst 14 females. The longest residence lasted 7 years. The largest harem contained 35 females - a total of 83 langurs, if all immatures were included.

Tough Luck for Bachelors

Various factors provide harem holders with an edge over their male band competitors. Harems occupy small ranges in the vicinity of ponds or wells where local people can easily find them. Almost all troops receive huge donations of vegetables, fruit and wheat cakes. This allows a harem holder to eat large amounts of food quickly and devote much of his time to watching for invaders. Additionally, the open landscape with its scattered bushes and thin tree cover makes it difficult for bachelors to sneak up to the females. However, even if a bachelor could successfully invade a troop, his chances of reproducing are slim. The ample food supply allows females to give birth year round. Consequently, only one or two females will be fertile on any given day in a particular troop - and a harem resident can easily concentrate on mate-guarding those.

Bachelors are also severely disadvantaged because for them, foraging is a challenging task. Harems occupy the good spots, forcing bands to criss-cross large areas in search of edible vegetation and water. Feeders will rarely locate these nomads. Moreover, it is difficult for the males to become familiar with their extensive ranges, which contain many hazards. Bachelors, already weakened by food and water stress, are therefore prone to fatal falls out of trees, road accidents, electrocution from power lines or predation by packs of feral dogs.

Males have little choice but to trade the relative comfort and security of their natal troops with the perils of bachelor life when a new harem holder takes over. Typically, cohorts of juvenile siblings are ousted together - often along with their defeated father. However, these closely knit units of relatives will quickly thin out - given the risky bachelor life - and brothers hardly ever mature together to become prime males and thus contenders for a harem. One would otherwise expect brothers to join forces, defeat a harem holder and share the females, as happens sometimes in lions. Such cooperation is virtually non-existent at Jodhpur. Bachelors will jointly invade a female troop, but only the highest ranking band member may challenge the current resident in an all-out fight.

Why are males willing to follow a high-ranking band fellow into battle at all, if he will turn against them as soon as he establishes himself as the new resident amongst the females? Bachelors seem to benefit from band life primarily because they find safety in numbers: external dangers such as preventive attacks by harem holders or

ambushes by predators are diluted.

Harem troops – a peaceful life

In contrast to the hardships experienced by the males, life for the females of a troop is characterised by peaceful interactions. Females do not leave the troops in which they were born, so are surrounded by a network of close relatives: mothers, daughters, aunts, sisters, and grandmothers. The hallmark of these monkeys is cooperation. Females come together to defend their resources – a favourite sleeping tree, or a popular provisioning spot – from neighbouring troops. They also take turns to care for one another's babies, and spend much of their time grooming through each other's fur. It pays each female to help her troop mates – because they are so closely related, to help another female is actually helping their shared genes to survive.

Escape From the Seraglio

The analysis of the one-male system at Jodhpur allows some predictions about the conditions prevailing at sites where multiple males live with the females. For example, a lack of provisioning by local people and strong climatic seasonality should lead to a relatively strict mating period. To guard multiple fertile females is very difficult for a single male, however strong. A densely forested environment will additionally hamper efforts to monopolise females.

Finally, it would be expected that extra-troop male mortality is lower in areas less influenced by human activity. This would allow siblings to grow up together, paving the way for a joint residency in female troops.

The Gruesome Logic of Infanticide

An account of langur life would be incomplete without highlighting a most infamous event: the killing of babies by new harem residents. At least half of all infants present during resident male changes at Jodhpur are attacked, and one third are bitten to death. A widely accepted theory maintains that a nursing female is less fertile and that the violent removal of her infant shortens the waiting time until a new male can sire his own offspring. The average female at Jodhpur gives birth to about 17 infants - and will see four of them killed by males.

Interestingly, females could virtually wipe out incidences of infanticide if they synchronised their menstrual cycles. This would bring about multi-male multi-female troops and thus increase the number of sexual partners per female. The corresponding confusion of paternity makes it less beneficial for males to kill infants since they would run the risk of killing their own progeny. Langurs at Jodhpur don't do this - probably because an increased number of males decreases the available food for females, thus lengthening the interval between births. Such conditions might lower the net reproduction per female more than will the presence of infanticidal males. In a way, females are choosing the lesser of two evils.

2.2 DATA AND HYPOTHESES ABOUT LANGUR SOCIOECOLOGY

The findings of many thousands of hours of behavioural observation and data collection form the basis for the preceding article. However, many of the hypotheses proposed have not yet been investigated using genetic data since these were unobtainable until recently. In FIGURE 2.1, diagrams of the socioecology of langur populations at both Jodhpur, India and Ramnagar, Nepal are presented. Ramnagar is home to another population of langurs, living in multi-male groups, which was studied between 1990 and 1997 by observers from the University of Göttingen (Koenig and Borries, 2001), using very similar measures for behavioural patterns and ecology to those employed at Jodhpur. Genetic studies have recently been used to address questions of paternity and mating success in these multi-male groups (Launhardt, 1998), which provide a comparison for the social system at Jodhpur. FIGURE 2.1 demonstrates the potential interplay between environment, observed behaviours and genetic relatedness that has been suggested for both populations, making it apparent that there are many

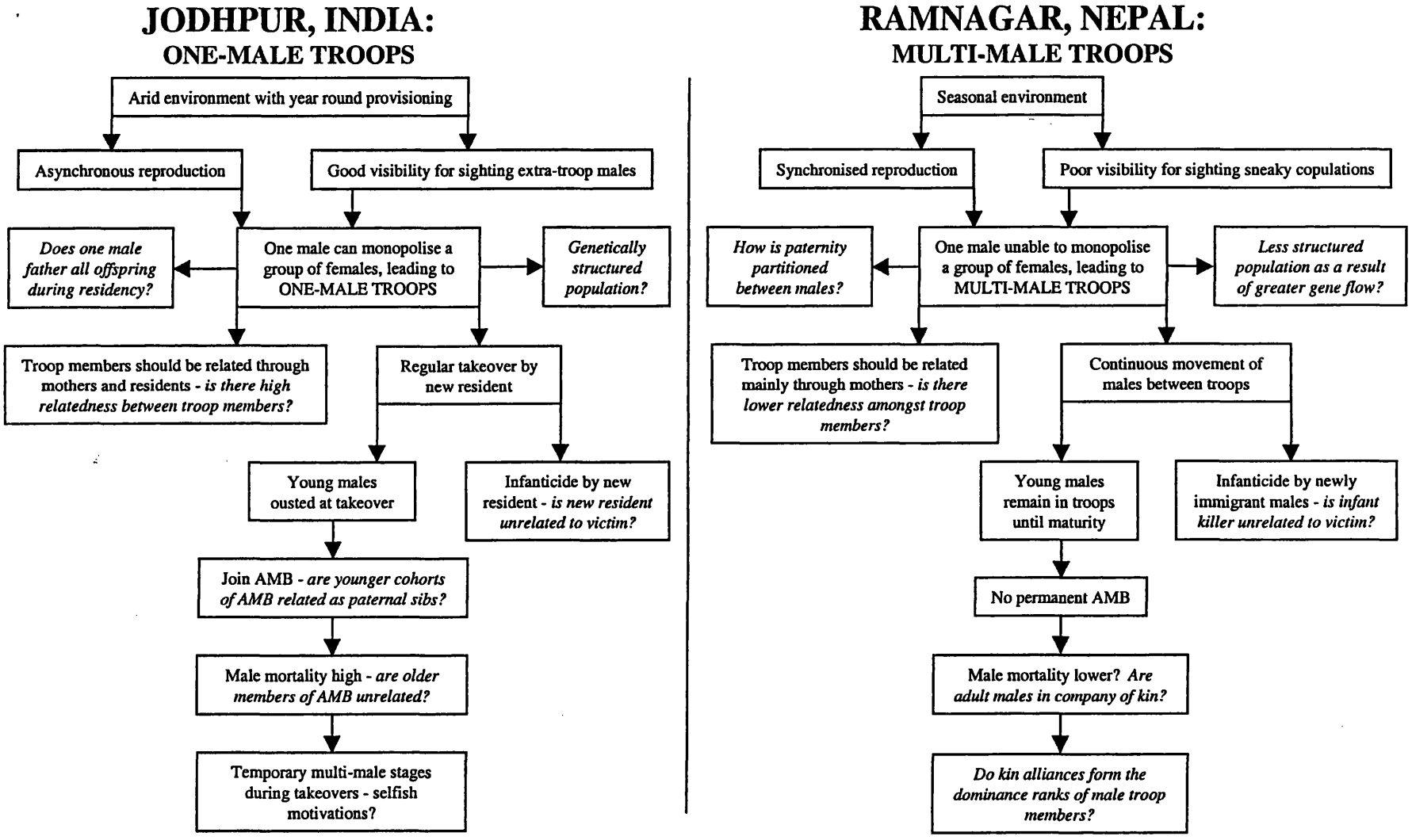


FIGURE 2.1 Comparison of Hanuman langur socioecology at Jodhpur, Rajasthan and Ramnagar, Nepal. Italics indicate questions that can only be answered by genetic investigation. AMB = all male band.

assumptions that have been made about the langurs' behaviour and genetic relationships that can only be tested with genetic data. The background for these hypotheses, many of which have been sketched in the reprinted article, will now be outlined in greater detail and the genetic implications explored in this chapter.

2.2.1 One male can monopolise a troop of females

The density and distribution of the female langurs, and hence the males, at Jodhpur is heavily influenced by humans. Females at Jodhpur exhibit year round menstrual cycling, and there is no discrete birth season (Sommer and Rajpurohit, 1989), probably because the provisioning and crop-raiding that occurs frees them from the constraints of the natural environment and allows them to be fertile all year round (Harley, 1985). In contrast to Jodhpur, the studied population of langurs at Ramnagar, Nepal, receive no provisioning and are dependent upon the natural seasonal environment for their food. Their reproduction is closely linked to the availability of food, and females only come into oestrus in the months following the monsoon, when food is abundant; conceptions are limited to this period (Koenig *et al.*, 1998). At other times of the year the females' physical condition is poor and they do not exhibit oestrus cycling. Combined with later age at first births, longer gestation, longer weaning and longer interbirth intervals at Ramnagar, the difference that provisioning can make is well demonstrated: a female living for 20 years at Jodhpur could produce 12 infants, compared to six in the unprovisioned Ramnagar population (Koenig and Borries, 2001).

Seasonal births are thought to have a profound effect on the ability of a male to monopolise females. At Jodhpur, where the females breed asynchronously, it is thought that a single male should be able to monopolise a troop of females. A resident male should be able to mate guard only one, or a few, females who are in oestrus simultaneously, and prevent access to fertile females by extra-troop males. However, if the females cycle synchronously in a seasonal environment, such as at Ramnagar, a single male will be unable to defend several oestrus females from competitors. In such a situation, there is little point in any one male attempting to exclude rivals from a troop, and a multi-male situation is likely to arise.

Additionally, the landscape around Jodhpur is open, and long distance visibility is good; resident males can see all male bands (AMBs) approaching, so it is highly unlikely that an extra-troop male could mate with an oestrus female unseen. In a

densely wooded environment visibility is poor and males may copulate with females unobserved.

Population density has also been suggested to explain the variation in occurrence of one-male troops across the langur's range (Moore, 1985). This hypothesis suggests that at low densities, multi-male troops will form. This would at first seem counter-intuitive, as at low population densities a resident should easily be able to oust the few intruders encountered. However, the strategies of the extra-troop males must also be considered. As troops are also more scarce, the all male bands' travel time between troops that can be invaded is increased, to the point where it becomes too costly to move between potential troops, and bands should stay with one troop for most of the time, waiting for the resident to fail and taking any available opportunities for sneak copulations (Moore, 1999). This model is similar to that of Srivastava and Dunbar (1996), who found that range size of the troops, and hence search time of the all male bands for those troops, was critical in determining the occurrence of one-male or multi-male troops. It may also be less costly for the resident to tolerate other males within the troop, rather than constantly having to oust them. He can adopt other tactics that will still ensure his superiority within the troop. For example, at low densities when troops appear multi-male, they may not be reproductively multi-male. Males may contest within the troop for matings; dominant males may peripheralise subordinate males, or even oust them from the troop for part of the year; this is observed in low density populations in the Himalayas (Bishop, 1979).

As the density increases, the advantages of monopolising should become greater and competition for females should increase, leading to one-male troops. AMB challengers should travel between troops, rather than concentrating on one, and make perfunctory challenges to the resident males who will normally drive them off. This is the situation observed at Jodhpur, where troops appear one-male for 95% of the time. As densities increase, the defence of the females is likely to become too energetically expensive for one male as intruder pressure increases; he will be forced to share access to the troop with other males, and troops should return to a multi-male, multi-female system. At these high densities, the breeding system would also be expected to be truly multi-male, as the alpha male will not be able to defend all mating opportunities.

This density dependent explanation for the occurrence of one-male troops is based on data from 23 study sites (Moore, 1999); the variation in the occurrence of one-male

versus multi-male troops across the subcontinent is shown in FIGURE 2.2. However, density figures and observations of seasonal breeding cannot reveal the *de facto* breeding system. To establish whether a resident male may have monopoly of troop females in a one-male troop, or how paternity is partitioned in a multi-male troop, DNA analyses are needed. For example, at Ramnagar, where 76% of troops are multi-male, paternity testing showed that the alpha male fathers 57% of the offspring (Launhardt *et al.*, 2001). This proportion represents more than the other troop males altogether, suggesting that at low densities even with synchronous breeding alpha males can dominate reproduction. Conversely, at high population densities, DNA studies would be able to reveal if paternity is randomly distributed between troop males when there is high intruder pressure and single males cannot monopolise females. At Jodhpur, with intermediate population density and female asynchrony, it would be predicted that female monopolisation would be found. This can be tested by DNA analyses in observed one-male troops. Examining relatedness, cohorts of infants should be related as paternal half sibs if fathered by the same resident. Additionally, if infants can be assigned to mothers, then the remaining paternally inherited alleles should correspond for all those infants assumed to belong to the same paternal cohort. The resident male's genotype may also be directly compared to the offspring's genotypes; if he has been in the troop since their conception, and can monopolise the females, he will not be excluded as the father of any offspring. Conversely, if he has taken over the troop recently, his genetic exclusion as a potential father of any offspring would support the observation that sneak copulations are rare at Jodhpur, and that a new male is unrelated to the offspring of a troop.

The hypothesis that a resident has monopoly of a troop at Jodhpur has wide-ranging implications for the troop members' relatedness and their behaviour towards one another. Hypotheses arising from this include:

- adult females, which are not observed to disperse, are related both through their mothers and their fathers, leading to a high relatedness which provides a genetic background for the evolution of cooperative behaviours.
- an incoming male is unrelated to the troop members, so if he kills infants, they are not his own; this supports the idea that infanticide is a sexually selected behaviour.
- incoming males oust young males at takeover because they represent competitors as they mature.

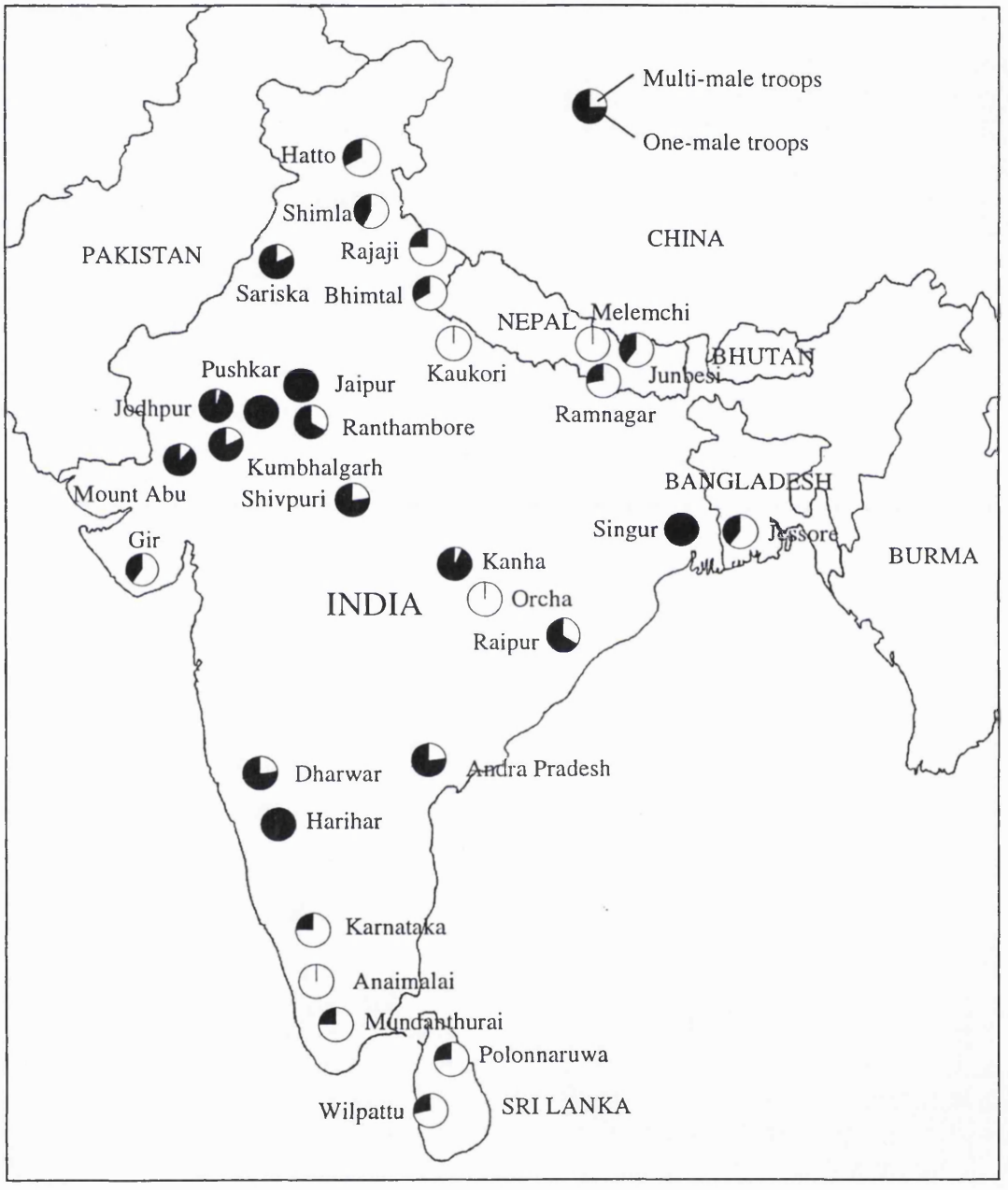


FIGURE 2.1 Distribution of one-male, multi-female and multi-male, multi-female troops across the Indian subcontinent. Based on an idea by Newton, 1988, and adapted from Sommer, 1996, and Moore, 1999.

- the offspring of a troop form paternal sib cohorts, fathered by successive residents, and are therefore related by at least 0.25 on average.

These hypotheses form the basis for the analysis of microsatellite data from Jodhpur langurs carried out in this thesis, and will be expanded upon in the following sections.

2.2.2 Adult females in a troop are closely related

The female philopatry of the langurs should lead to the presence of many mother – daughter dyads in the troop, related by 0.5. Half-sisters, borne by the same mother but with different fathers, should be common, with an average relatedness of 0.25. Full sisters are also a possibility, should a male sire infants with the same female twice. The troop should become a group of related females; mothers, daughters, aunts, cousins and more distant relatives. Generally, there should be a relatively high level of relatedness between the females of a troop in comparison to individuals randomly chosen from the population, because of the lack of female dispersal.

In addition to the relatedness resulting from matrilineal inheritance, there is also a paternal contribution to the relatedness of group members. If all individuals of a troop were fathered by different males, then there would be no paternal contribution to relatedness. If however, the resident has monopoly of matings with the females during his tenancy, as is hypothesised, there will be a paternal contribution to relatedness of cohort members. Infants sired would be paternal half sibs, in addition to any relatedness that results from their mothers' relationships. In this way, each new resident should effectively 'draw together' the relatedness of the troop, by an input of common paternity into each paternal cohort.

The maternal and paternal components of relatedness of troop members should therefore combine to give relatedness values within sampled troops that are significantly higher than if those groups of individuals had been randomly selected from the population. The concentration of relatively related individuals within troops should provide an environment for the expression of cooperative behaviours, including allogrooming, home range defence, and allomothering.

ALLOGROOMING: the daily life of a troop is characterised by relatively peaceful social interactions. One of the behaviours that characterises social primates is allogrooming. The function of grooming others is still debated. It has an obvious hygienic function, as the sites most frequently groomed are those inaccessible to the groomee (Borries,

1992). However, time spent grooming increases not as a function of body size, as would be expected were it purely a hygienic behaviour, but as a function of group size (Dunbar, 1991), suggesting that the amount of grooming relates to the number of grooming partners there are available, giving it a social function. Colobines as a group spend less time grooming one another than cercopithecines (Dunbar, 1991), which can be attributed to their smaller body size (meaning fewer parasites), differing ecologies, or differing social structures, such as smaller groups having to devote less time to maintaining social relationships.

Adult females spend approximately 6% of their day grooming one another, and < 1% grooming immatures and residents (Borries *et al.*, 1994). Females receive 97% of their grooming from other females. Nearly all potential grooming dyads between troop females will be observed. On average, female-female grooming episodes occur every 5.7h/ individual, whereas female-male grooming only occurred every 39.4h/ individual, and male-female every 900.8h/ individual. Adult males therefore play little active role in the troop grooming network.

If grooming has a benefit to the recipient, then it will increase the groomer's inclusive fitness if she directs grooming to close kin. By grooming members of her own troop, a female can assume all her partners are related individuals. It would also be expected that grooming should be directed to those members of highest reproductive value – the youngest, and most dominant females. This is exactly what is found. There is also a preference for first degree relatives, and mothers preferentially groom daughters. All these observations support the hypothesis that grooming increases inclusive fitness.

The fact that every group female grooms every other group female should serve to reinforce the cohesion of the group, whether the individual grooming dyads are close kin or more distantly related. This creates a strong network of social relationships within the troop, which can be drawn upon in the event of intertroop encounters. Grooming is also thus a means to induce individuals to cooperate with others in their social group, benefiting all the individuals of their troop in the long term as they defend their resources together.

BETWEEN GROUP COMPETITION: the female Jodhpur langurs fulfil almost perfectly the predictions for strong between group competition (van Schaik, 1989). It would seem that in the Jodhpur habitat, it is advantageous for females to group together to defend resources from neighbouring groups. Resource defensibility is high, as it is clumped in

its distribution (limited water or specific provisioning sites) and visibility is good across the landscape, so neighbouring troops encroaching into the home range can easily be seen. Each group member will gain from their cooperation in defending joint resources from neighbouring groups. The proposed high level of relatedness of troop members could account for the resource defence seen. If they are closely related, the benefits of cooperation in defence are greater as descendant and non-descendant kin will gain.

Encounters between troops are usually situations of home range defence. The landscape around Jodhpur is well suited for avoidance of neighbouring troops – sparsely vegetated country and high vantage points means that sighting opponents is easy, and males have loud vocalisations that can be heard over great distances. Nevertheless, troops often encounter one another at the boundaries of their ranges, which may overlap by 20% (Borries, 1993). When two troops do meet there may either be hostility or peaceful coexistence. Sometimes troops may forage alongside one another so that they are indistinguishable from a single troop. At other times, they may fight fiercely, especially if there is something immediately obvious to contest, such as a provisioning site. Two important points are to be noted regarding intertroop encounters. First, they are ritualised displays, indicating ability to fight rather than actually engaging in physical violence. Females progress through a series of increasingly strong threatening behaviours, from facing an opponent and biting the air, to lunging and slapping at them, but rarely do serious injuries occur. Secondly, they involve a wide range of troop members. Though intertroop encounters are often initiated by male vocalisations, it is the females who fight alongside one another, presenting a firm united front to any potential rivals (Hrdy, 1977: p149). The males will avoid fighting in these situations as injuries incurred here could seriously diminish their ability to defend their females from incoming males.

ALLOMOTHERING: a third cooperative behaviour seen in langur troops is allomothering. In langurs, along with many other primates, it is common for an infant to be cared for by a female other than its mother. This will often include holding, carrying and grooming the infant, but it is extremely rarely reported that an infant is allowed to suckle by an allomother (Launhardt *et al.*, 2001). Allomothering has been suggested to be desirable for the mother, because it enables her to forage freely and therefore more efficiently without being encumbered by an infant (Sommer, 1989). A clear link between allocare and decreased interbirth interval has been found in primates (Ross and MacLarnon, 2000). Allocare is linked to higher growth rates and earlier weaning than

in comparable species with no allocare. There is no significant correlation between age at weaning and infant mortality, and it has therefore been assumed that the main benefit to allomothering is to allow rapid weaning and a consequent reduction of interbirth intervals. The primary benefit is thus to the mother, leading to a conflict of interest – the infant would rather stay with its mother and receive a higher level of care, whilst the mother prefers to leave her offspring with a carer. The salient point is that allocare is extremely unlikely to evolve if the females are not closely related – it may benefit a female to be without her offspring for some time but there is rarely any benefit for a carer unless they improve their inclusive fitness. Kin selection is thus the most likely explanation for the occurrence of allomothering, whereas other explanations, such as "learning to mother" or allocare helping to form social bonds that can be drawn upon in conflicts, whilst not depending upon relatedness to explain the occurrence, are less robust in the face of evidence (Ross and MacLarnon, 2000). The high incidence of infanticide in Hanuman langurs (35% of infants are victims of witnessed or presumed infanticide, Sommer, 1994) might also have contributed to the evolution of allomothering to the extent observed in langurs – a newborn may be removed from its mother within the first hour after birth, and spend up to 50% of their first day away from their mothers (Hrdy, 1977). Ross and MacLarnon (2000), however, failed to find a significant correlation between weaning age and estimation of vulnerability to infanticide.

2.2.3 Infanticide is a sexually selected strategy

Genetic evidence of monopolisation of females by the resident would also provide support for the hypothesis that infanticide in langurs is sexually selected. Infanticidal behaviour has been recorded in many populations including Dharwar (Sugiyama, 1965), Jodhpur (Sommer, 1994 for review), Mount Abu (Hrdy, 1974), Kanha (Newton, 1986) and Ramnagar (Borries, 1997). Hrdy (1974) proposed that infanticide is explained by sexual selection. An incoming resident has only a limited time to take advantage of his exclusive mating opportunities whilst he can monopolise the females. Females with suckling infants will be experiencing lactational amenorrhea, and hence will be unable to conceive. The time until the infant is weaned is effectively wasted reproductive time for the new male. If residents monopolise mating opportunities, infants are very unlikely to be the progeny of the new resident. A mother's lactational amenorrhea will cease if the new male kills her infant, and she will recommence oestrus cycling earlier than she would have done had she weaned her infant normally.

For infanticide to be attributed to sexual selection, there are a number of conditions that must be fulfilled:

- the interbirth interval (IBI) of the female is shortened
- the infanticidal male is not related to the infants he kills
- the infanticidal male fathers the next offspring of that female.

Data from 16 years of observation of the Jodhpur population shows that 55/112 infants were attacked or killed immediately after takeover (Sommer, 1994). Females may return to oestrus as little as one day after losing an infant (mean 11.2 ± 7.2 days); this resumption of cycling seems to be fastest in species that are most prone to infanticide (van Schaik, 2000). A reduction of interbirth interval of up to 4.4 months following infanticide was recorded at Jodhpur; this represents a reduction of 26% (Sommer, 1994). A similar figure is seen for the Ramnagar population, which is an unprovisioned, seasonally breeding population (Borries, 1997). The older the infant that is killed, the smaller is the reduction in IBI, to a point where infanticide no longer makes any difference to IBI; this limit is calculated as six months in the Jodhpur population (Sommer, 1994). 67% of recorded infanticide victims were below this age. In Ramnagar, where breeding is seasonal, infanticide will still reduce IBI up to the age of 16 months (Borries, 1997); 88% of infanticides (inferred or observed) occur below this age.

It seems that at takeover, the new resident does not preferentially attack the youngest infants, but will attack all unweaned infants; the older ones may be better able to resist. There is, however, a male bias in the sex of infants attacked (Sommer, 1994). Given that the average tenure of a male is 27 months, but that 37% of residencies last over 36 months (Sommer and Rajpurohit, 1989) the resident male may have the opportunity to mate with the female infants in the troop before the end of his tenure, as first conception occurs at an average age of 36 months. Alternatively, it may be that the residents concentrate their efforts on removing young males. These individuals would otherwise grow up into competitors of the resident's own offspring, in particular his sons. It is therefore advantageous to remove them at an earlier age when they present little risk.

The one-male troop organisation observed in the population at Jodhpur suggests that males should have very high paternity certainty. 95% of offspring are conceived during stable tenures (Sommer and Rajpurohit, 1989), so it would seem a new resident can assume with high certainty that he has not fathered any of the troop infants. In 5% of

infanticide cases, a mother associated with several males including the attacking male around the time of conception, so a male may have killed his own progeny (Sommer, 1994). In 96% of cases the attacking male went on to associate or copulate with the female during her first return to oestrus. Because of further inter-male competition, he may have been ousted before the female conceived again, but in 71% of cases, the male was presumed to have exclusive access to the female when she conceived subsequently (Sommer, 1994).

Genetic data provide a much more robust way of testing paternity than observation alone, as has already been demonstrated in the long term study of langurs at Ramnagar in Nepal (Borries *et al.*, 1999a). Genetic samples were obtained from attacking males, presumed victims of infanticide and their mothers' subsequent offspring. In 16 cases of witnessed and presumed infanticide attacking males were unrelated to infants (Borries *et al.*, 1999a). In this multi-male population, attacks were only made by males that had recently joined the troop. Most of them committed infanticide in the first month of their tenure, killing infants that had been conceived, if not born, before they joined the troop. Thus, the males were likely to be using previous sexual access to the infant's mother as a cue for paternity. This was supported by the finding from Ramnagar that infants may be protected from infanticidal attacks by males that were in the troop during their conception, and were therefore potential fathers, even if genetically excluded (Borries *et al.*, 1999b). Finally, in all (n = 4) cases where a subsequent infant was born to a mother who had lost an infant to infanticide, the attacker was found to be the infant's father (Borries *et al.*, 1999a).

Similar genetic methods are used in this thesis to analyse paternity in Jodhpur. Genetic analysis of paternity allows direct measure of the relationships between residents and troop offspring. For infanticide at Jodhpur to be supported as a sexually selected reproductive strategy, it must be demonstrated that a resident male monopolises the females of the troop, giving him high paternity certainty of offspring conceived during his residency. This can be shown through relatedness comparisons between cohorts of infants and potential fathers, or by exclusion analysis of paternity. Cohorts of infants should be related by 0.5 to a long term resident who has maintained his monopoly, and lower relatedness than this would be indicative of extra-troop paternity. Conversely, a new resident should be related to the infants by 0, as he should not have had the opportunity to mate sneakily with their mothers. Similarly, it would be expected that a new resident could be excluded by allele comparisons as the father of the infants of the

troop, and may commit infanticide to his advantage, whereas a long term resident would be expected to share alleles at every locus with each offspring, indicating probable paternity.

Lack of relatedness of the new resident to the juvenile males of the troop would also account for the observed departure of young males from the troop following takeovers. When a new male takes over a troop at Jodhpur, very often young males leave at the same time (76%, Rajpurohit and Sommer, 1993), at an average age of 24 months, whereas those that leave during undisturbed periods are older (around 39 months). The majority of those that left at takeover were chased out. It is suggested that males are forced out by the new residents as they represent either future competitors for mates or current competitors for food with the male and his future offspring. Evidence from genetic methods that the incoming resident is unrelated to the males he ousts will support these competitive hypotheses. Conversely, if a new resident is in fact related to some of the males, for example by sneak copulations, then he would not be expected to oust them from the group as the high mortality of bachelor males would result in the loss of many of his genetic descendants.

2.2.4 Cohorts of offspring are paternal half sibs

It should be found that all the offspring sired by a resident are paternal half sibs and are therefore related by at least 0.25. They should also have a component of relatedness from their mothers, who are likewise related through previous residents and female relatives. This relatedness of cohorts is likely to be of importance not only to the future social behaviour of females as they mature, as described above, but also to the emigrating juvenile males. 85% of ousted juvenile males are estimated to have left their natal troop in the company of paternal sibs (Rajpurohit *et al.*, 1995). It might therefore be assumed that kin relations influence the social behaviour of AMB members. However, though it may influence the initial choice of AMB, with young males supporting one another after they are ousted, the high rate of mortality, and the frequent transfers of individuals between groups means that three years after migration, it is unlikely that any male is still in the company of half sibs. This hypothesis can be tested by examining the relatedness of age cohorts of AMBs at Jodhpur. It would be expected that the relatedness of cohorts should decrease with increasing age. It may be possible to identify paternal sibs – dyads of a similar age, related by 0.25 but not necessarily sharing alleles at all loci. According to the hypothesis, these dyads should

occur amongst the younger males, but should not be found in males above around six years of age.

In contrast with the Jodhpur population, at Ramnagar langurs live in multi-male groups and it has been shown that paternity is shared between males. This should lower the relatedness of cohorts, as they do not all share a common father. It might be expected that there would be less advantage within a troop for kin selected behaviours, though as female transfer is not observed at Ramnagar (Koenig and Borries, 2001), the females presumably receive some benefits from staying in their natal troop. The effect on males might be more profound. Natal males are not ousted at a very young age, perhaps because a degree of paternity certainty means that the adult troop members would not risk ousting their own offspring, or that adult males are preoccupied with competing with their peers in the troop. Therefore the males remain in their natal troops until six years old (Koenig and Borries, 2001). After this, they transfer into other troops to breed – there are no permanent AMBs. These groups of males are only found concurrently with one-male troops, and are characteristic of the Jodhpur population.

2.2.5 Competition and cooperation in all male bands

AMBs are quite different in their composition and behaviour from the troops. Whereas the troops comprise philopatric females, bands are constantly changing in their membership (Rajpurohit *et al.*, 1995). Troops remain in cohesive units during the course of a day's travel, whereas males may form subgroups, which are often formed along lines of age class. They may regroup in the evening, or some subgroups may remain separate for several days before returning to the main group. Whereas females have the defence of shared resources as a clear motivation for forming groups, it is harder to identify the reasons for males grouping together. It is proposed (Rajpurohit *et al.*, 1995) that males ousted from their natal troops join AMBs because they gain from the knowledge that the older band members have of finding food and avoiding danger in the area; in return, the older males, who are of an age and physical condition when they may become residents, benefit from the buffering effect of other group members during takeovers, who deflect some of the defensive aggression of the resident away from the band's highest ranking males.

Young males move between bands frequently. After two years only 33-55% of younger adults and subadults were found in their same bands, as opposed to 60-80% of older males (Rajpurohit *et al.*, 1995). The younger males are assumed to transfer frequently

so they can assess their potential rivals and the condition of neighbouring harem residents. Therefore, along with juveniles migrating into the AMB, and ousted residents rejoining them, the AMBs are characterised by a constantly changing membership.

In their search for females, males roam over much larger areas than individual troops. They are therefore relatively unfamiliar with the terrain, especially as they move between bands and so between ranges. This leaves them prone to unpredictable hazards, such as predation, or falls from trees or rock faces to a greater extent than females. Moreover, there are numerous deaths such as road accidents and electrocution that can be attributed to the human influence on the environment (Rajpurohit and Sommer, 1991). In addition to 'spectacular' deaths, AMB members are believed to suffer mortality because they are probably marginalised into poorer habitats by the harem residents (Rajpurohit *et al.*, 1995). Resources are likely to be poorer, and provisioning less frequent, as the bands are difficult to locate because of their larger ranges. This is likely to contribute to the high male mortality at Jodhpur, which results in an adult sex ratio of 1:6 M:F (A. Chhangani, pers. comm.). There is a suggestion (Mohnot and Chhangani, 1999) that this ratio is becoming increasingly female biased as the human population increases, resulting in more human created hazards and increased competition for food with livestock.

Generally it is the young adults that influence the daily pattern of behaviour of the bands (Moore, 1985). Subadults and juveniles seek out the company and follow adult males, rather than *vice versa* (Rajpurohit *et al.*, 1995). However, as a result of high mortality and frequent transfers, beyond six years old, males are extremely unlikely to be in the company of close relatives still (Rajpurohit and Sommer, 1993). Thus it is unlikely that kin selection is playing a role in the determination of the group's activities. The AMBs roam across the ranges of several troops, challenging the residents to test their ability to defend their position. The resident will fiercely defend his position, and the AMB members are likely to find themselves at several disadvantages to the troop resident (Rajpurohit *et al.*, 1995):

- Residents, in a small, well known, resource rich area, are at far less risk of starvation or accidental death.
- The energetic cost of finding food is low in troops, and it is possible that provisioned food is of greater nutritional value than a forage dependent diet. A

resident's condition improves within days of taking over a troop, becoming visibly bulkier (Rajpurohit *et al.*, 1995).

- Males must constantly re-establish grooming and dominance relationships within the AMB as membership changes. This is expensive in terms of time and energy. Dominance is maintained by displacements over food, females or indeterminate causes. The hierarchy is established according to age and size, with prime adult > young adult > old adult > subadult > juvenile. Grooming bouts are shorter than in females; also lower ranks groom up the hierarchy more often than *vice versa* (Rajpurohit *et al.*, 1995). This suggests that grooming is not performed to strengthen mutual social bonds, as hypothesised in females, but in order to appease or gain favour with more dominant individuals.

Nevertheless, eventually the time will come when the invading males are stronger than the resident, and are able to oust him. During these takeovers fighting may be extremely severe, resulting in sometimes fatal wounding of the resident. The most serious aggression takes place between the resident and the dominant male of the band, though all band members will be active in antagonising the male, which may ultimately weaken him.

Once the male is ousted, the troop will often enter a multi-male phase. This will last for anything from a few days to several months (11-119 days, Sommer, 1988), before the dominant male ousts his former supporters in his campaign, and takes over the residency. Takeovers require the involvement of the whole AMB, with only the dominant male ultimately benefiting; there must be other factors that induce the other members of the all male band to participate. Sommer (1988) has suggested several reasons why males may be induced to act together, including:

- Kin selection may induce related males to cooperate with one another at takeover. However, it is unlikely that the adult males of a band are closely related, owing to the dynamic structure of the bands, so there would be little genetic benefit in supporting peers.

- Game theory would predict that closely matched individuals should cooperate, then once established within the troop can then compete with one another to see who will become resident. However, at Jodhpur male hierarchies are well established

(Sommer, 1988) and only the alpha male of the troop would be expected to become resident of the troop.

- Subordinates may help dominant males in the takeover for selfish reasons. If the dominant male is effectively removed from the band hierarchy when he becomes a resident, the remaining males should all rise one rank in the new hierarchy.

- During takeovers, many members of an incoming AMB get the chance to copulate. Females copulate with all ranks of males, and it is seemingly impossible for one male to monopolise matings at this point. During prolonged male changes 62% of matings were by subordinates. Thus subordinates should benefit from a longer takeover, whilst the high ranking males should make them as rapid as possible. It must be stressed that the number of offspring conceived during these multi-male periods is <5% of all births (Sommer and Rajpurohit, 1989). However, for some males this will be their only opportunity for reproduction, however small, and any exposure to females is practice for a time when they may become troop residents themselves.

- The youngest individuals, in particular juveniles, may be safer from predation and attacks by conspecifics if they follow older AMB members – even if this leads them into the turmoil of takeover.

The fact that females may act promiscuously during takeover is likely to serve as a stimulus for competition between the males (Sommer, 1988). Many females soliciting copulations will make males compete more strongly for them, inducing the males to compete to the best of their true ability. This should be a way of obtaining the best resident for the troop. The stronger the resident, the more likely he will be to defend the troop for an adequate time for females to be able to conceive, rear and wean offspring before another takeover, reducing the chances of losing young to infanticide.

The langur male strategy of takeover contrasts with observations of lions, whose social organisation is superficially similar. Male lions cooperate to take over prides of females, but then remain together to defend the pride. Mating success is shared between males, related or unrelated (Packer *et al.*, 1991a). In contrast, langurs do not form permanent coalitions to take over harems at Jodhpur. There would be reduced benefits from the sharing of matings between a coalition if no males were related.

Male lions only ever make up coalitions of two or three unrelated individuals, whereas the larger coalitions comprised related individuals (Packer *et al.*, 1991a). This situation is determined by the distribution of mating success – in smaller coalitions, all males breed successfully, but in larger coalitions studied at least one male failed to breed. If a male who fails to breed is in a related coalition, he will still gain inclusive fitness through the success of his relatives; however, if a male in an unrelated coalition failed to breed, he would not have gained from helping to defend the pride, but succeeded in allowing more future competitors to be fathered.

Thus the seemingly similar social structures of the langurs and the lions differ in some crucial aspects. Langur males are assumed not to form related coalitions because there is no opportunity to do so, and they do not form unrelated coalitions because they do not need to; one male can defend a troop successfully from rivals. The cooperation of the band to takeover a troop appears to be driven proximately, by the mating opportunities for each individual. Once within a troop, the dominant male turns on his former allies and ousts them, gaining all mating opportunities for himself. It seems therefore more useful to ask why unrelated lions should form coalitions. The larger the coalition in lions, the more effectively they can gain and maintain residence in a pride; single males are not able to defend a pride effectively. However, this benefit is countered, for males with no relatives, by the cost of missing out on any reproductive success were they to join in a large coalition. Thus forming smaller coalitions with guaranteed reproduction, despite shorter tenures, is the better option.

Genetic analysis of relatedness of age cohorts in Jodhpur's AMBs would reveal whether the male members are related, and how this relatedness is distributed between age classes. From observations, it would be expected to reveal some relatedness between the youngest troop members, contrasting with no relatedness of the older cohorts of band members, which would explain the non-existence of alliances taking over troops. Unrelated males will act selfishly, using other members of the AMB to aid them in gaining residencies, before ousting them and maximising the propagation of their own genes.

At the multi-male site of Ramnagar in Nepal, it might be expected that more males, and therefore more sibs, would survive to adulthood, because of the length of time spent in natal troops, and the lower human impact on the environment that is responsible for much of the male mortality at Jodhpur. Groups of brothers might then stay together

when entering breeding troops. Male relatives could cooperate to displace unrelated males from oestrus females, or form an alliance to dominate a hierarchy, to the benefit of their common genes.

The data set on the langurs of Jodhpur can benefit greatly from detailed genetic investigation to either support or refute explanatory hypotheses that have arisen from observations. By clarifying issues such as breeding monopoly, female relatedness and band relatedness, a genetic basis for the evolution of many observed behaviours can be explored. Furthermore, such data can be contrasted with other populations, such as that of Ramnagar (which will be frequently referred to in this thesis), which inhabit different habitats with varying social organisations, in an attempt to establish how genetic and social structure may impact upon one another. Such data has until recently been virtually impossible to collect on a large scale, but recent advances in technologies now mean that a large amount of data can be obtained without harm to the population.

2.3 GENETIC STUDIES OF WILD PRIMATES

Questions about paternity and relatedness and how they interplay with the social structure of langurs can only be resolved through accurate identification of individuals using genetic markers. The use of genetic techniques, especially in primatology, has increased rapidly over the last decade, to tackle issues related to conservation biology, phylogenetics, population genetics, and social behaviour. This has been as a result of two main discoveries. The first is an increase in the number and types of genetic markers available for different types of study. The second is the development of the polymerase chain reaction (PCR) which enables the amplification of a single strand of DNA to give millions of identical copies, which can then be visualised or sequenced, depending upon the application.

The various types of genetic markers available for population studies are reviewed in Parker *et al.* (1998). The markers of choice for individual identification are currently microsatellites. Microsatellites are variable number tandem repeat (VNTR) markers; that is, they comprise a di-, tri- or tetra-nucleotide unit repeated a variable number of times, e.g. $(CA)_n$. They are highly polymorphic (Amos *et al.*, 1993). Since they are non-coding DNA, they do not affect an organism's phenotype and are therefore not subject to selection. They follow a pattern of Mendelian inheritance, and so are ideal for studying paternity and kinship, genetic variation, population structure and gene flow

(Bruford and Wayne, 1993). Additionally, microsatellite primers developed for one species can often be used in the study of related species (Primmer *et al.*, 1995). The more closely related the taxa, the more likely microsatellites are to cross-hybridise. For example, around 50% of human microsatellites are successful in great apes (Wolff *et al.*, 1991) and fewer in more distantly related species (Ely *et al.*, 1992). Not only does this mean a reduction in the amount of time that would have to be spent in the development of species-specific markers, but it allows cross species comparison of related groups.

Microsatellites are in the region of 100-400 base pairs long, giving them two advantages over minisatellites, which can be up to 50kb long. First, they are an ideal size for use as a template for amplification by PCR, which is not efficient for templates over 10kb (Bruford *et al.*, 1996). They are also more likely to survive degradation of DNA intact, making them suitable for screening forensic, ancient or non-invasive samples (Bruford and Wayne, 1993). A combination of microsatellite and PCR knowledge has now been applied to several non-invasive studies of wild animals, including: seals (Reed *et al.*, 1997); mountain lions (Ernest *et al.*, 2000); bears (Kohn *et al.*, 1995); and primates (chimps, Morin *et al.*, 1993; bonobos, Gerloff *et al.*, 1999; Hanuman langurs, Launhardt *et al.*, 1998; savannah baboons, Smith *et al.*, 2000).

2.3.1 Non-invasive sampling

Until recently, the collection of sufficient material for genetic analysis involved invasive sampling, to get tissue containing large amounts of high quality DNA, such as skin, muscle or blood. These procedures are stressful and dangerous for the animals being sampled, as it may require restraint, sedation or even death of the animal. This is clearly not always compatible with many long term primate field studies, as years of work to habituate the subjects could be instantly undone. Invasive collection may also require the presence of trained veterinary staff, a resource not always available to fieldworkers. Overall, this practice is time-consuming, technically difficult, stressful, and obviously necessitates locating the animals, often the hardest task of all when studying rare, elusive or nocturnal animals. The advent of PCR has meant that DNA no longer needs to be extracted in microgram quantities, thus opening up a variety of non-invasive sources of DNA, including feathers, shed hair, sloughed skin, saliva in food wadges, faeces and urine (Morin and Woodruff, 1996; Bricker *et al.*, 1996). DNA from these sources must conform to two conditions before it can form a viable sample type for genetic work (Morin and Woodruff, 1996):

- it must contain sufficient target DNA that can be extracted without contamination, e.g. by human DNA during collection, extraction and PCR.
- it must be amplifiable, without substances that might inhibit PCR.

2.3.2 Problems of low quality DNA

Typically for PCR, nanogram to microgram quantities of DNA are used as a starting template, corresponding to 300 – 300 000 copies of the target sequence (Taberlet *et al.*, 1996). A single gram of faeces may contain millions of sloughed intestinal mucosal cells (Albaugh *et al.*, 1992), making it potentially an ideal DNA source. However, the amount and quality of DNA extracted from a sample is highly variable. For example, Morin *et al.* (2001) found an average DNA concentration of 192 pg/μl in extracts from chimp faecal samples, with a range of 0-2550 pg/μl. Using the estimate in Taberlet *et al.* (1996) of 7pg DNA per cell, this suggests that 1 gram of faeces contains between 0 and over 700 000 cells (mean = 55 000). However, it should be remembered that the amount of DNA measured does not indicate its quality or its source. The DNA may be too degraded for amplification, or could be derived from gut microbes, or animals and plants consumed by the sampled individual (Kohn *et al.*, 1995). The distribution of these cells throughout the faeces is unlikely to be even. Some studies suggest homogenising the samples when they are collected (Wasser *et al.*, 1997), whilst others recommend using only the outside layer (Reed *et al.*, 1997) and techniques have been developed which work by washing DNA from the surface of the faeces (Flagstad *et al.*, 1999), as this is in contact with the gut wall.

Studies using faeces as a DNA source have had widely varying success rates for DNA amplification. Result range from 20% success rate (Pyrenean brown bear, Taberlet *et al.*, 1997) to 100% (baboon, Frantzen *et al.*, 1998) with an average success rate of 69% (see Frantzen *et al.*, 1998 for review). Authors have also differed in their recommendations of how many samples per individual should be collected to provide independent replicates for reliable genotyping (e.g. three to six replicates, Frantzen *et al.*, 1998, versus two replicates, Goossens *et al.*, 2000), and how many extractions per sample should be performed (three extracts/ sample, Goossens *et al.*, 2000; ten extracts, Smith *et al.*, 2000). These figures are either derived from rigorous empirical testing (Goossens *et al.*, 2000) or by continued extraction until PCR success is achieved (Smith *et al.*, 2000). However, some samples may never prove successful, no matter how many extractions are performed, and this eventuality should be borne in mind when working with faecal samples.

ALLELIC DROPOUT: a successful PCR does not necessarily lead to the correct genotype. The small amounts of template DNA can result in a phenomenon known as allelic dropout (Taberlet *et al.*, 1997). When such low amounts of DNA are present, there is a risk that only one allele will amplify successfully, giving rise to a false homozygote. This may be because of extreme degradation, preferential amplification, or the pipetting of only one allele into the PCR (Taberlet *et al.*, 1999). Launhardt *et al.* (1998) found that allelic dropout occurred more frequently in samples that were generally difficult to amplify, indicating a low amount of template DNA. Allelic dropout can result in mis-genotyping an individual, and producing an excess of homozygotes at a population level. Rates of allelic dropout vary between studies, e.g. Smith *et al.* (2000) report a rate of 9% whilst Gagneux *et al.* (1997) experience false homozygotes in 31% of cases. It is recommended by Taberlet *et al.* (1996) that to combat the problem of false alleles, a 'multiple tubes' approach to PCR is taken, and a protocol is given for the number of PCR repeats that should be performed to give 99% genotype confidence; at least three, and often seven or more. This modelled result is in contrast to the empirical results of Goossens *et al.* (2000), where at least four positive PCRs are recommended to give 99% accuracy. However, in a large scale screening of a population it is rarely financially viable or within the time scale of the project to follow the multiple tubes recommendations to the letter (Gagneux *et al.*, 1997; Kohn *et al.*, 1999). Alternative assessments of reliability are often made, such as comparisons of mother–offspring genotypes (Smith *et al.*, 2000), examination of within study error rates (Gagneux *et al.*, 1997), or comparison of genotypes obtained with those from high quality DNA sources e.g. blood or tissue from the same individuals (e.g. Bayes *et al.*, 2000; Launhardt, 1998). Recently, a 5' nuclease assay has been developed (Morin *et al.*, 2001) which can accurately quantify the amount of amplifiable single copy DNA, allowing samples to be sorted prior to screening for likelihood of success and reliability.

FALSE ALLELES: a second problem of microsatellite amplification with low quantities of DNA is the occurrence of false alleles, which are suggested to be the result of slippage by the *Taq* polymerase in the first cycles of the PCR as they often appear one repeat unit smaller, or occasionally larger, than the actual allele. This artefact is then amplified in the same quantities as the true allele(s), and will have the same peak profile in an electropherogram as the true allele. These may give rise to false heterozygotes, or even to individuals appearing to have three alleles at a locus. Their existence, as with

allelic dropout, can only be confirmed by repeated PCRs of the sample (Taberlet *et al.*, 1996).

Often if markers from another species are being used, amplification will only be achieved by lowering the annealing temperature for the primer. This can lead to a further problem of non-specific primer binding, another case where three peaks may be seen. In this case more than one locus may be amplified; this can lead to mis-allocation of relatives and underestimates of relatedness (Smith *et al.*, 2000).

Non-specific amplification can be overcome by using a thermostable *Taq* polymerase (e.g. AmpliTaq Gold, Perkin-Elmer) that is activated by an initial incubation for 10 minutes at 95°C. This prevents amplifications from non-specific primer binding events during PCR set-up, using a hot-start PCR protocol (Birch, 1996). This type of polymerase is also more robust and allows a greater number of cycles to be performed, which is again advantageous when using such a small amount of target DNA (Taberlet and Luikart, 1999).

Another primer-binding problem, not restricted to low amounts of template DNA, is that of null alleles. These are caused by mutations in the flanking primer sequences, so that one of the primers fails to bind to the template DNA, resulting in non-amplification of that allele (Pemberton *et al.*, 1995, Paetkau and Strobeck, 1995). Null alleles can lead to a heterozygote deficiency in a population, and the apparent non-inheritance of parental alleles in some offspring (Bruford and Wayne, 1993).

2.3.3 Inhibition of faecal DNA samples

Faeces contains many compounds that can be inhibitory to PCR, for example bile salts and bilirubin (Cheah and Bernstein, 1990). Additionally in the case of folivores, the high mature leaf content of their diet will increase the amount of plant polysaccharides present in the faeces, which also inhibit PCR (Adams and Demeke, 1992). These compounds are structurally similar to nucleic acids, and will often not be removed by laboratory DNA extraction protocols; they can then interfere with the action of DNA polymerases (Peist *et al.*, 2001). Various food substances such as tea and cocoa, which contain polyphenolics, also inhibit PCR (Brooke *et al.*, 1997). The effects of inhibitory compounds can be mitigated by the addition of adsorption matrices such as bovine serum albumin (BSA), cellulose, potato starch or potato flour (Deuter *et al.*, 1995), which have a higher affinity for the inhibitor than the *Taq* or template DNA (McGregor *et al.*, 1996). The addition of 0.2mg ml^{-1} BSA was found to increase the PCR success

rate in 12/19 cases (Launhardt *et al.*, 1998). Previously inhibited faecal extracts have also been cleaned of inhibitors using glassmilk (Wasser *et al.*, 1997).

Alternatively, a compound called polyvinylpolypyrrolidone (PVPP) will bind polyphenolic inhibitors and remove them from solution (Jobes *et al.*, 1995). Protocols for extraction of DNA from plant material have been used for faeces (Launhardt, 1998) in an attempt to remove polysaccharides during extraction. QIAGEN (QIAGEN GmbH, Hilden, Germany) have developed a commercial kit that is designed to remove inhibitory compounds early in the extraction procedure. They are bound to an insoluble matrix and spun down to leave an inhibitor-free DNA preparation.

2.3.4 Condition of samples

Reed *et al.* (1997) found that the appearance of the faeces collected, affected by its age, its exposure to water, and diet, was a good indicator of whether the DNA extraction would be successful. Goossens *et al.* (2000) also suggest that factors such as humidity and exposure to sunshine may also affect the extraction success. Launhardt *et al.* (1998) advise that because DNA content and inhibitor concentration is likely to vary between samples, several samples should be collected per individual.

2.3.5 Sample storage

Storage methods for faeces have been evaluated by Frantzen *et al.* (1998) and Wasser *et al.* (1997). Frantzen *et al.* (1998) conclude that DMSO/EDTA/Tris/salt solution (DETs) is most effective at preserving nuclear DNA, but also state that for short (<200 bp) fragments of nuclear DNA, and for mitochondrial DNA, storage in ethanol, freezing at –20°C and drying are all equally as good as preservation methods. Wasser *et al.* (1997) evaluated several different storage conditions, and concluded that storage in silica beads was most effective at preserving DNA, followed by freeze drying and Drierite, a commercially available $CaSO_4$ desiccant. Field studies must also take account of the practicalities of different storage methods. In practice, most studies have used ethanol for storage (Goossens *et al.*, 2000; Gerloff *et al.*, 1999; Launhardt, 1998; Smith *et al.*, 2000) with one (Morin *et al.*, 2001) using silica beads. This bias towards ethanol is most likely to be because it is most easy to obtain ethanol in the field, cheap, and preserves DNA adequately for most studies.

2.4 NON-INVASIVE PRIMATE MICROSATELLITE STUDIES

Microsatellites replaced protein polymorphisms as the marker of choice for individual identification in the early 1990s (see Martin *et al.*, 1992, for review). Initially, protein polymorphism studies concentrated mainly on the assignment of paternity by exclusion analysis, often focussing on captive groups of primates. Wild primates provided a greater range of questions to be answered, such as the occurrence of extra group paternity, partitioning of reproductive success, relatedness of social groups, and population structure (e.g. de Ruiter, 1994). These studies depended on invasive techniques of sample collection, which limited the range of species that could be investigated to those abundant and robust enough to withstand sampling. However, with the advent of non-invasive techniques of DNA extraction from samples such as hair, and later on faeces, the number of studies of wild primates increased dramatically. Non-invasive microsatellite studies were first used to assign paternity to two offspring in a Gombe community of chimpanzees (Morin *et al.*, 1994). Since then, the development of methods for extraction of DNA from faecal samples has further increased the power of genetic identification, as samples can be assigned to known individuals if defecation is observed. This means that behavioural observations can be contrasted with genetic data, for example in mother-offspring interactions, and records of copulations. In some cases this may lead to radically revised views of mating systems.

Primate species which have been studied by non-invasive techniques to date include:

- Baboons (*Papio cynocephalus*): Smith *et al.* (2000) used faecal DNA samples from 29 baboons to screen 29 human primers for their potential application to genotype a population for the identification of half-sisters. Only 41% of the primers amplified successfully, of which half were monomorphic. After the exclusion of a further locus, which showed non-Mendelian inheritance, five primers remained which were useful for determining relatedness between individuals.

- Bonobos (*Pan paniscus*): Gerloff *et al.* (1995) extracted and successfully amplified DNA from samples from 33 individuals at five human microsatellite loci. These loci were then used to type 36 bonobos from one community, analysis of which concluded that dispersal was female biased, and that therefore the observed high degree of sociality and cooperation was most likely to be the result of reciprocity or mutualism (Gerloff *et al.*, 1999).

- Chimpanzees (*Pan troglodytes*): since the publication of Morin *et al.*'s (1994) first non-invasive study, a number of studies have examined paternity and social structure in different chimpanzee populations (e.g. Constable *et al.*, 2001; Vigilant *et al.*, 2001; Gagneux *et al.*, 1999). These have considered aspects of paternity (including extra-group paternity), community structure, and relatedness. Interestingly, high levels of extra-group paternity were described in one group (Gagneux *et al.*, 1999) a finding which was then retracted (Vigilant *et al.*, 2001). This demonstrates that although genetic data may seem more dependable than observational data, it still demands careful interpretation and replication before it should be accepted.

- Hanuman langurs (*Presbytis entellus*): a detailed genetic study of a population of langurs in Ramnagar, Nepal, was carried out by Launhardt (1998). 178 individuals were sampled and genotyped, from groups that had been studied from 1990 to 1997, resulting in over 37000h of data. Genetic and behavioural data were combined to draw conclusions about mating and reproductive success and infanticide in this population. For example, as discussed above, it was found that reproductive success in multi-male, multi-female groups was biased towards the dominant male, who fathered more infants that all the other male residents (Launhardt *et al.*, 2001). The Ramnagar population has produced a large quantity of data pertaining to the multi-male structure of this population, and it will be referred to throughout this thesis as a contrasting social organisation to the one-male situation at Jodhpur.

The Ramnagar study of langur genetics, supported by several years of field data, has added a new dimension to the interpretation of these monkeys' social lives. Hypotheses that have been formulated through detailed recording and analysis of data have shed light on several areas of langur social behaviour. This thesis provides a contrast to the Ramnagar study. The Jodhpur and Ramnagar populations represent extremes of langur social organisation; 95% of Jodhpur troops are one-male, whereas 72% of Ramnagar troops are multi-male. However, the Jodhpur study is based upon a relatively short period of time with no supporting behavioural data, whereas Ramnagar couples genetic sampling with thousands of hours of field data. These studies complement one another, and advance the investigation of the genetic causes and consequences of the social organisation of this extremely adaptable species.

3. SAMPLING AND SCREENING OF A FREE-RANGING LANGUR POPULATION

Summary

The city of Jodhpur, Rajasthan, and its surrounding plateau, is home to nearly 2000 Hanuman langurs. Collection of faecal samples was carried out here in five months from the end of November, 1999 to the middle of April, 2000. Samples were collected from eight troops and six all male bands (AMB), and stored in ethanol.

DNA was extracted from samples in duplicate using the QIAamp ® DNA Stool Mini Kit (QIAGEN GmbH, Hilden, Germany, catalogue number 51504). Samples were genotyped using eight human microsatellites that had been found to be amplifiable and polymorphic in Hanuman langurs. A touchdown PCR protocol was used, and strict genotyping criteria followed.

Altogether, 227 faecal samples were collected, of which 89 were ultimately genotyped sufficiently to be used in data analysis. The typed individuals were members of five troops and one AMB. It was found that there was great variation in the success of extraction and genotyping of samples. The samples that worked most successfully, with consistent good amplification, were those from one troop (Nimba) and the AMB (Bhadreshwar), both of which were sited far from the city, and were rarely provisioned. It is suggested that a more naturally derived, folivorous diet forms faeces that are less likely to contain PCR inhibitors, and may have a more fibrous consistency that effectively abrades cells from the gut wall, so the samples contain more DNA. Conversely, many of the samples collected from other AMB members failed to amplify. These samples were often from defecations that took place under stressful conditions, such as encounters between bands, when males appeared to void their bowels as a reflex reaction to a tense situation. It is hypothesised that these samples contained little or no DNA because either (i) they contained so much water that the DNA was diluted; (ii) DNA was degraded in storage because the ethanol was diluted to ineffective concentrations by the excessive water in the faeces or (iii) high concentrations of ribonucleases in the foregut are still active in the faeces because of its accelerated passage, and have broken down the small amount of DNA present.

This study has demonstrated the applications of non-invasive sampling to wild populations, illustrating its advantages in terms of ease of collection, but also

highlighting the disadvantages. Recommendations for future studies with respect to the condition of the sample can be derived from these findings.

3.1 MATERIALS AND METHODS

The city of Jodhpur is in Rajasthan, Northwest India, on the edge of the Thar Desert. The climate is extremely dry, with 90% of its annual rainfall of 360mm falling in the monsoon season (July to September). Maximum temperatures of 50°C occur during May/June, and minimum temperatures of 0°C are experienced around dawn during December and January. The city itself is located on a red sandstone plateau which covers an area of around 85km^2. The natural vegetation of the area is xerophytic open scrub, the most common plant species being *Prosopis juliflora*, *Euphorbia caducifolia* and *Acacia senegal.*

The langur population of Jodhpur is geographically isolated. It comprises nearly 2000 individuals (TABLE 3.1), organised into harem troops and all male bands (AMB). Fieldwork was carried out in the area surrounding Jodhpur between 20/11/99 and 12/4/00. Sampled troops were chosen according to their size, the ease of access to the areas where they were commonly found, and their location in relation to other troops distributed across the plateau.

Individuals were classified according to sex and age class. A standard age class classification was used (TABLE 3.2). Individuals were identified according to size, coat colour, and identifying marks such as pigmentation, scars and scabs, moles, and injuries such as broken or missing tails or teeth.

Location	AM	AF	SM	SF	JM	JF	WM	WF	WU	CM	CF	CU	BM	BF	BU	TM	TF	TU	Total
Daijar	1	36	0	1	0	11	4	4	0	1	1	0	3	3	2	9	56	2	67
Beriganga	1	40	0	0	0	8	5	8	2	0	0	0	4	3	2	10	59	4	73
Nimba-Nimbri	1	51	0	4	0	6	4	7	3	1	0	0	3	4	2	9	72	5	86
GSK Nimba	1	10	0	1	3	3	0	0	0	0	0	1	1	2	1	5	16	2	23
Mandore dev.	1	30	0	2	4	5	2	2	0	1	0	0	3	4	0	11	43	0	54
Mandore fort	1	46	0	1	10	5	3	3	0	0	0	0	2	2	1	16	57	1	74
Mandore OS	1	20	0	0	0	1	2	3	0	0	0	0	1	0	0	4	24	0	28
Mandore Tp	1	71	0	5	7	12	6	7	2	2	1	1	5	4	5	21	100	8	129
Mandore Ns	1	61	0	1	3	4	7	9	2	0	2	1	4	5	1	15	82	4	101
Balsamand	1	13	0	2	0	0	3	2	0	0	0	0	2	2	1	6	19	1	26
Kaga North	1	22	0	0	3	2	3	4	0	0	1	0	2	3	1	9	32	1	42
Kaga South	1	24	0	0	2	3	5	6	0	1	0	0	3	4	2	12	37	2	51
Kaga South A	1	3	0	0	0	1	2	2	0	0	0	0	0	0	0	3	6	0	9
City (JM)	1	15	0	0	0	0	1	1	0	0	0	0	2	2	1	4	18	1	23
City (GM)	1	18	0	0	6	3	3	2	0	1	0	0	3	2	0	14	25	0	39
City Pachatia	1	7	0	0	3	1	1	1	0	0	0	1	1	1	1	6	10	2	18
Chandpol Ck	1	10	0	0	0	0	2	2	0	0	0	0	1	1	0	4	13	0	17
Chandpol Ds	1	16	0	0	0	4	3	4	0	1	1	0	0	0	0	5	25	0	30
Guptganga	1	40	3	5	4	7	8	10	1	0	0	1	2	2	2	18	64	4	86
Soorsagar Bg	1	9	1	2	1	2	2	3	0	0	1	0	1	2	0	6	19	0	25
Kailana Canal	1	11	0	1	3	2	0	0	0	2	1	0	2	2	0	8	17	0	25
Kailana 1	1	7	2	1	2	2	1	1	0	0	0	1	0	0	0	6	11	1	18
Kailana 2	1	20	0	2	5	3	2	2	0	1	1	0	3	2	2	12	30	2	44
Bijolai	1	5	0	0	0	0	1	1	0	0	0	0	1	0	0	3	6	0	9
Bheembharak	1	37	0	3	11	7	6	6	2	1	1	0	3	3	2	22	57	4	83
Sidhnath East	1	19	0	4	2	5	2	2	0	1	0	0	1	1	0	7	31	0	38
Sidhnath West	1	27	0	2	5	4	5	4	0	1	1	0	2	2	1	14	40	1	55
Filterhouse	1	7	0	1	0	0	1	1	0	0	0	0	0	0	0	2	9	0	11
Filterhouse S	0	4	0	1	0	0	1	0	0	0	0	0	0	0	0	1	5	0	6
Kadamkandi E	1	16	0	2	7	6	2	1	0	0	0	0	1	2	0	11	27	0	38
Kadamkandi SE	1	26	0	3	9	9	2	1	0	0	1	0	2	2	2	14	42	2	58
Kadamkandi W	1	17	2	3	7	6	2	2	1	0	1	0	2	2	0	14	31	1	46
Bhadreshwar	1	39	0	6	3	6	5	5	0	3	2	1	5	4	3	17	62	4	83
Chonkha Klgt	1	12	0	2	0	2	0	0	0	0	1	0	2	2	1	3	19	1	23
Chonka S M	1	9	0	1	7	2	4	2	0	0	1	0	0	2	0	12	17	0	29
Chonka mines	1	10	0	0	3	2	3	3	1	0	0	0	1	1	2	8	16	3	27
Arna East	1	28	0	2	6	8	4	4	0	0	0	1	1	1	1	12	43	2	57
Arna West	1	39	0	1	4	5	2	2	0	0	0	0	1	0	1	8	47	1	56
TOTAL	37	875	8	59	120	147	109	117	14	17	17	8	70	72	37	361	1287	59	1707

TABLE 3.1 1999 census of Jodhpur troops. A = adult, S = subadult, J = juvenile, W = whitecoat, C = changing coat, B = blackcoat; M = male, F = female, U = unknown; T = total. Data from A. Chhangani, pers. comm.

Location	OA	AM	YA	SA	JII	JI	Total
Daijar	2	3	1	2	8	0	16
Beriganga	0	0	0	0	0	0	0
Beriganga	0	0	0	0	0	0	0
Mandore N	2	13	2	5	9	0	31
Mandore S	1	2	1	2	3	1	10
Kaga-Balsamand	1	5	0	2	0	0	8
City-Fullalao	0	3	3	2	1	0	9
Chandpol, Soorsagar	2	2	0	2	2	0	8
City- Gaddi	2	4	0	0	0	0	6
Soothla (Guru ka talab)	0	2	0	1	2	0	5
Sidhnath	0	2	1	3	5	1	12
Chopasani	1	5	1	0	3	1	11
KK- Golasani Chonkha	0	0	0	0	0	0	0
Arna	0	11	0	6	3	2	22
Bhadreshwar KK	2	9	2	7	12	9	41
Balsamand	0	0	0	0	0	0	0
Chopasani HB	0	1	0	0	0	0	1
Chonkha Kalighati	0	1	1	1	0	0	3
BKK gas Agency	1	3	2	0	0	0	6
Sardarpura 5th Road	0	4	2	3	2	0	11
TOTAL	14	70	16	36	50	14	200

TABLE 3.1 (cont.) 1999 census of Jodhpur all male bands. O = old, A = adult, M = male, S = subadult, JII =juvenile II, JI = juvenile I. Data from A. Chhangani, pers. comm.

TABLE 3.2 Age classifications of langur monkeys (after Rajpurohit *et al.*, 1995). NB. These classifications may vary between studies; for example, in the 1999 census (A. Chhangani, pers. comm.) they include a changing coat classification between infant I (called "black coat") and infant II ("white coat").

Age class	Female	Male
Infant I	"black coat" from birth to about 5 months old, at completion of coat colour change from black to white.	
Infant II	"white coat" from about 5 to 15 months, after completion of weaning	
Juvenile	15 months to about 3 years i.e. time of menarche	15 months to at least 4 years; testes internal in younger and descended in elder juveniles; glans of penis generally not visible
Subadult/ young adult	From regular cycling to birth of first infant; not >4yrs (not included in Rajpurohit *et al.*, 1995)	About 4 to 6 years of age; glans usually visible, ischial pads still undeveloped; canine teeth not fully erupted, capable of copulatory behaviour
		About 6 to 7/8 years of age; glans always visible; ischial pads small and pale; canines fully erupted; not full size
Adult	From birth of first infant, or at least 4 years old; weight 11-12kg	From 7/8 years onwards; full size, weight 18-19kg; generally smooth face with few scars ischial pads pink, often puffy

The groups sampled are detailed below. Their locations are shown in FIGURE 3.1.

3.1.1 Troops

Kailana

This troop occupied a home range some 8 km from the city. The main feature of this area is the 3 km long Kailana lake, an artificial reservoir fed from the Indira Gandhi Canal, which brings water from the Himalayas to the arid regions of Rajasthan. The Kailana troop studied (Kailana I in 1999 census and previous studies) roosted in large trees in the garden of a empty guest house that was next to the central dam of the lake. During the time they were studied, they spent much of their time in the *Prosopis* trees growing on the dam itself. Apart from this area of vegetation, the environment was

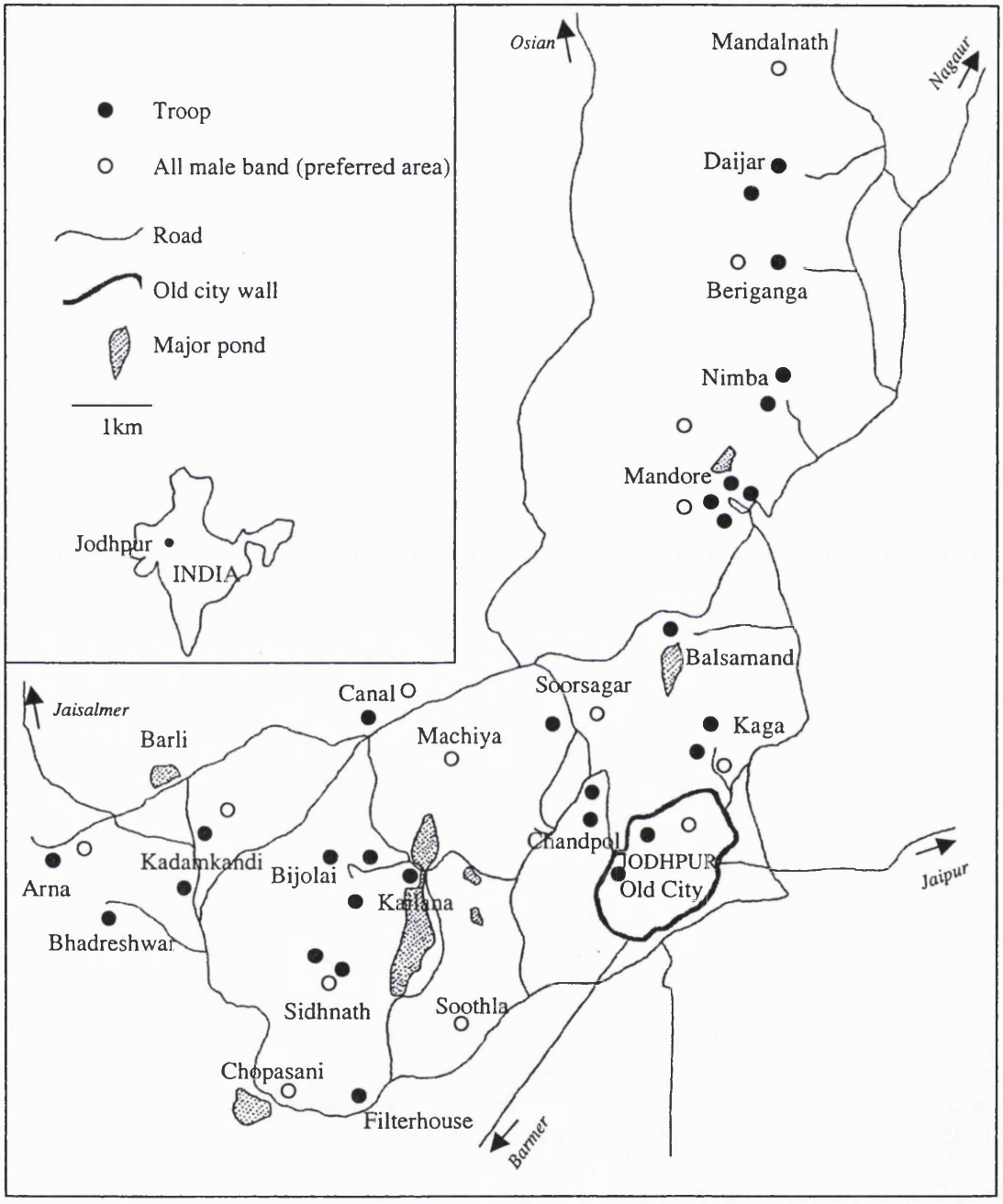


FIGURE 3.1. Location of harem troops and all male bands around Jodhpur, Rajasthan. Adapted from Rajpurohit *et al.*, 1995.

rocky and barren. The troop received a large amount of provisioning, despite the fact that it was far from the city. People would come throughout the day specifically to feed the langurs, and families visiting the public gardens by the lake would also bring food for the troop.

Kailana troop was notable because of the presence of several adult/ subadult males, a situation that had been stable for some years (A. Chhangani, pers. comm.). However, during the course of the sampling period, these males were ousted by a visiting AMB, and the resident was replaced; when the troop was visited later in the study period, there was only a resident male accompanying the troop.

Bijolai

Bijolai was named after the maharajah's hunting lodge, now derelict, which formed the sleeping site of this troop. Next to the lodge was a permanent artificial lake, and there were *Prosopis* trees surrounding the lodge. The site was further from the main road, and towards a military installation, so visits by people to provision the monkeys were less frequent than at Kailana. On public holidays, however, families would come and picnic, and bring food for the langurs. Generally, Bijolai troop was more timid than Kailana, possibly because of less human contact.

Filterhouse

Filterhouse troop lived around the waterworks at the southern end of Kailana lake. The habitat was very rich and green, with large fig and neem trees as well as more scrubby vegetation on the ground. The troop was very arboreal, spending the majority of the day in the fig trees surrounding the canals. They were only observed to come near to the ground to feed when provisioned on the roof of an outbuilding. On the whole, provisioning was relatively infrequent, once or twice a day, possibly because the resident male had a reputation for being extremely aggressive.

Chandpol A and B (Chandpol Ds and Ck)

These two troops (also referred to as Chandpole troops in some previous studies) occupied neighbouring home ranges right in the centre of the city in the old town. At night they roosted in large fig trees on opposite sides of a playing field. Occasionally in the evening they would encounter one another at one edge of the field, and fierce disputes would occur between them. During the day they would forage in separate directions. Chandpol A generally moved east towards Jodhpur fort, travelling across rooftops in a predictable route. Chandpol B would travel in the opposite direction,

south into the newer area of the city. Both troops would follow a circular route during the day, which varied little, except for occasions when they encountered AMBs. In the evening, both troops were provisioned near their sleeping sites; Chandpol A especially received a lot of food from regular feeders. Both troops were believed to have experienced takeovers in the six months prior to sample collection, and had new residents (A. Chhangani, pers. comm.).

Kaga A and B (Kaga N and S)

These two troops roosted close together on steep sandstone cliffs on the eastern edge of the Jodhpur plateau. During the day they generally travelled down into the Kaga area of the town to the west of the Mandore Road. They were provisioned a great deal; large sacks of food would be brought up to their roosting sites for them in the morning and evening, when often fierce disputes between the troops would occur over access to the food.

Nimba (Nimba GSK)

About 15km north of Jodhpur, beyond Mandore, this troop lived in the area surrounding a social workers' training centre. The compound was full of mature trees, and the langurs spent most of their time in these trees or on the roofs of the buildings. Only a few of the buildings were inhabited and it was generally a very quiet area. The troop was not often provisioned, but derived most of their food from the trees, or from the gardens surrounding the temple on the site.

3.1.2 All male bands (AMB)

The all male bands studied do not necessarily correspond to those surveyed in the census. The dynamic nature of these bands and the short time in which they could be found in order to sample them meant that they could be subgroups of recorded AMBs, or entirely different AMBs from those recorded in the census 6 months earlier. All male bands were sampled opportunistically. Because they range over such wide areas, they are difficult to find, and were often only located when they were interacting with another AMB or with a troop, and were quite conspicuous. Conversely, when attempts were made to find known AMBs, often several days' survey proved fruitless. This was the case at Daijar, Beriganga, City-Fullalao, Kadamkandi and Chopasani.

Kailana

This band interacted with Kailana troop for at least two weeks (5/12/99 – 20/12/99) eventually ousting the male resident and other males in the troop. By 18/2/00 there was only one resident male; he had been a member of the AMB seen interacting previously.

Chandpol

The adult males in this band were encountered when interacting with Chandpol A on 10/12/99. Whilst the resident male kept them at bay, four very young juvenile males vocalised from distant roofs. These young males moved off with the adults after this encounter. One adult stopped and turned in response to a distress call from a juvenile. In the following days, this band was encountered again. On one occasion only three juveniles were seen to remain; on another, one of the juveniles had been electrocuted, and died.

Kaga

A band of five adults was first encountered interacting with the Kaga troops in the morning of 25/2/00. They were followed throughout the day until dusk, when they roosted in trees next to the wholesale vegetable market on the Mandore Road. At first light the next day they were relocated on top of a water tower in the market. When they moved off from here they appeared agitated, often vocalising towards the east. They did not appear interested in eating as they travelled. The reason for their disquiet became apparent when Old Kaga AMB was sighted.

Old Kaga

When the Kaga males entered the area of Kaga to the east of the Mandore Road (26/2/00), Old Kaga male band appeared to challenge them. No AMB had previously been recorded in this area. It appeared to comprise around 14 individuals. During the interactions of the bands, nine of these males were sampled in the space of 15 minutes.

Mandore

In the census, Mandore males were divided into two bands, Mandore N (31 members) and Mandore S (10 members). On the first day in Mandore (10/3/00), a band of 18 members was encountered to the south of Mandore. Over the next two weeks, these individuals were seen in a much larger group of up to 40 individuals that would gather in the public gardens at Mandore. 36 of these were sampled. It may be that these were two separate bands that mixed together during the day, drawn to the Mandore Gardens by the large amount of provisioning they received there.

Bhadreshwar

These males were located on the evening of 11/4/00 as they settled to roost on the roof of a community building in Bhadreshwar. They were relocated in neighbouring fields the following day at first light. Samples were collected from unidentified individuals as they defecated soon after waking. The band size was estimated to be around 25 individuals. As 20 samples were collected it was decided not to sample again from this band, as it would have involved managing to follow them, identifying all individuals and sampling from all males again once they had been identified.

3.1.3 Sample collection

Groups of langurs were followed from first light either until they moved off from their initial sleeping or feeding sites on their daily travel and could no longer be followed, or until they settled to doze at around midday. They were then relocated in the mid afternoon (around 15:30) and followed until they went to roost at dusk. The langurs tended to defecate first thing in the morning, as they woke, then again in the late morning, before dozing into the afternoon. They would often defecate again in the evening. However, there was wide variation in the times when defecation would occur. Langurs would also defecate in response to social stimulus. If an intertroop encounter occurred, as often happened between the Chandpol or Kaga troops in the evening, individuals were likely to defecate more frequently. This was also true when males encountered one another – either AMBs sighting or interacting with each other, as happened when Kaga and Old Kaga males were sampled, or when an AMB interacted with a resident, either at a distance, or when they entered the troop. For example, the resident male of Chandpol B was surveying the town from the roof of a house high up by the city wall to the south of Chandpol. On sighting a male band, he sat toothgrinding towards them (an indication of aggression), before defecating copiously. Similarly, when Kaga and Old Kaga males encountered one another, although they stayed at least 25m apart, with the furthest males over 100m away, almost all the males in both bands were seen to defecate, even those individuals that had done so only an hour previously.

Samples were collected as soon as possible after defecation, when the langur had been positively identified, and had moved away from the faeces, and access to the faeces was possible. Often, although defecation was most predictable at first light, it was difficult to identify from which individual faeces had come, when the langurs were huddled together high in the roosting trees. Because the langurs used the same sites each day, it was quite likely that the ground onto which the faeces fell was covered in old faeces

from many individuals. It was therefore necessary to take precautions to avoid contamination from this, by only sampling from the upper side of the faeces that had not come into contact with the ground. Samples were collected in universal tubes with an integrated spoon in the lid (Sterilin; catalogue number 128SC). These were chosen as they made it possible to handle the samples into the wide-mouthed tubes without touching them or needing extra equipment, such as wooden tongue depressors or latex gloves (Launhardt, 1998). Tubes were labelled with the individual's ID, the group, the date, and the time in pencil. Samples were then covered in 100% ethanol and stored at ambient temperature in the field; on return to the lab they were stored at –70°C.

3.1.4 Extraction

DNA extraction was carried out according to the protocol for isolation of DNA from stool for human DNA detection outlined in the QIAamp® DNA Stool Mini Kit (QIAGEN GmbH, Hilden, Germany, catalogue number 51504). The faecal sample was placed on fresh tissue paper and as far as possible, the outside of the faeces was preferentially used for the extraction, separated using a scalpel blade. Each sample was extracted twice; extractions were carried out in a fume hood, cleaned before and after with bleach and Virkon™ (Fisher, catalogue number HYG-205-230B). Extractions were performed in batches of 12. Extractions were also carried out on blood samples of *P. entellus thersites*, obtained from the blood bank of the Institute of Zoology, London, according to the QIAamp® DNA Blood Mini Kit. Each extraction produced 200μl eluate, which was stored at –20°C.

3.1.5 Microsatellite screening

Initially human primers were used to screen *P. entellus thersites* blood extracted DNA, using 14 primers (TABLE 3.5) including those being tested in the lab to see if they were amplifiable in baboons, and those established by Coote and Bruford (1996) and Launhardt (1998) to be amplifiable in Hanuman langurs. This was increased to 24 primers (TABLE 3.5) when DNA extracted from faeces collected from Jodhpur langurs by V. Sommer in November 1998 was included in the screening process. Primers chosen for genotyping the Jodhpur population were those polymorphic in the faecal samples.

3.1.6 Sample screening

PCRs were carried out in batches of 36 reactions. A PCR cocktail was prepared, comprising: 175μl H_2O, 40μl 10x AmpliTaq Gold PCR buffer, 40μl of 25mM $MgCl_2$,

40μl of 2mM dNTPs, 10μl of 10pmol forward and reverse primers, 4μl of 20mg ml^{-1} non-acetylated BSA, and 1.1μl of AmpliTaq Gold. 8μl of this master mix was added to each PCR reaction, along with 2μl faeces derived DNA. Each batch of 36 PCR reactions comprised 34 samples, a positive control of blood derived langur DNA, and a negative control with no sample DNA.

PCRs were carried out using a Perkin Elmer Gene Amp PCR System 9700. A PCR amplification of 55 cycles was carried out. (Initial denaturation at 94°C for 10 min, then a five cycle touchdown (Don *et al.*, 1991) starting at 94°C denaturing for 30 sec, 55°C annealing for 1 min, and extension at 72°C for 30 sec. The annealing temperature was lowered by 1°C each cycle. This was followed by 50 cycles of 94°C denaturing for 30 sec, 50°C annealing for 1 min, and extension at 72°C for 30 sec. After a final 10 min extension period at 72°C, the samples were held at 4°C.) The PCR products were visualised on a polyacrylamide gel using an ABI PRISM™ 377 DNA sequencer with standard marker GS350 TAMRA. Gels were analysed using Genescan™ Analysis 3.1.2 and Genotyper® 2.5 software.

Specific criteria were followed when screening and genotyping samples. For each locus, the PCR was carried out on each extraction at least three times. Electropherograms less than 100 fluorescent units in intensity were not scored. Gels were not scored if the negative control appeared contaminated. KL was genotyped at all loci.

Genotyping criteria

Owing to the scale of this project, it was not possible to type each individual as many times as recommended by Taberlet *et al.* (1996) when using small amounts of DNA, which recommends three PCR replicates to assign heterozygote status, seven replicates to assign homozygotes, and further PCR reactions if the result is still unclear. Instead, alternative criteria were used.

For homozygotes:

Individuals were typed as homozygous if they produced only one allele in three initial amplifications. This is the same condition for homozygotes as was used for langur faecal DNA samples from the Ramnagar population (Launhardt, 1998). From the complete data set, it is possible to calculate the probability that an individual that appears to be homozygous in the first three amplifications will reveal itself to be heterozygous in further amplifications: altogether, 554 complete genotypes were

derived from more than three PCRs. Of these, only 14 appeared to be homozygous from the initial three PCRs, but after further PCR, proved to be heterozygous. Therefore, across all loci, 2.5% (14/554) of genotypes typed as homozygous by only three PCRs could in fact be falsely genotyped.

For heterozygotes:

Individuals were typed as heterozygous if both of the alleles appeared at least twice in the first three PCRs (Taberlet *et al.*, 1996). A genotype was not considered resolved, however, until both alleles amplified together at least once. For example, for an individual with the genotype AB, the PCRs could show A, AB, and B for the individual to be scored as a heterozygote. If the genotype was not resolved after three PCRs, further PCRs were carried out until it was possible to score a consensus genotype. PCRs were repeated up to 15 times in an attempt to reach a consensus for some samples.

3.2 RESULTS

3.2.1 Sample collection

227 faecal samples were collected, 142 from troops (TABLE 3.3) and 85 from AMBs (TABLE 3.4) at Jodhpur.

For the troops, extractions were carried out in duplicate from all individuals of Kailana, Chandpol A, Chandpol B, Bijolai and Nimba. These were chosen as they were the most completely sampled troops, from widespread areas of the range of the langur population. Extractions were carried out from all members of the Kailana, Chandpol, Kaga, Old Kaga and Bhadreshwar AMBs. Six randomly chosen samples from Mandore AMB were also extracted.

3.2.2 Microsatellite screening

The results of microsatellite screening are shown in TABLE 3.5. Eight loci were chosen with which to genotype the Jodhpur samples. These were loci that had proved to provide consistent amplification with polymorphism in the Jodhpur population (TABLE 3.6). Allele frequencies are shown in FIGURE. 3.2.

TABLE 3.3 Composition of sampled troops and samples collected.

Troop		AM	AF	SM	SF	JM	JF	WM	WF	BM	BF	Total
Kailana	Present	4	7	2	1	1	3	1	0	0	0	19
	Sampled	4	7	2	0	1	3	1	0	0	0	18
Chandpol A	Present	1	14	0	1	2	5	4	2	1	0	30
	Sampled	1	12	0	1	2	4	3	2	1	0	26
Chandpol B	Present	1	7	0	0	0	2	0	2	0	0	12
	Sampled	1	6	0	0	0	2	0	1	0	0	10
Filterhouse	Present	1	7	0	0	1	0	0	0	4?	0	13
	Sampled	1	7	0	0	1	0	0	0	0	0	9
Bijolai	Present	1	5	0	0	1	1	1	1	0	0	10
	Sampled	1	5	0	0	1	1	1	0	0	0	9
Nimba	Present	1	10	1	3	3	0	3	2	0	0	23
	Sampled	1	10	1	2	3	0	3	2	0	0	22
Kaga A	Present	1	21	0	1	2	4	0	0	5?	0	34
	Sampled	1	16	0	0	1	3	0	0	0	0	21
Kaga B	Present	1	23	1	0	5	4	2	4	0	0	40
	Sampled	1	15	0	0	4	3	2	2	0	0	27

TABLE 3.4 Composition of sampled bands and samples collected.

AMB		AM	SM	JM	Total
Kailana	Present	6	2	0	8
	Sampled	6	1	0	7
Chandpol	Present	7	0	4	11
	Sampled	7	0	3	10
Kaga	Present	5	0	0	5
	Sampled	5	0	0	5
Old Kaga	Present				?14
	Sampled				9
Mandore	Present	26	6	9	41
	Sampled	22	4	8	34
Bhadreshwar	Present				?25
	Sampled				20

TABLE 3.5 Human microsatellites used to screen Hanuman langur blood and faeces DNA samples. ‡ used by Launhardt (1998) to type langurs from Ramnagar, Nepal: * established by Coote and Bruford (1996) to be amplifiable in langurs.

Locus	Blood samples (*P. entellus thersites*)	Faecal samples (*P. entellus entellus*)
D3S1766	Polymorphic	Polymorphic
D3S1768	Polymorphic	Monomorphic
D4S243	Polymorphic	Monomorphic
D6S271*	Polymorphic	Polymorphic
D7S503*	Polymorphic	Polymorphic
D7S817	Polymorphic	Not consistent
D12S67‡	Polymorphic	Not consistent
D12S375	Polymorphic	Polymorphic
D13S159*	Polymorphic	Not consistent
D14S306	Polymorphic	Polymorphic
D16S420*‡	Polymorphic	Polymorphic
D17S791*‡	Polymorphic	Polymorphic
SCA-1‡	Polymorphic	Not consistent
D17S804	Monomorphic	No amplification
D1S533	Not tested	No amplification
D1S550	Not tested	No amplification
D2S141*	Not tested	Monomorphic
D4S431	Not tested	No amplification
D5S1475	Not tested	Monomorphic
D10S611	Not tested	Monomorphic
D11S871	Not tested	Monomorphic
D13S317	Not tested	Not consistent
D16S402	Not tested	Not consistent
D21S141	Not tested	Monomorphic

TABLE 3.6 Characteristics of microsatellites used to screen the Jodhpur population; data analysed by CERVUS 2.0. k = number of alleles; N = number of individuals typed; hets = heterozygotes; homs = homozygotes; HWE = Hardy-Weinberg Equilibrium test; null freq = estimation of null allele frequency. See Chapter 6 for discussion.

Locus	k	N	Hets	Homs	H_O	H_E	HWE	Null freq
D3S1766	9	68	47	21	0.691	0.807	NS	0.0759
D5S1457	5	65	40	25	0.615	0.772	NS	0.1064
D6S271	3	75	37	38	0.493	0.496	NS	-0.0024
D7S503	8	82	53	29	0.646	0.554	NS	-0.0968
D12S375	3	70	35	35	0.500	0.493	NS	-0.011
D14S306	4	79	60	19	0.759	0.680	NS	-0.0556
D16S420	8	76	63	13	0.829	0.698	**	-0.104
D17S791	5	82	52	30	0.634	0.558	**	-0.0999

3.2.3 Sample screening

It proved impossible to genotype all individuals sampled, due to time and budgetary constraints and technical difficulties. Amplification was attempted from all extracts three times for each locus. If no successful amplification was observed, the sample was re-extracted. Most samples collected from troops amplified successfully to some degree, so that it was possible to genotype these individuals. The least successful troop to be typed was Bijolai, with only three individuals being typed successfully at all loci, and 25% (36/144) of all possible alleles failing to amplify. It is suspected that the content of the faeces, for example PCR inhibitors that the langurs may have eaten, could have affected the PCR success.

Success with the male bands was very limited. Of all the bands that were sampled, only one (Bhadreshwar) gave results suitable for genotyping for the majority of individuals. Nearly all individuals of the other four bands consistently failed to amplify, despite repeated PCRs and extractions, or gave results that were inconsistent, unreproducible, and showed non-specific amplification. As with the failed samples from Bijolai troop, it is thought that it was the content, or the quality of these samples that made amplification impossible.

For many of these unsuccessful samples, when they were removed from –70°C storage, both the faeces itself and the ethanol surrounding it (often looking like a homogenate)

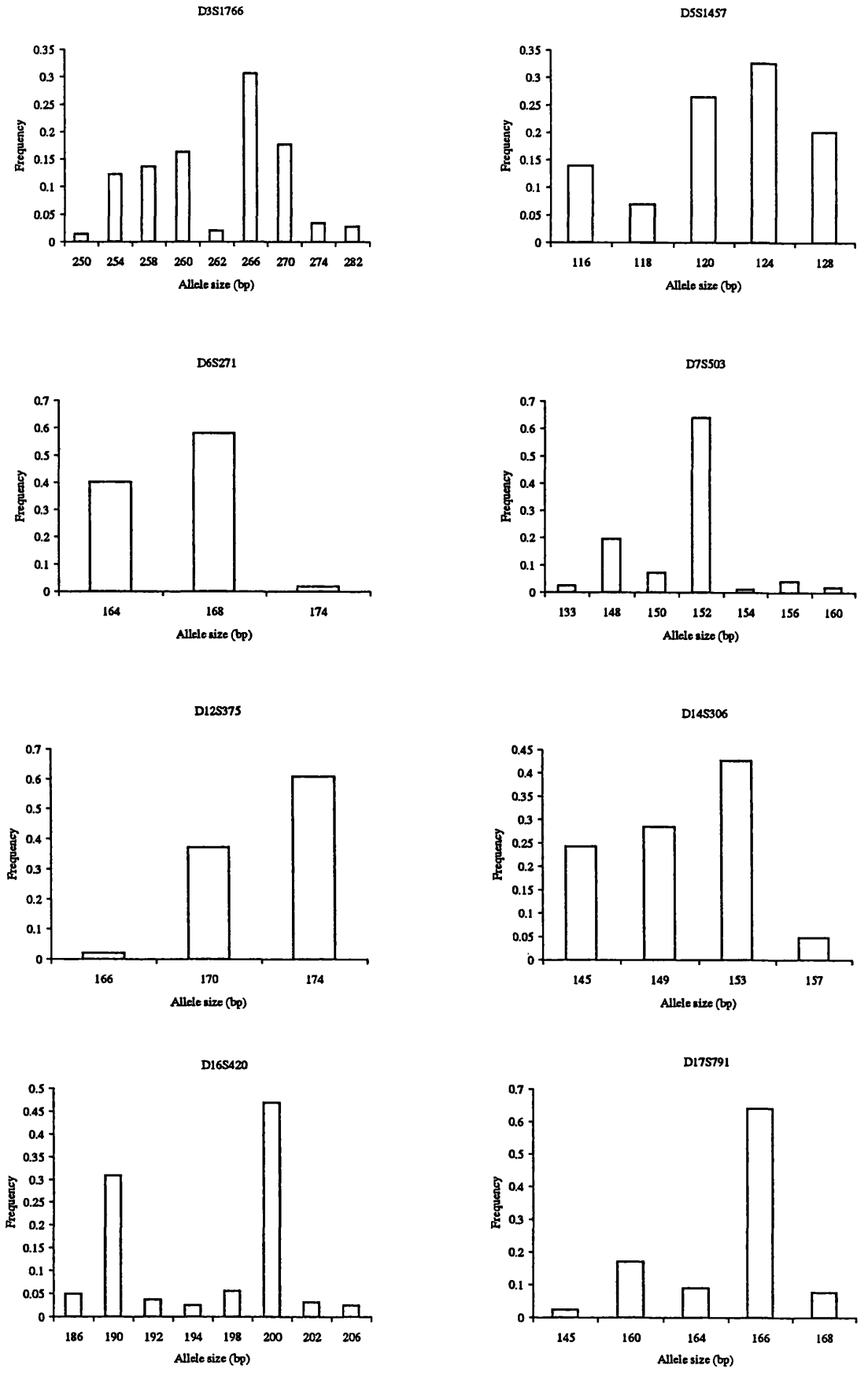


FIGURE 3.2 Allele size (bp) and frequencies of microsatellites used to genotype the Jodhpur population.

were frozen. They often remained semi-solid when on ice. The faeces itself was semi-liquid, and often appeared to be partially fermented, with bubbles of gas frozen into it. This was in contrast to samples that produced amplifiable extractions. These faeces were far more solid, and on removal from –70°C storage the surrounding ethanol was either clear and liquid, or defrosted very soon after removal. It was possible to identify the outside of the faeces and preferentially use that for extraction. Because it was possible to genotype so few individuals from the AMBs, any data from them, apart from Bhadreshwar AMB, were excluded from analysis.

The 89 genotypes obtained after screening are shown in TABLE 3.7.

The genotyping of these faecal samples proved extremely difficult, to the detriment of the quality and quantity of data that could be produced. It was not the case that there were differences between different extracts from the same sample; conversely, it was the case that if one extract amplified well, then the second would also amplify with similar success. If a sample failed to amplify with one extraction, further extractions did not usually produce any greater success, despite modifications to the extraction protocol such as increasing the amount of faeces used, increasing the incubation time with the lysis buffer, or centrifuging the sample and extracting from both the pellet and the supernatant. These results are in contrast to previous studies which have suggested that the distribution of DNA in the faeces is heterogeneous, and that there is likely to be great variation in replicate extracts from the same sample (Goossens *et al.*, 2000; Kohn *et al.*, 1995). The samples that worked most reliably were those where the outside of the faeces could be preferentially removed for extraction.

3.3 DISCUSSION

3.3.1 Faecal samples

The samples that worked most consistently were those from Nimba. These samples gave strong signals with amplification and provided unambiguous genotypes without having to perform many PCRs in addition to the initial three for each extraction. This troop was rarely provisioned and the langurs derived most of their food from leaves. This seems to contradict the expectation that a folivorous diet makes it more difficult to extract and amplify DNA from faeces, because of the presence of inhibitors in plant material (Adams and Demeke, 1992). Samples from Bhadreshwar AMB worked similarly well. This band ranged over a very rural area, far from the city, and it is

Group	Individual	D3S1766	D5S1457	D6S271	D7S503	D12S375	D14S306	D16S420	D17S791
Kailana	AM1	254/260	118/124	168/168	148/152	170/174	145/145	190/200	166/166
Kailana	AF1	254/274	120/124	•/168	152/156	170/174	•/149	190/200	160/166
Kailana	AF2	254/266	120/120	164/168	148/152	170/174	145/149	190/200	145/166
Kailana	AF3	•/•	120/124	164/168	148/152	170/174	149/149	190/200	160/168
Kailana	AF4	258/266	118/124	164/168	152/152	170/174	149/153	190/200	160/166
Kailana	AF5	•/260	120/124	168/168	148/152	170/170	145/149	•/200	166/166
Kailana	AF7	266/270	124/128	168/168	152/156	170/174	153/153	194/200	166/166
Kailana	AF8	•/262	124/128	168/168	152/160	170/174	149/157	190/192	164/166
Kailana	AM2	260/266	118/124	•/164	•/•	170/174	145/149	190/200	166/166
Kailana	AM3	•/•	124/128	•/168	148/156	170/174	149/153	190/200	160/166
Kailana	AM4	•/•	•/124	168/168	•/152	•/170	145/157	•/200	166/166
Kailana	SAM1	•/•	•/•	168/168	•/160	•/170	145/149	•/200	166/166
Kailana	SAM2	254/270	124/124	168/168	148/152	170/174	145/153	200/200	166/166
Kailana	JF1	•/•	•/120	168/168	148/152	•/•	145/145	•/190	160/166
Kailana	JF2	•/•	•/•	•/•	148/152	•/170	•/•	•/200	•/160
Kailana	JF3	254/260	118/124	168/168	152/152	170/170	145/149	190/200	160/166
Kailana	JM1	260/266	128/128	168/168	152/152	170/170	145/153	190/194	166/166
Kailana	WCM	260/260	124/124	164/168	148/152	170/170	145/149	190/190	166/168
Chandpol A	AM	•/270	116/124	164/164	152/152	•/174	•/153	198/200	•/166
Chandpol A	AF1	258/266	•/•	•/•	148/152	170/174	149/153	190/200	166/168
Chandpol A	AF3	254/266	116/120	164/168	148/152	174/174	153/153	190/206	166/166
Chandpol A	AF4	•/•	116/116	•/164	150/152	174/174	145/153	•/•	•/166
Chandpol A	AF5	266/270	118/118	164/168	•/148	174/174	145/153	198/200	166/168
Chandpol A	AF7	254/270	128/128	164/168	148/152	174/174	145/149	190/206	166/166
Chandpol A	AF9	•/•	118/128	•/164	148/152	174/174	•/153	•/200	164/166
Chandpol A	AF11	254/270	118/128	168/168	150/152	174/174	149/153	198/200	166/166
Chandpol A	AF12	266/270	128/128	168/168	150/152	174/174	149/153	200/206	164/166
Chandpol A	AF13	254/266	116/128	168/168	148/152	174/174	145/153	190/200	166/166
Chandpol A	AF14	266/266	120/120	164/168	150/152	174/174	145/153	200/200	164/166
Chandpol A	SAF1	260/270	124/128	164/168	148/152	170/174	149/153	200/200	166/166
Chandpol A	JF1	254/254	128/128	164/168	150/152	174/174	149/153	190/200	164/166
Chandpol A	JF2	266/270	120/120	164/168	•/148	174/174	145/153	198/200	166/168
Chandpol A	JF4	254/270	•/•	164/168	150/152	174/174	153/153	200/200	166/168
Chandpol A	WCF1	254/266	120/•	164/168	150/152	174/174	153/153	200/200	166/166
Chandpol A	WCF2	254/258	116/116	164/168	148/152	174/174	153/153	190/192	164/166
Chandpol A	WCM1	258/266	116/128	168/168	152/152	174/174	149/153	198/200	166/168
Chandpol A	WCM3	254/258	124/128	168/168	150/152	170/174	145/149	190/200	164/166
Chandpol A	WCM4	266/266	118/118	168/168	148/152	170/174	149/153	192/200	166/168
Chandpol A	BCM1	270/270	124/128	168/168	133/152	170/174	149/153	190/200	164/166
Nimba	AM	•/260	•/120	164/168	148/152	170/•	145/153	•/190	•/166
Nimba	AF1	266/266	124/124	164/164	152/152	174/174	149/153	186/200	166/168
Nimba	AF2	250/266	120/124	164/168	152/152	170/174	149/153	190/200	166/166
Nimba	AF3	266/266	124/124	164/168	152/152	170/174	149/153	•/200	166/166
Nimba	AF4	266/266	124/128	164/168	152/152	•/174	145/153	200/202	160/166
Nimba	AF5	266/270	116/128	164/168	152/152	•/174	149/153	190/200	160/166
Nimba	AF6	270/270	116/128	164/168	152/152	170/170	149/149	186/190	145/166
Nimba	AF7	266/266	120/128	168/168	152/152	174/174	145/153	186/200	160/166
Nimba	AF10	266/266	124/128	164/168	152/152	170/174	145/149	200/200	160/166
Nimba	SAM	260/266	120/124	164/168	150/152	170/170	145/145	186/200	164/166
Nimba	SAF2	262/266	116/120	164/164	152/152	170/174	145/149	200/202	166/166
Nimba	SAF3	•/260	116/120	•/164	•/152	•/174	•/153	186/186	166/166
Nimba	JM1	260/266	120/•	164/164	152/152	174/174	145/153	•/200	164/166
Nimba	JM2	•/266	120/•	•/164	152/152	•/174	153/153	190/202	166/166
Nimba	JM3	260/266	124/128	164/168	152/152	170/174	153/153	186/200	160/166
Nimba	WC1	260/270	120/128	164/168	148/152	170/174	149/153	190/200	164/166
Nimba	WC3	266/266	120/124	164/164	152/152	174/174	153/153	186/200	166/166
Nimba	WC4	250/260	120/120	168/168	148/152	170/174	153/153	190/200	166/166
Chandpol B	AM	•/266	•/•	164/168	150/152	•/•	•/153	•/•	•/•
Chandpol B	AF1	266/270	116/124	164/168	148/152	170/174	153/153	190/200	166/166
Chandpol B	AF2	270/270	116/128	164/168	152/152	166/174	149/153	190/200	166/166
Chandpol B	AF3	266/282	116/128	168/168	148/152	166/174	145/153	190/198	166/168
Chandpol B	AF5	266/266	120/124	168/168	148/152	174/174	•/145	200/206	•/166
Chandpol B	AF6	266/282	120/120	164/168	148/•	174/174	145/149	198/200	164/166
Chandpol B	AF7	254/270	116/128	164/168	150/152	174/174	145/149	190/190	145/166
Chandpol B	JF1	282/•	120/•	168/174	148/152	170/174	145/157	190/190	160/166
Chandpol B	JF2	•/•	120/124	168/174	148/152	170/174	145/157	190/198	160/166
Chandpol B	WCF1	•/•	120/•	•/174	•/152	•/•	•/149	•/200	•/166

TABLE 3.7 Genotypes of individuals analysed. • denotes missing genetic data.

Group	Individual	D3S1766	D5S1457	D6S271	D7S503	D12S375	D14S306	D16S420	D17S791
Bijolai	AM	260/270	120/•	164/168	150/152	170/174	153/157	190/200	160/160
Bijolai	AF1	260/260	124/•	•/•	•/•	•/•	•/145	•/•	•/•
Bijolai	AF2	•/274	116/128	•/168	152/152	•/174	•/153	192/200	145/166
Bijolai	AF3	266/270	124/124	168/168	152/152	170/170	145/157	190/200	166/166
Bijolai	AF4	•/•	•/•	•/•	152/156	•/174	•/145	•/190	166/166
Bijolai	AF5	270/274	120/120	164/168	152/152	174/174	145/149	190/200	160/166
Bijolai	JF	258/258	•/•	164/164	152/152	•/174	149/157	190/200	160/166
Bijolai	JM	•/262	•/124	•/164	•/152	•/•	149/153	190/192	•/160
Bijolai	WCM	260/274	120/124	164/164	152/152	170/174	149/157	190/200	160/160
Bhadreshwar	BM1	266/266	•/•	168/168	133/152	174/174	149/153	190/200	164/166
Bhadreshwar	BM2	•/260	•/•	168/168	152/152	•/170	149/153	•/•	160/168
Bhadreshwar	BM3	260/260	124/124	168/168	148/154	170/174	149/153	200/200	164/166
Bhadreshwar	BM4	254/258	124/124	168/168	133/152	170/174	149/153	190/200	166/166
Bhadreshwar	BM5	•/258	116/•	•/168	148/156	•/170	149/153	194/200	160/166
Bhadreshwar	BM6	258/258	•/124	164/168	148/156	•/170	145/153	194/200	160/166
Bhadreshwar	BM7	274/282	116/116	164/168	148/152	174/174	•/149	200/200	160/166
Bhadreshwar	BM8	258/270	120/120	164/164	•/152	170/174	153/153	190/200	166/168
Bhadreshwar	BM9	258/270	•/124	164/168	152/154	170/170	153/153	192/202	•/166
Bhadreshwar	BM10	258/260	120/124	164/164	152/156	170/170	145/149	190/202	160/166
Bhadreshwar	BM11	258/258	120/124	164/164	148/152	170/174	149/153	190/200	164/166
Bhadreshwar	BM15	258/258	•/124	•/164	133/160	•/•	145/153	•/190	160/160
Bhadreshwar	BM19	•/258	•/124	164/164	152/152	•/•	149/153	190/200	160/166

TABLE 3.7 (cont.)

suggested that they had a similar 'natural' diet. This compares with the population studied by Launhardt (1998) in which neither crop raiding or provisioning is observed; these samples presented little difficulty in typing; any inhibitory effects could be resolved by the addition of BSA (Launhardt *et al.*, 1998). Faeces formed from a natural diet may contain more fibrous matter that is abrasive to the wall of the gut, resulting in a greater amount of host DNA in the faeces. It may also be less aggravating to the gut than some provisioned foods, which may accelerate the progress of faeces through the gut; 'naturally' derived faeces may therefore have more time in contact with the gut wall and accrue more DNA.

It is possible that provisioned food somehow alters the normal digestive process for the langurs, making it less likely that DNA will be found in the faeces. Also, many 'human' foodstuffs contain polyphenolics that inhibit PCR (Brooke *et al.*, 1997). An example of this was suspected in Bijolai samples. The langurs were provisioned with a sackful of roasted chillies. Though they ate them without any apparent ill effects, the next day their digestion was clearly affected. One of the adult females sampled that day produced a liquid reddish-tinged faeces. When it came to extract this, it was noted that by the final stage of the extraction there was still a red residue left in the spin column. This sample gave consistently poor genotyping results. It seemed likely that there was some compound in the chilli that copurified with the DNA, inhibited the PCR reaction and was not counteracted by the addition of BSA.

Those samples that proved most difficult, or impossible, to achieve working extractions from were in the majority those from male bands. As these groups were only located when they were in encounters with other groups, with the exception of the Bhadreshwar AMB, their defecation was often apparently stress induced. This is a common reaction to 'violent mental stress', first documented by Cannon (1929). In a stressful situation, the sympathetic nervous system causes an increase in heart rate, dilation of the pupils, an increase in blood pressure, sweating, and diversion of blood flow from the viscera to skeletal muscles. This so-called 'Cannon's emergency reaction' is sometimes accompanied by sacral autonomic nervous discharge, which stimulate the voiding of the bladder, the rectum, or both. This is suggested to reduce the weight of an individual in preparation for flight (Tiger and Fox, 1972). The faeces that langurs produced in stressful situations was visibly different to normal faeces – it was far more liquid than the normal stools, which were relatively hard and dry, presumably because in an arid environment it is of paramount importance to conserve water.

It is possible that it was the composition of this kind of faeces that made DNA extraction so unsuccessful. The primary function of the colon, where faeces is stored prior to defecation, is the reabsorption of water. In the event that the colon is voided rapidly, prior to water reabsorption being complete, the water content of the faeces will be very much higher than in normal faeces. The increased water content could have resulted in the dilution of the ethanol used to fix the faecal DNA, resulting in the loss of its preservation properties and consequent degradation of DNA. It may also be the case that the faeces' accelerated passage through the gut in its semi-liquid form does not allow the contact time with the intestinal wall that is needed to collect sufficient cells. This might be exacerbated by the consistency of the faeces, which is possibly not abrasive enough to remove many gut cells.

The consistency of the samples could have reduced the efficacy of preservation. Normal samples which are actually relatively dry could have absorbed ethanol quickly. In contrast, the semi-liquid faeces would not have been able to absorb any ethanol, so ethanol only could penetrate the sample by diffusion. Especially in cases where the sample had slid to the bottom of the collection tube, only a small surface of the faeces relative to the total sample size was exposed. Underneath this surface, the DNA may still have been being degraded by still-active gut enzymes. In retrospect, if this type of sample was unavoidably collected, it is suggested that the ethanol and sample should be homogenised, to allow the ethanol to penetrate through the sample immediately.

Colobines have a high level of ribonuclease activity in the small intestine, which may be significant for DNA preservation. As a result of their ruminant digestion, colobines rely on the breakdown of fermenting microbes as an important source of nitrogen. They therefore have much higher ribonuclease activity in the small intestine than do primates without a forestomach (Beintema *et al.*, 1973). If in stress, the small intestine is rapidly voided, these ribonucleases will still be active in the faeces. They could therefore be breaking down the small quantity of gut-derived DNA that is contained in the faeces, before the ethanol is added.

In contrast to these unsuccessful langur samples, faecal samples from stressed orang-utans have given very successful DNA extractions (B. Goossens, pers. comm.). This difference may be due to the differences in gut biochemistry, as the orang-utan has low ribonuclease activity in the small intestine. The orang-utan also has a mainly

frugivorous diet, whereas the langurs, having quite an opportunistic diet in the city, may consume many types of food which inhibit PCR reactions.

The appearance of the faeces clearly correlated with whether it would be possible to extract and amplify DNA. As a result of repeated attempts to extract and amplify DNA from unsuccessful samples, the overall number of animals genotyped was reduced from the initial target figures of six troops and six bands. This highlights a problem with non-invasive DNA techniques. Considering the male bands alone, extractions were done from over fifty individuals. Each one of these was done in duplicate, totalling 100 extractions. Furthermore, some extractions were repeated. In addition to this, attempts were made to amplify all of the samples at most loci. This represents a large amount of time from which ultimately only thirteen males were successfully genotyped. For future studies it would be recommended that only fresh, solid samples be collected, if possible from individuals that are not often provisioned. However, this recommendation can only be given for this species, considering the vastly contrasting results from orang-utan faeces.

3.3.2 Microsatellites

Using human microsatellites for the genotyping of non-human primates is becoming common practice. It is often quicker to screen a range of human primers and establish which cross-hybridise and are polymorphic than to create and screen a library for species-specific primers. In using human microsatellites that cross-hybridise, there is a risk of human contamination from both the people that have collected the samples and those working with the samples in the lab. It is of paramount importance to eliminate contamination, and to screen for its occurrence by genotyping those individuals involved in working with the faeces. It might be the case that this problem could be solved by creating species-specific primers; however, time could also be spent isolating primers that later reveal themselves to cross-hybridise to humans.

Out of 26 human markers screened, eight proved consistently amplifiable and informative in the Jodhpur langurs. This compares favourably with Launhardt *et al.* (1998) who found that around one-third of the markers screened were polymorphic but only 16% were reliably informative. Interestingly, Launhardt *et al.* (1998) found that they obtained unreliable amplification when the amplified product was more than 200bp in length, which was attributed to the degradation of the DNA giving insufficient template. This was not found to be the case in this study, where D3S1766, with a size

range of 250-282 gave consistent amplification. This is possibly due to differences in the extraction technique.

The primers in this study were not highly polymorphic. The microsatellites screened initially were those that had proved to be successful in other non-human primates being genotyped in the study lab (including orang-utans, gorillas, chimpanzees, savannah baboons and red colobus), or had been shown to amplify successfully in Hanuman langurs (Coote and Bruford, 1996; Launhardt, 1998). Comparison of the number of alleles found in humans and non-human primates are shown in TABLE 3.8. This shows a disadvantage of using primers from one species to screen a population of a related species. It may be advantageous to screen a greater range of the nearly 8000 markers available, rather than concentrating on those that have had some success in non-human primates.

TABLE 3.8 Number of alleles for selected loci taken from various primate studies. Data from (a) Coote and Bruford, 1996; (b) this study; (c) Smith *et al.*, 2000; (d) Gerloff *et al.*, 1999; (e) von Segesser *et al.*, 1999.

Locus	Humans (*Homo sapiens*) (a)	Hanuman langur (*Presbytis entellus*) (b)	Savannah baboon (*Papio cynocephalus*) (c)	Bonobo (*Pan paniscus*) (d)	Barbary macaque (*Macaca sylvanus*) (e)
D6S271	13	3	15	6	
D7S503	7	8	13		9
D16S420	8	8			
D17S791	11	5		6	12

Within Hanuman langurs, there also seems to be large variation in the size range of the alleles in different subspecies. Three subspecies have now been screened: *P. entellus entellus* (Jodhpur), *P. entellus thersites* (Sri Lanka: Coote and Bruford, 1996) and *P. entellus ajax/ schistacea* (Ramnagar: Launhardt, 1998). The size range of some loci vary substantially between subspecies (TABLE 3.9).

The two loci that were studied in both Ramnagar and Jodhpur (D16S420 and D17S791) are both found to be shorter in the Ramnagar population. Moreover, there were indications from trials with D12S67 on the Jodhpur samples that its size range lay between 160-170, compared to 134-156 in Ramnagar. It might be assumed that a

TABLE 3.9 Size ranges (base pairs) and number of alleles in microsatellites in humans and different *P. entellus* populations. Data from Coote and Bruford, 1996 (a and d); this study (b); and Launhardt, 1998 (c).

Locus	Humans (a)	*P. entellus* population		
		Jodhpur (b)	Ramnagar (c)	Sri Lanka (d)
D6S271	144-202 (13)	164-174 (3)	-	172-178 (3)
D7S503	149-171 (7)	133-160 (8)	-	151-167 (5)
D16S420	175-197 (8)	186-206 (8)	164-184 (8)	195-201 (4)
D17S791	162-186 (11)	145-168 (5)	118-130 (5)	146-168 (5)

difference in sizing methods would account for this directional difference between studies, but Launhardt (1998) also ran human DNA control samples along with the langur samples. Such a divergence in the size range of the microsatellites represents at least three deletions or insertions (one for each locus) during the divergence of these two subspecies. Although these microsatellites show relatively little variability within populations, these differences in size range between populations suggest that across the whole subcontinental range of the species there may be great variation in these microsatellites. Thus these microsatellites will be very informative as markers for population genetic analysis.

3.4 CONCLUSIONS

3.4.1 Fieldwork

The collection of samples was relatively straightforward. The advantages of collecting non-invasive samples from this population were manifold. It involved no disturbance to the animals; samples were reliably assigned to known individuals; no form of paperwork under CITES agreements was needed to collect and transport faeces. In addition, the revered status of the langurs means that invasive sampling would not be tolerated by the Hindu population.

Care must be taken in the assignment of identity, as found in Bhadreshwar, where randomly collected samples assumed to be from 20 different individuals were later attributed to only 13 individuals, indicating duplicate sampling that could affect the

apparent relatedness of this group. Identification involves careful observation of the langur groups, so that monkeys may be individually recognised, which can be time consuming. However, despite these time demands, it was possible to collect more samples than were used in the screening of the population, making faecal sampling time and cost effective.

3.4.2 Primer screening

Screening a suite of human primers for their suitability for use in non-human primates has been used in many studies (Hanuman langurs, Launhardt, 1998; Savannah baboons, Smith *et al.*, 2000; chimpanzees, Vigilant *et al.*, 2001; gorillas, Clifford *et al.*, 1999). Although not all primers will cross-amplify, it is generally accepted that using human primers is quicker than creating a microsatellite library for each study species. One potential problem with using human microsatellites is that of human contamination. However, it should be noted that microsatellites isolated from the study species may well cross-hybridise with human DNA, so contamination will still be a risk. For example, Cooper *et al.* (1998) cloned microsatellites from chimpanzee DNA – out of 38 microsatellites, 28 amplified both chimp and human DNA. If possible, it would be advantageous to genotype using microsatellites which have different size ranges in humans and the study species. As the genetic distance between the study species and humans increases, there are likely to be a smaller number of microsatellites from which to choose, due to mutations in priming sites accumulating over time. Identifying a suite of suitable microsatellites from human primers therefore becomes more labour intensive. Nevertheless, human microsatellites have proved themselves an extremely useful tool in the screening of non-human primate populations, and are unequivocally recommended for similar studies.

3.4.3 Sample extraction and screening

The development of the QIAamp® DNA Stool Mini Kit has meant that DNA can easily be extracted from faecal samples from many species, often with excellent results. From samples that worked well, the electropherograms obtained in microsatellite amplification were as good as those obtained from blood extracted DNA. However, the greatest setback to this study was the failure of DNA extraction and amplification from many samples, especially those collected from all male bands. This has highlighted the necessity of exercising discretion in sample collecting, because there is great variability in the samples with respect to the amount and quality of DNA contained in them. Faeces can be a very good source of DNA for obtaining data about wild populations, but

the disadvantages, such as the failure of a proportion of samples, the inherent difficulties of amplifying small amounts of DNA, the risks of contamination when working with species closely related to humans, and the cost of multiple repeats in time and money must always be considered.

4. INVESTIGATING GENETIC RELATEDNESS AND SOCIAL STRUCTURE OF LANGUR GROUPS

Summary

Cooperative behaviours between langur female troop members, such as home range defence, allogrooming and allomothering, have been attributed to kin selection, and it is hypothesised that troop members are closely related in comparison to the rest of the population. In contrast, little cooperation is seen between all male band members; their competition for access to females, and the infanticide that can result, has been ascribed to their low relatedness. In this chapter, relatedness estimates of several troops and one band, and various subsets of troops (adult females, non-adults and adult males) are measured, using the programs RELATEDNESS and KINSHIP, based on Queller and Goodnight's (1989) index of relatedness. This index measures the similarity of an individual, or group of individuals, to another individual/ group of individuals, using their allele frequencies, scaled by the allele frequencies in the rest of the population. RELATEDNESS calculates values between groups of individuals, whilst KINSHIP measures pairwise relatedness and allows hypotheses about relationships to be tested.

Across the population, whole troops and the females of troops were found to have high relatedness values, as has been previously assumed and modelled for one-male, matrilocal troops. However, on a troop-by-troop basis, there was high variation in relatedness, with some groups exhibiting low relatedness values for whole troops and adult females. It is suggested that these low values are a cross-generation effect of successive resident males introducing new alleles to a troop, so that whilst a resident's offspring will be closely related as paternal sibs, the empirical relatedness of successive generations will be greatly decreased.

It is also demonstrated that the relatedness of the offspring of a troop to one another is contributed to both by their mothers' relatedness and by their father's contribution; troops where more than one resident is suspected to have fathered the offspring have the lowest non-adult cohort relatedness. Present residents are either related by high levels to their putative offspring, in troops where their residency was thought to have been stable for many years, or unrelated, where the resident was known to be new. Overall, the observations that troops are closely related groups of individuals are supported by the genetic data. Conversely, members of all male bands, particularly the young adults who control the bands' movements, are unlikely to be related, because of the constantly

changing membership and the high mortality rate suffered by the nomadic males. Genetic data from one AMB support this; the apparently cooperative behaviours seen must therefore be explained without recourse to kin selection.

Networks of KINSHIP estimates, which indicate relationships with significant likelihood, show the closely structured nature of the troops, where most individuals have relationships to at least one other troop member. These networks mirror the close grooming networks observed in troops, and give an impression of the close relatedness of the troops members. However, they are not entirely reliable as indicators of real genealogical relationships, as some known relationships are not represented by the KINSHIP networks.

4.1 INTRODUCTION

In many social mammals, females exhibit philopatry (Greenwood, 1980). Social groups are based around females and their offspring, with males dispersing before they reach maturity. The consequence of this pattern of dispersal is assumed to be the creation of a social group within which individuals share a high degree of relatedness, or coancestry. This provides a genetic background upon which cooperative social behaviours can be expressed. Members of female-bonded primate groups have been observed to support each other in conflicts (Hrdy, 1977) and tolerate each other more in competition over resources (Walters and Seyfarth, 1987). Cooperative behaviours between females are widespread, and are well illustrated by the Hanuman langur, which exhibits high levels of allogrooming, allomothering and cooperative home range defence within the female bonded troops (Hrdy, 1977). These behaviours are presumed to have arisen because of the high level of relatedness between individuals; having commonly inherited genes means that apparent altruism will benefit the actor.

Conversely, very little cooperation is seen in the langur all male bands. These comprise individuals who have dispersed from their natal troops, and are presumed not to be in the company of close relatives by adulthood (Rajpurohit and Sommer, 1993). As a result of this low relatedness, adult males compete with one another for access to females; there is no genetic incentive to form alliances with other band members. The infanticidal behaviour that has been observed in langurs is proposed as an extension of this male-male competition (Hrdy, 1974).

However, until the advent of genetic typing of individuals, it was not possible to identify whether the driving force for cooperative behaviour is kinship (Hamilton, 1964) or familiarity within a social group (Noë *et al.*, 1991). It is now possible to quantify relatedness among individuals. Particularly, estimators of relatedness can be calculated not just for dyads, but for groups of individuals, such as social groups. These estimators asymptotically equal the true degree of kinship (de Ruiter and Geffen, 1998). It is important to note that the Queller and Goodnight estimator of relatedness does not represent the absolute genetic similarity of the individuals under consideration, but how similar they are in comparison to the rest of the population under study.

This chapter examines the relatedness of groups and subgroups of langurs, and attempts to establish networks of relatedness that might characterise the social groups sampled, to examine how the social structure of the groups might have affected relatedness. It would be expected that the troops should have a relatively high level of relatedness, because they are philopatric. Conversely, the AMB should have a lower level of relatedness, as they comprise individuals from several troops and are not expected to be related to one another (apart from newly ousted residents and their male offspring).

4.2 QUELLER AND GOODNIGHT'S INDEX OF RELATEDNESS

89 langurs genotyped at a maximum of eight loci were used for relatedness analysis. These data were analysed using the program KINSHIP 1.3.1 to evaluate pairwise relationships and RELATEDNESS 5.0.8 to characterise relatedness values for various subgroups of the population. KINSHIP 1.3.1 and RELATEDNESS 5.0.8 are Macintosh based programs (available at http://gsoft.smu.edu/GSoft.html) which test hypotheses of pedigree relationships between pairs or groups of individuals, using data from single locus genetic markers such as microsatellites. These programs calculate relatedness statistics based on Queller and Goodnight's (1989) index of relatedness, R:

$$R = \frac{\sum_{x}\sum_{k}\sum_{l}\left(P_y - P^*\right)}{\sum_{x}\sum_{k}\sum_{l}\left(P_x - P^*\right)}$$

where x indexes the individuals in the data set, k indexes the loci, and l indexes the allelic position. P_x is the frequency within the current x individual of the allele found at x's locus k and allelic position l. In a diploid organism this value is either 0.5 or 1.0. P_y is the frequency of that allele in the 'partners' of x, those individuals to which x is being

compared; this value can be 0, 0.5 or 1.0 if only one individual is included in the definition, but will vary according to how many individuals the definition encompasses. P* is the frequency of that allele in the population as a whole. This equation allows the relatedness of two individuals or groups of individuals to be calculated when their pedigree is unknown, depending on how P_x and P_y are defined. It has been widely used in genetic studies of primate sociality, for example in chimpanzees (Vigilant *et al.*, 2001), baboons (Altmann *et al.*, 1996) and long-tailed macaques (de Ruiter and Geffen, 1998).

Unrelated individuals in a population may share alleles, depending upon the frequency of those alleles. If an allele occurs frequently then it is likely that two individuals will have this allele in common, although they may not be closely related. It is therefore necessary that the relatedness calculation takes account of the overall population allele frequency to control for this.

The power of loci to contribute to relatedness estimates depends on the number and frequency of alleles. For example, de Ruiter and Geffen (1998) suggested that protein coding loci may provide a better resolution of relatedness than microsatellites, in part because of the high variability of these loci. In previous studies, between five (Launhardt, 1998) and 16 (Constable *et al.*, 2001) microsatellite loci have been used in analyses of relatedness and paternity assignment in primates.

When using this relatedness calculation to estimate pairwise relatedness, as performed by KINSHIP, or comparisons of one individual to a group of others, using RELATEDNESS, it is important to be aware that it is subject to stochastic variation. The numerator and the denominator of the relatedness expression are both based on the allele frequency in an individual or set of individuals. If only one individual is being considered for either definition, then the allele frequency of P_x or P_y can only be 0, 0.5 or 1.0, leading to a lack of resolution in pairwise relatedness estimates in KINSHIP, or when either P_x or P_y are based on single individuals in RELATEDNESS. Relatedness values between individuals drawn from the same population are usually expected to lie between 0 and 1, but especially when either individual possesses a rare allele, a negative value for R may be obtained.

4.2.1 RELATEDNESS 5.0.8

For each langur troop, RELATEDNESS was used to calculate whole group relatedness, relatedness of particular subgroups within those troops, and the symmetrical relatedness

of the subgroups to one another. These relatedness calculations use a bias-corrected allele frequency. The bias correction is by group, and involves the exclusion of the *x* individual and all other members of *x*'s social group in the calculation of allele frequencies. This means that groups of individuals that are genetically similar to *x* will not bias the allele frequencies in their direction, which would give an underestimate of relatedness.

Under the rules of diploid genetics, $R_{A\leftrightarrow B} = R_{A\rightarrow B} = R_{B\rightarrow A}$. However, in any particular case estimates may vary markedly from one another, especially when either A or B comprises a single individual. In such a case, calculating symmetrical relatedness ($R_{A\leftrightarrow B}$) will give a better result, as the available information is used more thoroughly. This is done by including both A and B in the P_x definition, with P_y defined as all individuals other than the current *x* individual. This symmetrical relatedness calculation was used for comparing different subgroups of harem troops with one another, for example, comparing relatedness of adult females to the non-adults of a troop. For Bhadreshwar, the AMB, only the relatedness of the whole band could be calculated, because of the lack of data on the age of the sampled individuals.

Standard errors of relatedness were estimated by jack-knifing over loci (Queller and Goodnight, 1989). In this method, one locus at a time is dropped out of the relatedness estimation, and the resulting 'pseudo-values' used to calculate a standard error.

4.2.2 KINSHIP 1.3.1

For each of the social groups (five troops and one AMB) estimates of pairwise relatedness were calculated using KINSHIP, as well as likelihood ratios for different pairs of hypotheses. Initially, the primary hypothesis was set as R = 0.5, with a null hypothesis of R = 0.0. This tests the likelihood that individuals are first degree relatives (parent-offspring, full sibs) rather than unrelated. Secondly, the primary hypothesis was set as R = 0.5, and the null hypothesis as R = 0.25; this returns the likelihood that individuals are first degree relatives rather than second-degree relatives (e.g. half-sibs, grandmother–granddaughter). KINSHIP uses the hypothesised R values, the population allele frequencies and the genotypes of the two individuals tested to calculate the likelihood that this genotype combination could have resulted from the relationship hypothesised. It then calculates a ratio between the likelihoods of the primary and the null hypothesis: a high ratio will suggest the primary hypothesis should be accepted, whilst a low ratio rejects the primary hypothesis in favour of the null hypothesis. A

ratio rather than an absolute likelihood value for a hypothesis is reported because as more loci or alleles are added, the absolute likelihood of any genotype occurring decreases. The likelihood ratio therefore reports the relative likelihood of the primary and null hypotheses.

These ratios can then be flagged according to their significance, by using a simulation routine. This is because the ratios cannot be described by a standard distribution and must therefore be calculated for each pair of hypotheses.

Finally, for each group allele sharing scores were calculated by hand. The number of alleles shared by two individuals out of the total potential number of alleles shared was counted; in particular, it was noted whether each dyad could potentially be vertical first degree relatives (parent-offspring), sharing at least one allele at each locus.

From the KINSHIP output files networks of relationships within each group were created. Dyads that had significant likelihood ratios are joined, giving an indication of group structure according to relatedness. Additionally, each significant relationship is supported by the significance level, pairwise relatedness score and allele sharing value.

4.3 RESULTS

4.3.1 RELATEDNESS

The results of all relatedness calculations are shown in TABLE 4.1, and are plotted for each troop in FIGURE 4.1. The average R value for individuals within a troop was 0.17 ± 0.04; however, this varied widely between troops. Three troops (Kailana, Chandpol A and Nimba) had relatively high overall R values (0.21 ± 0.08, 0.20 ± 0.10, and 0.22 ± 0.09 respectively), whereas the remaining two troops had much lower values (Chandpol B, 0.00 ± 0.04, and Bijolai, 0.07 ± 0.07), which were more similar to that of the Bhadreshwar males (0.05 ± 0.08). This dichotomy between troops was seen again in the R values of the adult females of each troop. Although overall in the population the relatedness of troop adult females was 0.14 ± 0.07, when calculated separately, only Chandpol A and Nimba females had R values significantly different from 0 (0.26 ± 0.15 and 0.32 ± 0.17 respectively), in contrast with Kailana, Chandpol B and Bijolai (0.04 ± 0.08, 0.03 ± 0.06 and 0.02 ± 0.11 respectively).

TABLE 4.1 Relatedness values for troops and AMB at Jodhpur, and subgroups of troops, including standard errors. See FIGURE 4.1 for explanation of abbreviations.

Group	Name	n	Whole	AF	J/WC	AF to off.	AM to off.
Troop	Kailana	18	0.21±0.08	0.04±0.09	0.32±0.18	0.18±0.10	0.48±0.12
	Chandpol A	21	0.20±0.10	0.26±0.15	0.14±0.08	0.22±0.10	0.27±0.19
	Nimba	18	0.22±0.09	0.32±0.17	0.26±0.06	0.19±0.11	0.25±0.15
	Chandpol B	10	0.00±0.04	0.04±0.06	0.35±0.16	-0.11±0.10	-0.45±0.11
	Bijolai	9	0.07±0.08	0.02±0.12	0.49±0.22	0.00±0.16	0.27±0.20
	Troop average	76	0.17±0.04	0.14±0.07	0.27±0.07	0.14±0.05	0.28±0.08
AMB	Bhadreshwar	13	0.05±0.08				

The R value for non-adults in all troops was 0.27 ± 0.07. The values for individual troops were all positive, and in all troops except Chandpol A indicated a level of relatedness exceeding that of paternal sibs (0.25). However, when the relatedness of the adult females to the non-adults was calculated, again a bimodal distribution was seen. Three troops, Kailana, Chandpol A and Nimba, had high values of $R_{\text{adult females}\leftrightarrow\text{offspring}}$, whereas Chandpol B and Bijolai had low, even negative values.

In four out of the five troops the values of $R_{\text{resident male}\leftrightarrow\text{offspring}}$ were high, whereas in Chandpol B, R was strongly negative, suggesting an extremely low relatedness between this resident and the non-adults in the troop.

In Kailana, in contrast to other Jodhpur troops, there were four adult males resident. The R value for all the adult males resident in the troop was 0.29 ± 0.10, and 0.39 ± 0.11 if the two subadult males were also included, indicating a high degree of relatedness between all Kailana troop males.

4.3.2 KINSHIP networks

Results of KINSHIP analyses can be found in APPENDIX 1 (pairwise relatedness values); APPENDIX 2 (relationships with significant likelihood when tested with H_1 set at R = 0.5 against H_0 of R = 0.0); APPENDIX 3 (relationships with significant likelihood when tested with H_1 set at R = 0.5 against H_0 of R = 0.25), along with allele sharing scores (APPENDIX 4).

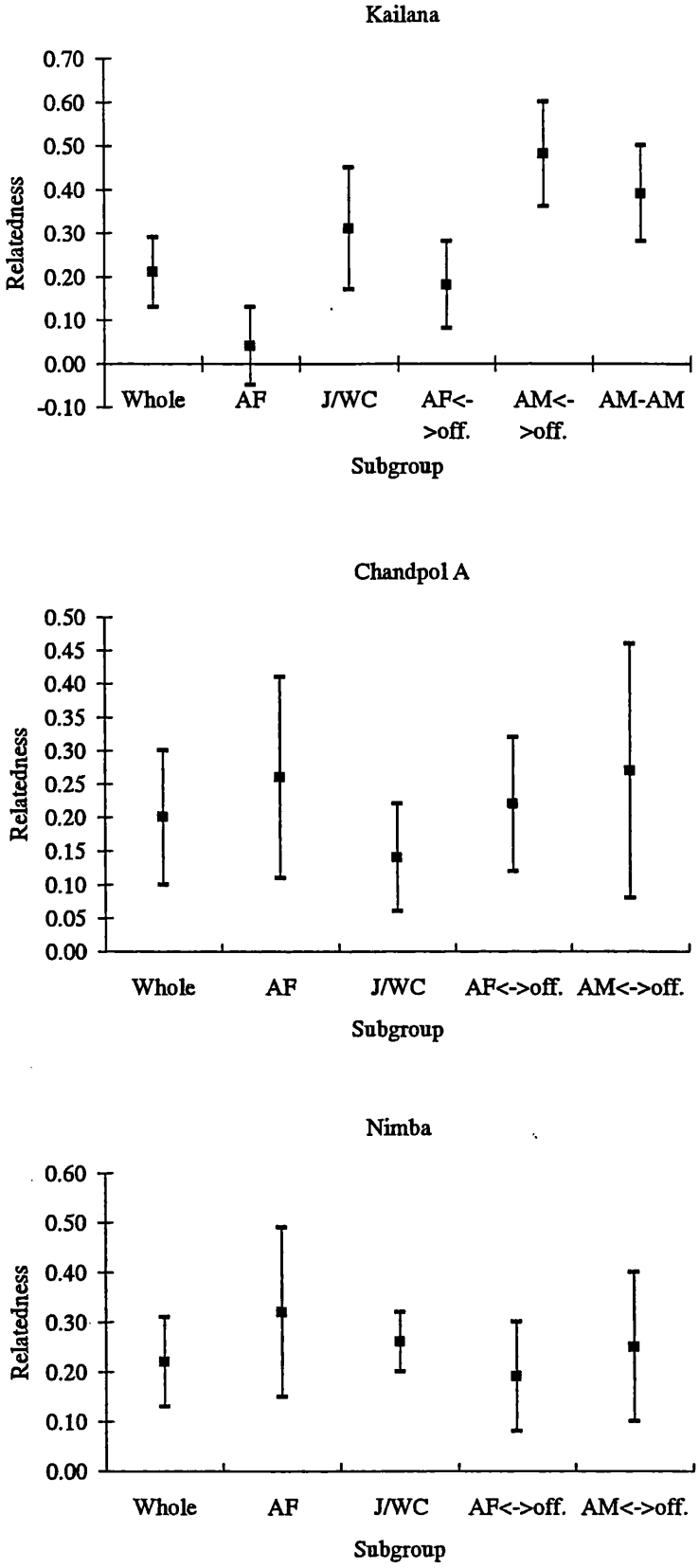
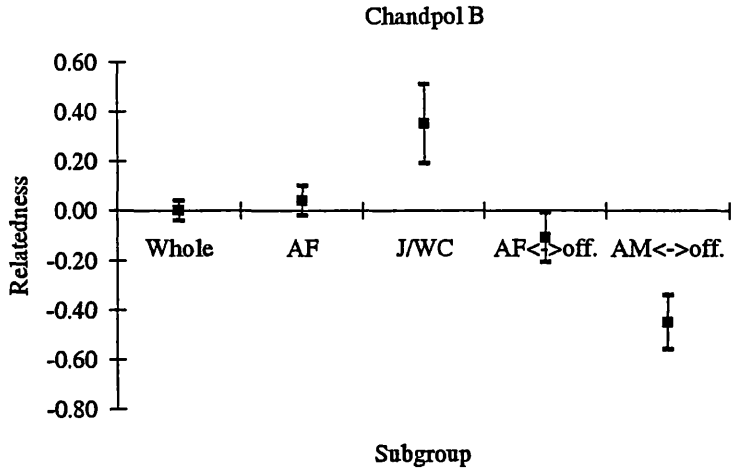
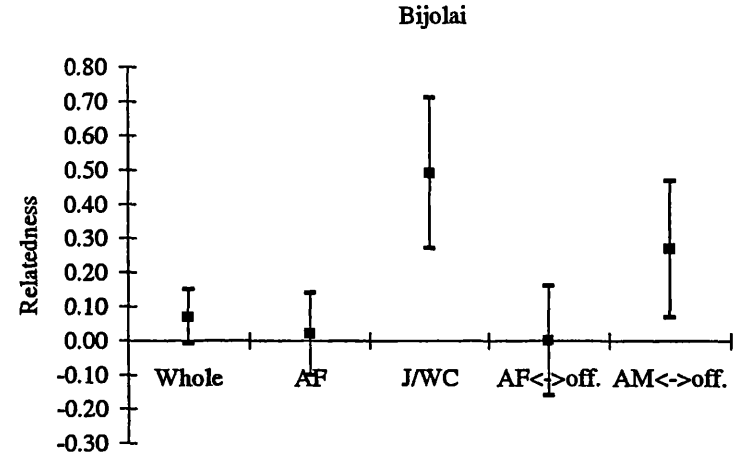


FIGURE 4.1 Relatedness of subgroups in troops. Whole = all individuals of troop included; AF = adult females; J/WC = non-adults (juveniles and white coats); AF <-> off. = symmetrical relatedness of adult females to non-adults; AM <-> off. = symmetrical relatedness of resident male to non-adults; AM-AM = adult males (Kailana only).





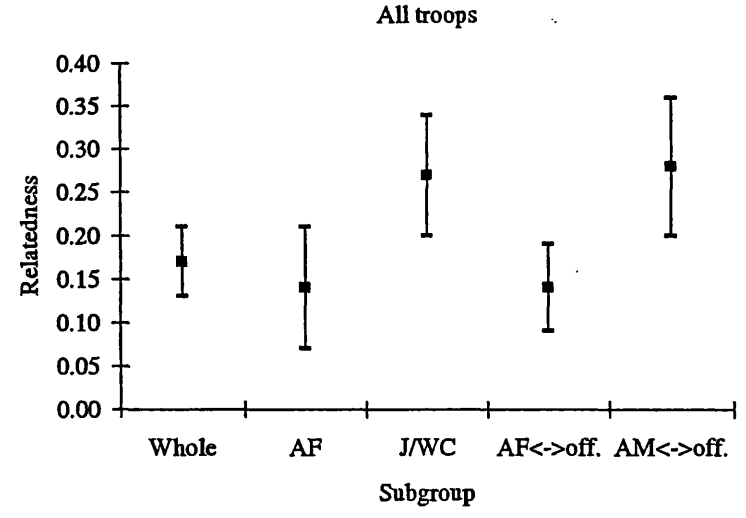


FIGURE 4.1 (cont.)

KINSHIP networks were drawn for Kailana, Chandpol B, Bijolai and Bhadreshwar (FIGURE 4.2a,d,e,f) using the dyads that had significant likelihood when H_1 was set at R = 0.5, against H_0 of R = 0.0. However, for both Chandpol A and Nimba this gave too many relationships with significant likelihood to produce a discriminating network. Chandpol A's network was hence drawn using the relationships with significant likelihood from the test of R = 0.5 against R = 0.25. This allowed a network of all individuals to be constructed (FIGURE 4.2b).

For Nimba, it was only possible to create a network of adult females from significant dyads from the test of R = 0.5 against R = 0.0 (FIGURE 4.2c(i)). Amongst all troop members there were many relationships that had significant likelihood at $p < 0.001$; all these closely related individuals shared an allele that was seen very infrequently in the rest of the population (186 at D16S420). Overall, there was a very high proportion of relationships with significant likelihood, which made producing an interpretable network extremely difficult. Therefore, the pairwise relatedness values and the likelihood calculations were repeated for Nimba, using allele frequencies calculated from Nimba only. This corrected for the large skew in significant likelihoods that was caused by the rare allele. A network was created from this data of dyads with significant likelihood from the test of R = 0.5 against R = 0.0 (FIGURE 4.2c (ii)).

For each troop, and for the AMB, it was possible to draw networks that comprised at least five individuals linked together by significant likelihood values. Conversely, in each group there was at least one individual that did not have a significant likelihood of relatedness to any other individual in the group. There was variation across the groups in how the significant likelihood ratio predicts the genetic relatedness of dyads. For example, in Chandpol A, there were 66 dyads out of a possible 210 with a significant likelihood ratio ($p < 0.05$) when tested at R = 0.5 against R = 0.0, but only 37 of these shared at least one allele at every locus. Similarly in Nimba, 14.4% (22/153) of all possible dyads were found to be significant at $p < 0.001$, but only 6.5% (10/153) shared alleles at every locus. Overall, the number of significant dyads that shared alleles at each locus varied between 96.6% in Kailana to 40.0% in Bhadreshwar (TABLE 4.2).

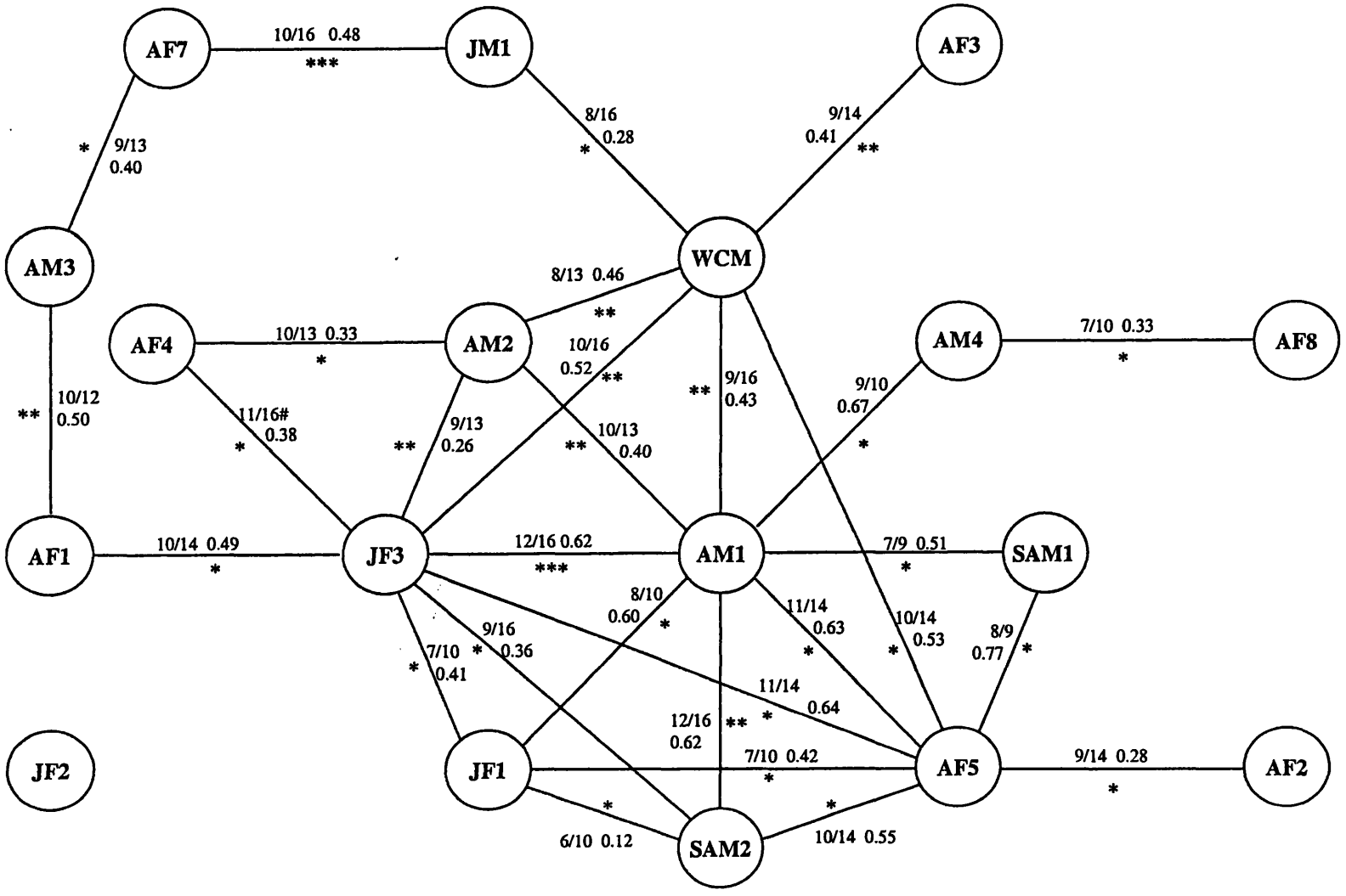


FIGURE 4.2 Kinship networks. For each dyad, allele sharing (x/y), pairwise relatedness values and significance levels of likelihood ratios for the hypotheses H_0 r=0 and H_1 r=0.5 are shown (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Allele sharing scores marked with # indicate that the dyad can be excluded as a parent-offspring relationship, as they do not share alleles at each locus. Lone individuals are not part of any significantly likely relationships.
(a) Kailana relationships.

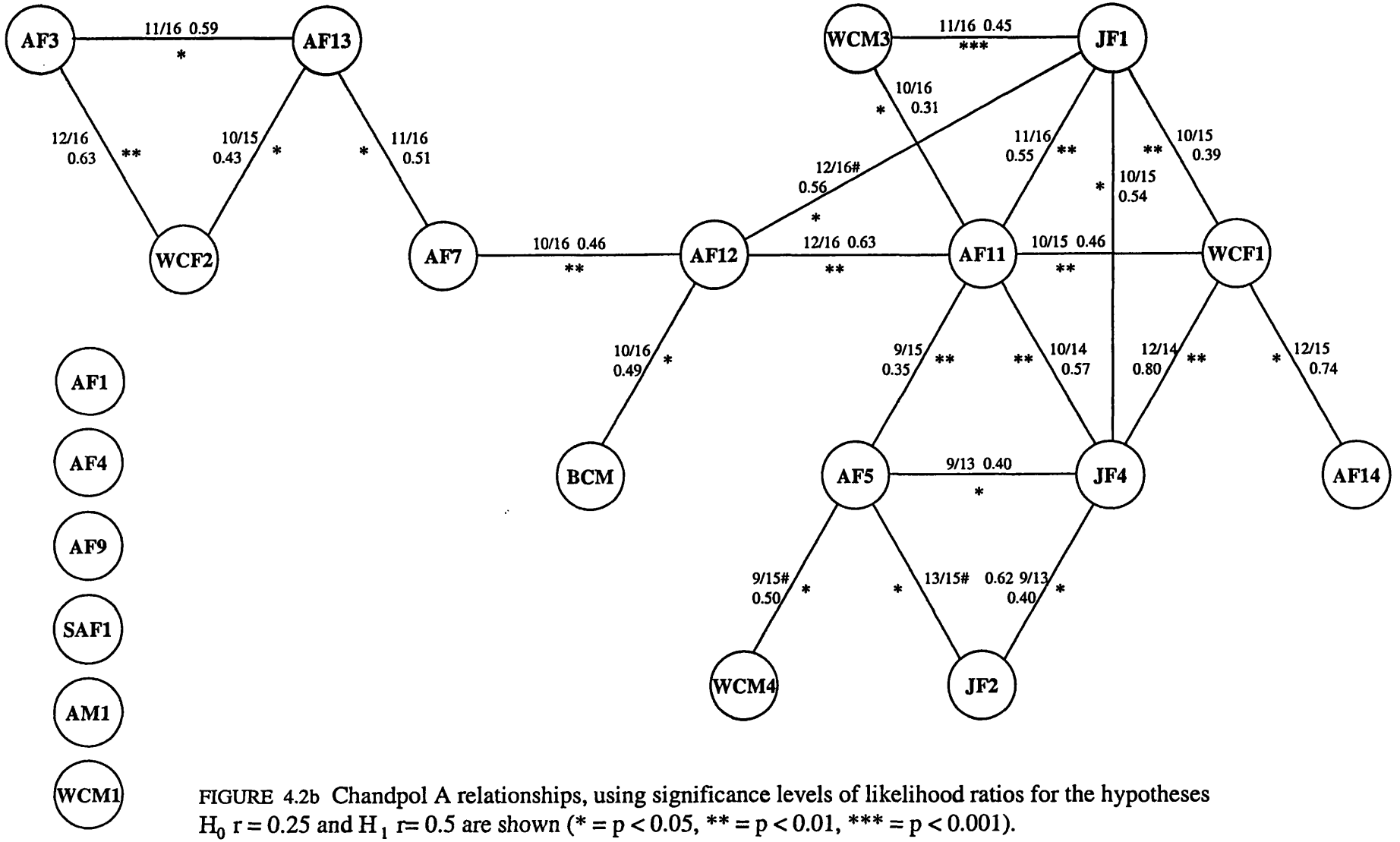


FIGURE 4.2b Chandpol A relationships, using significance levels of likelihood ratios for the hypotheses H_0 r = 0.25 and H_1 r= 0.5 are shown (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

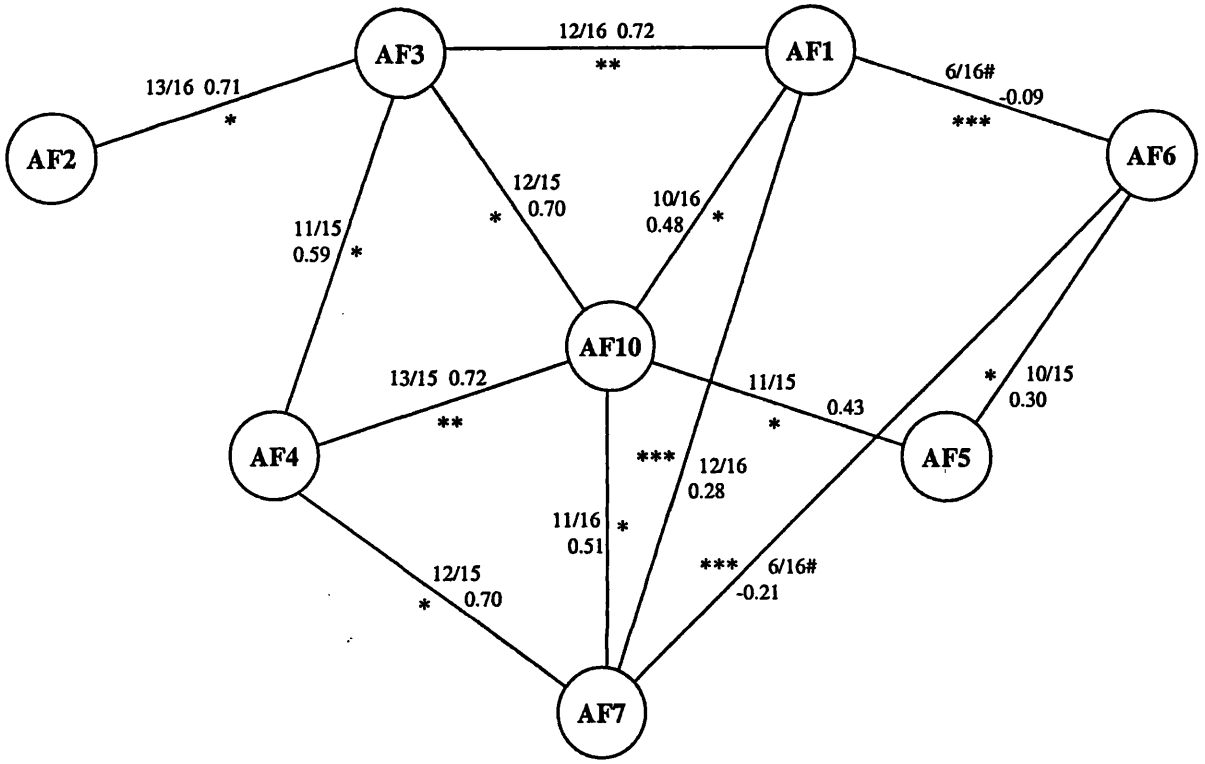


FIGURE 4.2c(i) Nimba adult female relationships (based on whole population allele frequencies).

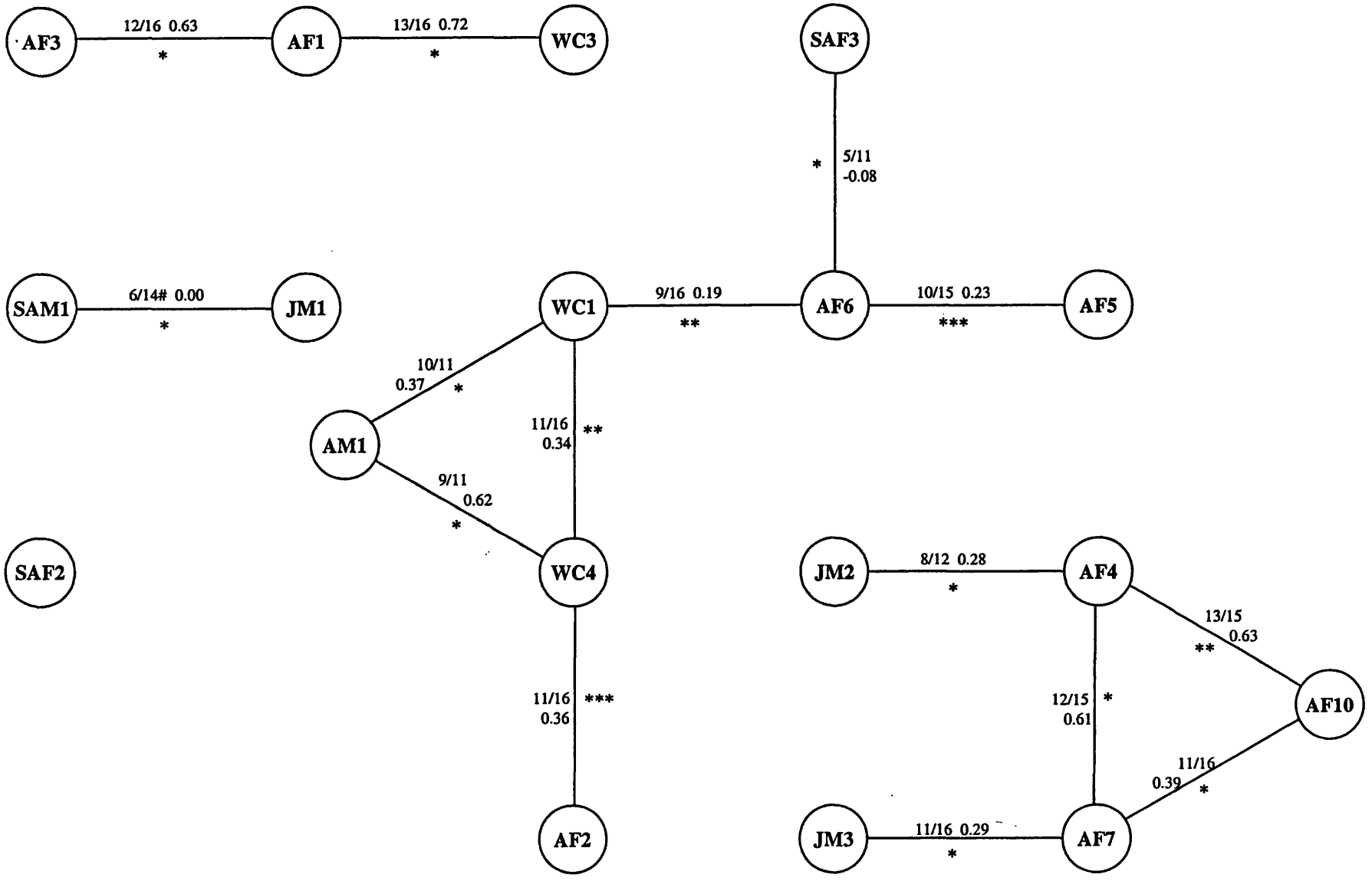


FIGURE 4.2c(ii) Nimba relationships (based on allele frequencies derived from Nimba alone).

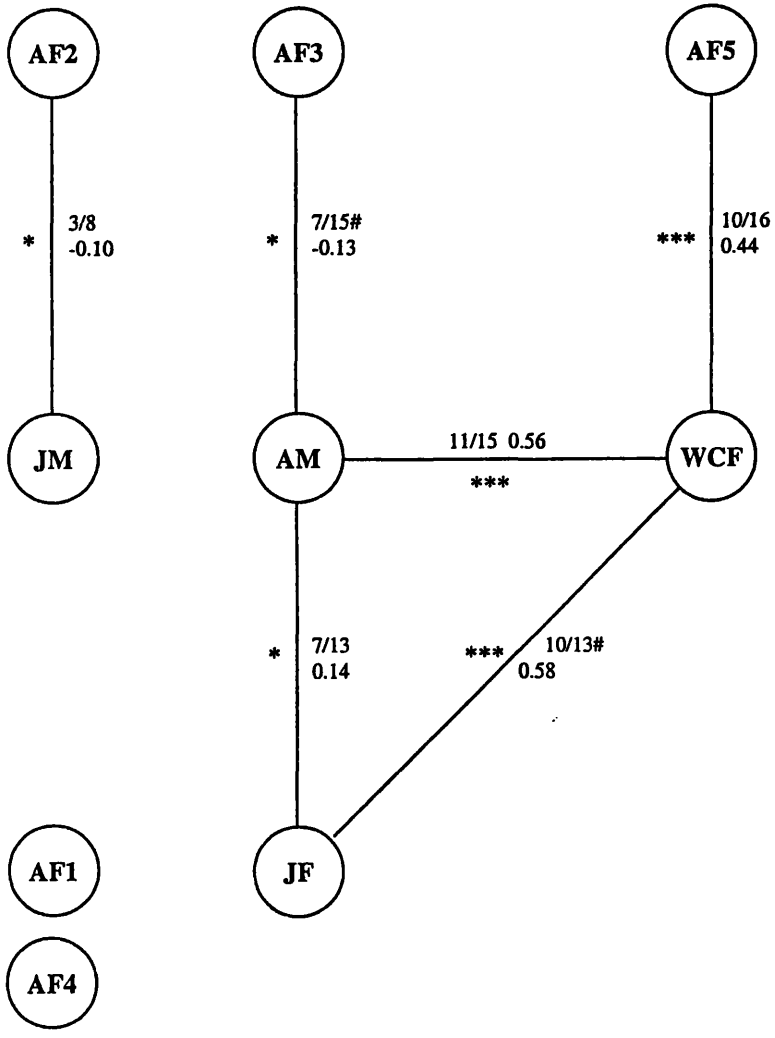


FIGURE 4.2d Bijolai relationships.

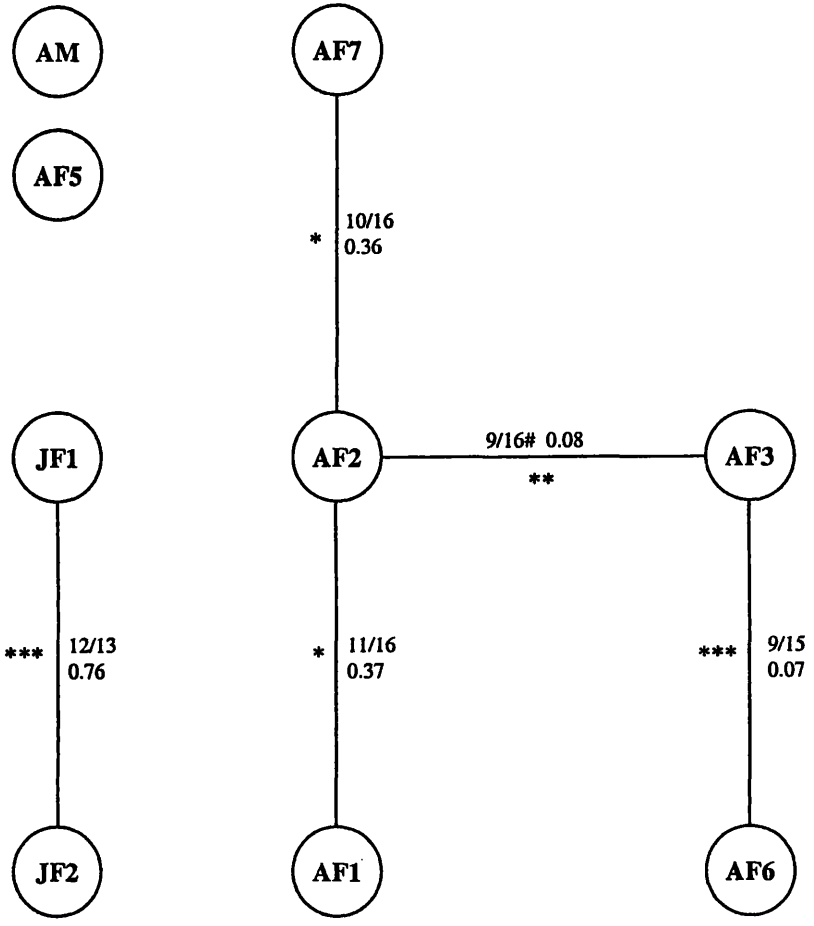


FIGURE 4.2e Chandpol B relationships.

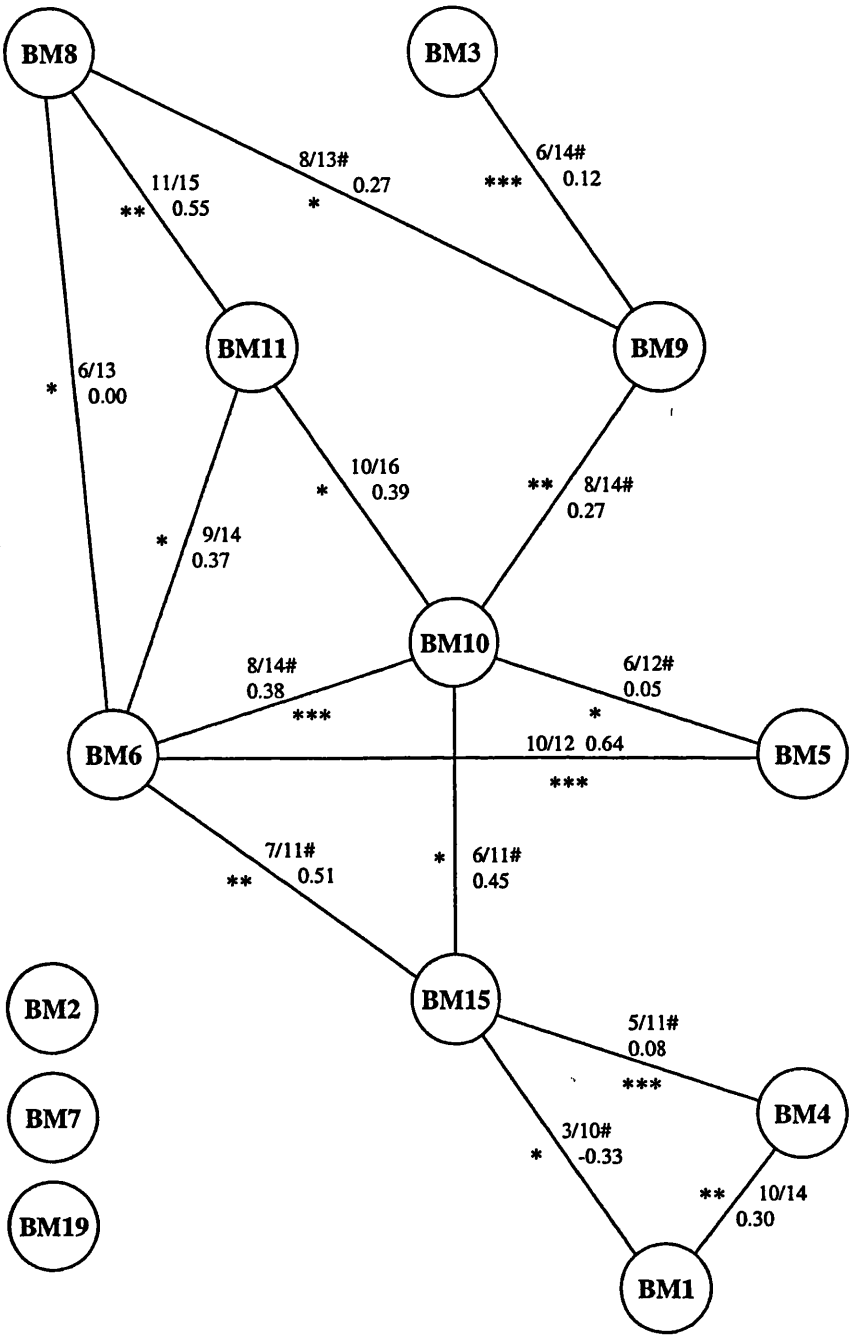


FIGURE 4.2f Bhadreshwar AMB relationships.

TABLE 4.2 The proportion of pairwise relationships in each troop and AMB which have significant likelihood and are potential parent-offspring relationships, based on allele sharing.

	Kailana	Chandpol A	Nimba	Chandpol B	Bijolai	Bhadreshwar
% of all dyads with significant likelihood (n)	19.0 (29/153)	31.4 (66/210)	28.8 (44/153)	11.1 (5/45)	16.7 (6/36)	19.2 (15/78)
% of all dyads with significant likelihood and share alleles at each locus (n)	18.3 (28/153)	17.6 (37/210)	19.0 (29/153)	8.9 (4/45)	11.1 (4/36)	7.7 (6/78)
% of significant dyads that share alleles at each locus (n)	96.6 (28/29)	56.1 (37/66)	65.9 (29/44)	80.0 (4/5)	66.7 (4/6)	40.0 (6/15)

Additionally, pairwise relatedness values were compared with the significance level of the likelihood ratio. This was carried out for both relatedness values for all dyads with significant likelihood, and for only those not excluded by allele sharing. For both data sets, the mean relatedness increased with increasing significance; the average relatedness values were higher when only non-excluded dyads were considered (FIGURE 4.3).

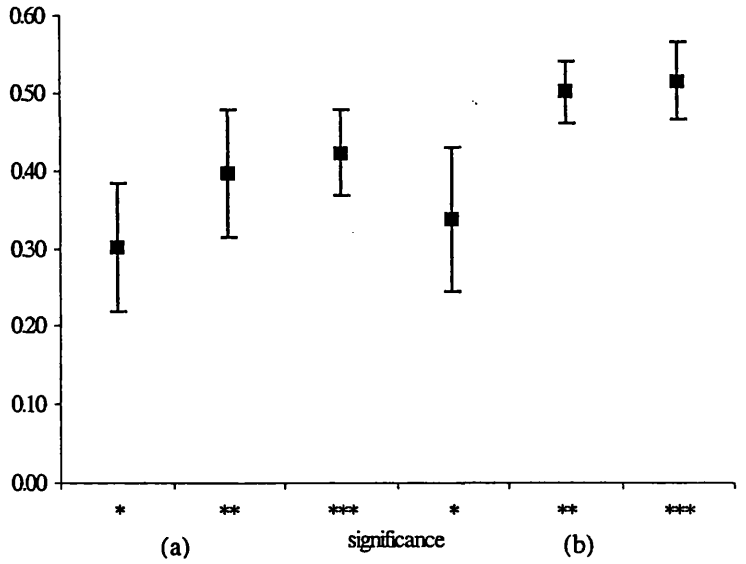


FIGURE 4.3 Relatedness scores of dyads with significant likelihood at *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ for (a) all dyads and (b) dyads of potential parent-offspring relationships based on allele sharing.

Kailana

This troop differed from the others in the presence of several adult and subadult males. All these males were connected to at least two other individuals in the troop, suggesting that they were related to other troop members. The key individual of Kailana was the resident male, AM1, located in the centre of the network. He was part of eight dyads with significant likelihood, a result equalled only by JF3, also partnered with eight other individuals. The adult females were dispersed throughout the network; there was only one dyad of adult females with significant likelihood between them, out of 21 possible. In contrast, the adult and subadult males grouped together, with all but one (AM3) connected to the resident.

Only one individual, JF2, was not joined to another; three were joined to only one other. The only known mother-offspring relationship, AF3-WCM, was represented in the network. Of all the dyads with significant likelihood, only one could be excluded as a parent-offspring relationship through allele sharing. All the relatedness values in the network exceeded 0.25, that of half-sibs, and the mean was 0.49.

Chandpol A

In Chandpol A, all individuals save one (SAF1) had relationships with significant likelihood with at least one other individual at $R = 0.5$ against $R = 0.0$, and 66 out of a possible 210 relationships had significant likelihoods, so the network was created using relationships with significant likelihood from the more stringent test of $R = 0.5$ against $R = 0.25$, differentiating between first and second degree relationships. This produced a clearer network, since more dyads were excluded from it. Six individuals did not form parts of any dyads with significant likelihoods, out of a total of 21 group members. There was a central individual, AF11, related to six other individuals, five of them at $p < 0.01$. There were only 3/22 dyads that could be excluded as potential parent-offspring relationships through allele sharing; the average R of all dyads was 0.52. Few mother-offspring relationships in the troop were known; AF12 was a probable candidate for the mother of BCM from behavioural observation, and this was borne out by the network. The only certain mother-offspring relationship was AF7-WCM3, and this was not represented as a dyad with significant likelihood in this network (it was significant at $p < 0.05$ under the less stringent hypothesis; pairwise relatedness = 0.21).

Nimba

Nimba adult females formed a very tightly interrelated group of individuals. All but one of them had relationships with significant likelihoods with two or more other adult females. AF10 formed the most dyads with significant likelihood, with five other females. There was also a high proportion (3/13) of highly significant ($p < 0.001$) dyads. However, two of these were not vertical first degree relatives, and had negative relatedness scores. These three females were the only adult females to have the rare 186 allele at D16S420, a fact which suggested that they are of common descent, even if not mother-daughter dyads. Throughout the whole troop, a likelihood ratio score of $p < 0.001$ seemed to be an indicator of possessing this allele, rather than more discriminating relatedness, when the hypotheses are tested using allele frequencies calculated from the whole population. It was because of the inflated relatedness scores and likelihood values this allele produced that the relatedness and likelihood ratios were recalculated for Nimba using only the troop's allele frequencies, in an attempt to give a more informative representation of relationships within the troop. This had the effect of creating a fragmented network of the troop. The largest subgroup comprised seven individuals, including the resident male, adult females and two white coats. A second subgroup comprised three adult females and two juvenile males; the rest of the troop divided into a trio, a dyad and a single subadult female, not related to any other individual with significant likelihood. Only one of the dyads did not share alleles at each locus, between SAM1 and JM1. The average relatedness of the dyads with significant likelihood was 0.46 when calculated using the whole population allele frequency, but fell to 0.37 when based on Nimba's allele frequencies only.

Chandpol B

This group had very few dyads with significant likelihoods. WCF1 was so incompletely genotyped that it was not possible for KINSHIP to calculate the likelihood ratios, hence it was not included in the network. The two juvenile females formed a discrete dyad unconnected to the other troop members. The adult females formed a distinct subgroup together, though one of the four dyads with significant likelihood joining them did not share alleles at each locus. The adult male did not feature in the network, not being related to any troop member with significant likelihood. The average R value of the dyads with significant likelihood was 0.33.

Bijolai

Bijolai had very few relationships with significant likelihood, but those that existed were highly significant (3/6 are $p < 0.001$). This was, as in Nimba, the result of a rare allele – 157 at D14S306 – that caused the individuals that had this allele to appear as very probable relatives. Bijolai comprised two subgroups, one containing AF2 and JM, the other including the resident male, two adult females and two offspring. The dyad AF2–JM was also assigned by CERVUS as a mother-offspring relationship (see Chapter 5), though behavioural observations showed AF4 to be the mother, a relationship not discounted by allele sharing. Two adult females remained without relationships with significant likelihood; these individuals were incompletely genotyped, which meant that not all likelihood ratios could be calculated for them. The average R of dyads with significant likelihood was 0.24.

Bhadreshwar AMB

Out of this group of thirteen males, ten could be formed into a continuous network; three were left without relationships with significant likelihood to any other. However, out of the 15 dyads, only six (39%) shared alleles at each locus, a far lower figure than was seen for any of the troops. The average R of the dyads with significant likelihood was 0.27.

4.4 DISCUSSION

4.4.1 Relatedness scores of subgroups

Troop relatedness

Female social groups are a reflection of females needs to maximise their reproductive output. Individual females' reproductive success is limited by access to resources and their avoidance of predation and intraspecific threats. These goals often require the formation of coalitions to outcompete rival groups (Wrangham, 1980). Hamilton's (1964) inclusive fitness theory predicts that it is more advantageous to cooperate with close relatives rather than with unrelated individuals. Also, because it is the females of a group that require reliable resources to rear their offspring, it is more advantageous for them to be the philopatric sex as they build up a familiarity, and therefore foraging efficiency, in an area. In contrast, the males disperse away from their natal group. They are most likely ousted from their natal group by new males as they represent, or will

grow to represent, competitors (Rajpurohit and Sommer, 1993). As a result, if they do gain a residency, it will be in another troop, so inbreeding is also avoided.

Female philopatry should lead to the females in a social group being related through the female line. Additionally, in a harem situation, one resident male should father a cohort of offspring, and his genetic input will link relatedness between female lines. Successive cohorts will therefore be paternal sibs (through their father), and also the offspring of paternal sibs (through their mothers). After generations, this should lead to a high degree of relatedness between all the individuals of a troop. The only troop member who is not likely to be closely related is the resident male if he is new to the troop and has not yet sired any offspring. In this case he is likely to be unrelated to all troop members, and have pairwise R values of around 0 with other troop members.

The overall relatedness score of all the Jodhpur troops was 0.17 ± 0.04. This supports the hypothesis that matrilineal troops are closely related – this value represents an average relatedness exceeding that of cousins. This compares with a value of 0.07 ± 0.04 in long-tailed macaque social groups (de Ruiter and Geffen, 1998) and 0.07 in Taï forest male (non-dispersing) chimps (Vigilant *et al.*, 2001). It thus appears that the relatedness of the Jodhpur troops is remarkably high. This could be attributed to the colobine social organisation. The long-tailed macaques live in strongly matrilineal groups, within which relatedness can be high (max. 0.34; de Ruiter and Geffen, 1998), but between which relatedness can be very low, and highly variable. The overall relatedness of a troop results from the combined relatedness of these matrilines. In contrast, the langurs are not obviously divided into discrete matrilines within troops. They have a more egalitarian dominance structure to their troops, which is not dependent on matrilineally based coalitions but on age (Borries *et al.*, 1991). A resident will sire offspring with many of the females of the troop, depending on the length of his residency. As a result, cohorts of individuals will be related by 0.25 through the resident. This paternal input from successive residents will maintain a high degree of relatedness between all members of the troop. The relatedness of male chimpanzees is not as high as that of the female langurs, despite that they are the non-dispersing sex, for they are the offspring of unrelated dispersing females who have joined the group. A single chimpanzee female cannot produce such a large number of related offspring as a langur male, and therefore one individual cannot raise the relatedness of a social group in the same way as a langur male.

However, this figure of high troop relatedness concealed a great deal of variation between troops which was revealed when individual troop values are calculated; close examination of relatedness of different subgroups was needed to explain the inter-troop differences. Kailana, Chandpol A and Nimba all had relatedness values of around 0.2, whereas Bijolai was only 0.07, and Chandpol B was 0.00. These values were low for troops that supposedly comprise close relatives. By breaking the troops down into subgroups, it was possible to investigate from where this disparity between the troops arose.

Adult females

The average R for the relatedness of the adult females of the troop was 0.14 ± 0.07. This falls within the estimates of average coefficients of relatedness of females in a one-male troop calculated by Seger (1977). He employs an iterative process to model the values of R within a one male troop, varying male tenure length and initial relatedness of females. Whether the females are initially unrelated (R = 0), or perfectly related (R = 1), whether the tenure of the male is 3 or 4 years and varies from one tenure to the next, adult females always tend to become related on average by at least 0.125, but by up to as much as 0.18.

However, when the relatedness of the adult females was considered separately, there was again large variation between troops. Chandpol A and Nimba had high values, resulting from the high relatedness of group females through both maternal and paternal lines. These figures were similar to those seen for the higher ranking matrilines in long-tailed macaques (de Ruiter and Geffen, 1998). High ranking macaque matrilines are thought to be highly related because the dominant male of the group preferentially mates with high ranking females; thus the relatedness values for high ranking matrilines should more closely reflect values that could be expected in a one-male troop. However, the langur troops' values were very high when compared to those of Seger's (1977) model. One explanation is that the troops were relatively newly formed. It is thought that Nimba did not exist as recently as 1988 (Vogel, 1988), though Chandpol A's history is unknown. Formation of new troops is rarely documented in the Jodhpur population, but it is likely to be by troop fission (Mohnot *et al.*, 1981). This could occur either along matrilineal lines – a mother and her female offspring – or on an age basis, with female paternal sibs leaving to form a new troop. Either case could result in founding females having higher relatedness than the overall value for the females. According to Seger's model, a troop initially related by 0.4, for example, may still be

related by 0.25 after 80 years. Although relatedness ultimately tends to between 0.125 and 0.18, it may take many generations to do so. Founding effect may account for the high relatedness of Nimba and Chandpol A females.

However, Kailana, Chandpol B and Bijolai all had extremely low values of adult female relatedness. This would account for the low levels of troop relatedness of Chandpol B and Bijolai, where the adult females formed the majority of the troop members (6/10 and 5/9 individuals respectively). In Kailana, the adult females comprised 7/18 troop members, so their low relatedness may not have had as great an effect on the overall R of the troop. These results for female relatedness do, however, beg the question of why these females appeared so unrelated, when it would be expected that there was a high level of relatedness in matrilineal groups.

There are two potential explanations, not mutually exclusive, for the Bijolai data. Only two out of five adult females were completely genotyped. The data that do exist are probably insufficient to give a good estimate of relatedness for five individuals. Additionally, Bijolai was decimated in 1969 by a suspected poisoning incident (Mohnot, 1971), reducing the troop from 82 to 11 individuals. It could be the case that these survivors were not as closely related as the entire group before it was reduced in size, and according to Seger's model, relatedness also takes some time to become established from unrelated individuals. This is highly speculative, in view of the lack of genotype data. However, lack of data was not the cause of the low relatedness scores in Kailana and Chandpol B. In these troops there were seven and six females genotyped almost completely.

For Kailana, a network of adult females was created by joining those individuals which shared alleles at every locus (FIGURE 4.4a), along with the pairwise relatedness values for each dyad. This network identified some outlying individuals, notably AF4 and more so, AF7. For every combination of three, four, five and six individuals from these females, the relatedness was calculated (TABLE 4.3). In these permutations it became clear from inspection, those groupings containing AF7 (and AF4 to a lesser extent) had lower relatedness values.

For the group of six adult females (excluding AF7) a putative pedigree could be constructed (FIGURE 4.4b). This network may not represent all real genealogical relationships, but it takes into account the observed allele sharing between individuals, pairwise relatedness scores, and their relative ages, as far as they were known. In

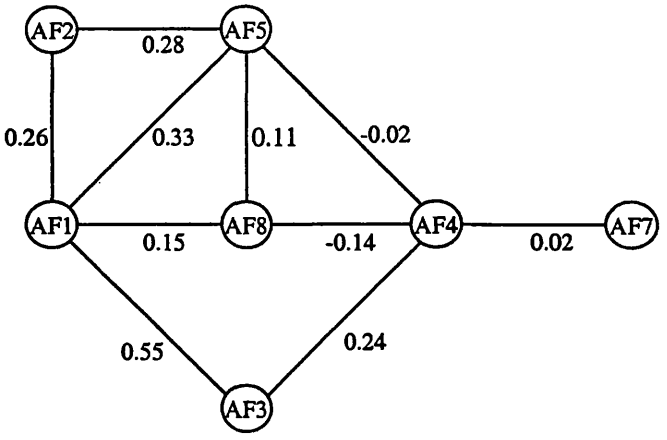


FIGURE 4.4a Network of Kailana adult female relationships that cannot be excluded as potential vertical first degree relatives by allele sharing.

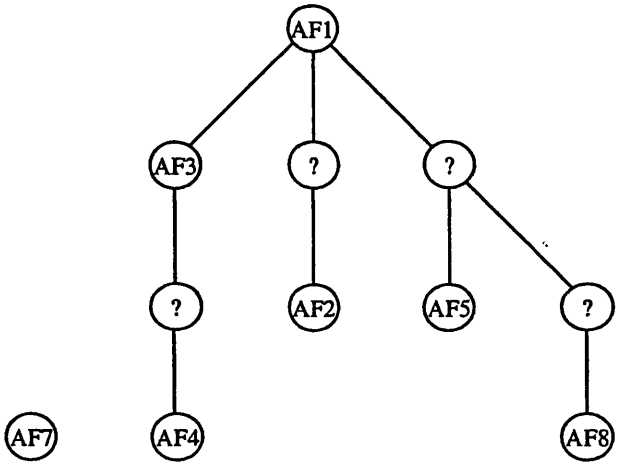


FIGURE 4.4b Putative pedigree of Kailana adult females, based on possible vertical first degree relationships and pairwise relatedness scores.

AF ID	R	AF ID	R	AF ID	R
1,2,3	0.35±0.20	1,2,3,4	0.20±0.14	1,2,3,4,5	0.19±0.13
1,2,4	0.08±0.10	1,2,3,5	0.29±0.17	1,2,3,4,7	0.00±0.07
1,2,5	0.29±0.13	1,2,3,7	-0.04±0.10	1,2,3,4,8	0.07±0.13
1,2,7	-0.10±0.12	1,2,3,8	0.11±0.17	1,2,3,5,7	0.08±0.10
1,2,8	0.02±0.13	1,2,4,5	0.15±0.10	1,2,3,5,8	0.16±0.14
1,3,4	0.24±0.20	1,2,4,7	-0.05±0.06	1,2,3,7,8	-0.04±0.10
1,3,5	0.27±0.19	1,2,4,8	-0.02±0.09	1,2,4,5,7	0.06±0.08
1,3,7	-0.12±0.12	1,2,5,7	0.10±0.12	1,2,4,5,8	0.07±0.09
1,3,8	0.14±0.22	1,2,5,8	0.14±0.12	1,2,4,7,8	-0.05±0.08
1,4,7	0.05±0.10	1,2,7,8	-0.07±0.13	1,2,5,7,8	0.06±0.13
1,4,8	0.03±0.11	1,3,4,5	0.18±0.14	1,3,4,5,7	0.06±0.10
1,5,4	0.13±0.13	1,3,4,7	0.00±0.08	1,3,4,5,8	0.11±0.13
1,5,7	0.15±0.23	1,3,4,8	0.09±0.16	1,3,4,7,8	-0.01±0.10
1,5,8	0.18±0.20	1,3,5,7	0.06±0.13	1,3,5,7,8	0.05±0.14
1,7,8	0.03±0.25	1,3,5,8	0.16±0.16	1,4,5,7,8	0.07±0.14
2,3,4	0.13±0.14	1,3,7,8	-0.05±0.14	2,3,4,5,7	0.01±0.07
2,3,5	0.22±0.21	1,4,5,7	0.09±0.13	2,3,4,5,8	0.04±0.11
2,3,7	-0.22±0.11	1,4,5,8	0.08±0.12	2,3,4,7,8	-0.11±0.07
2,3,8	-0.05±0.19	1,4,7,8	0.02±0.13	2,3,5,7,8	-0.02±0.10
2,4,7	-0.19±0.08	1,5,7,8	0.11±0.22	2,4,5,7,8	-0.02±0.10
2,4,8	-0.18±0.09	2,3,4,5	0.13±0.13	3,4,5,7,8	-0.01±0.10
2,5,4	0.07±0.08	2,3,4,7	-0.11±0.07	1,2,3,4,5,7	0.07±0.08
2,5,7	0.04±0.14	2,3,4,8	-0.03±0.13	1,2,3,4,5,8	0.11±0.11
2,5,8	0.05±0.11	2,3,5,7	0.00±0.09	2,3,4,5,7,8	-0.02±0.08
2,7,8	-0.21±0.14	2,3,5,8	0.08±0.14	1,2,3,4,7,8	-0.03±0.08
3,4,7	-0.12±0.08	2,3,7,8	-0.17±0.10	1,2,3,5,7,8	0.05±0.10
3,4,8	-0.03±0.15	2,4,5,7	0.01±0.08	1,2,4,5,7,8	0.03±0.10
3,5,4	0.08±0.14	2,4,5,8	-0.01±0.08	1,3,4,5,7,8	0.05±0.11
3,5,7	-0.07±0.15	2,4,7,8	-0.15±0.08		
3,5,8	0.07±0.16	2,5,7,8	0.00±0.14		
3,7,8	-0.21±0.16	3,4,5,7	0.00±0.09		
4,7,8	-0.03±0.13	3,4,5,8	0.03±0.12		
5,4,7	0.06±0.13	3,4,7,8	-0.09±0.10		
5,4,8	-0.01±0.11	3,5,7,8	-0.03±0.15		
5,7,8	0.08±0.26	4,5,7,8	0.03±0.15		

TABLE 4.3 Relatedness of permutations of females in Kailana troop. AF ID = ID number of adult females; R = relatedness of permutation, calculated by RELATEDNESS.

Kailana, AF1 appeared very much older than all the other females, and was accordingly placed at the head of the pedigree. If the relatedness values that would result from such a pedigree are calculated, the overall mean relatedness of these six females is 0.15, which is close to the relatedness value derived from the data for these six females (0.11 ± 0.11). However, when AF7 is included, this value is reduced to 0.04 ± 0.09. There are two possible explanations for the change in relatedness caused by this individual. AF7 could have immigrated into the troop from outside, and be unrelated to other troop members. Long-term behavioural studies of the Jodhpur population have witnessed extremely few female dispersers over a 30 year period (Makwana and Advani, 1981; Mohnot *et al.*, 1981); it is widely assumed to be the case that females remain in their natal troops throughout their lives. If the rest of the females in the troop were close relatives, then it would not be in their inclusive fitness interests to allow an unrelated female enter the troop - she would represent another competitor for limited food resources, with no kin selection benefits to the rest of the troop. Similarly it is unlikely that a female would leave her natal troop, where the benefits of kin selection are available to her. These costs may be countered to a degree by the value of having another troop member willing to defend resources from neighbouring troops.

A second way which would cause AF7 to appear so unrelated to the other adult females would be if she was the daughter of a resident male who fathered no other remaining offspring in the troop, and from whom AF7 had inherited alleles that were rare to this troop, or indeed in the population as a whole. For example, she had a 194 allele at D16S420, which no other Kailana adult female had, and was very rare in the population (f = 0.025). Additionally, she was homozygous for 153/153 at D14S306, an allele which occurred only once in the other adult females, in the heterozygote AF4. As a result of this, AF4 was the only female to which AF7 could be a vertical first degree relative (parent-offspring); the pairwise relatedness of these two females was 0.02. It is probable that these two alleles that were rare to the troop were critical in reducing the relatedness of AF7 to all the other adult females, and at the same time reducing the overall R of Kailana adult females. A similar case was seen in long-tailed macaques, where the addition of an unrelated individual to a group of three related individuals reduced the overall group relatedness by 50% (de Ruiter and Geffen, 1998). Unfortunately, the lack of records of male residents in the langur troops studied means that there is no demographic data to support the theory that changing residents bring rare alleles to the troop.

A similar situation is proposed for Chandpol B. There were also rare alleles in this troop, notably 282 at D3S1766, and 166 at D123S75, and 198 and 206 at D16S420. These could, again, have been introduced by a previous resident/ residents. It may be this fact that made, for example, the pairwise relatedness of AF1 to AF3 and AF5, 0.05 and 0.07, respectively, despite the fact that they shared alleles at each locus. A network drawn of females that shared alleles at each locus has one 'matriarch' to whom three other females are linked; two of these are linked to one other female (FIGURE 4.5a). In comparison to the seven Kailana females, this network is more open, with fewer potential parent–offspring relationships for each female; this may be as a result of a higher incidence of rare alleles.

A putative pedigree of adult females was constructed (FIGURE 4.5b) according to the incidence of possible parent–offspring relationships. Calculating the pairwise relatedness values for all female dyads, the overall relatedness was 0.26. This is in contrast to the value from the hypothetical Kailana pedigree, which matched the observed value closely. It seems to be that successive residents bringing different, and sometimes rare, alleles into the troop, can greatly reduce the relatedness of a subgroup across generations. Whilst it is the case that the regular replacement of the resident male who goes on to father a cohort of infants will contribute substantially to the relatedness of that cohort, it seems also possible that widely different alleles will greatly reduce the relatedness of one cohort, and therefore female generation, to the next. The relatedness of the adult females of a troop may therefore be very low, because a series of successive cohorts are being considered altogether. This process is similar to that suggested by Seger (1977), where parallel matrilines may develop within a troop, depending on the variance of male tenure. Separated matrilines may only rarely be linked by a common resident, boosting the relatedness between them, but in between these linking events, relatedness between these matrilines can be less than 0.001.

Non-Adults

The average relatedness of non-adults in the troops was 0.26 ± 0.06. This is extremely close to the theoretical value of 0.25 for cohorts of paternal half-sibs, when the mothers are unrelated. It would be expected that R might be higher than that of paternal half sibs, as cohorts of sibs should be related not only through their fathers, but also through their mothers, who are related, as seen previously. Indeed, de Ruiter and Geffen (1998) found that paternal half sibs in long-tailed macaques were related by 0.35, explained by taking into account the relatedness of the mothers (0.14) as well as the paternal half-sib

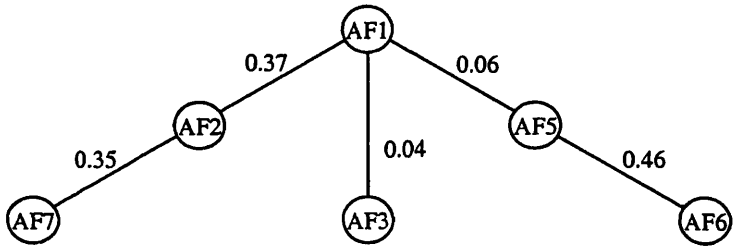


FIGURE 4.5a Network of Chandpol B adult female relationships that cannot be excluded as vertical first degree relatives.

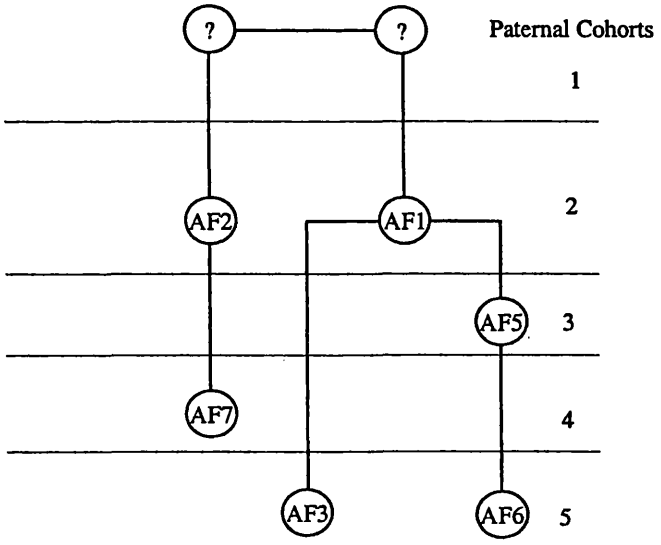


FIGURE 4.5b Putative pedigree of Chandpol B adult females, based on possible vertical first degree relationships and pairwise relatedness scores; dotted lines indicate succesive residencies.

figure of 0.25. Their theoretical calculation of paternal sib relatedness, 0.25 + 0.5 (0.14) = 0.32, was very close to the observed value. However, in the langurs there was wide variation between the different troops in the values of non-adult relatedness.

Kailana's non-adults had an average R to one another of 0.32. This suggests that there was a component of maternal relatedness that contributes to this value. de Ruiter and Geffen (1998) proposed that:

$$R_{\text{paternal sibs}} = R_{\text{paternal half sibs}} + 0.5\, R_{\text{reproductive females}} \quad (1)$$

This formula provided a close figure to that observed for paternal sibs in macaques, but the derivation is flawed, because relatedness through the mothers is mediated by two generation links, or $(0.5)^2$.

$$R_{\text{paternal sibs}} = R_{\text{paternal half sibs}} + 0.25\, R_{\text{reproductive females}} \quad (2)$$

In Kailana, the relatedness of the females was 0.04, so from equation (2), these non-adults should be related by 0.26. Returning to the idea that the adult females were more closely related, but their cohort value was reduced by one individual, the new value for $R_{\text{paternal sibs}}$, excluding AF7, was 0.25 + 0.25 (0.11) = 0.28, slightly higher, and closer to the observed value. In this case, it does not seem that the effect of the rare alleles in the maternal cohort had repercussions in the relatedness of the offspring, despite the fact that the rare allele had been inherited by AF7's offspring, JM1.

In Chandpol A, the relatedness of the non-adults was relatively low (0.14 ± 0.08). In a troop where the relatedness of the adult females was relatively high, this could be explained by the influence of successive residents. The age range of the non-adults spanned over four years, from black coats to sub-adults, and they were unlikely to have been fathered by the same resident, given that the average tenure is 27 months (Sommer and Rajpurohit, 1989). Additionally, if assigned mothers and offspring genotypes were compared, then there were up to four paternal alleles per locus indicated, suggesting that at least two residents fathered these offspring. Calculating relatedness for yet smaller subgroups of the non-adults, on an age basis, increased R values. This suggests that the non-adults of Chandpol A were the offspring of several consecutive resident males. For example, when SAF1, JF1 and JF4 were grouped, their relatedness was 0.35 ± 0.09. Similarly, when the three oldest white coats, WCF1, WCF2 and WCM1 were grouped, R = 0.27 ± 0.20. This is another example, as with the adult females, that successive males fathering offspring could cause lower values of R than would be expected for

subgroups of the troop. Another, non-genetic, indication that there had been a resident change in the midst of the conceptions of these non-adults was the observation that there were no subadult or juvenile males in the troop. It is possible that young males had been ousted during a takeover (Rajpurohit and Sommer 1993) and unweaned males that were present in the troop at the time of takeover were victims of male directed infanticide (Sommer, 1994). The perpetrator of this may have gone on to father the white coat males present in the troop at sampling.

Nimba non-adults had a higher relatedness than Chandpol A, at 0.26, but it was still not as high as would be expected if they were related as paternal half sibs. Their mothers' cohort was related by 0.32, so it would be expected that (from equation (2)) the paternal contribution to relatedness was 0.18. As a cohort sired by a single father should show a paternal contribution of 0.25, this suggests, as in Chandpol A, that these offspring had been sired by more than one resident. Indeed, if the oldest non-adult (SAM) was excluded from the subgroup, R increased to 0.32 ± 0.07. This agrees with the theoretical figure (0.25 + 0.25 (0.32) = 0.33) and suggests that it is indeed the case that all the offspring save the oldest subadult were fathered by the current resident. This is supported by parentage assignments (Chapter 5).

Chandpol B non-adults had a high R value (0.35 ± 0.16), though it must be noted that there were only three individuals. It was higher than would be expected for paternal sibs according to equation (2), because the relatedness of the adult females in this troop was so low; the expected figure for paternal sibs in this troop would be 0.25 + 0.25 (0.04) = 0.26. The inflation of this value was attributed to the rare alleles 174 at D6S271, and 157 at D14S306, which would make them statistically very different from the rest of the population, and indicated a common father, and to the stochastic variation of R when only three individuals were involved. Again, without knowing the genotype of the sire, or dates of resident changes, there was no supporting evidence for the theory that males introduce rare alleles to offspring cohorts.

Bijolai non-adults (n = 3) had a very high R value (0.49 ± 0.22), exceeding the expected value of 0.25 + 0.25 (0.02) = 0.26. This is suggested to be because of the effects of incomplete genotyping for both the adult females and for the non-adults, causing stochastic variation in the R values of both these subgroups. Additionally, rare alleles also play a part, as 2/3 non-adults had alleles 157 at D14S306 and 160 at D17S791.

Adult females to offspring

Intuitively, it would be expected that the relatedness of adult females to offspring should be 0.5. However, it must be remembered that this is the R value of a female to her own offspring; this is theoretically the highest value that the relatedness of females to offspring can be. (Empirically, R estimates may be higher than 0.5, if the mother and offspring share alleles by chance as well as by descent.) When testing all adult females' relatedness to the entire non-adult cohort, many of the relationships will be those of aunt↔niece/ nephew, or lesser degrees of relatedness, which will lower the overall value of R for these two subgroups. Hence, the actual value for $R_{\text{adult females}\leftrightarrow\text{offspring}}$ for all troops was 0.14 ± 0.05. As with R values for the entire troops, these values showed two distinct distributions, with values of around 0.2 in Kailana, Chandpol A and Nimba, and 0 to negative values in Bijolai and Chandpol B.

A calculation for the estimation of $R_{\text{adult females}\leftrightarrow\text{offspring}}$ is derived here:
In Kailana, there were 7 adult females and 7 offspring. There were therefore 49 pairwise relationships. Of these, 7 were actual mother-offspring relationships, which should have had R = 0.5. The remaining 42 relationships had values of around 0.21, the average relatedness for the troop. $R_{\text{adult females}\leftrightarrow\text{offspring}}$ should therefore be close to:

$$((7 \times 0.5) + (42 \times 0.21)) / 49 = 0.25$$

The negative value for Chandpol B is explained by the rare alleles that the offspring had. These were not found in the adult female subgroup, and had presumably been inherited from their father. A similar situation is proposed for Bijolai, compounded by incomplete genotyping and resulting small sample size.

Adult males to offspring

Adult males are related to their offspring by 0.5. This should be the value obtained for a single resident male and his putative offspring. If the value is lower than this, it would indicate that he did not fathered all the offspring included, either because despite a long tenancy he failed to maintain a mating monopoly, or because some or all of the offspring present were those of a previous resident.

Again, there was great variation across troops. Kailana had the highest value, of 0.48. This agreed with what is known of this troop's history, where the male had been resident for up to six years. This R value suggests that he successfully maintained his

monopoly, despite the presence of other adult males in the troop, a result supported by parentage analysis (see Chapter 5).

The relatively high value (0.27) of $R_{\text{adult male}\leftrightarrow\text{offspring}}$ in Chandpol A is surprising, given the troop's history. The resident male was known to have been new to the troop, joining it after the conception of all the non-adults present. Nevertheless, this high value of R would seem to indicate that he fathered at least some of the offspring present. However, allele sharing data conflicts with this conclusion, as he could be excluded as the father of all but two of the offspring. It is the allele sharing data rather than simulated relatedness figures that should be primarily relied on as a indicator of paternity. This led to another possibility, that the male was related to a previous resident, and therefore was a more distant relative of the offspring. This is extremely difficult to prove, as the identity or fate of the previous resident was not known. Also, as shown earlier by $R_{\text{offspring}}$, it was unlikely that all the offspring shared the same father. It would seem implausible that this male could have been related to all previous residents. Another possible explanation is that the resident male of Chandpol A had no particularly uncommon alleles, which meant that he was statistically similar to a large number of the offspring.

A similar figure for $R_{\text{adult male}\leftrightarrow\text{offspring}}$ was found in Nimba, where it is suggested to be the case that the resident fathered some, but not all, of the non-adults, which could account for the reduced R value. In this troop the resident could only be excluded as the father of one non-adult, SAM1, when the mother's genotype was taken into consideration (Chapter 5). The value may also be reduced from the expected value because of the incomplete genotyping of the male (only 3/8 loci completely genotyped, 11 alleles available for comparison).

The Chandpol B resident had a more extreme lack of genetic data. However, it is possible to see why he appeared to be completely unrelated to the non-adults of the troop (R = -0.45). At one completely typed locus, D6S271, the non-adults possessed a vary rare allele that did not occur in the adult females of the troop and must therefore have been inherited from their father. The adult male did not have this allele, so could be excluded as the father of these individuals, confirming what was inferred from behavioural observations of this troop. There were no young males present, which was indicative of a takeover having recently occurred. In addition to this, Chandpol AMB that was seen in the vicinity contained four very young males, who, it was thought, may

have recently joined the AMB in the company of their father, the ousted resident of Chandpol B. This group transfer was thought to have happened very recently prior to observation/ sampling, as two out of the four young males had disappeared from the AMB two weeks later; one was known to have been electrocuted (A. Chhangani, pers. comm.). This is very much in keeping with the expected high mortality suffered by males as soon as they leave the security of their natal troop (Rajpurohit and Sommer, 1991).

Another indication that the resident of Chandpol B may have been new was the abortion of a foetus by AF7. She was observed to miscarry on 22/12/99 (B. Rajpurohit, pers. comm.), after which she carried the foetus for two days. Miscarriage associated with male change has been reported in the Jodhpur population (Agoramoorthy *et al.*, 1988), and is explained as either a male reproductive strategy, or as a female counterstrategy to later infanticide.

The Bijolai resident had an intermediate level of relatedness with the non-adults (r = 0.27). It was probable that he was the father of all three non-adults. He could only be excluded as the father of B-JF, at one locus (D3S1766) where the juvenile was typed as a homozygote. From examination of the presumed mother's genotype (AF1; Chapter 5), this mismatch was attributed to a possible genotyping error in the juvenile. Rare alleles at D16S420 and D17S791 would indicate a very likely match with the resident if locus D3S1766 is disregarded.

Thus it can be seen that there is great variation in the relatedness of a resident male to the non-adults of a troop, as would be expected, given the occurrence of male replacement in this langur population. The assumption that resident males have monopoly of access to fertile females, as a result of the asynchronous cycling of the females of the troop, and other ecological benefits accrued by the troop resident (Rajpurohit *et al.*, 1995) is upheld by this genetic data.

Bhadreshwar AMB

The low relatedness of the males (0.05 ± 0.08) was as would be expected for a group of individuals with constantly changing membership. The age classes of the sampled individuals were not known, which limits the interpretation of the data. It was expected that the AMB comprised individuals from several natal troops, introducing a wide range of alleles from across the population into one group. The only possible relatives within the AMB might have been a recently ousted resident accompanied by his sons, who

would therefore be paternal sibs (Rajpurohit *et al.*, 1995). Although these groupings are observed in the field, it is not long before the cohort is reduced, due to emigration and mortality. Generally, by 38 months after emigration, no male is thought to be still in the company of relatives (Rajpurohit *et al.*, 1995). The older ranks of an AMB should thus consist of unrelated males, with an average relatedness of zero. The only way in which the relatedness of an AMB is expected to exceed zero is by the presence of an ousted resident and paternal sib subgroup.

The relatedness of the male band can be contrasted to the relatedness of female chimpanzees in social groups. Females are the dispersing sex in chimpanzees, analogous to the langur males. They have been found to have low levels of relatedness in their social groups (0.004, 0.045 and –0.003 in three separate groups; Vigilant *et al.*, 2001). It can be inferred from this genetic data that chimpanzee females also comprise groups of unrelated individuals, though again there is the possibility that mother-offspring pairs, with high relatedness, could be concealed by the unrelatedness of the rest of the group females. A case of this is seen in the dispersing males of long-tailed macaques (de Ruiter and Geffen, 1998). In this species, related males disperse together, or consecutively into the same group. However, the presence of other, unrelated males in their new groups results in the overall male relatedness being markedly reduced.

These primate examples are in contrast to that of the African wild dog. In this species of social, pack-living carnivore, both sexes disperse from their natal pack, but importantly, sexes remain with their peers. This results in a relatedness of 0.28 ± 0.08 (females; n = 8 packs) and 0.35 ± 0.09 (males; n = 5 packs) (Girman *et al.*, 1997). The resulting genetic make-up of the group provides an incentive for individuals to cooperate in hunting, provisioning and protection of the young, to whom all adult individuals of the group are related. This seems to compensate for the fact that only 10% of reproductive success is achieved by subdominant individuals.

It seems to be the case that the dispersing males will cooperate, either in sharing matings or in defence of young, if they are related (wild dogs), but will compete with one another if unrelated (for example, in the langurs). An intraspecies comparison is provided by the African lion. The males of this species are the dispersing sex, and form coalitions to take over prides of females. Males will only form large coalitions with relatives; subordinates will benefit from the reproductive success of related dominant

individuals. Coalitions of unrelated individuals are always small in size, where each male can be assured of gaining direct reproductive success (Packer *et al.*, 1991a).

The relatedness of the patrilineal, non-dispersing chimpanzee males has also been measured (Vigilant *et al.*, 2001). They show a low level of relatedness, not statistically different from that of the dispersing females and suggesting that cooperation observed between them is not governed by kin selection (Mitani *et al.*, 2000). This implies that there must be factors other than kin selection that encourage cooperation in chimps – perhaps that a smaller number of males cannot defend a group of females, or cannot hunt successfully.

The male chimps and lions differ from the male langurs at Jodhpur in their need for social allies. In Jodhpur, a single male is able to defend a troop from incoming AMBs. Although an alliance of two or more males might be able to defend a troop for longer, the asynchronous cycling of the females means that a single male can still monopolise breeding, and there will be no reproductive benefit to the subordinate males unless they are related to the dominant male. However, as noted previously, langurs are presumed almost never to reach adulthood in the same AMB as their brothers, an assumption now supported by the low level of genetic relatedness found in the Bhadreshwar AMB.

This study of AMB relatedness only represents a fraction of what could be examined, had sampling and genotyping been more successful. From behavioural observations of the Jodhpur langurs, it would be expected that the younger cohorts of the AMB could be shown to be closely related to one another, as they are presumed to be recently ousted paternal sibs. An indication of this is given by the pairwise relatedness value of 0.24 of two juvenile males observed in the Chandpol AMB, the value that would be expected of presumed paternal sibs. Unfortunately, these were the only two band members successfully genotyped. Observations indicated that two more similar aged males might also be their paternal sibs. One of the adult males could have been their father, a recently ousted resident. These statements remain speculation in the face of missing genotypes.

4.4.2 KINSHIP networks

Langur troops are characterised by close social relationships. Dominance hierarchy in females is not fiercely enforced, but is rather egalitarian as a result of its age-related basis rather than a nepotistic basis (Borries *et al.*, 1991). This is not to say that no competition occurs at all between the females – displacements and squabbles can occur

over resources such as food and shade – but that troop life is more often characterised by peaceful interactions, such as grooming. Borries *et al.* (1994) showed that grooming relations exemplified the nature of a troop, with grooming given and received both up and down the hierarchy, reinforcing relationships as a tight social network. These relationships might be expected to be reflected at a genetic level, with close relatives forming the core of the troop, with more peripheral individuals, perhaps including sub-adult males and resident males, being excluded from the social network and also in their fewer genetic relationships with the rest of the troop. Networks of troops produced from KINSHIP data support this to some degree.

The Kailana network was quite unusual, in that it centred on the resident male. This seems rather unexpected, until it is considered that he was resident for several years, and also had a high degree of relatedness to the males that were, unusually, present in the troop. He was therefore likely to be related to many/ all of the non-adults of the troop, having fathered them. The more unusual feature of this network was that very few of the possible adult female dyads had significant likelihood under the hypotheses tested by KINSHIP. It would be expected that at least some would be mother-daughter dyads, but if they were, they were not highlighted as having significant likelihood by KINSHIP. As was seen in the network of females sharing alleles at each locus, most of the relationships did not exceed 0.25 in pairwise relatedness. The putative pedigree (FIGURE 4.4b) suggested that it might be the absence of a number of females that led to the low relatedness of the remaining individuals.

In contrast, the proportion of relationships with significant likelihood between Chandpol A females was much higher (42% of possible dyads, as opposed to 5% in Kailana). This reflected the high relatedness of females of this troop. Even when a network was drawn of relationships with significant likelihood under the more stringent test of hypotheses, many of the adult females were still joined; those females that were excluded were those most incompletely genotyped (AF1, AF4, AF9). The adult male also had no relationships with significant likelihood at this level, explained by his recent immigration into the troop – he could not yet have fathered any offspring as resident so was unlikely to be a relative of any troop member.

Nimba females also displayed a very tight network of relationships, where all but one female were attached to at least three other females. This shows the high relatedness of the members of Nimba could not be attributed solely to the presence of a rare allele at

D16S420, as only three of the females possessed it. This allele was probably inherited from a common ancestor and was unique to Nimba in this sampled population. Other loci must also have contributed to the high relatedness of the Nimba females, an effect that disappeared when the allele frequencies of Nimba alone were used. This illustrates the importance of the reference population used in relatedness calculations (de Ruiter and Geffen, 1998). Generally, the reference population from which allele frequencies are drawn should be the same as that from which the individuals under test are taken, but should not be related to these individuals. If too many close relatives are used in the reference population, the estimates of relatedness will be reduced (e.g. Vigilant *et al.*, 2001). So, for example, when allele frequencies of the whole population were used, within which 186 at D16S420 was very rare, all those individuals that possessed it would be given a high estimate of relatedness to one another. This was most likely to be a reflection of true relatedness, but for the purposes of dyadic discrimination with a social group, using these frequencies concealed any finer details.

For this reason Nimba KINSHIP analysis was also carried out using only allele frequencies calculated from the members of Nimba. Although the relationships based on alleles rare to the population but common in Nimba were devalued, more distinctive relationships, perhaps based on alleles rare to the troop but common in a certain subset of relatives, could then be pinpointed.

In practice, this analysis fragmented the troop, presumably because important information about relatedness was devalued to such an extent. Nevertheless, dyads and triads of females still grouped together along with offspring, and it might be assumed that these were matrilines within the troop. Furthermore, all offspring that were joined unambiguously to one female were also assigned those females as their most likely mothers in parentage analysis (see Chapter 5).

The Bijolai network reiterated the importance of complete genotyping, as the two individuals that were incompletely typed were those that apparently have no relationships with significant likelihood within the troop, despite the fact that they were both observed with offspring in the field. The rest of the network did not show great interrelation of group members, though this was to be expected with a small sample of individuals open to stochastic variation in relatedness.

Chandpol B's quite open network with only four relationships with significant likelihood between six females was not what would be expected for a small, matrilineal

troop. This result reflected that of RELATEDNESS analysis, where a very low value of relatedness was calculated for the troop females. Both these results were attributed to the effect of subsequent residents breaking down the relatedness between cohorts.

All male bands comprise nomadic males that move from one band to another, rarely in the company of other males (Rajpurohit *et al.*, 1995). Additionally, mortality is high, and it has been suggested that a combination of these two factors should result in no close relatives being in the same band by the time they are young adults (Rajpurohit *et al.*, 1995). It was therefore not expected that Bhadreshwar would form a continuous network – the extent of relationships was surprising. However, the relationships did appear fundamentally different from those of the troops. A much higher proportion of them were excluded as possible parent-offspring relationships through allele sharing, and the mean relatedness of dyads with significant likelihood was only 0.27. It could be that the band did contain some groups of paternal sibs, with or without their father, before they further dispersed from the first band into which they immigrated. The ages of sampled individuals were unknown, which reduced the extent to which the data could be interpreted.

4.5 CONCLUSIONS

The differences in behaviour seen between the female troops and the male bands are reflected at a genetic level. Average values of relatedness calculated from the sampled troops for the whole troops and subsets of them were close to those that would be expected from theoretical calculations. However, all values had large standard errors, and the variation between troops was very great. This is attributed to the small troop sizes – the results that are closest to the theoretical calculations are from Chandpol A and Nimba, the largest and most completely genotyped troops. In fact, the troops genotyped were smaller than the average sized langur troop at Jodhpur (A. Chhangani, pers. comm.), because of the difficulties of sufficiently identifying and sampling from an equivalent number of average sized troops. The unforeseen problems encountered in the genotyping meant that it would only have been possible to genotype fewer larger groups. Additionally, genotyping difficulties greatly reduced the amount of data obtained from the all male bands, which prevented any comparison between all male bands, or even detailed analysis within the male band genotyped.

In spite of the limitations of the available data, this study has proved informative about group structure and substructure through relatedness. Wide variation in relatedness of different troops has been revealed, which is attributed to the influence of the genetic input of successive residents creating fluctuations of relatedness through age cohorts. These results are compatible with behavioural observations of troop dynamics.

Kinship networks reveal how tightly individuals of social groups are interrelated, though they are only considered to be of limited value in revealing actual pedigree relationships. For example, known relationships are not always shown, and not every dyad shown can possibly be a first degree relationship. Therefore, it is recommended that such a representation of a social group is used only as a guide to the relatedness structure.

5. ASSIGNMENT OF PARENTAGE AND EVALUATION OF METHODS

Summary

The Jodhpur langurs form almost exclusively one-male multi-female troops. Behavioural research suggests that the resident troop male has a monopoly of the mating opportunities with the troop females and fathers all the infants conceived during his residency. This is also one of the predictions that arises from the hypothesis that infanticide is a sexually selected behaviour. If a resident male has reproductive monopoly, then his successor will be unrelated to the non-adults, and infanticide will be a successful reproductive strategy.

The genetic data were analysed both manually and using CERVUS 2.0, a parentage assignment program. Maternity and paternity were assigned using these methods; in the majority of cases maternity was not possible to assign through observation, particularly because of the prevalence of allomothering in langurs. It was found that most offspring could be assigned a mother from their natal troop, through a combination of manual and CERVUS analysis. However, CERVUS was not found to be a useful tool for paternity assignment, because (i) it attributes equal mating opportunity to each male, which is unlikely; (ii) only a small percentage of Jodhpur's adult males were sampled and (iii) CERVUS discounts loci typed for only one allele, which can nevertheless be informative in non-invasive studies. Therefore paternity assignments were made by exclusions alone on the basis of allele sharing. On this basis, it was found that new residents could be excluded as the father for the majority of non-adults in their troops, whilst males in more stable residencies could be assigned paternity for the majority of non-adults of their troops.

5.1 INTRODUCTION

In a wild animal population it is often difficult to assign parentage accurately. Behavioural observations are not always reliable as true indicators of parentage. For example, birds that pair monogamously may indulge in extra-pair copulations, the extent of which can now be quantified using genetic identification methods, e.g. Birkhead *et al.*, 1990. Extra-pair copulations can be difficult to observe in many species, even in many hours of observations. These difficulties are compounded in the observation of group living animals, where it may not be possible to observe all

individuals at all times. The open landscape around Jodhpur, and the harem organisation of the troops, offers the observer the best chance of witnessing any extra-troop copulations. Even so, it cannot be said for certain that a resident male can guard the troop females constantly through his tenure. Non-invasively collected genotypes should provide a means of assigning paternity with confidence to a male, and excluding other males as fathers, allowing a better understanding of the social structure of the langurs, and its flexibility.

The simplest way of assigning paternity is by exclusion. If an adult male shares an allele at every locus with the putative offspring, he is considered a potential father; if he can be excluded at one or more loci, then he cannot be the father. However, in many cases, exclusion alone does not result in unambiguous assignment of paternity, as several individuals may still be included as potential fathers. Statistical methods are therefore used to assign paternity to one male over the others. In this chapter, a commonly used likelihood based method of paternity assignment, CERVUS, is assessed for its usefulness in parentage allocation in this langur population. This Windows based computer program (Marshall *et al.*, 1998; available to download at http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html), written for the investigation of paternity in red deer (*Cervus elaphus*), has been used in many studies of parentage (e.g. Damaraland mole-rats, Burland *et al.*, 2002; chimpanzees, Constable *et al.*, 2001; grey seals, Wilmer *et al.*, 1999) where exclusion alone has provided ambiguous results. Here, the results of CERVUS are both combined and contrasted with the findings of exclusion analysis alone.

Unusually for a study of social animals, maternity in the langurs was rarely clear cut in this field situation. This resulted from the early independence of the offspring, the prevalence of allomothering (in which numerous individuals other than the mother handle an infant, often for extended periods), and the relatively short amount of time that could be spent observing each troop. As a result, maternity was assigned by exclusions of potential mothers' genotypes, supported by behavioural observations where possible. Again, the data was also analysed by CERVUS and the results compared to, and combined with, the exclusions to assess the value of CERVUS in the analysis of such a data set.

The results of CERVUS are compared with those of manual exclusions by allele sharing. Likelihood analysis of parentage is suggested to be a great improvement in the

assignment of parentage, allowing statistical confidences to be given in assignment when exclusions cannot differentiate between candidate parents. However, as the analysis presented here demonstrates, genetic data should not be considered in isolation from demographic data.

5.2 CERVUS 2.0

CERVUS 2.0 is a Windows based program used to assign parentage when one or both parents are unknown. Analysis comprises three stages:

1. Genotype data for offspring and potential parents are used to calculate population allele frequencies.

2. These allele frequencies are used in a simulation step. This simulation randomly generates parental genotypes from the population allele frequencies, and offspring genotypes are derived by Mendelian sampling of the parental alleles. Genotypes of unrelated individuals are also generated. Various parameters that characterise the data set are then reflected in the simulation, such as the existence of unsampled candidate parents, incomplete genotyping, and incorrectly typed individuals. Each generated genotype is considered as the potential parent, and an LOD score (the sum of $\log_e$ likelihood ratios at each locus) is generated. A negative LOD score indicates that the candidate parent is less likely to be the true parent than a randomly chosen individual; a positive LOD score indicates that it is more likely than a randomly chosen genotype to be the true parent. However, negative LOD scores can also occur when the parent and offspring share very common alleles at every locus. The most likely candidate parent will have the highest LOD score.

   This simulation is carried out both with and without one parent being known. A delta (Δ) score is then used to assess the reliability of assigning parentage to the most likely parent. Δ is defined as the difference between the highest and the second highest LOD scores, i.e. the scores of the most likely and second most likely candidates. However, the significance of the Δ score cannot be assessed using a standard distribution such as Chi-squared. CERVUS therefore runs this simulation to calculate Δ for a large number (usually 10000) of parentage tests, and then compares the distribution of Δ for tests in which the true parent was the most likely candidate with the distribution of Δ for tests where the most likely candidate was not the true

parent. It identifies a value of Δ at which, for example, 95% of simulations exceeding this score are obtained by true fathers. CERVUS allows relaxed (80%) and strict (95%) confidence levels to be set. It also indicates the number of simulations that exceed these values, giving an indication of the success rate of parentage assignment that can be expected from the data set. These simulations are carried out for cases where one parent is known, and neither parent is known, and critical values for Δ calculated for both cases. As would be expected, the Δ criteria for confidence levels are higher and the success rate of assignment is lower when no parent is known. The parameters used in the simulation of the Jodhpur population are shown in TABLE 5.1.

TABLE 5.1 Criteria used in simulation of parentage analysis by CERVUS for the Jodhpur langur data set.

Parameter	Value used
Number of candidate parents	10
Proportion of candidate parents sampled	0.9
Proportion of loci typed	0.81
Rate of typing error	0.01
Number of tests	10000
Relaxed confidence level	80%
Strict confidence level	95%

3. The final step is that of parentage analysis. This module uses a list of all offspring along with known parents (if available) and candidate parents. Maternity and paternity are assigned in different analysis runs. CERVUS calculates an LOD score for each candidate parent, then calculates a Δ score from the LOD scores of the two most likely parents. The Δ criteria calculated in the simulation are then used to assign a confidence level to the most likely parent (95%, 80% or most likely).

5.3 METHODS

5.3.1 Maternity assignment by exclusion

Potential mothers were compared with potential offspring's genotypes. If they had no matching alleles at one or more loci, then they were excluded as potential mothers. In many cases, this led to more than one female being assigned as potential mothers through allele sharing. The results of this exclusion analysis were used as one of the inputs into CERVUS for maternity analysis.

Additionally, if the resident could not be excluded as the father, the non-excluded females' genotypes were compared with the resident-offspring genotype combination.

5.3.2 Maternity assignment using CERVUS

Two different analyses were run using CERVUS, to assess how parentage assignment changes with different inputs. In these analyses there was no known parent ID input (which would state the father if known), so the probability of assigning a mother with confidence was quite low (13% chance of obtaining 95% confidence level; 47% chance of obtaining 80% confidence level). Initially, for each offspring, all the adult females of their natal troop were included as candidate mothers. Secondly, the non-excluded females were used as candidate mothers for each offspring. This was in an attempt to differentiate between the different females who could all be considered as possible mothers through allele sharing.

5.3.3 Paternity assignment

The resident male of every troop was compared with his putative offspring in that troop. The male was also compared with the genotypes of the offspring and assigned mother, to establish whether the three-way match had no exclusions.

CERVUS was also used in the allocation of paternity. Several analyses were performed, each time using all the residents plus the Bhadreshwar AMB members as candidate fathers. Four different inputs of known mothers were used:

1. no known mother assigned
2. known mothers assigned by CERVUS from all candidate mothers
3. known mothers assigned by CERVUS from non-excluded candidate mothers
4. known mothers assigned by combining exclusions, CERVUS and behavioural/ demographic observation.

The results of the maternity and paternity assignments by different methods and inputs were assessed, and a protocol for parentage assignment for this population was devised (see FIGURE 5.1).

5.4 RESULTS

5.4.1 Maternity assignment

A summary of all CERVUS analysis and manual exclusions is found in TABLE 5.2a. Using both CERVUS and manual exclusion analysis 31/32 offspring could be assigned mothers from their natal troops. The only offspring not to have been assigned a mother, WCF1 in Chandpol B, was extremely data deficient, and neither CERVUS nor exclusion analysis could be carried out.

Using CERVUS alone, 30/32 offspring could be assigned mothers, the remaining two offspring being data deficient. Of the successful assignments, 44% were with 80% confidence when all troop females were considered, and 9% with 95% confidence. This was slightly lower than expected from simulations, which predicted success rates of 47% for 80% confidence and 13% for 95% confidence. When maternity was analysed using only non-excluded mothers as candidate parents, the success rate increased to 50% at 80%, though remained unchanged at 95% confidence with only 9% success. Additionally, six maternal assignments changed with the removal of excluded females from the candidate mothers. Furthermore, there were mismatches in mothers assigned by CERVUS and those assigned through manual genotype comparisons. This was immediately apparent in Kailana, where only two out of six assignments by CERVUS agreed with mothers allocated through allele exclusions (taking account of the resident's genotype) and behavioural observation. The converse was true for Nimba offspring, where eight out of nine CERVUS assignments agreed with maternities allocated by manual analysis.

The three maternity assignments that were given at a 95% confidence level in both CERVUS analyses were also those that were confirmed manually and support behavioural observations of presumed mother-offspring pairs. In total, CERVUS assigned eleven maternities (five at 80% confidence) that were overridden by observations of age and associations, and incompatibilities with the non-excluded resident's genotype.

Offspring	Non-excluded mothers	Assigned mother (a)	Con.	Assigned mother (b)	Con.	Can resident be excluded as father?	Potential mothers if resident is father	Assigned mother (c)	Cervus assignment disagrees with manual assignments
K-SAM1	K-AF1,2,4,5,8	K-AF5	+	K-AF5	+	no	K-AF8	K-AF8	yes
K-SAM2	K-AF1,5,7	K-AF5	+	K-AF5	+	no	K-AF7	K-AF7	yes
K-JF1	K-AF1,2,5	K-AF5	+	K-AF5	+	no	K-AF1	K-AF1	yes
K-JF2	K-AF1,2,3,4,5,7,8	?		?		no	K-AF1,3,4	K-AF4	yes
K-JF3	K-AF1,3,5,8	K-AF5	-	K-AF5	-	no	K-AF1,3	K-AF3	yes
K-JM1	K-AF7	K-AF7	*	K-AF7	*	no	K-AF7✱	K-AF7	
K-WCM	K-AF3,5,8	K-AF3	+	K-AF3	+	no	K-AF3	K-AF3	
CA-SAF1	CA-AF9,11,12	CA-AF7	-	CA-AF9	-	no	none	CA-AF9	
CA-JF1	CA-AF2,7,9,11,13	CA-AF12	-	CA-AF11	+	yes	none	CA-AF11	
CA-JF2	CA-AF1,14	CA-AF5	+	CA-AF14	+	yes	none	CA-AF14	
CA-JF4	CA-AF4,5,9,11,12,13	CA-AF11	-	CA-AF11	-	no	CA-AF4,5,11,12	CA-AF5	yes
CA-WCF1	CA-AF1,4,5,9,11,12,13,14	CA-AF14	-	CA-AF14	-	no	CA-AF14	CA-AF14	
CA-WCF2	CA-AF1,3,4,13	CA-AF3	+	CA-AF3	+	yes	none	CA-AF3	
CA-WCM1	CA-AF1,4,9,12,13	CA-AF5	-	CA-AF1	-	yes	none	CA-AF4	yes
CA-WCM3	CA-AF1,7,9,11,13	CA-AF12	-	CA-AF11	+	yes	none	CA-AF7	yes
CA-WCM4	CA-AF1,5,9	CA-AF1	-	CA-AF1	-	yes	none	CA-AF1	
CA-BCM1	CA-AF7,9,11,12	CA-AF12	+	CA-AF12	+	yes	none	CA-AF12	
N-SAM	N-AF4,10	N-AF10	-	N-AF10	-	no	none	N-AF10	
N-SAF2	N-AF2,5	N-AF2	-	N-AF2	-	no	N-AF2,5	N-AF2	
N-SAF3	N-AF6,7	N-AF6	-	N-AF6	-	no	N-AF6,7	N-AF6	
N-JM1	N-AF1,2,3,4,5,10	N-AF1	-	N-AF1	-	no	N-AF1,2,3,4,5,8,10	N-AF1	
N-JM2	N-AF2,4,5	N-AF4	-	N-AF4	+	no	N-AF2,4,5	N-AF4	
N-JM3	N-AF1,2,3,4,5,7	N-AF7	-	N-AF7	-	no	N-AF1,2,3,4,5,7,8	N-AF7	
N-WC1	N-AF5,6	N-AF6	-	N-AF6	-	no	N-AF5,6	N-AF5	yes
N-WC3	N-AF1,2,3,4	N-AF1	+	N-AF1	+	no	N-AF1,2,3,4,8	N-AF1	
N-WC4	N-AF2	N-AF2	*	N-AF2	*	no	N-AF2	N-AF2	
CB-JF1	CB-AF3,7	CB-AF3		CB-AF3		no	none	CB-AF3	
CB-JF2	CB-AF6	CB-AF6	+	CB-AF6	+	no	none	CB-AF6	
CB-WCF1	?	?		?		?	?	?	
B-JF	B-AF1✱,2,4	B-AF5	+	B-AF2		no	B-AF1,2,4	B-AF1	yes
B-JM	B-AF1,2,4,5	B-AF2	+	B-AF2	+	no	B-AF2,4	B-AF4	yes
B-WCM	B-AF1,5	B-AF5	*	B-AF5	*	no	B-AF5	B-AF5	

TABLE 5.2a Results of maternity analysis. Assigned mothers (a): CERVUS input included all troop females as candidate mothers ; assigned mothers (b): CERVUS input included all non-excluded troop females; assigned mothers (c): assigned through exclusion, CERVUS and observation. Con. = confidence: * denotes assignment with 95% probability; + denotes assignment with 80% probability; - denotes most likely parent. ✱ denotes an alleleic mismatch where one individual is a homozygote at that locus.

Offspring	Can resident be excluded as father?	Is resident excluded when mother's genotype considered?	Assigned father (d)	Con.	Assigned father (e)	Con.	Assigned father (f)	Con.	Assigned father (g)	Con.	Cervus assignment disagrees with manual assignments
K-SAM1	no	no	K-AM1	+	K-AM1	-	K-AM1	+	K-AM1	+	
K-SAM2	no	no	BM4	+	K-AM1	+	BM4	+	K-AM1	*	
K-JF1	no	no	BM15	+	K-AM1	-	BM15	+	K-AM1	+	
K-JF2	no	no	?		?		?		?		
K-JF3	no	no	B-AM	-	K-AM1	+	B-AM	-	K-AM1	+	
K-JM1	no	no	K-AM1	+	K-AM1	-	K-AM1	+	K-AM1	+	
K-WCM	no	no	K-AM1	+	K-AM1	-	K-AM1	+	K-AM1	+	
CA-SAF1	no	yes	BM3	*	BM3	+	BM3	*	BM3	*	yes
CA-JF1	yes	yes	BM19	+	BM1	-	BM19	-	BM19	-	yes
CA-JF2	yes	yes	BM8	+	BM8	+	BM8	+	BM8	+	yes
CA-JF4	no	no	BM8	*	BM8	+	BM8	*	BM4	+	yes
CA-WCF1	no	no	BM4	+	BM4	-	BM4	+	BM4	+	yes
CA-WCF2	yes	yes	BM11	+	BM11	+	BM11	+	BM11	+	yes
CA-WCM1	yes	yes	BM2	-	BM2	+	BM2	+	BM2	+	yes
CA-WCM3	yes	yes	K-AM1	*	BM4	-	BM15	+	BM3	*	yes
CA-WCM4	yes	yes	BM2	+	BM2	+	BM2	+	BM2	+	yes
CA-BCM1	yes	yes	BM4	*	BM1	-	BM4	*	BM4	*	yes
N-SAM	no	yes	B-AM	+	BM3	-	B-AM	+	B-AM	+	yes
N-SAF2	no	no	CA-AM	+	BM10	+	CA-AM	+	CA-AM	+	yes
N-SAF3	no	no	BM8		BM7	-	BM8		BM8		yes
N-JM1	no	no	CA-AM	+	CA-AM	+	CA-AM	+	CA-AM	+	yes
N-JM2	no	no	BM8	-	BM9	+	BM8	-	BM8	-	yes
N-JM3	no	no	BM19	-	BM19	-	BM19	-	BM19	-	yes
N-WC1	no	no	BM11	-	BM11	+	BM11	-	BM11	+	yes
N-WC3	no	no	BM19	+	BM19	-	BM19	+	BM19	+	yes
N-WC4	no	no	BM8	-	BM8	+	BM8	-	BM8	-	yes
CB-JF1	no	yes	B-AM	+	K-AM1	-	B-AM	+	B-AM	+	yes
CB-JF2	no	yes	B-AM	-	BM15	-	B-AM	-	B-AM	-	yes
CB-WCF1	?	?	?		?		?		?		
B-JF	no	no	CA-AM	-	BM19	-	BM10	-	BM19	-	yes
B-JM	no	no			BM9	-			BM9	-	yes
B-WCM	no	no	B-AM	-	BM19	-	B-AM	-	B-AM	-	

TABLE 5.2b Results of paternity exclusion analysis and CERVUS paternity assignment. Assigned father (d): no known mother in CERVUS input; assigned father (e): assigned mother (a) as known mother; assigned father (f): assigned mother (b) as known mother; assigned father (g) assigned mother (c) as known mother. Con. = confidence: * denotes assignment with 95% probability; + denotes assignment with 80% probability; - denotes most likely parent.

5.4.2 Paternity assignment

The exclusion of the resident from paternity (TABLE 5.2b) varied greatly between troops. The first set of exclusions noted only whether or not it was possible for the resident male of each troop to have sired the offspring of that troop, by allele sharing at each locus. This method of paternity assignment was therefore more considerate of the opportunity for the residents to have mated, rather than using a likelihood measure calculated simply from the alleles they possessed. When comparing offspring/ candidate father genotypes alone it appeared that the resident of Chandpol A was the only male who could not have sired all the offspring in his troop. However, when the assigned mother (TABLE 2, assigned mother (c)) was taken into account, Chandpol B's resident was also excluded as the father of all of the offspring in that troop, and Nimba resident was excluded as the father of the oldest non-adult in Nimba, SAM.

CERVUS paternity assignments were carried out using several different inputs for the known mothers; however, there was not a great deal of variation between the results of the analyses. There was a much higher success rate for paternity allocation when there was a known parent ID input – between 63 and 70% at 80% confidence, depending upon the identities of the known mother used. For non-adults where the resident was excluded as the father, CERVUS did not allocate that resident paternity in any case. However, in cases where the resident could not be excluded, CERVUS did not assign the resident as the most likely father from all the sampled males. For example, none of the offspring of Nimba were assigned the Nimba resident as their father by CERVUS, despite the fact that he could only be excluded as a potential sire in one case (SAM). CERVUS attributed the paternity of Nimba's nine non-adults to five different males, none of them troop resident.

The most rigorous test of paternity by CERVUS was that using the known mothers assigned by exclusions, behaviour and CERVUS together (TABLE 2, assigned mother (c)). Of the 30 individuals assigned fathers in this test, only seven of them were to their troop residents, six of these in Kailana. The other non-adults had a wide range of adult males assigned as their fathers. The difference between the average LOD score for residents and the non-residents assigned paternity (3.28 ± 1.12 and 1.42 ± 0.92 respectively) was significant ($t = 4.44$, d.f. = 27, $p < 0.01$). Additionally, there was only 1/41 (2.4%) mismatch of alleles in the resident – assigned mother – offspring allocation (Kailana: AM1–JM1) whereas there were 15/104 (14.4%) mismatches in the non-resident - assigned mother – offspring allocations.

5.5 DISCUSSION

5.5.1 Maternity

Maternity assignment by exclusion

Langurs remain within their natal troops at least until they are weaned. After this, the males may leave or be ousted (Rajpurohit and Sommer, 1993), but the females almost invariably stay in their troops for the rest of their lives. Therefore it would be expected that non-adults in a troop could all be assigned at least one adult female as their mother. If it was the case that all females in the troop could be excluded as the mother of a non-adult, it would have to be assumed that (i) the mother was present in the troop but had not been sampled; (ii) the mother had died or left the troop; (iii) there was a genotyping error which led to mismatch at one or more loci. In the whole sample set, there was only one infant (Bijolai: JF) that could be excluded as the offspring of all troop females, despite all adult females having been sampled. However, observations showed that she often associated with AF1 in what appeared to be a mother-offspring relationship. These two individuals mismatched at D3S1766, where AF1 was typed as 260/260, and JF as 258/258; this seems likely to be a scoring error, and that JF should be 260/260. If this locus was ignored, paternity could also be assigned to the troop resident; it seems very likely that he was the juvenile's sire as he had been resident in the troop at the time of conception, and shared other rare alleles with the juvenile. Overall, the only offspring that could not be assigned to a female was Chandpol B's WCF1 because of incomplete genotyping that meant that no mother could be included with certainty.

Maternity assignment by CERVUS

Of the CERVUS assignments, only 11/30 were flagged with 80% likelihood. This was in part because of the incomplete proportion of loci typed. CERVUS simulations showed that with only 81% of loci genotyped, as was the case, only 47% of true parents could be identified with 80% confidence; this figure rises to 68% when 90% of loci are genotyped. Five mothers assigned by CERVUS with > 80% likelihood were discounted, on the basis of demographic or behavioural observation.

Additionally, in the initial CERVUS analysis when all troop females were included as candidate mothers, in six cases the assigned most likely mother had a mismatch at one locus, when other females had no mismatches (TABLE 2; assigned mother (a)). All potential mothers with exclusions were therefore left out of the following CERVUS analysis (TABLE 2; assigned mother (b)). This is in contrast to Constable *et al.* (2001) in their paternity assignment of chimps, where they allocated paternity to the most likely

candidate chosen by CERVUS despite one locus mismatching. This locus was estimated to have null alleles, which could lead to parent-offspring mismatches; one of the mismatching pair was an apparent homozygote in each case. It is not stated whether other less likely parents had no mismatches.

It might be expected that relatives of the true mother might have a confounding affect on maternity assignment by CERVUS. However, as shown by Marshall *et al.* (1998) even the presence of full sibs of the true parent being included in the set of candidate parents does not greatly reduce the likelihood of identifying the true parent. Kailana troop maternity allocations seem to contradict this. AF5 could not be the mother of all the offspring to which she was assigned; even if it was not the case that the resident male fathered all these offspring, it simply is not possible that she could have borne all these infants, as they were too close in age to be sequential births, given an interbirth interval of 16.7 months (Sommer, 1994). This suggests that she was assigned most likely maternity because she shared common alleles with all of the offspring. In the relationship network of the troop (FIGURE 4.2a) she had a significant likelihood of relationships with seven individuals, four of whom were the non-adults to whom she was assigned maternity. It seems possible that AF5 had a genotype of common alleles that made her closely related to many members of the troop and resulted in her being the most likely maternal candidate.

The CERVUS maternity assignments therefore often conflicted with what was known, or possible, from observations of the troops. The results of this study clearly show that some of those maternities assigned with 80% confidence could not be true mothers. Therefore it is suggested that CERVUS is only used as a final test for discriminating between several non-excluded mothers, each of whom could equally be mothers after their age, any behavioural observations, probability of being mother to another non-adult of the troop and, for non-excluded residents, compatibility with the resident's genotype have all been taken into consideration. For example, in Chandpol A, many females could not be excluded for several offspring, and CERVUS was used to allocate the most likely mother. In some cases, for example AF11 in Chandpol A, females were assigned as most likely mothers to two offspring that were too close in age to have been consecutively borne by that female; hence the female was assigned as mother to the offspring with the highest LOD score, and the next most likely mother assigned to the second offspring (in this case, AF5 to JF4).

5.5.2 Paternity

In this study, the harem organisation has been assumed to give a high degree of paternity certainty, with at least 95% of a troop's history characterised by a one-male situation, during which the resident is expected to have monopoly of mating opportunities (Sommer and Rajpurohit, 1989). Troops are geographically isolated (Rajpurohit and Mohnot, 1988), the landscape is open, affording good visibility to the resident, and harem males are extremely defensive of the females of their troop. Hence it is unlikely that a non-troop male could sneak a successful copulation during a stable residency. If the sampled resident was in the troop at the time of conception, he would be expected to be the most likely paternal candidate. Paternity assignments are considerate of this social structure and the mating opportunities available to the various males.

Paternity assignment by exclusion

Comparing resident males' genotypes with those of their potential offspring produced a clear patterning of paternity non-exclusions. For troops Kailana, Nimba and Bijolai, the current resident could not be excluded as a potential father for all offspring (except Nimba SAM). After consideration of the females' genotypes, the resident of Chandpol A was excluded for 8/10 offspring, and in Chandpol B, the male could be excluded from all offspring. These results are all in accordance with what was known of the troop histories, with presumed stable long term residencies in Kailana, Nimba and Bijolai contrasting with recent male replacements in both the Chandpol troops. Furthermore, in the three stable residencies, no extra-troop paternity was detected, as would be indicated by exclusion of the male for one offspring in the midst of the age range of other offspring attributed to the male. The only non-adult for which a resident could be excluded is Nimba SAM (after the consideration of the assigned mother's genotype). This male was the oldest non-adult of the troop, so he may have been the last remaining non-adult offspring of the previous resident. This cut-off point between excluded and non-excluded offspring, assuming no extra-troop conceptions, allows estimation of the time of the last resident takeover. SAM, excluded as a son of the resident, and SAF2, for whom the resident was not excluded, were similarly aged at about three years old, so takeover could be assumed to have been around the time of their conception, 3 1/2 years prior to sampling.

Paternity assignments by CERVUS

CERVUS paternity assignments were made under several conditions for 30/32 offspring. From these assignments alone, there would seem to be much extra-group paternity in this population. In all troops save Kailana, a male other than the resident was assigned as most likely father when no mother is assigned. Even when the strictest assignment of known mothers was applied (TABLE 5.2a, assigned mother (c)), only 7/30 infants were assigned current residents as their father. The fact that the resident was not assigned paternity could be as a result of several factors.

The resident of the troop, and father of the non-adults, may have recently been replaced. This was known to be true, from observations, for both Chandpol troops. This is the simplest explanation for the non-assignment of paternity to the resident in a completely sampled and genotyped population. However, there are other reasons that stem from the completeness of the sampling and genotyping data that could also affect paternity assignment, and must not be overlooked.

CERVUS makes the assumption that all males have equal reproductive success; it cannot account for the variation in mating opportunities available to different males. For example, it would seem from observation that the residents have far greater opportunities for mating than do non-troop males, who would have to travel into the troop's home range, identify oestrus females and successfully mate with them out of sight of the protective resident (Rajpurohit *et al.*, 1995).

At the time of sampling there were 120 old and adult males in the Jodhpur population (A. Chhangani, pers. comm.). Therefore only 1/6 (21) of all possible fathers were sampled. The CERVUS simulation takes into account the fact that some potential fathers may be unsampled, which is the case in this and many other studies of wild animal populations, and attributes equal reproductive success to these individuals. A CERVUS simulation was run with a more realistic proportion of candidate parents typed (0.17), causing the success rate of allocating parents with 80% certainty to fall dramatically, to only 7%. Only two males were assigned with 80% certainty using this simulation; one of these was the Kailana resident to SAM2. This was the only known mother – candidate father – offspring comparison that was compared at all eight loci with no mismatches. The other was of BM4 to Chandpol A BCM1, despite one mismatch and a large distance between the groups, which might have precluded mating opportunities.

The lack of significant assignments illustrates the need for comprehensive sampling of candidate parents.

The allocations of paternity by CERVUS must therefore be considered in the context of the population's structure. Even if it was the case that, for example, the male of Chandpol A had recently been replaced, it seems unlikely that the non-adults of the troop were fathered by a total of seven different males as assigned by CERVUS, who then all regrouped in the same all male band, Bhadreshwar. Despite the fact that the majority of these assignments were made with 80% confidence, they were ultimately discounted as true fathers.

The residents of troops Kailana, Nimba and Bijolai were known to have been residents for some time, and therefore it would be assumed that they had fathered most, if not all, the non-adults of their troop. This was true for CERVUS' assignments for Kailana, but in Bijolai only one out of three offspring was assigned to the resident, and none of the Nimba offspring. This was in part because CERVUS considered only individuals typed at four or more loci. CERVUS will not consider partially typed loci at which only one allele has been identified (T. Marshall, pers. comm.). This meant that the resident of Nimba was not even considered as a potential father for any of the sampled non-adults. However, the manual exclusions for paternity showed markedly different results, in which the Nimba resident could only be excluded as father for one of the troop's non-adults (SAM). In addition, his genotype was compatible with that of the assigned mothers to produce the observed offspring genotypes. From the raw genotype data it is seen that the resident and 6/9 troop non-adults shared an allele 260 at D3S1766, which was not found in any troop female. This strongly suggests a common paternal inheritance of this allele, yet because the resident was only typed for one allele at this locus, this information was disregarded by CERVUS.

This seems to be a drawback in CERVUS' method. Data from loci that are only typed for one allele can potentially be highly informative. For example, a potential mother may be 152/156 at a certain locus, and the offspring 148/•. It is possible, then, that this female may be the mother. However, if the potential father is 152/160 at this locus, then one of these candidate parents must be wrong, as the offspring must have inherited the 148 allele from one of its parents. However, data from all completely typed loci might not indicate this exclusion. This type of incomplete data that is nevertheless informative is not considered by CERVUS, and can lead to conflicting conclusions.

Marshall *et al.* (1998) stress the importance of maternal sampling, stating that 50% more loci are needed to assign paternity with similar confidence if the maternal genotype is unknown. This is true in some cases in this analysis; in Kailana, only two out of the six paternities assigned to the resident are with 80% confidence, whereas when the known mothers are considered, all are assigned with at least 80% confidence.

Ultimately it must be decided what weight must be given to CERVUS assignments in the final allocation of paternity. When factors such as the potential access of candidate fathers to troop females, the known residencies of troop males, and the possible incomplete genotyping of candidate males are all considered, it becomes apparent that in this study, CERVUS assignments should be considered as no more than a guide, that may be used as confirmation of non-excluded troop males as potential fathers, but not in the ultimate allocation of paternity. Implausible allocation of paternity by CERVUS is attributed to the small number of candidate fathers sampled and genotyped. If the resident male could not be considered as a potential father, it seems unlikely that any of the other sampled males could more likely be the father. A more likely conclusion to draw would be that the males of Bhadreshwar, accounting for 13/21 sampled males, were assigned 18/30 paternities by chance. This was upheld by the lack of 80% confidence allocations when a realistic proportion of sampled males was used in the parentage simulation.

This conclusion is further supported by paternity assignments within Kailana. For these non-adults, all four adult members of the troop were considered as candidate parents, along with all other residents and AMB members. However, none of the troop males other than the dominant resident was assigned paternity to any offspring under any known mother condition. This was despite the fact that the males of Kailana were all closely related, potentially as father-son/ sibs ($R = 0.29 \pm 0.10$) and therefore would have similar genotypes. It was more likely that the adult males were half-sibs of the resident, judging by their ages, and "half sibs of the parents...have only modest effects on paternity inference even when present in large numbers" (Marshall *et al.*, 1998). Overall, it seems that in this group where there was an obvious paternal candidate, CERVUS was able to discriminate between him and close relatives, assigning paternity in agreement with behavioural expectations.

This study is lacking in data from more AMBs. It would be expected, if further AMBs had been genotyped, that these individuals would also randomly be assigned paternity in

accordance with the proportion of long-term residents: band members. However, it would also be expected, as more of the AMB members were genotyped, that eventually an ousted resident of one of the troops would be sampled. If this resident had had a monopoly of mating access to the females of the troop, paternity would be expected to be allocated to this individual for a cohort of non-adults. As Constable *et al.* (2001) state, "the question of prevalence of extra group conceptions in chimpanzees will only fully be resolved when extra-community males are also genotyped." The assignments made by CERVUS can only be as good as the data provided.

Assuming that resident exclusions alone as indications of paternity are more reliable than assignments of CERVUS, it is concluded that genetic data supports the observations of Sommer and Rajpurohit (1989). They assume that insemination of troop females by extra troop males is absent, or very rare, given the isolation of most of the troops, and the open habitat that provides good visibility. The only time when a male that is not resident may possibly mate with a troop female is during the instability of takeover. Sommer and Rajpurohit (1989) found only 4.7% of infants were conceived during these times, and probably only 0.7% of infants (1 conception out of 143) was achieved by a low-ranking male who did not go on to become the harem holder. The genetic exclusions seem to corroborate these observations.

These findings support the hypothesis that there is only one profitable mating strategy for males around Jodhpur – to gain residency of a harem and monopolise the matings (Sommer and Rajpurohit, 1989). The situation in Kailana thus requires explanation. Here, though there were four adult males, all the paternity was attributed to the dominant male. The other males must have had some incentive to stay within the troop, rather than seek troops of their own. A multi-male situation is very rare in Jodhpur – only three troops were observed to have two to four adult males for between 17-28 months between 1983 and 1988 (Sommer and Rajpurohit, 1989). The additional adult males in Kailana were clearly discounted by CERVUS as possible fathers, and could be excluded in many cases as possible fathers, suggesting that although they were present in the troop they did not sire any offspring. This is in contrast to the situation in multi-male troops at Ramnagar, Nepal (Launhardt, 1998) where mating success was shared, albeit disproportionately, between several males of a troop. It seems to be the case that for some reason the dominant male was tolerating the other adult males in the troop whilst preventing them from having successful matings. Srivastava *et al.* (1986) report that in one apparently multi-male troop observed at Jodhpur, the only fully adult male

monopolised mating opportunities with the females. In Kailana, all the adult males seemed similarly aged and battle scarred. If it was the case that the dominant male could monopolise matings, then he may have tolerated the presence of the other adults because of their group advantage in defending the troop from takeover by other males. The males were seen to engage in defensive interactions with the dominant male against an incoming AMB; but they were also quick to desert, and were not sighted with the troop after the first day of takeover. In contrast, the dominant male did not give up the troop until the third day of interactions with the male band. Nevertheless, the aid that the extra males gave in defence may have been important in maintaining the exceptionally long residence of the dominant male.

The high level of provisioning that occurred at Kailana was probably also important in the maintenance of this multi-male situation. There were a great number of provisioners that came regularly to Kailana both in the morning and the evening, to the point where they would call to wake sleeping langurs to persuade them to come and take food. There was clearly no shortage of food; if there had been, then it is possible that the males would have had many more agonistic interactions over access to food, which would have eventually resulted in the extra males being ousted. The resident male would have defended his access to food to maintain his physical condition, needed to assert his dominance and defend his harem. The females would also be less willing to tolerate extra males in a poorer environment, though how they might manipulate redundant males to leave is unclear, as sexual dimorphism makes physical conflict unfavourable.

The main conundrum in Kailana seems to be why the other males should have stayed with the resident. If they were not having any direct reproductive success, then it seems counterintuitive that they should have stayed in the troop at all, rather than leave to find their own residencies. It is possible that they were simply biding their time before moving out to compete for their own troops, in what was a better provisioned environment than that of an AMB. By remaining in the troop, they would have no chance of experiencing contact with other residents in takeovers, which seem necessary to gauge the condition of current residents, but they would have been benefiting from indirect reproductive success, as all the troop adult males had a high R value (0.29 ± 0.10), so the resident's offspring would have shared some genes with the other adult males. Still, the adult males were only likely to be related as paternal half sibs, therefore would only be related to the resident's offspring by 0.125. It seems unlikely

that kin selection provides an adequate explanation for the presence of the extra adult males in Kailana troop, although it could provide a partial answer. It is more likely that the high level of provisioning of the site enabled the resident to tolerate these related males whilst they were not a threat to his reproductive success.

5.5.3 Paternity and infanticide

Male langurs compete with one another for reproductive success. A male will attempt to increase his reproductive success at the expense of another male's. In several langur populations it has been observed that some newly incoming males into a troop (harem or multi-male multi-female) commit infanticide (Dharwar, Sugiyama, 1965; Mt Abu, Hrdy, 1977; Gir Forest, Newton, 1986; Jodhpur, Sommer, 1987; Ramnagar, Borries, 1997). Debate has raged over whether these observations have an adaptive cause or are anthropogenic, as a result of overcrowding and habitat destruction (Sommer, 2001). In any case, several reports of infanticide (Newton, 1986; Borries, 1997) have made it clear that infant killing is not restricted in its distribution to only those areas where the humans have greatly influenced the environment, which might have led to overcrowding, and 'pathological' killing of infants. Infanticide occurs in undisturbed habitats, and in those where troops are multi-male. The important factors in the decision to commit infanticide are the paternity certainty of the resident male, and the opportunity of mating with the mother after her offspring has been killed and she has resumed cycling.

The Jodhpur exclusion data suggest that resident males are likely to have high paternity certainty, and the corollary of this is that non-residents are very unlikely to have fathered offspring in troops in which they are not resident. As a result of this, incoming residents may commit infanticide without the risk of killing their own offspring. This supports the hypothesis that infanticide is a sexually selected behaviour in langurs.

Furthermore, once a male has established himself as resident in Jodhpur, it is apparent that he has almost exclusive access to females of the new troop. Committing infanticide in this situation will ensure he establishes this monopoly as quickly as possible after takeover, without having to wait for his predecessor's, and competitor's, offspring to wean.

Infanticidal males must be sure that they are not attacking their own infants. They appear to use mating access as an indicator of paternity. This is a clear discriminator at Jodhpur, where the resident apparently has a monopoly of mating access. At Ramnagar,

Nepal, males who committed infanticide were also only those that had immigrated into a multi-male troop since the previous mating season (Borries *et al.*, 1999a). Newly incoming males were observed to attack or kill only infants unrelated to them and go on to father subsequent offspring (Borries *et al.*, 1999a), even in this multi-male situation. It seems to be the case that male langurs have the capacity to discriminate between pregnant and potentially fertile females, and only copulations with the latter are taken as a cue for paternity (Borries *et al.*, 1999b). By being promiscuous when fertile, females can induce troop males other than the fathers to act as infant protectors (Borries and Koenig, 2000). Although this situation is not found at Jodhpur, it appears that all male langurs use mating access to fertile females as their cue for paternity.

5.6 CONCLUSION

Genetic data made the assignment of maternity a possibility in this study, demonstrating that it is not always necessary to have accurate observations of behaviour to identify related individuals. It is also clear that maternity assignment must be approached with caution. In a number of cases, non-adults had several females assigned as possible mothers based on allele sharing, and only CERVUS could be used to identify the most likely mother. However, it must be borne in mind that CERVUS was clearly incorrect in some assignments of most likely parent, a problem that could only be resolved in the future by increasing the number of microsatellite loci used in genotyping.

In the case of paternity assignment, it is suggested that for this data set, likelihood estimation using CERVUS was not appropriate. The low proportion of the population's males that were sampled, coupled with the probable variation in mating opportunities between residents and band members, led to assignments that appeared quite arbitrary. A more logical pattern of assignments was revealed when only residents were considered with the offspring of their troop. Paternity in this case was allocated to the resident in troops where the tenure was thought to have been stable for some time, but not to the newly established residents of Chandpol A and Chandpol B troops, supporting the behavioural observations of researchers that residents have monopoly of access to females during their tenure.

A decision-making flow chart for parentage assignment is shown in FIGURE 5.1. This is specific to this study and the quantity and quality of data it has generated. For example, the use of CERVUS is not an option for paternity assignment, as it might have been had a

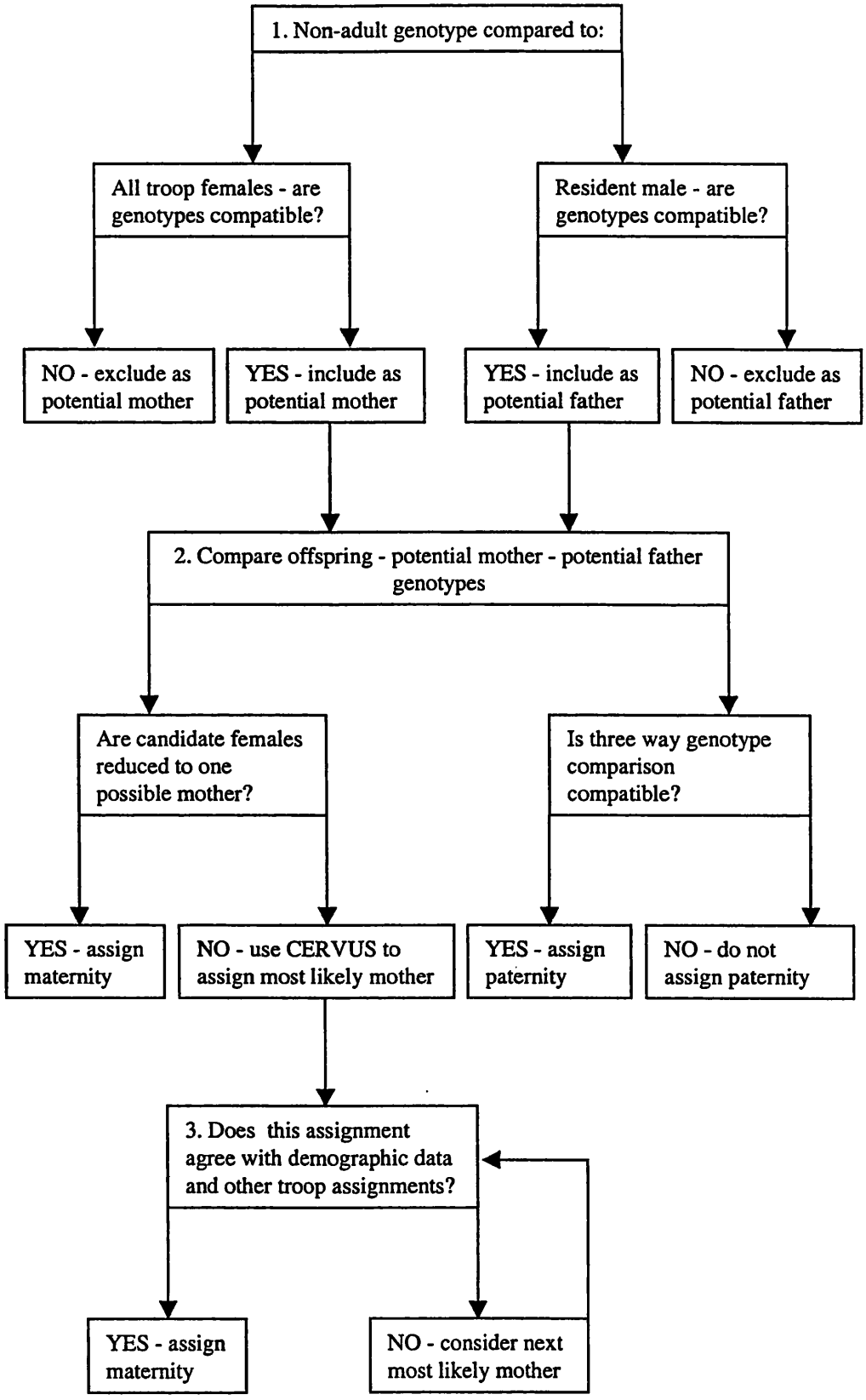


FIGURE 5.1 Parentage assignment flow chart for this study. Note that in step 2. there may be no potential father, if the resident male shares no alleles with the non-adult.

greater proportion of adult males been genotyped. Also, it demonstrates how maternity and paternity assignment are interlinked. This is a point particular to this study, where maternity was not unambiguously determined through observation. In the allocation of maternity, the genotype of the resident, if not excluded, can be used to reduce the number of candidate mothers who are non-excluded. Conversely, if the resident is not excluded, but on comparison of his genotype with the potential mother(s) and offspring he is incompatible, then he is excluded from paternity.

It is clear from the examination of this data set that genetic parentage analysis is a useful tool in the description of wild populations. However, study of genetic data in isolation from other information has been shown to lead to erroneous conclusions. There is a general risk of believing the genetic data to be more robust, or reproducible, or more powerful than observations, when the actual fact is that faecal genotyping is still a developing technique, vulnerable to failures and mistakes, and that to unquestioningly accept its results as more reliable than observation can lead to 'discoveries' that will later be unceremoniously refuted (e.g. Gagneux *et al.*, 1999, followed by Vigilant *et al.*, 2001). It is vitally important that the genetic data is only used in conjunction with what is known of the population's geographic and demographic structure.

6. POPULATION GENETICS OF JODHPUR LANGURS

Summary

Genetic variability within and between groups was studied using Wright's F-statistics (1951) to describe the population genetic structure, which results from the effects of migration of individuals between subgroups of the population, and breeding structure. F-statistics describe the relationships between the observed and expected heterozygosities of individuals at the level of the individual, the subpopulation and the population. It is hypothesised that the Jodhpur population should show a high level of differentiation between groups, the result of reproduction being monopolised by a single male in each group. In turn, high intergroup differentiation should mean that a new troop resident would have different allele frequencies to resident females; their resulting offspring will exhibit an excess of heterozygotes.

Both these predictions are supported by the data. The Jodhpur troops show an extremely high level of outbreeding within troops (negative F_{IS}) as would be produced by successive one-male residencies. There is also strong intergroup differentiation (positive F_{ST}) suggesting that female philopatry combined with polygyny create a highly structured population. This population structure means that neighbouring troops become sufficiently differentiated from each other that it benefits individuals to direct cooperative behaviours towards members of their own troop to whom they are genetically similar. High heterozygosity within troops may also be advantageous in the adaptation of langurs to new habitats, as they have a variability upon which selection can act to colonise new environments.

6.1 INTRODUCTION

In population genetics, a population refers not to an entire species but to a group of individuals living within an area where any member can potentially mate with any other. The level at which population genetics is studied must be defined from the outset. In this study, it is assumed that the langurs living at Jodhpur represent a geographically, and genetically, isolated population, the nearest langurs being found 100km to the south. Within the Jodhpur area, it is assumed that distance is no barrier to mating, and that a male born at one extreme of the geographic range could gain a

residency at the most distant limit of the range from his natal troop. Hence, Jodhpur could be considered to be one continuous potentially randomly mating population.

However, the Hanuman langur population at Jodhpur is highly geographically structured. As has been explained, the langurs live in social groups that either comprise females and their offspring along with one resident male (troops), or all male bands (AMB) comprising all age classes of males save the very young. The troops remain within well-defined home ranges, and there is very little female movement between them (Makwana and Advani, 1981; Mohnot *et al.*, 1981). In contrast, the AMB move over larger areas, and membership is constantly changing. The lack of movement of females between troops might be expected to lead to high relatedness. The mating system is thought to be polygynous, with the resident male at any time being assumed to have monopoly of mating access to the females of the troop. The genetic structure of the Jodhpur population is investigated here using the microsatellite data from five troops and one AMB.

The genetic structure of a population results from the effects of mutation at the level of the genome, migration of individuals between social groups, random genetic drift, particularly in small populations, selection, and breeding structure. Genetic structure of a population at a particular time can be described by Wright's (1951) fixation indices or F-statistics, F_{IT}, F_{IS} and F_{ST}. These F-statistics describe the relationships between observed and expected heterozygosities of individuals at different population levels: I = individual, S = subpopulation and T = total population. The definitions are to some degree arbitrary, and defined for each study; in this case, the subpopulations are the troops and all male bands, and the population is the geographically isolated langur population of Jodhpur from which the samples were drawn.

The values of heterozygosities used in the calculation of F-statistics are as follows (italics denote means):

H_I is the observed heterozygosity of an individual, averaged over all groups;

H_S is the expected heterozygosity of an individual, based upon the allele frequencies in that individual's group, averaged over all groups;

H_T is the expected heterozygosity of an individual, based upon the average allele frequencies over all groups.

The F-statistics are then as follows:

$$F_{IT} = \frac{H_T - H_I}{H_T}$$

$$F_{IS} = \frac{H_S - H_I}{H_S}$$

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

Hardy Weinberg Equilibrium (HWE) describes the expected frequencies of genotypes given the observed allele frequencies within the population, under a number of simplifying assumptions, the most important of these being that mating within the population is random (Hartl and Clark, 1997). Deviations from the expected values of heterozygosities and therefore of F-statistics are expected to be indicative of departure from the assumptions of HWE, and suggest non-random mating and population substructure.

F_{IT} (range –1 to 1) measures the deviation from HWE at the whole population level. This does not take account of the effect that population substructure may have. In reality, the distribution of allele frequencies might be highly partitioned into subgroups but be balanced across all groups, so that the total population measure gives no indication of inbreeding or outbreeding. It is only when the F-statistics that take account of subgroups of the population, F_{IS} and F_{ST}, are also used that deviations from HWE may be revealed.

F_{IS} measures the deviation from HWE within a group, i.e. in a subset of the population, such as a langur troop in the Jodhpur population. Values range from –1 to 1; a positive F_{IS} indicates an excess of homozygotes in the group, as a result of either inbreeding (close relatives mating together; Hartl, 2000); assortative mating (individuals choosing to mate with partners on average more genetically similar to them than a randomly selected mate, on the basis of phenotypic characters); the occurrence of null alleles (nonamplifying alleles leading to an apparent excess of homozygotes; Pemberton *et al.*, 1995); or because of the Wahlund effect (Wahlund, 1928). The Wahlund effect is an observed deficit of heterozygotes because of an unknown substructure of the sample. Variance in allele frequencies in the subgroups of the population from which the sample is drawn will give rise to a lower heterozygosity than would be expected from the observed allele frequencies of that sample.

F_{ST} is a measure of population substructure; it measures the inbreeding due to the correlation among alleles because they are found in the same subpopulation. Values of F_{ST} are found between 0 and 1. If $F_{ST} = 0$, then there is no population substructure, suggesting that individuals mate randomly between subunits of the population, and there is no variance in allele frequencies between subunits. F_{ST} will reach one when each subpopulation is fixed and therefore homozygous for alternative alleles. Such a case would be indicative of highly structured population with little or no gene flow between subunits. F_{ST} gives a measure of the heterozygote deficiency relative to that expected under HWE because of known or assumed population subdivision, analogous to the Wahlund effect giving rise to heterozygote deficiency within subgroups because of unknown population structure.

Although the interpretations of F_{ST} values of 1 and 0 are straightforward, as are F_{IT} and F_{IS} values of –1, 0 and 1, it is difficult to give biological meaning to intermediate values. For example, in real populations, F_{ST} will not be 1 for totally differentiated populations, mainly due to the effect of polymorphism, which greatly reduces the expected value of F_{ST}. This is a common result of using microsatellite markers, which are specifically chosen for their polymorphism, and hence their information content. It is highly improbable that, having chosen markers for their variability, they will then be fixed in every subgroup. Therefore it is necessary to be aware that an apparently low value of F_{ST}, such as 0.05, may be highly significant in actual terms of population differentiation (Wright, 1978). The interpretation of F-statistics can be based upon permutation procedures (as used by GENETIX, the software used for the following analysis), where real data is compared to a large number of simulated data sets produced from the genotypes of the population, leading to an unbiased P-value for that particular test.

6.2 METHODS

Genetic diversity within each group was measured by calculating the average number of alleles per locus and the observed (H_O) and expected (H_E) heterozygosities using GENETIX software program (Belkhir *et al.*, 1996-1997; available at http://www.univ-montp2.fr/~genetix/genetix.htm). Wright's F-statistics were used to identify within and between group structure. The F-statistics were estimated using Weir and Cockerham's (1984) method, which is less dependent on the number of samples (number of groups and number of individuals within the groups) than alternative methods (e.g. Nei and

Chesser, 1983). It was hypothesised that female philopatry and resident monopoly within the troops should lead to outbreeding within each troop (negative F_{IS}) and differentiation between troops (positive F_{ST}). Additionally, F_{IS} and F_{ST} were calculated for the females of the troops only, and the non-adults of the troop only, and compared to the troop F-statistic values, as it is suggested that the partitioning of genetic variance can be very different between pre- and post-dispersal age classes (Basset *et al.*, 2001).

The GENETIX program uses a permutation approach to test the estimated F-statistics for their departure from the null hypothesis (for F_{IT} and F_{IS}, this assumes Hardy–Weinberg equilibrium; for F_{ST}, no substructure i.e. no difference between different social groups). 10000 permutations were carried out for each analysis.

Linkage disequilibrium (LD) is a measure of non-random association between two alleles or loci, caused by real association between loci, when they are physically linked on one chromosome, or when there is selection on multilocus genotypes. Apparent LD can also be caused by population substructure, with the presence of subgroups in some samples. Linkage equilibrium may eventually be obtained under random mating, but will take longer than attainment of HWE for alleles of a single gene. If two loci are closely linked, it effectively means that the information obtained is equivalent to only one locus. Linkage disequilibrium is here measured using the average correlation coefficient as defined in Garnier-Géré and Dillmann (1992) which is applied by GENETIX, based on the correlation coefficients between all pairs of alleles and tested using 1000 permutations in GENETIX. Genotypes analysed by GENETIX were those of individuals typed at four or more loci, reducing the overall sample size to 80.

6.3 RESULTS

6.3.1 Genetic variation and Hardy-Weinberg equilibrium

All eight loci used to genotype the population were polymorphic, with between three (D6S271 and D12S375) and nine (D3S1766) alleles per locus across all groups. There was less variation in the average number of alleles across groups, from 3.1 (Bijolai) to 3.9 (Kailana and Bhadreshwar AMB) (TABLE 6.1). Expected heterozygosities ranged from 0.17 (D7S503 in Bijolai) to 0.87 (D3S1766 in Bijolai).

		D3S1766	D5S1457	D6S271	D7S503	D12S375	D14S306	D16S420	D17S791	Total	NA
Kailana	H_E	0.81	0.69	0.29	0.60	0.48	0.70	0.62	0.53	0.59	
n=15	H_O	0.90	0.71	0.33	0.79	0.71	0.71	0.85	0.60	0.70	3.875
	F_{IS}	-0.12	-0.04	-0.13	-0.35	-0.53	-0.02	-0.38	-0.11	-0.20**	
Chandpol A	H_E	0.76	0.78	0.46	0.62	0.22	0.61	0.67	0.52	0.58	
n=21	H_O	0.78	0.50	0.56	0.89	0.25	0.79	0.79	0.68	0.66	3.625
	F_{IS}	-0.03	0.37*	-0.22	-0.46**	-0.12	-0.31	-0.18	-0.32	-0.13*	
Nimba	H_E	0.61	0.74	0.51	0.22	0.51	0.64	0.71	0.51	0.56	
n=17	H_O	0.53	0.79	0.63	0.24	0.54	0.65	0.93	0.63	0.61	3.500
	F_{IS}	0.13	-0.07	-0.24	-0.08	-0.06	-0.01	-0.22	-0.22	-0.09	
Chandpol B	H_E	0.74	0.80	0.57	0.58	0.51	0.78	0.69	0.59	0.66	
n=8	H_O	0.67	0.86	0.75	0.86	0.63	0.86	0.75	0.71	0.76	3.750
	F_{IS}	0.11	-0.07	-0.40	-0.48	-0.25	-0.11	-0.09	-0.22	-0.17*	
Bijolai	H_E	0.87	0.79	0.53	0.17	0.57	0.78	0.62	0.62	0.62	
n=6	H_O	0.80	0.50	0.40	0.17	0.50	1.00	1.00	0.50	0.61	3.125
	F_{IS}	0.22	0.40	0.27	-0.04	0.14	-0.30	-0.58	0.31	0.06	
Bhadreshwar	H_E	0.78	0.67	0.52	0.74	0.53	0.59	0.69	0.68	0.65	
n=13	H_O	0.56	0.33	0.27	0.82	0.50	0.82	0.82	0.91	0.63	3.875
	F_{IS}	0.33*	0.52	0.49	-0.08	0.07	-0.40	-0.20	-0.22	0.06	
Total	H_E	0.81	0.77	0.50	0.55	0.49	0.68	0.70	0.56	0.63	
	H_O	0.69	0.62	0.49	0.65	0.50	0.76	0.83	0.63	0.65	
	F_{IS}	0.16**	0.17*	0.00	-0.20**	-0.01	-0.12	-0.20**	-0.13*	-0.03	

TABLE 6.1 Observed (H_O) and expected (H_E) heterozygosities, and departures from Hardy-Weinberg equilibrium (F_{IS}) for all groups and for all loci. NA = average number of alleles per locus in each group. *** $p < 0.001$, ** $p < 0.01$,* $p < 0.05$.

6.3.2 Linkage disequilibrium

When all the populations were analysed together, significant linkage disequilibrium (LD) was observed in 12 out of 28 possible pairwise allele combinations (TABLE 6.2). Across groups, significant LD was seen in 14 out of 168 pairwise comparisons.

There was no particular trend seen in the distribution of significant LD, with significant values distributed evenly across the 28 pairs of loci. LD observed in this study did not show any trends such as significant LD for certain pairs of loci, therefore it was assumed that none of the loci were linked, as might be expected for human microsatellites derived from different chromosomes.

6.3.3 Population structure

The overall F_{IT} value for the population was –0.016, -0.038 for the troops alone; neither of these values was significant, suggesting that the population fulfilled HWE expectations. However, the overall F_{IS} value for the Jodhpur troops was –0.125, significantly different from the null hypothesis ($p < 0.001$), indicating that the troops contained more heterozygotes than would be expected under HWE. This significant difference was preserved, though the value of F_{IS} was less negative (F_{IS} = -0.098, $p < 0.001$) when the AMB was included.

When subgroups of the troops were examined the adult females of a troop had, on average, a more negative F_{IS} than the entire troop, whereas the non-adults' F_{IS} was less negative than the troop F_{IS} (TABLE 6.3; FIGURE 6.1). However, taken troop by troop, there was great variation seen. In Kailana, Chandpol A and Nimba, female F_{IS} was more negative than that of the non-adults. In Bijolai, the adult female F_{IS} was positive and much higher than the troop value, and in Chandpol B, the non-adults F_{IS} was –0.63, an extremely low value compared to other non-adult, and troop values. It is important to bear in mind the small sample sizes involved in these calculations are likely to produce large stochastic variation.

Loci	Kailana	Chandpol A	Nimba	Chandpol B	Bijolai	Bhadreshwar	All groups
D3S1766/D5S1457	0.28	0.30	0.39**	0.57	0.00	0.85	0.13
D3S1766/D6S271	0.35	0.17	0.27	0.62	0.99*	0.33	0.13
D3S1766/D7S503	0.34	0.23	0.34	0.97	0.51	0.30	0.11
D3S1766/D12S375	0.39	0.26	0.32	0.33	0.96	0.48	0.16*
D3S1766/D14S306	0.50	0.15	0.31	0.60	0.60	0.43	0.11
D3S1766/D16S420	0.43	0.21	0.37	0.78	fixed allele	0.28	0.10
D3S1766/D17S791	0.33	0.22	0.30	0.79	0.99	0.44	0.12
D5S1457/D6S271	0.27	0.25	0.35	0.30	0.00	0.90	0.15
D5S1457/D7S503	0.24	0.19	0.32	0.58	fixed allele	0.48	0.09*
D5S1457/D12S375	0.08	0.41*	0.23	0.54	0.00	0.70	0.20
D5S1457/D14S306	0.23	0.34*	0.22	0.50	0.00	0.98	0.10
D5S1457/D16S420	0.28	0.25	0.22	0.49	fixed allele	0.42	0.10
D5S1457/D17S791	0.25	0.21	0.35*	0.67	0.91	0.55	0.10
D6S271/D7S503	0.18	0.17	0.28	0.48	0.17	0.33	0.13
D6S271/D12S375	0.20	0.41	0.32	0.41	0.67	0.47	0.07
D6S271/D14S306	0.41	0.38	0.10	0.80*	0.77	0.20	0.15
D6S271/D16S420	0.37	0.09	0.22	0.38	fixed allele	0.24	0.13
D6S271/D17S791	0.50	0.09	0.23	0.84	0.80	0.13	0.11
D7S503/D12S375	0.29	0.28	0.38	0.50	0.00	0.43	0.10
D7S503/D14S306	0.58*	0.11	0.27	0.77	0.96	0.28	0.08
D7S503/D16S420	0.55	0.30	0.23	0.34	fixed allele	0.44	0.12
D7S503/D17S791	0.43	0.24	0.23	0.74	0.54	0.27	0.11
D12S375/D14S306	0.21	0.40	0.40	0.54	0.96	0.40	0.15
D12S375/D16S420	0.40	0.24	0.25	0.25	fixed allele	0.73*	0.18**
D12S375/D17S791	0.18	0.10	0.27	0.51	0.57	0.17	0.11
D14S306/D16S420	0.62**	0.12	0.23	0.44	fixed allele	0.31	0.11
D14S306/D17S791	0.52*	0.07	0.26	0.78**	0.66	0.36	0.14
D16S420/D17S791	0.50	0.23	0.25	0.53	fixed allele	0.25	0.10

TABLE 6.2 Linkage disequilibrium. *** $p < 0.001$, ** $p < 0.01$,* $p < 0.05$. All other values are non-significant.

TABLE 6.3 F_{IS} values calculated for subgroups of troops. AF: adult female; J/WC: juveniles/ white coats. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; all other values are not significant.

Troop	F_{IS} (whole troop) (n)	F_{IS} (AF) (n)	F_{IS} (J/WC) (n)
Kailana	-0.20 (15)***	-0.26 (7)**	-0.02 (4)
Chandpol A	-0.13 (21)**	-0.18 (11)*	-0.11 (9)
Nimba	-0.09 (17)	-0.15 (8)	-0.09 (8)
Chandpol B	-0.17 (8)*	-0.14 (6)	-0.63 (2)
Bijolai	0.06 (6)	0.14 (3)	-0.11 (2)
Total	-0.13 (67)***	-0.16 (35)***	-0.11 (25)*

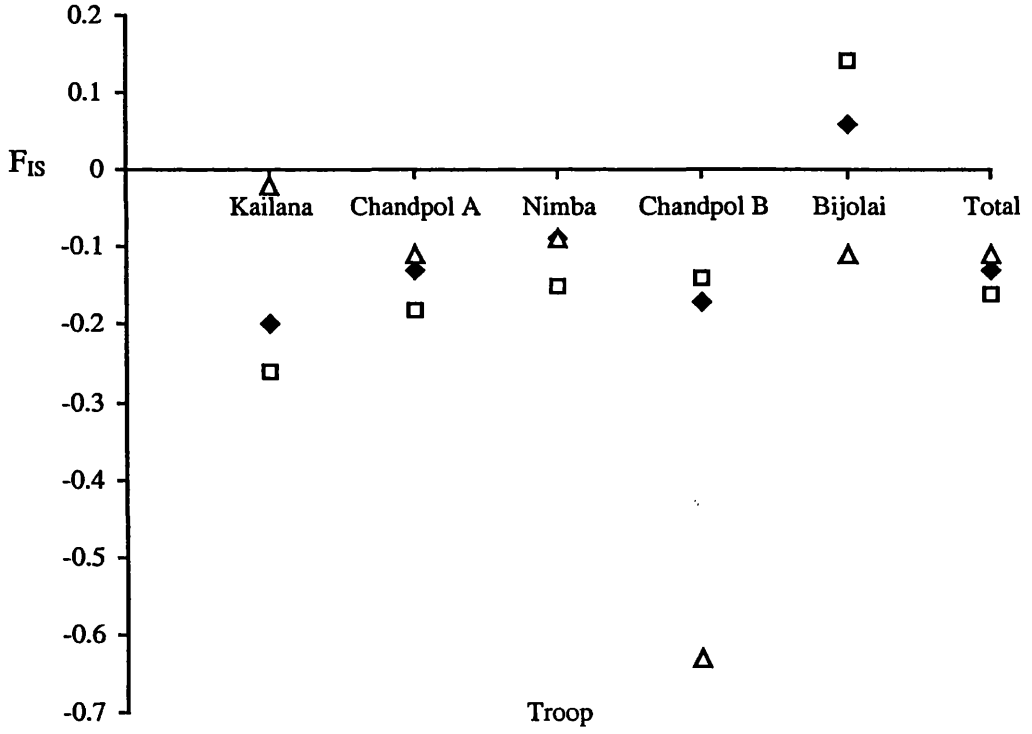


FIGURE 6.1 F_{IS} values of subgroups of troops. ◆ whole troop; □ adult females; Δ juveniles/ white coats.

The average F_{ST} was high and significant (0.077, $p < 0.001$), an effect that was little reduced with the inclusion of the AMB (0.075, $p < 0.001$). Pairwise analysis of F_{ST} values between groups did not suggest that any particular group was responsible for this

between group differentiation (TABLE 6.4). The F_{ST} values for subgroups of the troops were 0.075 ($p < 0.001$) for the adult females, and 0.146 ($p < 0.001$) for the non-adults. A summary of the F-statistics for various subgroups of the Jodhpur population is shown in TABLE 6.5.

TABLE 6.4 Pairwise values of F_{ST}; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

	Chandpol A	Nimba	Chandpol B	Bijolai	Bhadreshwar
Kailana	0.110***	0.095***	0.058**	0.060*	0.067**
Chandpol A		0.072***	0.021	0.111***	0.087***
Nimba			0.047*	0.041*	0.069***
Chandpol B				0.040	0.062*
Bijolai					0.003

TABLE 6.5 F-statistics for the Jodhpur population. AF: adult females; J/WC: juveniles/ white coats. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Subgroup	Troops & AMB	Troops only	AF	J/WC
F_{IS}	-0.098***	-0.125***	-0.163***	-0.112*
F_{ST}	0.075***	0.077***	0.075***	0.146***
F_{IT}	-0.016	-0.038	-0.11**	0.050

6.4 DISCUSSION

6.4.1 Genetic variation

Chesser (1991) modelled the effects of female philopatry and male polygyny on the partitioning of genetic variation within populations. He found that in a finite population, the more males that breed in a troop, the closer the situation is likely to be to panmixis, hence heterozygosities will approach those of HWE. Conversely, in a one-male troop, an unrelated breeding male represents an extreme subunit of the population introduced to the troop, the resulting progeny will appear outbred, and heterozygosities will exceed HWE expectations. Genetic variability in the Jodhpur langurs was reasonably high, with an average H_E of 0.63. This was very close to the variability seen at Ramnagar, Nepal, the only other langur population to have been studied using

microsatellites, where the average H_E was found to be 0.59 (Launhardt, 1998). Observed heterozygosities were extremely close to that expected in the Ramnagar population. In contrast, the observed heterozygosities of the Jodhpur population were, when examined by loci or group, higher in many cases than the expected values (TABLE 6.1), a difference perhaps because of the differing social organisations of these two populations. Ramnagar troops usually contain more than one breeding male, in contrast to the almost exclusively one-male organisation seen at Jodhpur. The F-statistics of the Jodhpur population also support the one-male breeding situation, as shown below.

6.4.2 Divergence from HWE among breeding groups

F_{IT} values for the troops were not significantly different from zero, indicating that the populationwide genotypic frequencies conform to panmictic proportions, as would occur with HWE. However, the relationship between the F-statistics, $[1-F_{IT}] = [1-F_{ST}][1-F_{IS}]$ (Storz *et al.*, 2001), is such that a positive F_{ST} can be counteracted by a negative F_{IS} to give an F_{IT} approaching zero. By examining F_{IT} alone, it could be wrongly concluded that the population is randomly mating. In order to describe the population structure fully, F_{IS} and F_{ST} must also be calculated, to take into account the population subdivision.

6.4.3 Divergence from HWE within breeding groups

One unrelated resident represents the smallest possible sample of total population genetic variability that could be introduced to a troop, and the variance between his allele frequencies and those of the troop females will be most extreme, leading to the most outbred offspring (Chesser, 1991). Negative F_{IS} values were observed for all but one of the troops, indicating an excess of heterozygotes in these groups, suggestive of outbreeding. Bijolai had an F_{IS} value that is positive and non-significant. The apparent difference of this troop to the other troops may have been a stochastic sample effect (seen for example in alpine marmots, Goossens *et al.*, 2001).

Behavioural studies of the langurs are supported by this interpretation of genetically outbreeding groups. Sons are never observed to remain in their natal groups to maturity to mate with relatives (although it is not known if they may return to their natal troops to breed; observations suggest this is unlikely). Female langurs are philopatric, and gene flow between social groups is through the change of male residents. A new resident introduces new alleles to the troop, and his male progeny will in later generations distribute their mothers' alleles to other troops as they in turn gain

residencies. In this way the potentially detrimental effects of inbreeding may be avoided.

Negative F_{IS} values have been interpreted in several species as evidence for the avoidance of inbreeding (alpine marmot, Goossens *et al.*, 2001; red howler monkeys, Pope, 1992; black tailed prairie dog, Foltz and Hoogland, 1983; rhesus macaques, Melnick *et al.*, 1984). All these species show female philopatry and male dispersal, in common with the Hanuman langur. Severe inbreeding is associated with reduced reproductive success, manifest as increased spermatozoa mutations, reduced litter size, increased juvenile mortality (see Pusey and Wolf, 1996, for review). The detrimental consequences of inbreeding are suggested to have led to the evolution of mechanisms by which inbreeding can be avoided, including kin recognition and dispersal. In fact, observations suggest that inbreeding is rare in large wild mammal populations, though it has been documented in some species (e.g. olive baboons, Packer, 1979; lions, Packer *et al.*, 1991b).

However, intrasexual competition might be a better proximate explanation than inbreeding avoidance for the occurrence of male juvenile dispersal in langurs (Moore, 1993), including those at Jodhpur. Inbreeding avoidance would suggest that emigrations were voluntary, or that there was some sexual attraction towards members of other troops. Observations contradict these predictions – males generally leave before they reach maturity, making it unlikely that attraction caused them to emigrate. Additionally, 76% of emigrations were as the result of the intolerance of a new resident, actively chasing out the immature males (Rajpurohit and Sommer, 1993). An adult male taking over a troop may force out young males because they will otherwise mature to compete with him for the troop's females, or he may oust them because they represent competitors for food with him and his future offspring. Thus, intrasexual competition for either food or mates provides a better, or at least more parsimonious proximate explanation for langur male dispersal than inbreeding avoidance, though it should be noted that incest avoidance will be a consequence of the dispersal of one sex.

Chesser (1991) shows that mating of an unrelated male with related females should produce an excess of heterozygotes in the progeny. In fact, the F_{IS} values of the non-adults of each troop were on average less negative than that of the females, suggesting a more homozygous subgroup. The only troop in which an extreme case of outbreeding

was seen in the non-adults was in Chandpol B, where the two non-adults had an exceptionally negative F_{IS} of -0.63.

The F_{IS} of the offspring in the remaining troops mirrored that of red howlers, where the offspring had a less negative F_{IS} than the females (Pope, 1992) (FIGURE 6.2). In the howlers, this was attributed to the demographic structure of the smaller social units, where the adult females of the group were probably fathered by the same male, and therefore be a most extreme case of outbreeding, whereas the offspring were more likely to be the progeny of several residents, and so represent a less outbred subgroup (Chesser, 1991). However, in the langur troops, it was probably the case that the adult females were the progeny of several residents, whereas the non-adults present were more likely to be the progeny of one, or a few, residents, so the reversed values of F_{IS} might be expected.

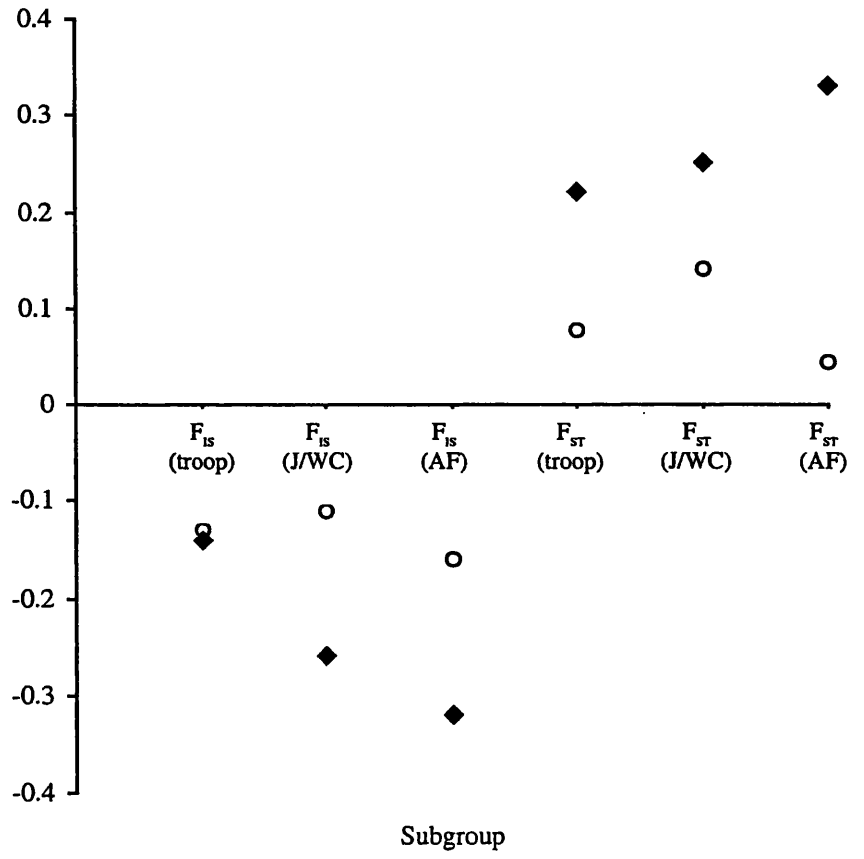


FIGURE 6.2 F_{IS} and F_{ST} values for subgroups of troops for o langurs and ◆ red howler monkeys.

It must be borne in mind that F-statistics have been developed to describe populations. They may not be appropriate for dealing with the small sample sizes involved in the analysis of subgroups of one-male troops, which are by their nature not good approximations to randomly breeding populations. It is probable that the small sample sizes involved in the calculation of F_{IS} for subgroups are vulnerable to stochastic variation, and any inferences drawn from the F-statistics should not be overinterpreted, at least until future studies can produce larger sample sizes. Until more data is available it will not be possible to identify significant trends in the F_{IS} values of subgroups of troops, or explain the factors that contribute to these trends.

The F_{IS} value of the Bhadreshwar AMB was significantly different from that of the troops and not significantly different from zero, suggesting that in contrast to the females, it was not an outbred group. The observed heterozygosity within the AMB was also lower than expected. Sampling from an AMB is the equivalent of sampling a few individuals from each of a range of troops, as AMBs comprise males who have joined the band from several different troops. Such a combination of individuals derived from troops with differing allelic frequencies is likely to produce a Wahlund effect – an apparent deficit of heterozygotes. Similar Wahlund effects have been observed in studies where sampling has unintentionally combined several breeding groups as the lowest hierarchical level of population structure, e.g. Goossens *et al.* (2001). Because an AMB is not a breeding group, and membership is constantly changing, the F_{IS} value will always be less negative than the troops from which the band members originate.

6.4.4 Population structure

The significant F_{ST} values for the population are suggestive of population substructure. The troops of Jodhpur exhibit a well-defined geographical structure. Geographical models of genetic differentiation (e.g. Wright, 1978) suggest that low immigration in such populations will result in an increase in consanguineous matings, leading to a loss of genetic diversity within groups. Genetic differentiation between groups (F_{ST}) will increase. In the Jodhpur population, although F_{ST} was high, F_{IS} was significantly negative. In fact, the langur troops' differentiation is primarily derived from the differentiation of gene frequencies between male and female gametes within troops (Pope, 1992). Unrelated resident males mating with genetically different females will increase the variance in gene frequencies among the troops (hence F_{ST}) but decrease the correlation of gametes within the troops (so reducing F_{IS}). The negative values of F_{IS}

and positive values of F_{ST} are maximised by the polygynous organisation of the troops and sex biased dispersal (Chesser *et al.*, 1993).

A troop comprises a non-random subset of the alleles present in the entire population, as demonstrated by the significant deviation of F_{ST} for troop females, troop non-adults and entire troops from expected under the conditions of HWE. A single male per generation will increase the coancestry (probability of alleles being identical by descent), and relatedness, of the resulting females of the troop, so increasing the substructuring of the entire population. If more males were present in a troop at any one time, the differentiation between subgroups and the excess heterozygosity within the troops would be decreased (Chesser, 1991). This is perhaps what gives rise to heterozygosities close to the expected values in the Ramnagar population, where there are often multiple males breeding within the troop (Launhardt, 1998).

The F_{ST} value for all the non-adults was much greater than that of the females, and for the troops as a whole. The females showed less population substructure than the offspring. This is perhaps because the offspring represented only one (or few) cohort(s) whereas the females represented several generations of outbreeding. This would lead to the offspring appearing more highly structured than the females. The non-adults could be considered as an instantaneous sample of population substructure, whereas the females represented several generations of gene flow, and their substructuring was consequently reduced. Again, these results contrast with the red howlers, where the F_{ST} value of the adult females exceeded that of the offspring. The cause of this was interpreted by Pope (1992) as the direct opposite of that in langurs; that female howlers could be the progeny of as few as one male resident, whereas the offspring were more likely to be the result of several females and males, thereby reducing substructuring. The F_{ST} values of subgroups are thus apparently variable between species depending upon their group size and composition, and length of male residency.

This also demonstrates that F_{ST} values can vary widely depending upon the time at which sampling is carried out relative to dispersal. Basset *et al.* (2001) found that in simulations, F_{ST} values were always higher when genotypes were sampled before dispersal than after. In this study, the multi-generational females of a troop were the equivalent of an 'after dispersal' sample, as they represented the genetic variance that exists in a social group after generations of gene flow between troops. Conversely, the non-adults represented the troop's situation 'before dispersal', a single generation of

offspring before they had left their natal units. Drawing these parallels, the findings of Basset *et al.*'s (2001) simulations agree with the results of this study for the F_{ST} values of the subgroups. Dispersal of males between troops causes, over time, a reduction in substructuring, revealed in the reduced F_{ST} of the females, whereas at any particular moment in time, represented here by sampling the non-adults who had not yet dispersed, substructuring in the population is more extreme. Each troop's non-adults were closely related (Chapter 4) and were likely to be paternal sibs, accentuating population substructure. This variation in F_{ST} highlights the importance of knowing the age class of the individuals sampled if genetic differentiation in a population is to be detected and correctly interpreted.

A comparison of F-statistic values in several primate species is shown in TABLE 6.6. It can be seen that the langurs, with the exception of the red howlers, have the most extreme values of F_{ST} and F_{IS} of all the primates listed. This may be partially due to their smaller sample size, but can also be attributed to the polygynous mating system, and dominant male dispersal of the langurs and howlers; all the cercopithecines listed live in multimale groups, leading to a less structured population.

TABLE 6.6 F-statistics for five different primate species. R = relatedness. Data from (a) this study; (b) de Ruiter *et al.*, 1994; (c) Kawamoto *et al.*, 1982; (d) Rogers, 1989; (e) Melnick *et al.*, 1984; (f) and (g) Pope, 1992. Adapted from de Ruiter *et al.*, 1994.

Species	No. of groups	No. of loci	F_{ST}	F_{IS}	F_{IT}	R	Average group size
Hanuman langur, *Presbytis entellus* (a)	5	8	0.08	-0.14	-0.05	0.17	15
Long-tailed macaque, *Macaca fascicularis* (b)	6	11	0.05	0.02	0.06	0.08	38
Long-tailed macaque, *Macaca fascicularis* (c)	5	4	0.02	0.09	0.11	0.04	40
Tanzanian yellow baboon, *Papio h. cynocephalus* (d)	4	4	0.05	0.07	0.12	0.09	48
Rhesus macaque, *Macaca mulatta* (e)	5	5	0.04	-0.09	-0.05	0.08	42
Red howler, *Alouatta seniculus* (f)	14	9	0.23	-0.16	0.10	0.41	9
Red howler, *Alouatta seniculus* (g)	4	8	0.14	-0.06	0.09	0.26	9

6.4.5 Evolution of social structure

Since Hamilton (1964) outlined the concept of inclusive fitness, evolutionary biologists have examined the genetic consequences of social organisation. Dispersal and breeding strategies have profound influence upon the genetic structure within, and hence between, social groups (Chesser, 1991). Different reproductive patterns, for example polygyny and sex biased dispersal, will result in genetic partitioning within populations that is very different from that which would be expected under panmictic conditions.

Originally it was believed that dispersal was the major factor influencing coancestry within groups (e.g. Chesser and Ryman, 1986). A higher rate of dispersal was assumed to lead to greater outbreeding and a reduction of coancestry within a social group, which would negate the benefits of social behaviour. Attention was therefore focussed on the optimal dispersal rate, trading off between the level of coancestry (equivalent to half the coefficient of relatedness between two individuals) needed to benefit social behaviours and the cost of inbreeding (Chesser and Ryman, 1986). This view failed to take into account the fact that populations are more than just randomly mating subpopulations; assumptions of random mating and equal dispersal are not always justified (Sugg *et al.*, 1996). The combining of behavioural ecology with the 'classical' view of population genetics led to the development of models based on breeding groups (e.g. Chesser, 1991), demonstrating that parents may confer a high degree of relatedness upon their offspring without concurrently conveying inbreeding. These models show coancestry in social systems to be maintained primarily by the philopatric sex; coefficients of relatedness of between 33 and 40% can be maintained even with random male dispersal (Chesser *et al.*, 1993). In some 65% of mammalian taxa, female philopatry and polygyny are observed (Greenwood, 1980), suggesting that these behaviours have evolved for some selective advantage over panmictic mating.

The driving force for certain breeding and dispersal behaviours is to increase the coancestry among individuals within groups in relation to the coancestry between individuals from different breeding groups. This will result in a situation where altruistic acts within the breeding group will benefit the genes of that group's members, over the genes of other breeding groups. Female philopatry increases the level of coancestry within a breeding group to a much higher level than that observed with female dispersal (Chesser, 1991), suggesting that female philopatry has evolved to increase the potential for cooperation between the breeding females of the group; for example, langur females will benefit from cooperating with kin in home range defence

and care of offspring. The increased coancestry of offspring as a result of female philopatry is proposed to be only of secondary importance, an artefact of female non-dispersal. Additionally, female philopatry removes the cost of dispersal for females, which arises from moving into unknown territory away from established resources.

Chesser's (1991) model also emphasises that evolution of female philopatry and polygyny are not independent. There is no advantage to female philopatry if there are multiple males mating within each breeding group. In this situation, philopatry confers extremely little increase in the coancestry of a breeding group. In an extreme case, gene flow mediated by multiple males will become so high that there will be little subpopulation differentiation as the population approaches panmixia, despite the philopatry of the females. If this happens, then there is no genetic benefit to the females cooperating with their group members rather than with other individuals outside their troop. However, in groups where females are philopatric, despite the presence of multiple males within the group, there is often differential reproductive success within the males, making the breeding system tend towards being more effectively monogamous. Such a case is seen in the multimale langur troops at Ramnagar, Nepal, where the dominant male of a troop achieves 57% of the paternity on average (Launhardt *et al.*, 2001). In this situation, the advantages to the females of cooperation within the group are presumably still valid; unfortunately F-statistics for the Ramnagar population are not available for comparison.

In other social organisations, where dispersing males might have more equal reproductive success, coancestry of the group will be low, F_{IS} is likely to be more positive, and there will be less genetic incentive to stay and cooperate within a breeding group. In such a situation females might more readily disperse from their natal group and join with other females, if ecological factors allow. For example, data from Taï forest chimps (Vigilant *et al.*, 2001) indicate F_{IS} values that are not as significant as that of the polygynous langurs (TABLE 6.7); they do, however, show significant substructuring ($F_{ST} = 0.019$, $p < 0.001$). In the case of the Taï chimps, females leave their natal groups and disperse away from relatives, whereas males stay within their natal communities, and many of them will have an input into the paternity of that group; for example, in one community, the paternity of 23 offspring was distributed between at least six males. A reduction in consanguinity of the communities results, and F_{IS} is less significant (indicative of more panmictic mating), so there is less incentive for the

females to remain in their natal groups because the benefits of kin selection are decreased.

TABLE 6.7 F_{IS} values for three subgroups of Taï forest chimpanzees. Data from TABLE 3 of Vigilant *et al.*, 2001, available on the PNAS website, http://www.pnas.org.

Population	North	Middle	South	Combined
F_{IS}	-0.028 (n.s.)	-0.049 (n.s.)	-0.023 (n.s)	-0.028 ($p<0.05$)

Finally, Pope (1992) suggested that the high level of heterozygosity observed in the red howlers may provide a mechanism for adaptation to new environments, since there is variability within groups upon which selection can act to form populations adapted for new habitats. This genetic variability might be particularly important in folivores dealing with an array of secondary compound in plants, many of which may be potential toxins. New troop formation is not often observed among the langurs of Jodhpur, but it must happen at times, mostly through troop fission. As a result, there has been an increase in the number of troops at Jodhpur over the last 20 years, from 29 to 38 harems. The high heterozygosity within troops and the variation between troops might be an important factor in the langurs' ability to colonise and exploit a variety of habitats.

6.5 CONCLUSION

The langur population at Jodhpur was highly structured, as indicated by positive F_{ST} values for the troops. Each troop was apparently outbred, with negative F_{IS} values, whereas the AMB had a less negative, non-significant F_{IS} value. These findings support the behavioural observations that the population is structured in discrete troops, between which gene flow is mediated by dispersing males. Relatedness of troop members is increased, and inbreeding is prevented, by regular resident male changes, who confer common ancestry to their offspring.

The F_{IS} of the AMB is a result of the Wahlund effect. Bands comprise individuals derived from several troops whose alleles are not reassorted by mating in this unisex group. Such a situation results in an excess of homozygotes in the group.

The F-statistics of the Jodhpur population describe an extreme of primate social organisation. Structuring of the population and outbreeding of groups are greater than in other primates. Such a social system, with polygyny and male dispersal gives rise to a situation where there are great genetic incentives to cooperating within troops.

7. CONCLUSIONS, RECOMMENDATIONS AND FURTHER RESEARCH

7.1 CONCLUSIONS

Several hypotheses that have developed from long term behavioural studies of the Jodhpur langurs have been tested in this thesis using microsatellite data obtained from faecal DNA sampling. The results provide a new perspective on langur social behaviour and open up many possibilities for future research. Furthermore, it is also possible to compare Jodhpur langurs with the contrasting multi-male population from Ramnagar, Nepal, and discover how differing social organisations are reflected in the genetic compositions of social groups.

1. *Females cooperate within a troop because they are, on average, closely related.*
Theoretically, cooperation between group living animals could be attributed to reciprocal altruism, as group living fulfils the conditions necessary for this: individuals are together for a long time, so they can recognise individuals and they have time over which acts can be reciprocated. The fact that female philopatry creates a social unit within which relatedness is higher than average is therefore not a prerequisite for the occurrence of cooperative behaviours. However, where cooperative acts occur between philopatric group members, there will be additional effects of increased fitness benefits for those involved. Therefore, the explanation of reciprocal altruism need not be invoked if the group members are found to be related, as kin selection provides a more parsimonious solution.

On average, the results of RELATEDNESS showed troop females, non-adult troop members, and troops as a whole to comprise closely related individuals. However, large intertroop variation was also apparent, with some troops' females appearing no more related than individuals randomly selected from the population. The low relatedness of some troops is attributed to the effect of successive residents. Each resident may introduce new alleles to the troop, causing his offspring to be closely related, but simultaneously causing them to appear extremely different to their mothers. This finding has demonstrated how data can produce results which, taken at face value, can be misleading if other factors, such as female philopatry, multigenerational troops, and successive residents are not considered. Low empirical relatedness is not reflected in how the troop members behave towards one another. The pairwise relatedness of a dyad that is calculated from genetic data may be extremely low as a result of one member having a rare allele at one locus; nevertheless, they may still be mother and

daughter. Thus, langur behaviour should be considered to be mediated by absolute, not empirical relatedness.

2. *Male band members are, on average, not closely related, so cooperation is not driven by kin selection.*

The only AMB that was adequately genotyped had a very low level of relatedness between members, indicating that they were unlikely to be close relatives. However, several of them could have been first degree relatives, as shown by the KINSHIP network created, and many of these shared alleles at each locus, implying that they could be father-son relationships. It is not unlikely that this was the case, as ousted residents and young males from the same troop (possible sons, and therefore paternal sibs) have been observed to migrate into the same AMB. Overall, despite the potential presence of close relatives, the band relatedness was low, suggesting that it is unlikely that the males should support of one another for kin selected reasons. It is more likely that in takeover situations when members appear to be supporting one another, they are in fact acting for proximate egotistical reasons: each male wants to get his own residency, and to achieve this, he must selfishly use his fellow band members. A dominant band member will benefit from the rest of the band buffering attacks by a resident, and subordinate band members will accompany a dominant male entering a troop, primarily because there is safety in numbers; males do well to remain with one another rather than become solitary, especially for the youngest males. Further reasons for the male members to stay together during takeovers are possibly because they will rise in the band hierarchy once the band's alpha male has become a troop resident, or because the takeover of a band provides a rare opportunity for mating – though conception is unlikely, practice at copulation might also be valuable.

3. *Resident males father all offspring during their tenure.*

Genetic data from Jodhpur showed that in three of the sampled troops, resident males were potential fathers of all of the youngest troop members. These residents were known, or suspected, to have held their residencies for over three years. In two other troops residents could be excluded as fathers for most offspring; these were known to be residents that had taken over the troop in the last three months. Findings presented here, based on exclusions of residents alone, support the hypothesis that a resident has monopoly of access to the troop females and fathers virtually all the offspring conceived during his residency.

Inclusion or exclusion of the current resident was the extent of practicable analysis of this data, as data from AMBs was so scarce. Had sampling been more extensive from AMBs, it might have been possible to identify previous residents that had fathered several consecutive offspring.

The finding that residents father all (or almost all) offspring during their residency also provides at least indirect support for the hypothesis that infanticide is a sexually selected behaviour. Critics of this hypothesis have argued that infanticide is an indirect consequence of the generally heightened aggression during takeover, or is aberrant, pathological behaviour (Curtin and Dolhinow, 1978). Jodhpur paternity assignments would support the case that infant killing is adaptive behaviour directed specifically at unrelated unweaned infants: newly incoming males should not be the father of those infants they kill, and should then have a monopoly of the females whilst they are resident.

4. *The Jodhpur population shows significant genetic variability because of its grouping and mating system.*

The langur population is subdivided into discrete units of troops and AMBs. The observations that the females are philopatric and breed with only one male at a time suggest that the population should show great genetic subdivision. This is illustrated by the high F_{ST} values of the troops. Additionally, F_{IS} values are significantly negative, showing that the troops are outbred as a result of a regular influx of new residents' alleles.

The F-values calculated describe a population ideal for the evolution of social behaviour within troops. Population subdivision makes cooperative behaviours beneficial to inclusive fitness of the actor when directed within a troop, members of which are more similar to the actor than members of neighbouring troops. Apart from the potential costs of dispersal, it is therefore also advantageous to stay within the troop and cooperate with relatives rather than emigrate to other troops, where cooperative behaviours will not similarly increase inclusive fitness. However, the young males are forced to disperse by the resident; they represent competitors for food within the troop, and could also compete with the resident's own sons in the future (Rajpurohit and Sommer, 1993). Troop females are present or future breeding partners of the resident; they remain in familiar company and surroundings. In this way, female philopatry and polygyny is perpetuated.

7.2 EVALUATION OF METHODS

This study sampled a genetic snapshot of the Jodhpur langur population. It was not intended to involve a detailed behavioural study, but simply to survey selected troops and bands from the area and collect non-invasive samples of genetic material from them. The detailed behavioural studies of the population had been conducted when genotyping from faeces had not yet been developed as a method to supplement field studies. The challenge lay in determining how much could be revealed from this sampling method, in contrast to other genetic studies which directly compliment long-term behavioural studies (e.g. Vigilant *et al.*, 2001; Launhardt, 1998).

The problems encountered during fieldwork on a day-to-day basis were those of identification and sample collection. It was difficult to identify individuals of an entire group accurately within two to three weeks, during which sample collection occurred. At the beginning of a sampling period, many animals could not be identified when they defecated, so samples were not taken, whereas towards the end, when the animals were easier to identify, much time would be spent waiting for particular individuals to defecate. Additionally, defecation could take place in inaccessible places, such as over water, or on roofs to which there was no access.

An entire age class, black-coat infants, proved almost impossible to sample. Only once was a black-coat seen to defecate, and the amount of faeces was so minimal that it was extremely difficult to separate it from the dusty ground upon which it fell. Only a single extraction was possible, from which genotyping was successful. Apart from this, no other defecation was seen, and it was suspected that the blackcoats generally defecate onto the caregiver holding them at the time. Additionally, both mothers and allomothers spend much time inspecting and cleaning the ano-genital region of their charges.

The most problematic samples to collect were those of the adult males. The troops were always easily located within a prescribed home range, though it was not always possible to follow the troops throughout this area. However, the AMBs were extremely difficult to find. The fact that they were mostly located during noisy, energetic encounters had two results. First, it was more difficult to get close to the groups and identify them, and they would often quickly leave the area afterwards, making following difficult, especially in urban areas. Secondly, many of the males would defecate during these encounters. In the field, this seemed advantageous, as many of the band members could

be sampled at the same time, which reduced the need to follow them. However, these faeces voided in stressful situations later proved to yield very poor quality DNA extractions, probably because of the dilution or degradation of the genetic material present by excessive water or high nuclease concentrations.

7.3 RECOMMENDATIONS FOR SAMPLE COLLECTION

A longer field study period could improve the sample collection. It should allow more time to be spent with each troop, enabling better identification of individuals, and possibly duplicate sampling of individuals. However, in this study it was generally found that samples worked adequately if they were solid when collected, and it is suggested that it is more important to collect a single good quality sample than to concentrate on collecting two samples of any quality. With this in mind, it would be worth considering the diet of the groups from which the samples are collected, in particular the amount of provisioning that they receive. Samples that were most successful were those from groups that had received little provisioning, perhaps confirming why Launhardt (1998) did not report such difficulties with genotyping from the unprovisioned Ramnagar groups. However, since many of the hypotheses about the social behaviour of the langurs at Jodhpur are based upon their provisioned nature it is imperative that samples are collected from groups which experience varying levels of provisioning.

Returning to troops six months after they were first encountered would allow samples to be obtained from the youngest members of the troop, that were impossible to collect when they were black coats. These individuals are of particular interest as, if it is the case that a resident has mating monopoly of access to troop females, blackcoats could be indicators of recent resident history in a troop.

More time would also allow larger troops to be sampled. All the troops ultimately genotyped in this study were smaller than average. Larger troops should be less vulnerable to stochastic variations in results. Also, the potential of a resident to monopolise a larger troop could be investigated; genotyping of all troop members could reveal extra–troop paternity, and maybe a consequential lowering of overall relatedness.

A longer field study, or one involving more researchers, would have better chances of locating AMBs when they were not interacting with other bands or troops. This would

hopefully lead to more observation time of bands during more peaceful day-to–day activities, making it possible to identify individuals and class them by age. This information, along with the possibility of collecting good quality faecal samples would allow much more detailed investigation of the AMB relationships.

Another recommendation is to have more than one fieldworker collecting samples. The advantages of only one person having to identify the group members, rather than two people having to reach a consensus on identity, are likely to be outweighed by the benefits of having two observers watching for defecation, as the troops and bands may often spread out over large areas and it is not always possible for one person to observe all individuals at all times.

7.4 RECOMMENDATIONS FOR SAMPLE SCREENING

Screening of samples in the lab could be improved by the development of multiplex PCR systems, whereby more than one locus is amplified in the same reaction. A multiplex system can take some time to optimise, as relative primer concentrations need to be adjusted, and it is dependent on the quality and amount of template DNA, but once a system has been developed it can reduce the amount of time taken to screen samples, and therefore increase the number of samples that can be screened. This method also reduces consumable costs, and removes the step of multi-loading samples, which can introduce error and contamination. It would also be possible to use more loci in the same amount of screening time, which would increase the power of the analysis.

7.5 FUTURE POSSIBILITIES FOR RESEARCH

Relatedness of troops

Contrasting the Jodhpur and the Ramnagar troops' relatedness would determine whether this R value is affected by social organisation. Ramnagar troops are expected to be less related because of the multi-male situation, leading to: lower F_{ST}; less genetic incentive to cooperate with members of the same social group than those of other groups; more female transfer; or the formation of a dominance system where female kin support one another and rank is inherited. Neither female transfer nor such 'matrilineal dominance' is seen at Ramnagar (Koenig and Borries, 2001). It would be informative to compare measures of cooperative and competitive behaviours, such as allogrooming and

displacement frequencies, from the Ramnagar troops with their genetic relatedness, and contrast these with the Jodhpur results to see if there are correlations between the relatedness of the troop members and their propensity to cooperate or compete with one another.

Paternity

Paternity assignments in more troops would be possible with a larger data set. Typing of more extra-troop males might allow identification of previous residents, or of extra-troop fathers, now in AMBs. Typing more AMB members would also provide more complete data for CERVUS to analyse, hopefully producing more accurate paternity assignments. Comparison of these CERVUS results could also be made with Ramnagar data analysed by CERVUS (previous studies e.g. Launhardt, 1998, have only used exclusions to assign paternity), with the results of both populations compared with those assigned by exclusion. It is likely to be the case that Ramnagar paternity assignments by CERVUS would be more successful, as a larger proportion of the population's males will have been sampled.

Infanticide

In the long term, it would be desirable to sample from langurs involved in infanticides witnessed at Jodhpur (the attacker, the victim, and the subsequent offspring of the victim's mother). Such data would compliment that of Borries *et al.* (1999) which directly demonstrated that in the Ramnagar population, attackers were not related to their victim, but in several cases fathered the subsequent offspring.

Detailed description of AMB structure

Improved identification and sampling of Jodhpur's AMB members would allow a new aspect to the analysis carried out in this thesis. Not only could the overall relatedness of the bands be calculated, but also the relatedness of different age cohorts, to see if relatedness decreased with increasing age within bands. Such findings would support the observation that young males emigrate from their natal troops with paternal sibs, so that the youngest AMB cohorts are formed of close relatives, but with time these groups of brothers are split up, through emigration and high mortality, so that the older cohorts contain no close relatives (Rajpurohit and Sommer, 1993); relatedness should be approximately 0. Additionally, it would be possible to analyse paternity of newly emigrant young males; it may be that their father(s) could be identified in the same AMB amongst the old members.

Ramnagar males may suffer lower mortality than the Jodhpur males; they stay in their natal troops until much later (the average age of emigration is six years as opposed to two years at Jodhpur; Koenig and Borries, 2001), and presumably benefit from kin selection and knowledge of the natal troop's home range until this age. They then leave their natal troops and soon join a breeding troop – permanent AMBs are not found at Ramnagar. It is unlikely that the hazards to which Jodhpur males are exposed – dangers of traffic, electricity wires, and unfamiliar habitats – are frequently encountered in Ramnagar, and the habitat is also possibly more homogeneous, therefore more predictable. Overall, it might be expected that male mortality is not so great at Ramnagar than it is at Jodhpur. The consequences of this may be two-fold: there will be more males competing for females, which might be another reason for the observed multi-male situation; there may also be the opportunity for paternal sibs to grow up and emigrate together, and for coalitions to take over troops. It would be informative to quantify both male mortality and the relatedness of males in Ramnagar troops, to explore the opportunities for coalitions to be established, and whether the Ramnagar males ever do so.

Population origin

Sampling from neighbouring populations to Jodhpur could help to determine from where the Jodhpur population originated. Nearest present day populations are 100km to the south, in the Kumbhalgarh Wildlife Sanctuary, and 150km to the south east, at Pushkar. Genetic drift in the Jodhpur population may have decreased the variation relative to the population founders, but it might be possible to ascertain which neighbouring population the Jodhpur langurs more closely resemble (cf. Ciofi and Bruford, 1999). It would also be interesting to compare Jodhpur with another larger population of one-male troops, which might have greater genetic variation, leading to an even more structured population.

Overall, this study has answered several questions about the genetic structure of Jodhpur's social groups, and speculated upon how these findings may explain the behaviours observed in Jodhpur's langurs. It has also highlighted an important methodological consideration that has not been highlighted in previous similar studies, the finding that the condition of the sample has dramatic effects on the success of DNA extraction. Researchers embarking on future sampling of Jodhpur langurs, or those of

similar colobine populations are strongly encouraged to consider these findings before the start of sample collection.

Data analysis programs have also been evaluated for their applicability to such a data set. It is concluded that the outputs of packages such as RELATEDNESS, KINSHIP and CERVUS should not be taken at face value, but considered in the light of what is known of the behaviour of the study species. Genetic data may be seductive in its quantitative nature; nevertheless, it should not result in the premature rejection of many years of painstakingly collected behavioural data, but rather used to compliment it.

Finally, the present study has provided an idea of how future genetic analysis of langurs at Jodhpur and at other study sites could further increase the knowledge of these monkeys' social lives and population organisation. The long tale of Hanuman and his incarnations is beginning a new chapter.

8. EPILOGUE

The genetic data presented in this thesis suggest that there is a correlation between the level of relatedness in a langur group and the level of cooperative behaviours seen within it. The high level of relatedness observed within the troops, which typically consist of one male and multiple females with infants, could explain the high level of cooperative behaviours seen between troop members, and conversely the low level of cooperation between male band members could be the result of their low average relatedness. However, observation of a correlation does not necessarily indicate a cause and effect relationship. Even if it were the case that the cooperation and relatedness were linked, relatedness could be a result of, not the cause behind such behaviour. Moreover, it is not possible to conclude from this data alone whether kin selection is the driving force behind this pattern, as this is intertwined with proximate factors such as philopatry, dispersal and familiarity of individuals. Individuals who remain within their natal groups all their lives will be more related to other group members than to randomly drawn members of the population. Can cooperating individuals distinguish between close relatives and unrelated individuals, or are they merely using a rule of familiarity when deciding whether to cooperate?

8.1 KIN SELECTION OR FAMILIARITY?

Further investigation of these questions would require an in-depth comparison of different langur populations with varying social organisations. To establish whether there is truly a causal relationship between cooperation and relatedness, it is necessary to compare groups' relatedness with the amount of cooperative behaviour observed within them, for example between the Ramnagar and Jodhpur populations, with their contrasting social structures. Furthermore, it would be informative to carry out detailed observational studies of the groups alongside genetic profiling. It may be the case that cooperative behaviours are more common amongst first degree relatives, which could indicate kin recognition leading to discrimination between troop members. Alternatively, observations could indicate that there is no link between relatedness of dyads and the frequency of cooperation, suggesting either that cooperation is determined only by group membership and thus through familiarity, or that langurs can recognise distant relatives as related individuals. In a study of the distribution of allogrooming (Borries *et al.*, 1994) it was found that grooming was preferentially directed towards first degree relatives, but also that it occurred between all troop

members. This finding might suggest that though kin selection may have a role to play in the distribution of cooperative behaviours, all troop members judge less discriminate cooperation to be a worthwhile activity.

8.2 COOPERATION VERSUS COMPETITION

It is believed that the difference in social interactions observed between female troops' members and those of all male bands of langurs are indicative of their variance in relatedness. All group living individuals are subject to within group competition as members compete for resources. Males are suggested to contest fiercely for the non-shareable resources of access to females, in part because of the lack of relatedness between the members. The females, in contrast, are believed to cooperate because they are related, yet because they do not disperse, group members living within a finite area and should also compete for resources within the group – so-called scramble competition. In social insects, for example, it has been found that when dispersal is limited, increasing competition between related individuals counteracts any effect that increased relatedness might have on altruistic behaviours (Queller, 1992). The influences of increased relatedness and increased competition are intertwined, making assessment of the role of kin selection a difficult task.

The langurs could be considered as an ideal mammalian species within which to examine the conflicting roles of dispersal and competition. Troops, comprising of mostly non-dispersed, and therefore related females, could be contrasted with dispersed, unrelated males. Observations of competitive behaviour would allow a comparison of similar sized groups of individuals that are related or unrelated. However, given the different priorities of the two sexes that form contrasting majorities in the two group types – males competing most fiercely for mating opportunities and females for food – it could not be considered a fair comparison of the effect of relatedness on competition.

Levels of competition within a troop could be manipulated by providing varying levels of resources. Langurs in the Jodhpur population are dependent to varying extents on provisioned food, over which much within group competition occurs. Competition within a group could be increased by reducing the amount of provisioning to a troop, or decreased by providing excessive quantities of food. If competition is a counteracting force to cooperation in the langurs, it might be expected that reducing pressure might increase the amount of cooperation between all troop members; conversely, under

harsher conditions, competition might reduce cooperative behaviours across the troop, or limit them to between close relatives.

The corollary of resource manipulation would be the alteration of relatedness within a troop, so that the role of genetic similarity in competition could be measured. At a similar resource availability, competition in two groups of the same size should be equal. If the relatedness of one group of individuals is high, it might be expected that they compete less fiercely, or that cooperative behaviours are apparent. However, manipulation of relatedness of individuals within a troop would not be a simple task; the movement of individuals between troops, as well as being ethically questionable, would be likely to create so much social disruption that observation of competition and cooperation would become impossible to measure.

The complexity of social interactions and the compounding effects of differing ecologies on various langur groups and populations make it extremely difficult to tease apart the varying factors that might influence to occurrence of cooperation and competitive behaviours. The data in this thesis brings a quantitative approach to the measurement of relatedness within groups. However, it is still unclear whether relatedness is a result of non-dispersal and subsequent cooperation due to kin selection, or conversely, whether relatedness promotes kin selection. Detailed observations of group behaviour coupled with knowledge of relatedness of interacting individuals could shed light on the importance of kin selection in cooperation of group living langurs.

Further investigation of the importance of relatedness in reducing competition in a resource-limited group could be measured by contrasting troops of differing average relatedness, or alternatively by varying the resource availability for a single troop. Such experimental manipulation could not be carried out in total isolation from other influences in the langurs' lives, such as incursion by male bands or takeovers. Now that quantification of relatedness has become possible for the langurs, as demonstrated in this thesis, it now becomes imperative that the costs and benefits of altruistic behaviours, as outlined in Hamilton's rule, should also be accurately resolved.

APPENDICES

KAILANA

	AM1	AF1	AF2	AF3	AF4	AF5	AF7	AF8	AM2	AM3	AM4	SAM1	SAM2	JF1	JF2	JF3	JM1	WCM
AM1	-																	
AF1	0.17	-																
AF2	0.18	0.25	-															
AF3	-0.19	0.55	0.32	-														
AF4	-0.03	0.16	-0.17	0.24	-													
AF5	0.63	0.33	0.28	0.18	-0.03	-												
AF7	0.08	-0.01	-0.38	-0.52	0.03	0.17	-											
AF8	0.06	0.16	-0.28	-0.06	-0.13	0.11	0.00	-										
AM2	0.40	-0.03	0.15	0.04	0.33	0.32	-0.19	-0.21	-									
AM3	0.13	0.50	-0.23	0.20	0.03	0.25	0.40	0.16	-0.12	-								
AM4	0.67	0.31	0.00	-0.14	0.20	0.79	0.44	0.33	0.23	0.11	-							
SAM1	0.51	0.31	0.14	-0.12	-0.19	0.77	0.25	0.33	0.28	0.23	0.65	-						
SAM2	0.62	0.20	-0.03	-0.15	0.01	0.55	0.50	-0.01	0.06	0.31	0.79	0.55	-					
JF1	0.60	0.26	0.51	0.06	-0.16	0.42	-0.19	0.04	-0.08	0.05	0.23	0.19	0.12	-				
JF2	-0.17	0.18	0.01	0.56	0.27	0.41	-0.30	-0.55	-0.29	0.29	0.23	0.04	0.18	0.05	-			
JF3	0.62	0.49	0.09	0.24	0.38	0.64	0.01	0.19	0.26	0.17	0.68	0.47	0.36	0.41	0.58	-		
JM1	0.34	-0.07	-0.07	-0.37	0.06	0.40	0.48	0.21	0.06	0.10	0.46	0.35	0.11	0.30	-0.04	0.44	-	
WCM	0.43	0.13	0.04	0.41	0.07	0.53	-0.21	0.13	0.46	0.17	0.35	0.16	0.12	0.27	0.10	0.52	0.28	-

APPENDIX 1 Pairwise relatedness values from KINSHIP

CHANDPOL A

	AM	AF1	AF3	AF4	AF5	AF7	AF9	AF11	AF12	AF13	AF14	SAF1	JF1	JF2	JF4	WCF1	WCF2	WCM1	WCM3	WCM4	BCM1
AM	-																				
AF1	-0.08	-																			
AF3	0.31	0.16	-																		
AF4	0.69	-0.24	0.57	-																	
AF5	0.20	0.12	0.19	0.20	-																
AF7	0.11	-0.13	0.33	0.23	0.13	-															
AF9	0.52	0.18	0.34	0.36	0.55	0.25	-														
AF11	0.24	-0.15	0.23	0.17	0.35	0.44	0.30	-													
AF12	0.02	-0.09	0.15	0.04	0.10	0.46	0.34	0.63	-												
AF13	0.03	0.14	0.59	0.39	0.26	0.51	0.26	0.50	0.41	-											
AF14	0.09	0.00	0.26	0.35	0.21	-0.15	0.41	0.11	0.30	0.24	-										
SAF1	0.36	0.12	-0.06	-0.07	0.05	0.22	0.41	0.32	0.23	0.14	-0.07	-									
JF1	0.08	-0.08	0.25	0.25	-0.07	0.55	0.55	0.55	0.56	0.42	0.14	0.13	-								
JF2	0.16	0.12	0.35	0.15	0.62	0.09	0.29	0.13	0.05	0.22	0.54	-0.01	-0.12	-							
JF4	0.58	0.05	0.34	0.63	0.40	0.06	0.65	0.57	0.42	0.31	0.46	0.36	0.54	0.40	-						
WCF1	0.36	0.09	0.58	0.46	0.20	0.02	0.51	0.46	0.29	0.42	0.74	0.24	0.39	0.44	0.80	-					
WCF2	0.32	0.12	0.63	0.66	0.08	0.12	0.40	0.11	0.04	0.43	-0.02	-0.18	0.31	0.03	0.32	0.22	-				
WCM1	0.21	0.39	0.20	0.21	0.14	0.14	0.17	0.51	0.53	0.48	0.17	0.05	0.25	0.09	0.33	0.25	0.26	-			
WCM3	-0.40	-0.10	-0.31	-0.26	-0.35	0.21	-0.29	0.31	0.33	0.26	-0.08	-0.02	0.45	-0.44	-0.04	-0.10	-0.08	0.16	-		
WCM4	-0.26	0.54	0.05	-0.32	0.50	-0.18	0.14	0.26	0.19	0.22	0.12	0.00	-0.16	0.10	0.04	0.11	-0.05	0.33	-0.12	-	
BCM1	0.09	-0.12	-0.20	-0.43	-0.13	0.17	-0.08	0.37	0.49	0.10	-0.19	0.35	0.19	-0.20	0.18	-0.11	-0.09	0.13	0.34	-0.03	-

APPENDIX 1 (cont.)

NIMBA

	AM	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF10	SAM	SAF2	SAF3	JM1	JM2	JM3	WC1	WC3	WC4
AM	-																	
AF1	-0.34	-																
AF2	0.24	0.42	-															
AF3	-0.14	0.72	0.71	-														
AF4	-0.28	0.56	0.29	0.59	-													
AF5	-0.27	0.31	0.31	0.30	0.47	-												
AF6	0.07	-0.09	0.12	0.02	-0.25	0.30	-											
AF7	-0.16	0.28	0.22	0.31	0.70	0.43	-0.21	-										
AF10	-0.29	0.48	0.37	0.70	0.72	0.43	0.04	0.51	-									
SAM	0.38	0.00	0.04	0.18	0.04	-0.46	-0.01	0.06	0.27	-								
SAF2	0.13	0.34	0.42	0.39	0.32	0.23	0.18	0.08	0.39	0.22	-							
SAF3	0.31	0.36	0.22	0.10	0.06	0.16	0.05	0.20	-0.19	0.07	0.35	-						
JM1	0.15	0.48	0.32	0.26	0.44	0.25	-0.30	0.37	0.31	0.24	0.54	0.53	-					
JM2	0.29	0.46	0.57	0.39	0.47	0.35	-0.13	0.35	0.06	-0.13	0.51	0.55	0.61	-				
JM3	0.04	0.45	0.31	0.51	0.58	0.37	-0.02	0.47	0.41	0.10	0.02	0.46	0.31	0.35	-			
WC1	0.43	-0.20	0.11	-0.11	-0.29	0.18	0.23	-0.15	-0.16	-0.10	-0.06	0.12	0.23	0.10	0.03	-		
WC3	-0.02	0.79	0.53	0.65	0.57	0.34	-0.19	0.46	0.33	0.03	0.45	0.63	0.69	0.81	0.56	-0.04	-	
WC4	0.63	-0.29	0.44	0.03	-0.17	-0.09	-0.21	0.19	-0.28	-0.05	-0.11	0.28	0.14	0.35	0.15	0.36	0.15	-

CHANDPOL B

	AM	AF1	AF2	AF3	AF5	AF6	AF7	JF1	JF2	WCF1
AM	-									
AF1	0.56	-								
AF2	-0.07	0.37	-							
AF3	0.16	0.05	0.08	-						
AF5	0.16	0.07	-0.21	0.25	-					
AF6	-0.27	-0.26	-0.34	0.07	0.47	-				
AF7	-0.32	-0.12	0.36	0.09	-0.10	-0.13	-			
JF1	-0.55	-0.25	-0.29	0.14	0.04	0.18	0.04	-		
JF2	-0.85	-0.30	-0.61	-0.01	0.22	0.06	-0.21	0.76	-	
WCF1	-0.55	-0.24	0.20	-0.50	0.03	0.24	-0.23	0.12	0.04	-

APPENDIX 1 (cont.)

BIJOLAI

	AM	AF1	AF2	AF3	AF4	AF5	JF	JM	WCM
AM	-								
AF1	0.02	-							
AF2	-0.19	-0.31	-						
AF3	-0.13	0.39	-0.10	-					
AF4	-0.40	1.00	-0.11	0.24	-				
AF5	0.34	-0.10	0.14	-0.08	0.44	-			
JF	0.14	-0.23	-0.09	-0.17	0.13	0.41	-		
JM	0.20	0.02	-0.10	-0.15	-0.28	0.05	0.36	-	
WCM	0.56	0.31	-0.14	-0.06	-0.27	0.44	0.58	0.57	-

BHADRESHWAR

	BM1	BM2	BM3	BM4	BM5	BM6	BM7	BM8	BM9	BM10	BM11	BM15	BM19
BM1	-												
BM2	-0.15	-											
BM3	0.10	0.31	-										
BM4	0.30	0.07	0.36	-									
BM5	-0.29	0.18	0.14	0.13	-								
BM6	-0.56	-0.14	0.17	0.22	0.64	-							
BM7	0.03	-0.25	0.03	-0.14	0.22	-0.23	-						
BM8	-0.32	-0.09	-0.35	-0.15	-0.19	0.00	-0.23	-					
BM9	-0.42	0.12	0.12	0.31	0.17	0.39	-0.49	0.27	-				
BM10	-0.69	0.10	-0.19	-0.11	0.05	0.38	-0.33	0.21	0.27	-			
BM11	-0.25	-0.33	-0.01	0.12	0.07	0.37	-0.05	0.55	0.20	0.39	-		
BM15	-0.33	-0.24	-0.21	0.08	0.08	0.51	-0.24	0.15	0.15	0.45	0.38	-	
BM19	-0.33	-0.04	-0.13	0.26	-0.09	0.31	0.06	0.43	0.29	0.52	0.71	0.51	-

APPENDIX 1 (cont.)

KAILANA

	AM1	AF1	AF2	AF3	AF4	AF5	AF7	AF8	AM2	AM3	AM4	SAM1	SAM2	JF1	JF2	JF3	JM1	WCM
AM1	-																	
AF1	N.S.	-																
AF2	N.S.	N.S.	-															
AF3	N.S.	N.S.	N.S.	-														
AF4	N.S.	N.S.	N.S.	N.S.	-													
AF5	*	N.S.	*	N.S.	N.S.	-												
AF7	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-											
AF8	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-										
AM2	**	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	-									
AM3	N.S.	**	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	-								
AM4	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	-							
SAM1	*	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	-						
SAM2	**	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-					
JF1	*	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	-				
JF2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	?	N.S.	?	?	N.S.	N.S.	-			
JF3	***	*	N.S.	N.S.	*	*	N.S.	N.S.	**	N.S.	N.S.	N.S.	*	*	N.S.	-		
JM1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	***	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-	
WCM	**	N.S.	N.S.	**	N.S.	*	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**	*	-

APPENDIX 2 Significant likelihood ratios for H_1 set at R=0.5, against Ho of R=0.0. *** $p < 0.001$, ** $p < 0.01$,* $p < 0.05$; ? = data defficient

CHANDPOL A

	AM	AF1	AF3	AF4	AF5	AF7	AF9	AF11	AF12	AF13	AF14	SAF1	JF1	JF2	JF4	WCF1	WCF2	WCM1	WCM3	WCM4	BCM1
AM	-																				
AF1	N.S.	-																			
AF3	N.S.	N.S.	-																		
AF4	N.S.	N.S.	*	-																	
AF5	N.S.	N.S.	N.S.	N.S.	-																
AF7	N.S.	N.S.	***	N.S.	N.S.	-															
AF9	N.S.	N.S.	N.S.	N.S.	*	*	-														
AF11	N.S.	N.S.	N.S.	*	***	*	*	-													
AF12	N.S.	N.S.	*	*	N.S.	***	*	***	-												
AF13	N.S.	N.S.	**	*	N.S.	**	N.S.	*	N.S.	-											
AF14	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	**	N.S.	-										
SAF1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-									
JF1	N.S.	N.S.	N.S.	*	N.S.	**	*	***	***	*	*	N.S.	-								
JF2	N.S.	N.S.	N.S.	N.S.	***	N.S.	N.S.	*	N.S.	N.S.	*	N.S.	N.S.	-							
JF4	N.S.	N.S.	N.S.	**	*	N.S.	N.S.	***	*	N.S.	*	N.S.	***	*	-						
WCF1	N.S.	N.S.	*	**	N.S.	N.S.	N.S.	***	*	*	**	N.S.	***	N.S.	***	-					
WCF2	N.S.	N.S.	***	*	N.S.	N.S.	N.S.	N.S.	N.S.	**	N.S.	N.S.	*	N.S.	N.S.	N.S.	-				
WCM1	*	*	N.S.	*	*	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	-			
WCM3	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	**	**	N.S.	*	N.S.	***	N.S.	*	*	N.S.	N.S.	-		
WCM4	N.S.	*	N.S.	N.S.	***	N.S.	*	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-	
BCM1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-

APPENDIX 2 (cont.)

NIMBA

	AM	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF10	SAM	SAF2	SAF3	JM1	JM2	JM3	WC1	WC3	WC4
AM	-																	
AF1	N.S.	-																
AF2	N.S.	N.S.	-															
AF3	N.S.	**	*	-														
AF4	N.S.	N.S.	N.S.	*	-													
AF5	N.S.	N.S.	N.S.	N.S.	N.S.	-												
AF6	N.S.	***	N.S.	N.S.	N.S.	*	-											
AF7	N.S.	***	N.S.	N.S.	*	N.S.	***	-										
AF10	N.S.	*	N.S.	*	**	*	N.S.	*	-									
SAM	N.S.	***	N.S.	N.S.	N.S.	N.S.	***	***	N.S.	-								
SAF2	N.S.	N.S.	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.	-							
SAF3	?	***	N.S.	N.S.	N.S.	N.S.	***	***	N.S.	***	N.S.	-						
JM1	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	-					
JM2	N.S.	N.S.	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	-				
JM3	N.S.	***	N.S.	*	*	N.S.	***	***	N.S.	***	N.S.	***	N.S.	N.S.	-			
WC1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-		
WC3	N.S.	***	*	*	*	N.S.	***	***	N.S.	***	N.S.	***	*	N.S.	***	N.S.	-	
WC4	N.S.	N.S.	***	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	-

CHANDPOL B

	AM	AF1	AF2	AF3	AF5	AF6	AF7	JF1	JF2	WCF1
AM	-									
AF1	N.S.	-								
AF2	N.S.	*	-							
AF3	N.S.	N.S.	**	-						
AF5	N.S.	N.S.	N.S.	N.S.	-					
AF6	N.S.	N.S.	N.S.	***	N.S.	-				
AF7	N.S.	N.S.	*	N.S.	N.S.	N.S.	-			
JF1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-		
JF2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	***	-	
WCF1	?	?	?	?	?	?	?	?	?	-

APPENDIX 2 (cont.)

BIJOLAI

	AM	AF1	AF2	AF3	AF4	AF5	JF	JM	WCM
AM	-								
AF1	N.S.	-							
AF2	N.S.	?	-						
AF3	*	N.S.	N.S.	-					
AF4	N.S.	?	N.S.	N.S.	-				
AF5	N.S.	N.S.	N.S.	N.S.	N.S.	-			
JF	*	N.S.	N.S.	N.S.	N.S.	N.S.	-		
JM	N.S.	?	*	N.S.	?	N.S.	N.S.	-	
WCM	***	N.S.	N.S.	N.S.	N.S.	***	***	N.S.	-

BHADRESHWAR

	BM1	BM2	BM3	BM4	BM5	BM6	BM7	BM8	BM9	BM10	BM11	BM15	BM19
BM1	-												
BM2	N.S.	-											
BM3	N.S.	N.S.	-										
BM4	**	N.S.	N.S.	-									
BM5	N.S.	N.S.	N.S.	N.S.	-								
BM6	N.S.	N.S.	N.S.	N.S.	***	-							
BM7	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-						
BM8	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	-					
BM9	N.S.	N.S.	***	N.S.	N.S.	N.S.	N.S.	*	-				
BM10	N.S.	N.S.	N.S.	N.S.	*	***	N.S.	N.S.	**	-			
BM11	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	**	N.S.	*	-		
BM15	*	N.S.	N.S.	***	N.S.	**	N.S.	N.S.	N.S.	*	N.S.	-	
BM19	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-

APPENDIX 2 (cont.)

KAILANA

	AM1	AF1	AF2	AF3	AF4	AF5	AF7	AF8	AM2	AM3	AM4	SAM1	SAM2	JF1	JF2	JF3	JM1	WCM
AM1	-																	
AF1	N.S.	-																
AF2	N.S.	N.S.	-															
AF3	N.S.	N.S.	N.S.	-														
AF4	N.S.	N.S.	N.S.	N.S.	-													
AF5	N.S.	N.S.	N.S.	N.S.	N.S.	-												
AF7	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-											
AF8	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-										
AM2	**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-									
AM3	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-								
AM4	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-							
SAM1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-						
SAM2	**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-					
JF1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-				
JF2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	?	N.S.	?	?	N.S.	N.S.	-			
JF3	**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-		
JM1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-	
WCM	**	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	-

APPENDIX 3 Significant likelihood ratios for H_1 set at R=0.5, against Ho of R=0.25. *** $p < 0.001$, ** $p < 0.01$,* $p < 0.05$; ? = data defficient

CHANDPOL A

	AM	AF1	AF3	AF4	AF5	AF7	AF9	AF11	AF12	AF13	AF14	SAF1	JF1	JF2	JF4	WCF1	WCF2	WCM1	WCM3	WCM4	BCM1
AM	-																				
AF1	N.S.	-																			
AF3	N.S.	N.S.	-																		
AF4	N.S.	N.S.	N.S.	-																	
AF5	N.S.	N.S.	N.S.	N.S.	-																
AF7	N.S.	N.S.	N.S.	N.S.	N.S.	-															
AF9	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-														
AF11	N.S.	N.S.	N.S.	N.S.	**	N.S.	N.S.	-													
AF12	N.S.	N.S.	N.S.	N.S.	N.S.	**	N.S.	**	-												
AF13	N.S.	N.S.	*	N.S.	N.S.	*	N.S.	N.S.	N.S.	-											
AF14	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-										
SAF1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-									
JF1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**	*	N.S.	N.S.	N.S.	-								
JF2	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-							
JF4	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	*	*	-						
WCF1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	**	N.S.	N.S.	*	N.S.	**	N.S.	**	-					
WCF2	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-				
WCM1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-			
WCM3	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	***	N.S.	N.S.	N.S.	N.S.	N.S.	-		
WCM4	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-	
BCM1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-

APPENDIX 3 (cont.)

NIMBA

	AM	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF10	SAM	SAF2	SAF3	JM1	JM2	JM3	WC1	WC3	WC4
AM	-																	
AF1	N.S.	-																
AF2	N.S.	N.S.	-															
AF3	N.S.	*	N.S.	-														
AF4	N.S.	N.S.	N.S.	N.S.	-													
AF5	N.S.	N.S.	N.S.	N.S.	N.S.	-												
AF6	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-											
AF7	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-										
AF10	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	-									
SAM	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-								
SAF2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-							
SAF3	?	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-						
JM1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-					
JM2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-				
JM3	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-			
WC1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-		
WC3	N.S.	***	N.S.	*	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	*	N.S.	N.S.	**	N.S.	-	
WC4	N.S.	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-

CHANDPOL B

	AM	AF1	AF2	AF3	AF5	AF6	AF7	JF1	JF2	WCF1
AM	-									
AF1	N.S.	-								
AF2	N.S.	N.S.	-							
AF3	N.S.	N.S.	N.S.	-						
AF5	N.S.	N.S.	N.S.	N.S.	-					
AF6	N.S.	N.S.	N.S.	N.S.	N.S.	-				
AF7	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-			
JF1	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-		
JF2	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	***	-	
WCF1	?	?	?	?	?	?	?	?	?	-

APPENDIX 3 (cont.)

BIJOLAI

	AM	AF1	AF2	AF3	AF4	AF5	JF	JM	WCM
AM	-								
AF1	N.S.	-							
AF2	N.S.	?	-						
AF3	N.S.	N.S.	N.S.	-					
AF4	N.S.	?	N.S.	N.S.	-				
AF5	N.S.	N.S.	N.S.	N.S.	N.S.	-			
JF	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-		
JM	N.S.	?	N.S.	N.S.	?	N.S.	N.S.	-	
WCM	**	N.S.	N.S.	N.S.	N.S.	**	*	N.S.	-

BHADRESHWAR

	BM1	BM2	BM3	BM4	BM5	BM6	BM7	BM8	BM9	BM10	BM11	BM15	BM19
BM1	-												
BM2	N.S.	-											
BM3	N.S.	N.S.	-										
BM4	N.S.	N.S.	N.S.	-									
BM5	N.S.	N.S.	N.S.	N.S.	-								
BM6	N.S.	N.S.	N.S.	N.S.	**	-							
BM7	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-						
BM8	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-					
BM9	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-				
BM10	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.	-			
BM11	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	N.S.	N.S.	-		
BM15	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-	
BM19	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	-

APPENDIX 3 (cont.)

KAILANA

	AM1	AF1	AF2	AF3	AF4	AF5	AF7	AF8	AM2	AM3	AM4	SAM1	SAM2	JF1	JF2	JF3	JM1	
AM1	-																	
AF1	9/14	-																
AF2	10/16*	10/14	-															
AF3	8/14*	10/12	10/14*	-														
AF4	9/16*	10/14*	10/16*	10/14	-													
AF5	11/14	8/12	9/14	8/13*	7/14	-												
AF7	9/16*	8/14*	7/16*	6/14*	9/16	8/14*	-											
AF8	8/15*	8/13	7/15*	7/14*	8/15	7/14	8/15*	-										
AM2	10/13	7/12*	9/13*	7/11*	10/13	8/11	7/13*	6/12	-									
AM3	7/13*	10/12	8/13*	9/13	10/13	7/12	9/13	8/13*	7/11	-								
AM4	9/10	6/8	6/10	5/10	6/10*	9/10	8/10*	7/10	6/8	5/9*	-							
SAM1	7/9	5/7	4/9	4/9*	5/9	8/9	6/9*	6/9	6/7	5/8	7/9	-						
SAM2	12/16	8/14	9/16*	7/14*	8/16*	10/14	11/16	7/15*	7/13	8/13	9/10	7/9	-					
JF1	8/10	6/8	7/10	6/10*	5/10*	7/10	4/10*	5/10*	3/7	5/9*	5/9	4/8	6/10	-				
JF2	4/5	4/5	4/5	5/5	4/5	4/5	3/5	2/5	2/3	4/5	3/5	2/4	4/5	3/4	-			
JF3	12/16	10/14	9/16*	8/14	11/16*	11/14	7/16*	8/15	9/13	8/13*	8/10	7/9	9/16	7/10	4/5	-		
JM1	9/16*	5/14*	7/16*	4/14*	8/16*	9/14*	10/16	7/15*	7/13*	6/13*	7/10	6/9	8/16*	6/10	2/5	10/16*	-	
WCM	9/16	7/14*	8/16*	9/14	8/16*	10/14	5/16*	7/15	8/13	7/13	6/10	5/9	8/16*	6/10	3/5	10/16	8/16	-

APPENDIX 4 Allele sharing. Dyads that can be excluded as first degree relatives at one or more alleles designated by *.

CHANDPOL A

	AM	AF1	AF3	AF4	AF5	AF7	AF9	AF11	AF12	AF13	AF14	SAF1	JF1	JF2	JF4	WCF1	WCF2	WCM	WCM	WCM	BCM1
AM	-																				
AF1	5/8	-																			
AF3	6/12*	7/12	-																		
AF4	6/8	4/7	7/10	-																	
AF5	7/11*	6/11	8/15*	6/9*	-																
AF7	5/12*	6/12*	11/16ˣ	6/10*	8/15*	-															
AF9	6/9*	6/8	7/11*	6/9*	8/10	7/11	-														
AF11	7/12*	6/12*	8/16*	6/10*	9/15	10/16ˣ	8/11	-													
AF12	6/12*	7/12	8/16*	6/10*	8/15*	10/16	8/11	12/16	-												
AF13	6/12*	8/12	11/16	6/10	9/15*	11/16	8/11	11/16	10/16	-											
AF14	6/12*	6/12	9/16*	8/10*	9/15*	7/16	8/11*	8/16	10/16ˣ	9/16*	-										
SAF1	8/12	8/12*	8/16*	5/10*	8/15*	10/16ˣ	8/11	10/16	9/16	9/16*	8/16*	-									
JF1	6/12*	7/12*	9/16*	7/10*	7/15*	11/16	9/11	11/16	12/16ˣ	10/16	10/16ˣ	9/16*	-								
JF2	7/11*	7/11	9/15*	6/9*	13/15ˣ	8/16*	7/10*	8/15*	8/15*	9/16*	11/15	8/15*	7/15*	-							
JF4	7/10	6/12*	9/14*	7/8	9/13	8/14*	7/9	10/14	9/14	8/15	10/14ˣ	9/14	10/15	9/13	-						
WCF1	6/11	6/12	12/14ˣ	7/9	8/14	8/15	7/10	10/15	9/15	10/15	12/15	9/15*	10/15	9/14	12/14	-					
WCF2	6/12*	7/12	12/16	7/10	7/15*	9/16*	8/11	7/16*	7/16*	10/15	8/16*	7/16*	10/16ˣ	7/15*	9/14*	9/15*	-				
WCM1	7/12*	8/12	8/16*	6/10	9/15*	7/16*	7/11	11/16ˣ	11/16	11/16	8/16*	8/16*	9/16*	9/15*	8/14*	8/15*	8/16*	-			
WCM3	5/12*	8/12	6/16*	5/10*	5/15*	8/16	6/11	10/16	10/16ˣ	10/16	8/16*	9/16	11/16	5/15*	7/14*	7/15*	8/16*	9/16	-		
WCM4	5/12*	10/12	7/16*	4/10*	9/15*	6/16*	7/11	9/16*	9/16*	9/16*	8/16*	9/16*	7/16*	8/15*	7/14*	7/15	7/16*	10/16ˣ	8/16*	-	
BCM1	7/12*	8/12*	6/16*	4/10*	6/15*	8/16	7/11	10/16	10/16	9/16	7/16*	11/16	10/16ˣ	6/15*	7/14	6/15*	7/16*	9/16*	12/16ˣ	9/16*	-

APPENDIX 4 (cont.)

NIMBA

	AM	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF10	SAM	SAF2	SAF3	JM1	JM2	JM3	WC1	WC3	WC4
AM	-																	
AF1	4/11	-																
AF2	8/11	10/16	-															
AF3	6/11	12/16	13/16	-														
AF4	6/11	10/15	10/15	11/15	-													
AF5	6/11	9/15*	11/15ˣ	10/15ˣ	11/15	-												
AF6	6/11*	6/16*	8/16	7/15*	6/15*	10/15	-											
AF7	6/11	12/16	9/16	9/15*	12/15	10/15	6/16*	-										
AF10	6/11	10/16	11/16	12/15	13/15	11/15	8/16*	11/16	-									
SAM	8/11	7/16*	9/16*	8/15	8/15	6/15*	7/16*	8/16*	9/16	-								
SAF2	6/11	9/16*	11/16	10/15	9/15*	9/15	7/16*	8/16*	10/16ˣ	8/16	-							
SAF3	6/8	6/11*	7/11*	6/11*	5/11*	6/11*	5/11	6/11	4/11*	6/11	7/11*	-						
JM1	7/11	10/14	9/14	8/14	9/13	8/13	4/14*	10/14ˣ	8/14	6/14*	10/14	7/9	-					
JM2	6/10	7/12*	10/12	8/12	8/12	8/12	5/12*	7/12*	6/12*	5/12*	9/12*	7/10	8/11	-				
JM3	7/11	10/16	11/16	11/15	12/15	11/15	8/16*	11/16	12/16ˣ	10/16ˣ	8/16*	7/11*	9/14	8/12*	-			
WC1	10/11	7/16*	11/16ˣ	9/15*	8/15*	11/15	9/16	8/16*	9/16*	9/16*	8/16*	7/11*	9/14	7/12	10/16	-		
WC3	5/11	13/16	11/16	11/15	10/15	8/15	5/16*	11/16ˣ	9/16*	8/16*	10/16ˣ	8/11	11/14	10/12	11/16	7/16*	-	
WC4	9/11	5/16*	11/16	8/15	6/15*	7/15*	5/16*	8/16*	9/16*	7/16*	7/16*	7/11*	7/14*	8/12	9/16*	11/16	8/16*	-

CHANDPOL B

	AM	AF1	AF2	AF3	AF5	AF6	AF7	JF1	JF2	WCF1
AM	-									
AF1	5/6	-								
AF2	4/6	11/16	-							
AF3	4/6	10/16	9/16*	-						
AF5	3/6	8/16	5/14*	7/14*	-					
AF6	3/5	7/16*	6/15*	9/15*	9/13	-				
AF7	4/6	8/16*	10/16	8/16*	6/14*	7/15*	-			
JF1	2/6	7/14*	5/14*	8/14	7/12*	7/13*	7/14	-		
JF2	2/5	8/14*	5/14*	8/14*	8/12*	7/13	6/14*	12/13	-	
WCF1	1/3	3/6	4/6	2/6	4/6	4/6	3/6	4/6	4/6	-

APPENDIX 4 (cont.)

BIJOLAI

	AM	AF1	AF2	AF3	AF4	AF5	JF	JM	WCM
AM	-								
AF1	1/4	-							
AF2	5/11*	0/3	-						
AF3	7/15*	2/4*	5/12*	-					
AF4	3/7*	1/1	3/7	4/7	-				
AF5	9/16*	1/4*	7/12*	8/16*	5/7	-			
JF	7/13	0/3*	5/10	6/13*	4/7	8/13*	-		
JM	5/9	1/3	3/8	3/9	2/4	5/9	5/8	-	
WCM	11/15	2/4	5/12*	7/16*	3/7	10/16	10/13*	6/9	-

BHADRESHWAR

	BM1	BM2	BM3	BM4	BM5	BM6	BM7	BM8	BM9	BM10	BM11	BM15	BM19
BM1	-												
BM2	5/10*	-											
BM3	8/14*	6/10*	-										
BM4	10/14	6/10*	10/16*	-									
BM5	5/11*	5/9*	7/12	7/12*	-								
BM6	4/13*	4/10*	7/14*	7/14*	10/12	-							
BM7	10/13	4/9	7/15*	6/15*	7/11	6/13*	-						
BM8	6/13*	4/9*	5/15*	8/15*	5/11	6/13	5/14*	-					
BM9	4/13*	4/7	6/14*	7/14*	5/11*	7/13*	4/13*	8/13*	-				
BM10	4/14	5/10*	5/16*	7/16*	6/12*	8/14*	5/15*	8/13*	8/14*	-			
BM11	8/14*	4/10*	8/16*	10/16*	7/12	9/14	7/15*	11/15	7/14*	10/16	-		
BM15	3/10*	2/8*	2/11*	5/11*	3/10*	7/11*	2/10*	4/10*	4/10*	6/11*	6/11*	-	
BM19	6/11*	5/9*	5/12	8/12*	6/11*	7/12*	6/11	8/11	6/11*	9/12	10/12	6/10*	-

APPENDIX 4 (cont.)

APPENDIX 5 **ABBREVIATIONS**

BSA	bovine serum albumin
bp	base pairs
HWE	Hardy-Weinberg Equilibrium
IBI	interbirth interval
kb	kilobase
LD	linkage disequilibrium
LOD	$\log_e$ likelihood
PCR	Polymerase Chain Reaction

AMB	all male band
AM	adult male
AF	adult female
SAM	subadult male
SAF	subadult female
JM	juvenile male
JF	juvenile female
WC	white coat
WCM	white coat male
WCF	white coat female
BCM	black coat male

B	Bijolai troop
BM	Bhadreshwar AMB
CA	Chandpol A troop
CB	Chandpol B troop
K	Kailana troop
N	Nimba troop

REFERENCES

Adams, R.P., and Demeke, T. (1992). The effects of plant polysaccharides and buffer additives on PCR. *BioTechniques* **12**:332-334

Agoramoorthy, G., Mohnot, S.M., Sommer, V., and Srivastava, A. (1988). Abortions in free-ranging Hanuman langurs (*Presbytis entellus*) - a male induced strategy? *Hum. Evol.* **3**:297-308

Albaugh, G.P., Iyengar, V., Lohani, A., Malayeri, M., Bala, S., and Nair, P.P. (1992). Isolation of exfoliated colonic epithelial cells, a novel, non-invasive approach to the study of cellular markers. *Int. J. Cancer* **52**:347-350

Altmann, J., Alberts, S.C., Haines, S.A., Dubach, J., Muruthi, P., Coote, T., Geffen, E., Cheesman, D.J., Mututua, R.S., Saiyalel, S.N., Wayne, R.K., Lacy, R.C., and Bruford, M.W. (1996). Behaviour predicts genetic structure in a wild primate group. *Proc. Nat. Acad. Sci. USA* **93**:5797-5801

Amos, B., Schlötterer, C., and Tautz, D. (1993). Social structure of pilot whales revealed by analytical DNA profiling. *Science* **260**:670-672

Axelrod, R., and Hamilton, W.D. (1981). The evolution of cooperation. *Science* **211**:1390-1396

Basset, P., Balloux, F., and Perrin, N. (2001). Testing demographic models of effective population size. *Proc. Roy. Soc. Lond. B* **268**:311-317

Bayes, M.K., Smith, K.L., Alberts, S.C., Altmann, J., and Bruford, M.W. (2000). Testing the reliability of microsatellite typing from faecal DNA in the savannah baboon. *Cons. Genet.* **1**:173-176

Beintema, J.J., Scheffer, A.J., van Dijk, H., Welling, G.W., and Zwiers, H. (1973). Pancreatic ribonuclease distribution and comparisons in mammals. *Nature New Biology* **241**:76-78

Belisle, P., and Chapais, B. (2001). Tolerated co-feeding in relation to degree of kinship in Japanese macaques. *Behaviour* **138**:487-509

Belkhir, K., Borsa, P., Chikhi, L., Goudet, J., and Bonhomme, F. (1996-1997). *Genetix 3.07, Windows™ Software for Population Genetics.* Laboratoire Génome et Populations, University of Montpellier II, Montpellier, France.

Birch, D.E. (1996). Simplified hot start PCR. *Nature* **381**:630

Birkhead, T.R., Burke, T., Zann, R., Hunter, F.M., and Krupa, A.P. (1990). Extra-pair paternity and intraspecific brood parasitism in wild zebra finches, *Taeniopygia guttata*, revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.* **27**:315-324

Bishop, N. (1979). Himalayan langurs: temperate colobines. *J. Hum. Evol.* **8**:251-281

Borries, C. (1992). Grooming site preferences in female langurs (*Presbytis entellus*). *Int. J. Primatol.* **13**:19-32

Borries, C. (1993). Ecology of female social relationships: Hanuman langurs (*Presbytis entellus*) and the van Schaik model. *Folia Primatol.* **61**:21-30

Borries, C. (1997). Infanticide in seasonally breeding multimale groups of Hanuman langurs (*Presbytis entellus*) in Ramnagar (South Nepal). *Behav. Ecol. Sociobiol.* **41**:139-150

Borries, C., and Koenig, A. (2000). Infanticide in hanuman langurs: social organization, male migration, and weaning age. *In* van Schaik, C.P. and Janson, C.H. (eds.) *Infanticide by males and its implications*, pp99-122. Cambridge: Cambridge University Press

Borries, C., Launhardt, K., Epplen, C., Epplen, J.T., and Winkler, P. (1999a). DNA analyses support the hypothesis that infanticide is adaptive in langur monkeys. *Proc. Roy. Soc. Lond. B* **266**:901-904

Borries, C., Launhardt, K., Epplen, C., Epplen, J.T., and Winkler, P. (1999b). Males as infant protectors in Hanuman langurs (*Presbytis entellus*) living in multimale groups – defence pattern, paternity and sexual behaviour. *Behav. Ecol. Sociobiol.* **46**:350-356

Borries, C., Sommer, V., and Srivastava, A. (1991). Dominance, age, and reproductive success in free-ranging female Hanuman langurs (*Presbytis entellus*). *Int. J. Primatol.* **12**:231-257

Borries, C., Sommer, V., and Srivastava, A. (1994). Weaving a tight social net: allogrooming in free-ranging female langurs (*Presbytis entellus*). *Int. J. Primatol.* **1**:421-443

Bourke, A.F.G., and Franks, N.R. (1995). *Social evolution in ants.* Princeton: Princeton University Press.

Bricker, J., Bushar, L.M., Reinert, H.K., and Gelbert, L. (1996). Purification of high quality DNA from shed skin. *Herpetol. Rev.* **27**:133-134

Brooke, C.L., Tice, G., Elliott, V.L., Zinck, G.T., and DiMarzio, N.L. (1997). Modification of the sample preparation protocol in the BAX™ system for screening/ *Salmonella* to permit detection of food matrices with inhibitory PCR effects. *8th European Congress of Clinical Microbiology and Infectious Diseases.*

Brown, J.L. (1980). Fitness in complex avian social systems. *In* H. Markl (ed.) *Evolution of social behaviour*, pp115-128. Weinheim, Verlag-chemie.

Bruford, M.W., and Wayne, R.K. (1993). Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development* **3**:939-943

Bruford, M.W., Cheesman, D.J., Coote, T., Green, H.A.A., Haines, S.A., O'Ryan, C., and Williams, T.R. (1996). Microsatellites and their application to conservation genetics. *In* Smith, T.B. and Wayne, R.K. (eds.) *Molecular genetic approaches in conservation*, pp278-297. Oxford: Oxford University Press.

Burland, T.M., Bennett, N.C., Jarvis, J.U.M., and Faulkes, C.G. (2002). Eusociality in African mole-rats: new insights from patterns of genetic relatedness in the Damaraland mole-rat (*Cryptomys damarensis*). *Proc. Roy. Soc. Lond. B* **269**:1025-1030

Cannon, W.B. (1929). Bodily changes in pain, hunger, fear and rage. New York: D. Appleton and Co.

Cheah, P.Y., and Bernstein, H. (1990). Colon cancer and dietary fiber – cellulose inhibits the DNA-damaging ability of bile acids. *Nutr. Cancer* **13**:51-57

Chesser, R.K. (1991). Gene diversity and female philopatry. *Genetics* **127**:437-447

Chesser, R.K., and Ryman, N. (1986). Inbreeding as a strategy in subdivided populations. *Evolution* **40**:616-624

Chesser, R.K., Sugg, D.W., Rhodes, O.E., Jr., Novak, J.M, and Smith, M.H. (1993). Evolution of mammalian social structure. *Acta Theriol.* **38** Suppl. 2:163-174

Ciofi, C., and Bruford, M.W. (1999). Genetic structure and gene flow among Komodo dragon populations inferred by microsatellite loci analysis. *Mol. Ecol.* **8**:S17-S30

Clifford, S.L., Jeffery, K., Bruford, M.W., and Wickings, E.J. (1999). Identification of polymorphic microsatellite loci in the gorilla (*Gorilla gorilla gorilla*) using human primers: application to noninvasively collected hair samples. *Mol. Ecol.* **8**:1556-1558

Constable, J.L., Ashley M.V., Goodall, J., and Pusey, A.E. (2001). Noninvasive paternity assignment in Gombe chimpanzees. *Mol. Ecol.* **10**:1279-1300

Cooper, G., Rubinsztein, D.C., and Amos, W. (1998). Ascertainment bias cannot entirely account for human microsatellites being longer than their chimpanzee homologues. *Hum. Mol. Gen.* **7**:1425-1429

Coote, T., and Bruford, M.W. (1996). Human microsatellites applicable for analysis of genetic variation in apes and old world monkeys. *J. Hered.* **87**:406-410

Curtin, R.A., and Dolhinow, P. (1978). Primate social behavior in a changing world. *Am. Sci.* **66**:468-475

Darwin, C. (1859). *On the origin of species.* London: Murray.

de Ruiter, J.R. (1994). Behaviour and genes in natural populations of long tailed macaques (*Macaca fascicularis*). Ph.D. thesis, University of Utrecht, Netherlands.

de Ruiter, J.R., and Geffen, E. (1998). Relatedness of matrilines, dispersing males and social groups in long tailed macaques (*Macaca fascicularis*). *Proc. Roy. Soc. Lond. B* **265**:79-87

de Ruiter, J.R., Haring, R., de Jong, G., Scheffrahn, W., and van Hooff, J. (1994). The influence of social structure on genetic variability and relatedness in populations with stable social groups: A computer simulation study based on paternity testing and population genetic analysis of *Macaca fascicularis*. pp107-130 *in* de Ruiter, J. *Behaviour and genes in natural populations of long tailed macaques* (*Macaca fascicularis*). Ph.D. thesis, University of Utrecht, Netherlands.

Deuter, R., Pietsch, S., Hertel, S., and Muller, O. (1995). A method of preparation of fecal DNA suitable for PCR. *Nuc. Ac. Res.* **23**:3800-3801

Don, R.H., Cox, P.T., Wainwright, B.J., Baker, K., and Mattick, J.S. (1991). 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nuc. Ac. Res.* **19**:4008

Dunbar, R.I.M. (1991). Functional significance of social grooming in primates. *Folia Primatol.* **57**:121-131

Ely, J., Sponeybarger, K., Manis, G.S., Morton, K., and Stone, W.H. (1992). Characterisation of single and multiple tandem repeat loci in rhesus monkeys (*Macaca mulatta*). Paper presented at the Second International Conference on DNA fingerprinting, Belo Horizonte, Brazil, November 1992.

Ernest, H.B., Penedo, M.C.T., May, B.P., Syvanen, M., and Boyce, W.M. (2000). Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Mol. Ecol.* **9**:433-441

Flagstad, Ø., Røed, K., Stacy, J.E., and Jakobsen, K.S. (1999). Reliable noninvasive genotyping based on excremental PCR of nuclear DNA purified with a magnetic bead protocol. *Mol. Ecol.* **8**:879-883

Foltz, D.W. and Hoogland, J.L. (1993). Genetic evidence of outbreeding in the black-tailed prairie dog (*Cynomys ludovicianus*). *Evolution* **37**:784-797

Frantzen, M.A.J., Silk, J.B., Ferguson, J.W.H., Wayne, R.K., and Kohn, M.H. (1998). Empirical evaluation of preservation methods for faecal DNA. *Mol. Ecol.* **7**:1423-1428

Gagneux, P., Boesch, C., and Woodruff, D.S. (1997). Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. *Mol. Ecol.* **6**:861-868

Gagneux, P., Boesch, C., and Woodruff, D.S. (1999). Female reproductive strategies, paternity and community structure in wild West African chimpanzees. *Anim. Behav.* **57**:19-32

Garnier-Géré, P., and Dillmann, C. (1992). A computer program for testing pairwise linkage disequilibria in subdivided populations. *J. Hered.* **83**:239

Gerloff, U., Hartung, B., Fruth, B., Hohmann, G., and Tautz, D. (1999). Intracommunity relationships, dispersal pattern and paternity success in a wild living community of Bonobos (*Pan paniscus*) determined from DNA analysis of faecal samples. *Proc. Roy. Soc. Lond. B* **266**:1189-1195

Gerloff, U., Schlötterer, C., Rassmann, K., Rambold, I., Hohmann, G., Fruth, B., and Tautz, D. (1995). Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA of wild living bonobos (*Pan paniscus*). *Mol. Ecol.* **4**:515-518

Girman, D.J., Mills, M.G.L., Geffen, E., and Wayne, R.K. (1997). A molecular genetic analysis of social structure, dispersal, and interpack relationships of the African wild dog (*Lycaon pictus*). *Behav. Ecol. Sociobiol.* **40**:187-198

Goossens, B., Chikhi, L., Taberlet, P., Waits, L.S., and Allainé, D. (2001). Microsatellite analysis of genetic variation among and within Alpine marmot populations in the French Alps. *Mol. Ecol.* **10**:41-52

Goossens, B., Chikhi, L., Utami, S.S., de Ruiter, J., and Bruford, M.W. (2000). A multi-samples, multi-extracts approach for microsatellite analysis of faecal samples in an arboreal ape. *Cons. Genet.* **1**:157-162

Greenwood, P.J. (1980). Mating systems, philopatry and dispersal in birds and mammals. *Anim. Behav.* **28**:1140-1162

Hamilton, W.D. (1964). The genetical evolution of social behaviour, I and II. *J. Theor. Biol.* **7**:1-52

Harley, D. (1985). Birth spacing in langur monkeys (*Presbytis entellus*). *Int. J. Primatol.* **6**:227-242

Hartl, D.L. (2000). *A primer of population genetics.* 3rd edition. Sunderland, MA.: Sinauer Associates.

Hartl, D.L., and Clark, A.G. (1997). *Principles of population genetics.* 3rd edition. Sunderland, MA.: Sinauer Associates.

Hrdy, S.B. (1974). Male-male competition and infanticide among the langurs (*Presbytis entellus*) of Abu, Rajasthan. *Folia Primatol.* **22**:19-58

Hrdy. S.B. (1977). *The Langurs of Abu.* Cambridge, MA.: Harvard University Press.

Hughes, T.H. (1884). An incident in the habits of *Semnopithecus entellus*, the common hanuman monkey. *Proceedings of the Asiatic Society of Bengal.* September, pp147-150.

Jobes, D.V., Hurley, D.L., and Thien, L.B. (1995). Plant DNA isolation: a method to effectively remove polyphenolics, polysaccharides and RNA. *TAXON* **44**:379-386

Kawamoto, Y., Ischak, M., and Supriatna, J. (1982). Gene constitution of crab-eating macaques (*Macaca fascicularis*) on Lombok and Sumbawa. *Kyoto University Overseas Report of Studies on Asian Primates* **2**:57-64

Koenig, A., and Borries, C. (2001). Socioecology of Hanuman langurs: the story of their success. *Evol. Anthro.* **10**:122-137

Koenig, A., Beise, J., Chalise, M.K., and Ganzhorn, J.U. (1998). When females should contest for food – testing hypotheses about resource density, distribution, size, and quality with Hanuman langurs (*Presbytis entellus*). *Behav. Ecol. Sociobiol.* **42**:225-237

Kohn, M., Knauer, F., Stoffella, A., Schröder, W., and Pääbo, S. (1995). Conservation genetics of the European brown bear – a study using excremental PCR of nuclear and mitochondrial sequences. *Mol. Ecol.* **4**:95-103

Kohn, M.H., York, E.C., Kamradt, D.A. Haugt, G., Sauvajot, R.M., and Wayne, R.K. (1999). Estimating population size by genotyping faeces. *Proc. Roy. Soc. Lond. B* **266**:657-663

Komdeur, J. (1992). Importance of habitat saturation and territory quality for the evolution of cooperative breeding in the Seychelles warbler. *Nature* **358**:493-495

Lack, D. (1966). *Population studies of birds.* London: Clarendon Press.

Launhardt, K. (1998). Paarungs- und Reproduktionserfolg männlicher Hanuman-Languren (*Presbytis entellus*) in Ramnagar/Südnepal. Ph.D. thesis, Georg-August-Universität, Göttingen.

Launhardt, K., Borries, C., Hardt, C., Epplen, J.T., and Winkler, P. (2001). Paternity analysis of male reproductive routes among the langurs (*Semnopithecus entellus*) of Ramnagar. *Anim. Behav.* **61**:53-64

Launhardt, K., Epplen, C., Epplen, J.T., and Winkler, P. (1998). Amplification of microsatellites adapted from human systems in faecal DNA of wild Hanuman langurs (*Presbytis entellus*). *Electrophoresis* **19**:1356-1361

Lee, P.C. (1987). Allomothering among African elephants. *Anim. Behav.* **35**:278-291

Ligon, J.D. (1991). Cooperation and reciprocity in birds and mammals. *In* Hepper, P.G. (ed.) *Kin recognition*, pp30-59. Cambridge: Cambridge University Press.

Makwana, S.C., and Advani, R. (1981). Social changes in the Hanuman langur, *Presbytis entellus*, around Jodhpur. *J. Bombay Nat. His. Soc.* **78**:1-3

Marshall, T.C., Slate, J., Kruuk, L.E.B., and Pemberton, J.M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**:639-655

Martin, R.D., Dixson, A.F., and Wickings, E.J. (eds.) (1992). *Paternity in Primates: Genetic tests and theories.* Basel: Karger.

McGregor, D.P., Forster, S., Steven, J., Adair, J., Leary, S.E.C., Leslie, D.L., Harris, W.J., and Titball, R.W. (1996). Simultaneous detection of microorganisms in soil suspension based on PCR amplification of bacterial 16SrRNA fragments. *BioTechniques* **21**:463-471

Melnick, D.J., Pearl, M.C., and Richard, A.F. (1984). Male migration and inbreeding avoidance in wild rhesus monkeys. *Am. J. Primatol.* **7**:229-243

Mitani, J.C., Merriwether, D.A., and Zhang, C. (2000). Male affiliation, cooperation and kinship in wild chimpanzees. *Anim. Behav.* **59**:885-893

Mohnot, S.M. (1971). Some aspects of social changes and infant-killing in the Hanuman langur, *Presbytis entellus* (Primates: Cercopithecidae), in Western India. *Mammalia* **35**:175-198

Mohnot, S.M., and Chhangani, A. (1999). Crisis of male-female sex ratio in natural population of Hanuman langur, *Semnopithecus entellus*, living in and around Jodhpur, India. Abstract, XXVI International Ethological Conference, Bangalore, India.

Mohnot, S.M., Gadgil, M., and Makwana, S.C. (1981). On the dynamics of the Hanuman Langur populations of Jodhpur (Rajasthan, India). *Primates* **22**:182-191

Moore, J. (1985). *Demography and sociality in primates.* Ph.D. thesis, Harvard University, Cambridge, MA.

Moore, J. (1993). Inbreeding and outbreeding in primates: What's wrong with "The dispersing sex"? *In* Thornhill, N.W. (ed.) *The natural history of inbreeding and outbreeding*, pp392–426. Chicago: University of Chicago.

Moore, J. (1999). Population density, social pathology, and behavioral ecology. *Primates* **40**:1-22

Morin, P.A., and Woodruff, D.S. (1996). Noninvasive genotyping for vertebrate conservation. *In* Smith, T.B. and Wayne, R.K. (eds.) *Molecular genetic approaches in conservation*, pp298-313. Oxford: Oxford University Press.

Morin, P.A., Chambers, K.E., Boesch, C., and Vigilant, L. (2001). Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). *Mol. Ecol.* **10**:1835-1844

Morin, P.A., Wallis, J., Moore, J.J., and Woodruff, D.S. (1994). Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. *Mol. Ecol.* **3**:469-478

Morin, P.A., Wallis, J., Moore, J.J., Chakraborty, R., and Woodruff, D.S. (1993). Non-invasive sampling and DNA amplification for paternity exclusion, community structure, and phylogeography in wild chimpanzees. *Primates* **34:**347-356

Nei, M., and Chesser, R.K. (1983). Estimation of fixation indices and gene diversities. *Ann. Hum. Genet.* **47**:253-259

Newton, P.N. (1986). Infanticide in an undisturbed forest population of hanuman langurs, *Presbytis entellus. Anim. Behav.* **34**:785-789

Nicolson, N.A. (1987). Infants, mothers and other females. *In* Smuts, B.B., Cheney, D.L., Seyfarth, R.M., Wrangham, R.W., and Struhsaker, T.T. (eds.) *Primate Societies*, pp343-357. Chicago: University of Chicago.

Noë, R., van Schaik, C.P., and van Hooff, J.A.R.A.M. (1991). The market effect: an explanation for pay off asymmetries among collaborating animals. *Ethology* **87**:97-118

Packer, C. (1979). Inter-troop transfer and inbreeding avoidance in *Papio anubis*. *Anim. Behav.* **27**:1-36

Packer, C., Gilbert, D.A., Pusey, A.E., and O'Brien, S.J. (1991a). A molecular genetic analysis of kinship and cooperation in African lions. *Nature* **351**:562-565

Packer, C., Pusey, A.E., Rowley, H., Gilbert, D.A., Martenson, J., and O'Brien, S.J. (1991b). Case study of a population bottleneck: lions of the Ngorongoro crater. *Cons. Biol.* **5**:219-230

Paetkau, D., and Strobeck, C. (1995). Microsatellite analysis of genetic variation in black bear populations. *Mol. Ecol.* **3**:489-495

Parker, P., Snow, A.A, Schug, M.D., Booton, G.C., and Fuerst, P.A. (1998). What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* **79**:361-382

Peist, R., Honsel, D., Twieling, G., and Löffert, D. (2001). PCR inhibitors in plant DNA preparations. *Qiagen News* **3**:7-9

Pemberton, J.M., Slate, J., Bancroft, D.R., and Barrett, J.A. (1995). Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Mol. Ecol.* **4**:249-252

Pope, T.R. (1992). The influence of dispersal patterns and mating system on genetic differentiation within and between populations of the red howler monkey (*Alouatta seniculus*). *Evolution* **46**:1112-1128

Primmer, C.R., Møller, A.P., and Ellegren, H. (1995). Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica*. *Mol. Ecol.* **4**:493-498

Pusey, A., and Wolf, M. (1996). Inbreeding avoidance in animals. *TREE* **11**:201-206

Queller, D.C. (1992). Does population viscosity promote kin selection? *TREE* **7**:322-324

Queller, D.C., and Goodnight, K.F. (1989). Estimating relatedness using genetic markers. *Evolution* **43**:258-275

Rajpurohit, L.S., and Mohnot, S.M. (1988). Fate of ousted residents of one-male bisexual troops of Hanuman langurs (*Presbytis entellus*) at Jodhpur, Rajasthan, India. *Hum. Evol.* **3**:309-318

Rajpurohit, L.S., and Sommer, V. (1991). Sex differences in mortality among langurs (*Presbytis entellus*) of Jodhpur, Rajasthan. *Folia Primatol.* **56**:17-27

Rajpurohit, L.S., and Sommer, V. (1993). Juvenile male migration from natal one-male troops in Hanuman langurs. *In* Pereira, M.E. and Fairbanks, L.A. (eds.) *Juvenile Primates: Life history, development, and behaviour*, pp86-103. New York and Oxford: Oxford University Press.

Rajpurohit, L.S., Sommer, V., and Mohnot, S.M. (1995). Wanderers between harems and bachelor bands: male Hanuman langurs (*Presbytis entellus*) at Jodhpur in Rajasthan. *Behaviour* **132**: 255-299

Reed, J.Z., Tollit, D.J., Thompson, P.M., and Amos, W. (1997). Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Mol. Ecol.* **6**:225-234

Rogers, J.A. (1989). Genetic structure and microevolution in a population of Tanzanian yellow Baboon (*Papio hamadryas cynocephalus*). Ph.D. thesis, Yale University.

Ross, C. and MacLarnon, A. (2000). The evolution of non-maternal care in anthropoid primates: a test of the hypotheses. *Folia Primatol.* **71**:93-113

Seger, J. (1977). A numerical method for estimating coefficients of relationship in a langur troop. *In* Hrdy, S.B. *The Langurs of Abu*, pp317-326. Cambridge, MA: Harvard University Press.

Sherman, P.W. (1977). Nepotism and the evolution of alarm calls. *Science* **197**:1246-1253

Sherman, P.W., Jarvis, J.U.M., and Braude, S.H. (1992) Naked mole rats. *Sci. Amer.* **267**:72-78

Silk, J.B. (2002). Kin selection in primate groups. *Int. J. Primatol.* **23**:849-875

Smith, K.L., Alberts, S.C., Bayes, M.K., Bruford, M.W., Altmann, J., and Ober, C. (2000). Cross-species amplification, non-invasive genotyping, and non-Mendelian inheritance of human STRPs in savannah baboons. *Am. J. Primatol.* **51**:219-227

Sober, E., and Wilson, D.S. (1998). Unto others: the evolution and psychology of unselfish behavior. Cambridge, MA.: Harvard University Press.

Sommer, V. (1988). Male competition and coalitions in langurs (*Presbytis entellus*) at Jodhpur, Rajasthan, India. *Hum. Evol.* **3**:261-278

Sommer, V. (1989). Infant mistreatment in langur monkeys - sociobiology tackled from the wrong end? *In* Rasa, A.E., Vogel, C., and Voland, E. (eds.) *The Sociobiology of Sexual and Reproductive Strategies*, pp155-193. London and New York: Chapman and Hall.

Sommer, V. (1994). Infanticide among the langurs of Jodhpur: testing the sexual selection hypothesis with a long-term record. *In* Parmigiani, S. and vom Saal, F. (eds.). *Infanticide and Parental Care*, pp155-193. London: Harwood Academic.

Sommer, V. (1996) *Heilige Egoisten. Die Soziobiologie indischer Tempelaffen.* Munich: C.H. Beck.

Sommer, V. (2000). The holy wars about infanticide. Which side are you on? And why? *In* van Schaik, C.P. and Janson, C.H. (eds.) *Infanticide by males and its implications*, pp9-26. Cambridge: Cambridge University Press.

Sommer, V. (2002). The holy Hanuman. *BBC Wildlife* **20**:50-56

Sommer, V., and Rajpurohit, L.S. (1989). Male reproductive success in harem troops of Hanuman langurs (*Presbytis entellus*). *Int. J. Primatol.* **10**:293-317

Srivastava, A., and Dunbar, R.I.M. (1996). The mating system of Hanuman langurs: a problem in optimal foraging. *Behav. Ecol. Sociobiol.* **39**:219-226

Srivastava, A., Mohnot, S.M., and Rajpurohit, L.S. (1986). Bisexual troops of Hanuman langur (*Presbytis entellus*) in a predominantly one-male troop habitat. *Abstracts, International Symposium on Primates – The New Revolution.* New Delhi, India, 26-31 December 1986, pp41-42

Storz, J.F., Bhat, H.R., and Kunz, T.H. (2001). Genetic consequences of polygyny and social structure in an Indian fruit bat, *Cynopterus sphinx*. I. Inbreeding, outbreeding, and population subdivision. *Evolution* **55**:1215-1223

Sugg, D.W., Chesser, R.K., Dobson, F.S., and Hoogland, J.L. (1996). Population genetics meets behavioral ecology. *TREE* **11**:338-342

Sugiyama, Y. (1965). On the social change of Hanuman langurs (*Presbytis entellus*) in their natural condition. *Primates* **6**:381-418

Taberlet, P, Camarra, J.-J., Griffin, S., Uhrès, E., Hanotte, O., Waits, L.P., Dubois-Paganon, C., Burke, T., and Bouvet, J. (1997). Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Mol. Ecol.* **6**:869-879

Taberlet, P., and Luikhart, G. (1999). Non-invasive genetic sampling and individual identification. *Biol. J. Linn. Soc.* **68**:41-55

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P., and Bouvet, J. (1996). Reliable genotyping of samples with very low DNA quantities using PCR. *Nuc. Ac. Res.* **24**:3189-3194

Taberlet, P., Waits, L.P., and Luikart, G. (1999). Noninvasive genetic sampling: look before you leap. *TREE* **14**:323-327

Tiger, L., and Fox, R. (1972). *The imperial animal*. London: Vintage/ Ebury.

Trivers, R.L. (1971). The evolution of reciprocal altruism. *Q. Review Biol.* **46**:35-57

van Schaik, C.P. (1989). The ecology of social relationships amongst female primates. *In* Standen, V. and Foley, A. (eds.) *Comparative socioecology: The behavioural ecology of humans and other mammals*, pp195-218. Oxford: Blackwell.

van Schaik, C.P. (2000). Infanticide by male primates: the sexual selection hypothesis revisited. *In* van Schaik, C.P. and Janson, C.H. (eds.) *Infanticide by males and its implications*, pp27-60. Cambridge: Cambridge University Press.

Vigilant, L., Hofreiter, M., Siedel, H., and Boesch, C. (2001). Paternity and relatedness in wild chimpanzee communities. *Proc. Nat. Acad. Sci.* **98**:12890-12895

Vogel, C. (1988). Sociobiology of Hanuman langurs (*Presbytis entellus*): introduction into the Jodhpur field project. *Hum. Evol.* **3**:217-226

von Segesser, F., Menard, N., Gaci, B., and Martin, R.D. (1999). Genetic differentiation within and between isolated Algerian subpopulations of Barbary macaques (*Macaca sylvanus*): evidence from microsatellites. *Mol. Ecol.* **8**:433-442

Wahlund, S. (1928). Zusammensetzung von Populationen und Korrelation-serscheinungen vom Standpunkt der Verebungslehre aus betrachtet. *Hereditas* **11**:65-105

Walters, J.R., and Seyfarth, R.M. (1987). Conflict and Cooperation. *In* Smuts, B.B., Cheney, D.I., Seyfarth, R.M., Wrangham, R.W., and Struhsaker, T.T. (eds.) *Primate Societies*, pp306-317. Chicago: University of Chicago Press.

Wasser, S.K., Houston, C.S., Koehler, G.M., Cadd, G.G., and Fain, S.R. (1997). Techniques for application of faecal DNA methods to field studies of Ursids. *Mol. Ecol.* **6**:1091-1097

Weir, B.S., and Cockerham, C.C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**:1358-1370

Wilkinson, G.S. (1984). Reciprocal food sharing in the vampire bat. *Nature* **308**:181-184

Wilmer, J.W., Allen, P.J., Pomeroy, P.P., Twiss, S.D., and Amos, W. (1999). Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halicherus grypus*). *Mol. Ecol.* **8**:1417-1429

Woolf, R., Nakamura, Y., Odelberg, S., Shiang, R., and White, W. (1991). Generation of variability at VNTR loci in human DNA. *In* Burke, T., Dolf, G., Jeffreys, A.J., and Woolf, R. (eds.) *DNA fingerprinting: Approaches and Applications*, pp20-38. Basel: Birchauser-Verlag.

Wrangham, R.W. (1980). An ecological model of female bonded primate groups. *Behaviour* **75**:262-300

Wright, S. (1951). The genetical structure of populations. *Ann. Eugen.* **15**:323-354

Wright, S. (1978). *Evolution and the genetics of populations. Vol. 4: Variability within and among natural populations.* Chicago: University of Chicago Press.

Wynne-Edwards, V.C. (1962). *Animal dispersion in relation to social behaviour.* Edinburgh: Oliver and Boyd.

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FI-NISHED!

[1]a very fine flat coated retriever