A GENETIC ANALYSIS OF COOPERATIVE BREEDING IN MEERKATS

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PREFACE

This thesis has been composed by me, is the result of my own research and contains no work done in collaboration except where stated otherwise. The text does not exceed 100,000 words. No part of this thesis has been submitted to any other university in application for a higher degree.

ABSTRACT

A GENETIC ANALYSIS OF COOPERATIVE BREEDING IN MEERKATS

Cooperative breeders live in groups which are characterised by skewed distribution of investment in the provision of offspring care. Subordinate 'helpers' carry out the majority of care-giving behaviours while dominants invest little or nothing. Caring for offspring involves behaviours which incur substantial costs to helpers in terms of energy expenditure and increased risk of mortality. Understanding how helpers accrue fitness through investment in offspring care has, therefore, presented a central problem to evolutionary biology. Helpers may accrue fitness in three ways: (1) directly, by increasing survival of own offspring; (2) indirectly, by increasing the reproductive success of kin, and (3) through enhancement of future reproductive success. The importance of these mechanisms was investigated in a cooperatively breeding mammal - the meerkat (*Suricata suricatta*) - a small carnivore inhabiting arid regions of southern Africa.

The distribution of fitness between group members was investigated by sampling and genotyping around 400 individuals from two study sites in South Africa for 6-12 microsatellite markers. This data was used first, to construct group pedigrees using parentage analysis to assign maternity and paternity to pups; and second, to calculate relatedness coefficients. Parentage analysis showed that up to 84% and 100% of pups were the offspring of dominant males and females respectively, demonstrating that subordinates rarely invested in their own offspring. Breeding success of subordinate males was predictable by the presence or absence of an unrelated opposite sex breeder, whereas all subordinate females appeared subject to suppression by the dominant female. The majority of subordinates accrued substantial indirect fitness from helping in their natal group. Relatedness measurements revealed that natal subordinates of both sexes, have on average, a relatedness of 0.29 to pups (not significantly different from $r=0.25$, i.e. that between half sibs). Non-breeding, immigrant males were unrelated to offspring and so gained no direct or indirect fitness from helping, but had a higher chance of obtaining direct fitness in the future either while subordinate or by gaining dominance.

In conclusion, direct fitness benefits are distributed strongly in favour of dominants which invest least in care-provision for young. Only a small proportion of subordinate helpers recruit to the dominant breeding population, due to constraints on breeding from suppression by dominants and/or high ecological constraints on successful dispersal. Helping is prevalent, therefore, as a means by which fitness can be acquired indirectly, by

providing care for related young in natal groups. The small proportion of males which disperse successfully from their natal group do not acquire indirect fitness from helping but increase the chance of breeding while subordinate and/or inheriting dominance in the future.

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CHAPTER 1

INTRODUCTION

1.1 The problem of altruism

> "Natural Selection is demanding, exacting, relentless. It is intolerant of weakness, indifferent to suffering.....But look carefully at nature and you will find that it doesn't always seem like that. ...Indeed, in some respects [animals] behave more like the moral paragons of Aesop - working dutifully for the sake of the community, noble in spirit and generous in deed - than the hard-bitten, self-seeking individualists that natural selection would seem to favour. Such behaviour poses a problem for the Darwinian view of nature. It has become the problem of altruism."
>
> (Cronin 1991, p253)

Given that altruistic behaviour doesn't conform to a Darwinian view of the world centred on the individual, how can the struggle for survival of the fittest accommodate selection for altruism? Helping in cooperatively breeding species represents a substantial cost. The action may involve delayed reproduction or permanent sterility, donation of food, significant loss of foraging time due to 'baby-sitting' and increased risk of predation or injury due to defence of young (Reyer 1984, Clutton-Brock et al. 1998). The mechanism by which such behaviour could have evolved is hard to explain in terms of individual selection, given the potential for reduced reproductive success.

To satisfy the definition of altruism as an *unselfish* act committed for the benefit of another, the actor must not receive a pay-off for its actions. It could be argued then, that the resolution of the problem of altruism is that it doesn't exist, as there is no such thing as an unselfish action. The 'paragons of Aesop' that Cronin (1991) refers to, appear so only because a selfish motivation for their actions is obscured. For simplicity, the word 'altruism' is often used to denote what might be better described as aid-giving behaviour.

In cooperatively breeding species, it is often unclear what benefits individuals receive as a result of aid-giving behaviours. Research into the evolution of social groups has attempted to show how fitness is distributed between donors and beneficiaries of aid-giving interactions. More specifically, in cooperative breeders, 'helpers' which invest in rearing offspring could enhance fitness in one or more of the following ways: (1) direct fitness - helpers may actually be breeding and, therefore, be enhancing their reproductive success by investing in their own offspring; (2) indirect fitness - by enhancing reproductive success of relatives, and (3) future fitness - helping may not affect fitness in the short term, but may lead to a greater chance of acquiring direct and/or indirect fitness in the future.

The work described in this thesis aims to investigate routes to fitness in a cooperatively breeding mammal, the meerkat (*Suricata suricatta*), using an analysis of genetic data. This chapter reviews the literature as an introduction to why and when different routes to fitness are important. Chapter 2 reviews information about the ecology and behaviour of the meerkat which are relevant to understand how selection has acted upon cooperative behaviours in this species. Chapter 3 describes the techniques involved in collecting genetic data with microsatellite markers. Chapter 4 describes how the microsatellite data was analysed both in order to infer parentage of offspring, and to measure relatedness between individuals or groups of individuals. Chapter 5 describes the results of applying these analyses to the question of who breeds in meerkat groups, answering the question: How is direct fitness distributed between group members? Chapter 6 describes the results of applying analyses of genetic data to the question: Does helping lead to indirect fitness, or to the enhancement of future fitness? Chapter 7 brings the results together in a general discussion.

1.2 Inclusive fitness

Hamilton (1964a,b) showed that animals are selected to maximise what he termed inclusive fitness. Inclusive fitness theory states that an individual can acquire fitness through the propagation of non-descendent kin by 'kin selection'. This conclusion represented a breakthrough in the study of sociality as it provided an explanation as to how behaviour that affects non-descendent kin can be subject to natural selection. Inclusive fitness theory accounts for the existence of helping behaviour and sterility by showing that an altruist stands to gain inclusive fitness if his actions enhance the reproductive success of his relatives, even if they incur a cost to himself.

'Hamilton's inequality' states that a gene for a behaviour will increase in frequency if:

$rb-c > 0$

where b is the benefit to the recipient, c is the cost to the actor in terms of number of offspring produced, and r is the relatedness between the two interactants. The degree of relatedness, therefore, is equal between parent-offspring interactants and full sibs (0.5), and equal between grandparent-offspring interactants and half sibs (0.25). There is widespread confusion about the application and measurement of inclusive fitness. First, consider Hamilton's (1964a) verbal definition:

> 'the animal's production of adult offspring...stripped of all components...due to the individual's social environment, leaving the fitness he would express if not exposed to any of the harms and benefits of that environment....and augmented by certain fractions of the harm and benefit the individual himself causes to the fitness of his neighbours. The fractions in question are simply the coefficients of relatedness..'

If components due to the social environment are not stripped from an individuals fitness then units of fitness will be double-counted, artificially inflating 'inclusive fitness' values (Grafen 1982, 1991). However, in the case of obligate communal breeders this stripping will leave reproductive individuals with zero inclusive fitness as any offspring produced will count only towards the fitness of helpers. This is paradoxical, because obviously a reproductive transmits more of its genes to subsequent generations than helpers. Creel (1990a) and Lucas et al. (1996) attempt to resolve this paradox by stripping the same, average effect from all individuals. However, as Queller (1996) explains, these attempts do not eliminate the double-counting error. Queller (1996) goes on to describe how the reproductive may retain the fitness conferred by the production of offspring. The decision of whether to breed or not breed by the dominant is different from the decision made by a subordinate and can be considered to depend on different genes. Queller (1996) suggests that reference to the inclusive fitness of an action is more accurate than the inclusive fitness of an individual.

Crucially, in order to measure inclusive fitness it is necessary to take into account the propagation of genes identical by descent indirectly through the production of non-descendent kin (indirect fitness) as well as that propagated directly through reproductive success (direct fitness). Cooperative breeders represent a special case in that the indirect component of inclusive fitness may be more important than the direct component. Helpers may rear large numbers of offspring, each of which contributes towards their inclusive fitness.

1.3 Direct fitness

1.3.1 Reproductive skew

Where there is selection for group-living, there is potential for a dominant to exploit the mutual benefits of the group to their own advantage. They may do this by shirking risky activities such as territory defence, or by monopolising reproduction. The extent to which dominants are able to do this will depend on what Vehrencamp (1984) terms the 'relative leverage' of dominants and subordinates. Cooperative breeders are predominantly singular breeders as opposed to plural breeders (many breeding pairs per group) suggesting that 'leverage' must often be in the dominants' favour.

1.3.1.1 Concession models

What determines leverage is a central question. Vehrencamp's (1984) model of reproductive skew predicts the maximum amount of fitness biasing possible in a group. The term 'reproductive skew' has been coined to describe this fitness bias or distribution of reproduction between members of a group. In highly skewed societies reproduction is monopolised by a few individuals whereas in low skew societies reproduction is shared more equally. The idea emerges that the subordinates' options outside the group limit the ability of a dominant to impose its optimum bias, or skew, on the rest of the group. The dominant must make reproductive concessions to subordinates (or 'staying incentives'), in terms of direct fitness, to prevent them from dispersing if constraints on doing so are weak. The cost to a dominant of relinquishing fitness may be less than that they would suffer if the subordinate were to leave.

Keller and Reeve (1994) expanded on Vehrencamp's model to include 'peace incentives', that is reduction in skew to lower the subordinate's incentive to gain reproductive control through fighting. The expanded skew model makes four predictions about the factors which affect the relative leverage that Vehrencamp refers to: (1) Productivity: Keller and Reeve (1994) suggest that if group productivity is high subordinates will be more likely to tolerate suppression to stay in the group, and skew will be high. (2) Harshness of ecological constraints: the greater the risk associated with dispersal the more likely subordinates are to tolerate suppression and accept lower staying incentives (Emlen 1982a, 1982b, 1991, Stacey & Lignon 1987, Rabenold 1985, 1990). (3) Fighting ability: skew will increase with greater asymmetry in fighting ability between dominants and subordinates as peace incentives will be lower. (4) Relatedness: skew will be higher in groups composed of relatives. This is because subordinates are receiving indirect fitness benefits from the reproduction of the dominant and so will tolerate suppression.

A possible fifth factor affecting the magnitude of incentive required to retain helpers is the physiological cost of reproduction (Creel & Creel 1991). When costs of breeding are high, the benefits of attempting reproduction as a subordinate are more likely to be outweighed. Creel & Creel (1991) show that reproductive suppression (high reproductive skew) is associated with costly gestation and post-natal investment in litter growth in communally breeding carnivores.

1.3.1.2 Conflict models

In the models of reproductive skew described above, dominants are expected to make reproductive concessions to retain subordinate helpers. For this reason, these models have been termed 'concession' models by Clutton-Brock (1998). Concession models attempt to provide a unifying framework to explain reproductive skew in all social taxa. However, it has been pointed out that assumptions of complete control by a dominant may be highly restrictive (Clutton-Brock 1998, Cant 1998, but see Creel & Waser 1991, Reeve et al. 1998). Specifically, it has been argued that in mammals and birds there may be negligible differences in the competitive ability of dominants and subordinates. In such cases, subordinates will not necessarily be selected to increase the fitness of the dominant by foregoing breeding opportunities. Instead, it is proposed that the degree of reproductive skew will result from the outcome of conflict for reproduction between group members. This idea has been distinguished from concession models by the term 'conflict model' by Clutton-Brock (1998).

1.3.1.3 Availability of unrelated mates

Reproductive skew may also depend on the relatedness structure of a group or population. Breeding with close relatives (inbreeding) can reduce fitness (Charlesworth & Charlesworth 1987) and so opportunities to breed may depend on the availability of unrelated members of the opposite sex to mate with (Michod 1993, Pusey & Wolf 1996). In many species, sex-biased dispersal of individuals from their natal area prevents close relatives from residing together as adults (Packer & Pusey

1993) in this way, individuals may increase the availability of unrelated potential mating partners (Koenig & Pitelka 1979). The importance of this factor in determining who breeds in a group has been shown to differ widely between species, however. Acorn woodpeckers (*Melanerpes formicivorus*) may forego breeding for years on the death of a breeder until the breeder is replaced by an unrelated immigrant (Koenig et al. 1998, Koenig et al., in press). Dispersive morphs which will only attempt to mate with non-colony members, have been discovered to exist at low frequencies in colonies of highly inbred naked mole-rats (*Heterocephalus glaber*) (O'Riain et al. 1996).

In contrast, Keane et al. (1996) show that inbreeding is not avoided in the dwarf mongoose (*Helogale parvula*) where average relatedness among potential mates is high. Mating patterns are random with respect to relatedness and dispersal has a limited effect in reducing relatedness between mates because relatedness is often high between adjacent groups. Young females are infrequent dispersers but are more likely to do so when the opposite sexed dominant is a close relative. Young males disperse at random with respect to the relatedness of the dominant female and are more likely to disperse to packs that contain genetically similar individuals. It is suggested that dispersal to packs containing relatives may be advantageous if levels of aggression towards related immigrants are lower, or if increased indirect fitness from helping outweighs any cost of inbreeding. It is also suggested that there may be geographical reasons favouring dispersal to particular territories. Inbreeding avoidance also did not provide sufficient explanation for mate choice in other cooperative breeders such as white-fronted bee-eaters (*Merops bullockoides*) (Wrege & Emlen 1994) or white-nosed coatis (*Nasua narica*) (Gompper et al. 1998).

1.4 Indirect fitness

In cooperative breeders, indirect fitness commonly accrues when offspring of the breeding pair delay dispersal from their natal group (Stacey & Koenig 1990, Creel & Creel 1991, Goldizen 1987). Understanding indirect fitness gain, therefore, also becomes a matter of understanding why individuals delay dispersal. Research on dispersal decisions in cooperative breeders hs revealed strong parallels in vertebrates (Emlen 1982a, 1987) and wasps (Brockman 1997, Field et al. 1998) with both groups of taxa being limited by breeding site availability. The decision to disperse is often dependent on the sex and age of an individual. While younger subordinates remain at the natal territory, older subordinates are more likely to disperse. Dwarf mongooses were shown to optimise dispersal strategies according to sex and age (Creel & Waser 1994). In such circumstances it is advantageous to be long-lived, and many cooperatively breeding species are long-lived relative to related non-social taxa, for example the stripe-backed wren (*Campylorynchus nuchalis*) (Rabenold 1990), the Arabian babbler (*Turdoides squamiceps*) (Zahavi 1990) and the splendid fairy wren (*Malarus splendens*) (Rowley & Russell 1990). Two compatible hypotheses have been proposed to account for delayed dispersal, namely the ecological constraints hypothesis and the benefits-of-philopatry hypothesis.

1.4.1 Ecological constraints

Attempts have been made to identify ecological conditions associated with species which delay dispersal from their natal territory. Emlen (1982a) proposed an ecological constraints model to resolve the apparent contradiction between two sets of observations: that many cooperative breeders are sedentary in stable, predictable habitats and that many cooperative breeders inhabit harsh, unpredictable environments.

Dispersing to reproduce may not be possible in either of the two habitat types described above. In the first instance, the habitat is saturated with established territory holders and in the second instance there is a substantial risk of mortality associated with dispersal. Habitat saturation may apply to a number of species, for example the Florida scrub jay (*Aphelocoma coerulescens*) (Woolfenden & Fitzpatrick 1984) and the Seychelles warbler (*Acrocephalus sechellensis*) (Komdeur et al. 1995) where vacant territories, suitable for breeding, are rarely available. A non-breeder must first attain sufficient age and experience before it is able to obtain and defend a territory and it may be better to wait in familiar habitat among relatives.

1.4.2 Philopatry

There are cases of animals failing to disperse even when adjacent territories become vacant, for example in the stripe-backed wren (Rabenold 1985) and the long-tailed tit (*Aegithalos caudatus*) (Hatchwell pers. comm.). This suggests that animals may be philopatric to their natal territories and may not take advantage of opportunities to disperse (Emlen 1982b). Factors promoting philopatry may include the advantage of inheriting a familiar territory, living in a group or the opportunity to increase indirect fitness (Creel & Waser 1994, see also below). Stacey & Lignon (1987) emphasise this point with a study on the acorn woodpecker, pointing out that although most species face ecological constraints and include a non-breeding proportion of their population, cooperative breeding is rare, occurring in 3% of 9000 species of birds. In their benefits-of-philopatry hypothesis, cooperative breeding may lead to habitat saturation, thus accounting for the link observed by Emlen (1982a).

1.4.2.1 Effect of helping on indirect fitness benefits

Direct fitness accrued by dispersing to breed is more likely to be outweighed by indirect fitness acquired by helping, if helping is a relatively efficient way of producing kin. For several species the number of young reared is positively correlated with the number of helpers, for example in white-fronted bee-eaters (Emlen & Wrege 1989) and dwarf mongooses (Rood 1990). Young also grow more slowly in smaller groups of dwarf mongooses (Creel & Creel 1991). Correlative evidence does not prove an effect of helping on survivorship, however, and in some studies helpers have been removed, as a more powerful test (Brown et al. 1982). Mumme et al. (1989) showed that control groups of Florida scrub jays, from which helpers hadn't been removed, were three times more successful than experimental groups. He attributed most of this difference in success to the effects of predation. However, in a similar study carried out in moorhens (*Gallinula chloropus*) (Leonard et al. 1989) no difference was found when helpers were removed and ecological and breeder quality variables were controlled for.

1.5 Future fitness

As discussed above, cooperative breeders are often long-lived, and optimum strategies for investing in indirect versus direct fitness may change over lifespans. Helping may not necessarily result in significant fitness benefits in the short term, fitness acquired by helping depends on being amongst kin which may not always be the case and opportunities to breed may be limited for subordinates. It has been demonstrated, however, that helping may still be adaptive if it enhances fitness acquired directly or indirectly in the future (Creel 1990b, Mumme et al. 1989).

1.5.1 Enhancement of future direct fitness by helping

1.5.1.1 Enhanced probability of survival of helper

Helping will increase the number of young reared and as survivorship is often positively correlated with group size, helping will enhance survivorship. Evidence for this exists from studies on the acorn woodpeckers (Stacey & Lignon 1987), dwarf mongooses (Rood 1990) and meerkats (Clutton-Brock et al., in press c).

1.5.1.2 Enhanced likelihood of becoming a breeder in the future

In cooperative breeders most animals that attain breeding status do so in their natal territories, (e.g. dwarf mongooses (Rood 1990), jackals (*Canis mesomelis*) (Moehlman 1979), hoatzins (*Opisthocomus hoazin*) (Strahl & Schmitz 1990) and Florida scrub jays (Woolfenden & Fitzpatrick 1984)). However, the opposite is observed in the pied kingfisher (*Ceryle rudis*) (Reyer 1984). It is not clear to what extent helping increases the chance of inheriting a territory. There is no study which compares acquisition of territories between helpers and non-helpers.

Helpers may also increase their chance of attaining breeding status if they raise more individuals with which to form coalitions in the future. For example, former helpers disperse with young they helped to raise in lions (*Panthero leo*) (Packer et al. 1988) and dwarf mongooses (Rood 1990).

1.5.1.3 Increased fecundity when breeding status is attained

This hypothesis works from the premise that experience gained as a helper will increase success as a breeder. There is limited evidence for this but Rowley & Russell (1990) found in splendid fairy-wrens that first time breeders with past helping experience were more successful.

1.5.1.4 Increased production of non-descendent kin

There is limited evidence to suggest that, if they are faced with a decision of who to help, helpers often favour the young of their close relatives. For example, in the white-fronted bee-eater (Emlen & Wrege 1988) which live in extended family groups containing individuals of variable relatedness. Helpers had a strong positive effect on the reproductive success of the breeder and there was a strong effect of relatedness on the probability of help being given, to the extent that if there were no close relatives in the group then non-breeders did not participate in helping at all. However, the amount of aid provided did not vary with relatedness. Relatedness has also been reported to be an important determinant of helping behaviour in the pied kingfisher (Reyer 1984) and the Seychelles warbler (Komdeur 1994). However, in many species care is provided irrespective of kinship such as the superb fairy-wren (Dunn et al. 1995), dwarf mongooses (Rood 1990) but as has been discussed, the lack of inclusive fitness benefit does not preclude the evolution of helping. The decision to preferentially help close kin depends on the ability to distinguish kin from non kin using genetic cues. There is evidence to suggest that vertebrates are able to recognise kin but some controversy over the relative importance of environmental versus genetic cues

1.5.1.5 Punishment avoidance

The importance of coercion and the avoidance of punishment as an incentive in animal societies was highlighted by Clutton-Brock & Parker (1995). It may pay subordinates to help, even in the absence of fitness benefits, to avoid punishment from a dominant. By doing so, subordinates may increase the probability of acquiring fitness in the future by remaining in the group and avoiding injury. Consider the example of the superb fairy wren (*Malurus cyaneus*) (Mulder & Langmore 1993) where helpers removed during the breeding season are attacked by the dominant male on their return, but not attacked if they are removed outwith the breeding season. The ability to coerce may increase with relatedness between subordinates and dominants. An offspring may share the same proportion of genes (0.5) with its siblings that it would with its own offspring. The parent, however, only shares 0.25 of its genes with any grandchildren. It should be easy, therefore, for a parent to coerce offspring into helping raise more siblings rather than reproduce, as the offspring's inclusive fitness will be unaffected. In the naked mole-rat helpers are highly related to their siblings (Jarvis et al. 1994).

1.5.1.6 Sexual selection

Helping behaviour in the Arabian babbler confounds explanations based on inclusive fitness, coercion or mutualism (Zahavi 1990). Non-breeding helpers have been documented to compete to help. Furthermore, dominant breeders attempt to interfere with helping attempts and will punish an individual that brings food to it, or the young. Zahavi (1990) suggests that bearing the burden of being a helper is an honest signal of fitness to potential mates and will, therefore, be maintained in the population by sexual selection in the same way as a long tail. Advertisement in this species is important as they are highly interactive, long-lived and individuals may influence their 'status' as well as their position in a hierarchy. For example, there may be a difference in dominance between a father and son ranked one and two in a hierarchy, compared to that between brothers (Zahavi 1990).

1.5.2 Future indirect fitness

Mumme et al. (1989) demonstrated that future effects on indirect fitness could represent a substantial proportion of total indirect fitness acquired over a lifetime (29-49%). However, this component of inclusive fitness has often been overlooked in studies of the effects on fitness accrued by helping.

1.5.2.1 Enhancement of breeder survival/productivity

In some species the provisioning of young is unaffected by the number of helpers but the workload, e.g. number of foraging trips per individual, is reduced (e.g. in the hoatzin (Strahl & Schmitz 1990), pied kingfisher (Reyer 1984), Florida scrub jay (Woolfenden & Fitzpatrick 1984), and splendid fairy-wren (Russell & Rowley 1988). In cooperatively breeding mammals, but not birds, the reproductive individual invests least in caring for the young, for example in mole rats, (Jarvis et al. 1994) and meerkats (Clutton-Brock et al. 1998). This may be outweighed by costly gestation, particularly in some species of carnivore. Creel & Creel (1991) found a significant positive relationship between the number of helpers and breeding female foraging time in the dwarf mongoose.

Helpers, therefore, may not just be lightening the load, they may be releasing a breeder from postnatal investment altogether. This could facilitate additional breeding attempts in a season or greater success in future years. Mumme et al. (1989) suggest that it is often more useful to view helping as aid-giving behaviour towards a breeder rather than towards the young, as this perspective emphasises the helper's increased *future* indirect fitness as well as the current indirect fitness. They reanalyse data from Florida scrub jays (Woolfenden & Fitzpatrick 1984), pied kingfisher (Reyer 1984) and splendid fairy-wrens (Russell & Rowley 1988) and show that future indirect fitness is enhanced by helping to the same extent as current indirect fitness.

In obligate communal breeders such as dwarf mongooses (Creel & Creel 1991) and meerkats (Clutton-Brock et al. 1998) reproduction is impossible in the absence of helpers. Many carnivorous mammals are obligate communal breeders for reasons discussed by Creel & Creel (1991), who show that breeding in these species can be costly in terms of gestation and postnatal investment relative to other species. They use the dwarf mongoose as a case-study to test the hypothesis that the energetic costs of reproduction and the degree of reproductive suppression evolve in parallel and costs may reach a point at which females require help to breed successfully. Pre- and post-natal litter growth rates are adapted to the normal social environment making it extremely difficult for parents without helpers to sustain the level of investment required.

1.6 Mutualism

Cooperative interactions can evolve, not from selection on one individual to help another, but from mutualism, where interactants benefit equally. Animals often carry out tasks which rely on the cooperation of others for success. There may be a mutual benefit to be had from cooperating with members of other species as in mixed-species colonies of ant species *Azteca constructor* and *Azteca xanthacroa* (Choe & Perlman 1997).

Hunting lions represent a classic example of animals cooperating to achieve a common goal (Packer & Ruttan 1988). However, if success is measured per individual as it should be, lions do better to hunt on their own (Packer et al. 1990). Living in a group confers other advantages such as increasing the ability to defend kills successfully against other lions or hyenas. A group of males is more successful at defending a harem of females than a solitary male and male offspring are produced in synchrony which may be to ensure that sons are members of large coalitions, maximising their chance of success in access to females in the future (Packer et al. 1988). Recent studies have revealed, however, that all is not as harmonious as it might seem, and in fact lionesses defending a pride's territory against intruders cheat on one another by hanging back (Heinsohn & Packer 1995). There are clearly benefits to be had from cheating and letting someone do the work for you.

1.6.1 Game theory - the prisoner's dilemma

A common metaphor used to assess the optimality of cooperative or cheating strategies in such situations is the two-person game of the prisoners dilemma. In the game two prisoners are faced with the simple choice of defecting or cooperating. If they both cooperate by not giving evidence against the other, neither prisoner will receive a full sentence. A defector who gives evidence against the other will receive the lightest possible sentence whilst the other will receive the longest possible sentence, commonly referred to as the Sucker's Payoff. If both defect, then they receive a lighter sentence than the Sucker's Payoff but a longer one than mutual cooperators. This game encapsulates a problem in trying to understand the adaptiveness of altruistic behaviour: it always pays to defect. If your partner co-operates you receive the lightest possible sentence by defecting, and if your partner defects you avoid the stiffest possible sentence by defecting.

Not only does the prisoner's dilemma leaves the problem of altruism unexplained, it has been suggested that the prisoner's dilemma is not a good model for cooperative behaviour in animals (O'Conner 1995, Mukherji 1996 but see Nowak et al. 1996). One way to make it more realistic may be to imagine a scenario where the game is played repeatedly between the same opponents (iterated prisoner's dilemma game). When animals are faced repeatedly with the decision to cooperate or defect with the same opponents, reciprocal altruism can evolve, as defectors risk retaliation in future rounds (Bendor & Swistak 1995). There is some experimental evidence that animals do not learn to co-operate in the iterated prisoner's dilemma game (Maynard Smith & Szathmary 1995) and it seems likely that animals such as lions are able to gather information about other participants which is not taken into account in the rules of the games (Legge 1996). Maynard Smith & Szathmary (1995) and Clutton-Brock (pers. comm.) suggest an alternative analogy to cooperating individuals, based on a rowing team. In this game, cooperation can be an evolutionarily stable strategy (as well as defecting). Whichever becomes more common in the first place will prevail as a robust strategy, and cooperation between relatives may spread to include non-relatives. Even if this does not always occur, cooperation must sometimes pay, contrary to the predictions of the prisoner's dilemma.

1.7 Helping behaviour in the absence of fitness benefits

In this review, it has been assumed until now that helping behaviour maximises some component of fitness, even if the exact mechanism by which this occurs is not obvious. There is one example, however, of helping behaviour occurring in the absence of any fitness benefits to the actor.

1.7.1 Deception

In some cooperatively breeding species, a breeder may elicit help from a non-relative that is deceived into expecting returns in indirect fitness (Conner & Curry 1995). To achieve this, advantage is taken of kin-recognition systems based on associations learned by young whilst being fed. For example, birds such as scrub jays

(Woolfenden & Fitzpatrick 1984) ‘adopt’ young from neighbouring groups. An extreme example of exploitation by deception can be seen in the white-winged chough (*Corcorax melanorphyrus*) (Heinsohn 1991) in which groups of adults go on kidnapping forays to neighbouring nests. The young they capture and raise as their own are then sequestered into the workforce of helpers for subsequent generations, but don’t impose an inclusive fitness cost by competing for resources with their kidnappers. Cooperation induced by deception is more common in interspecific interactions. Slave-making ants raise larvae from the nests of other species to become workers (Bourke & Franks 1995) and the same principles apply to cases of ‘altruism’ observed in bird species parasitised by cuckoos (Cronin 1991).

CHAPTER 2

STUDYING SOCIAL EVOLUTION IN MEERKATS

2.1 Introduction

Basic questions about the evolution of sociality have been tackled from different directions by biologists studying vertebrates and invertebrates. Research on sociality in vertebrates has come mainly from an ecological perspective (Emlen 1982a,b, Stacey & Koenig 1990, Solomon & French 1996). The study of social evolution on invertebrate systems, on the other hand, has concentrated on testing genetic bases of theories such as Hamilton's kin selection model (Hamilton 1964a,b). There are problems with using vertebrates systems to similar ends, as it is often impractical and/or unethical to test theory experimentally, or to obtain sufficient sample sizes for analyses. Difficulties also arise from the fact that vertebrates have relatively long life-spans, making it necessary to invest in long-term studies in order to measure vital parameters such as reproductive success over entire lifetimes.

With the advent of genetic techniques to clarify family structure, information on genetic relationships is now being used to study vertebrate as well as invertebrate social systems so research on different taxa has converged. This convergence has led to an increasing realisation that studies should incorporate both genetic analyses and ecological and behavioural information. Furthermore, direct comparison of different taxa can provide valuable insights into social evolution in general (McRae et al. 1997, Brockman 1997, Cahan et al. in prep). By collecting information from a wide range of taxa, we can aim to identify idiosyncratic differences associated with particular taxa, allowing common principles of social evolution to emerge.

2.2 The Meerkat Project

Meerkats offer a unique opportunity to understand how sociality has evolved in mammals and to increase understanding of how cooperative behaviours evolve. Meerkats are relatively visible and have comparatively small ranges. It has proven possible to habituate groups to the presence of a human observer and identify individuals from pelage patterns and/or facial marks. This makes it possible to follow individuals from birth to death and, in some cases follow dispersal events. Importantly, it is also possible to monitor breeding success as it is possible to obtain samples for genetic analysis with minimum interference from a large number of individuals for which other life history information is available.

Meerkats have been studied in the Kalahari basin, South Africa, since 1993 by Prof. T. H. Clutton-Brock, University of Cambridge (Clutton-Brock et al. 1998, Clutton-Brock et al. in press a-c, Clutton-Brock et al. in prep). The project extends from a previous study in the same area by Dr D. Macdonald, University of Oxford (Doolan & Macdonald 1996 1997).

One of two study sites is situated along the Nossob fossil river bed in the Kalahari Gemsbok National Park situated in the Northern Cape region of South Africa and another is situated on farmland near Van Zyls Rus approximately 120 km to the south east (Figure 3.1).

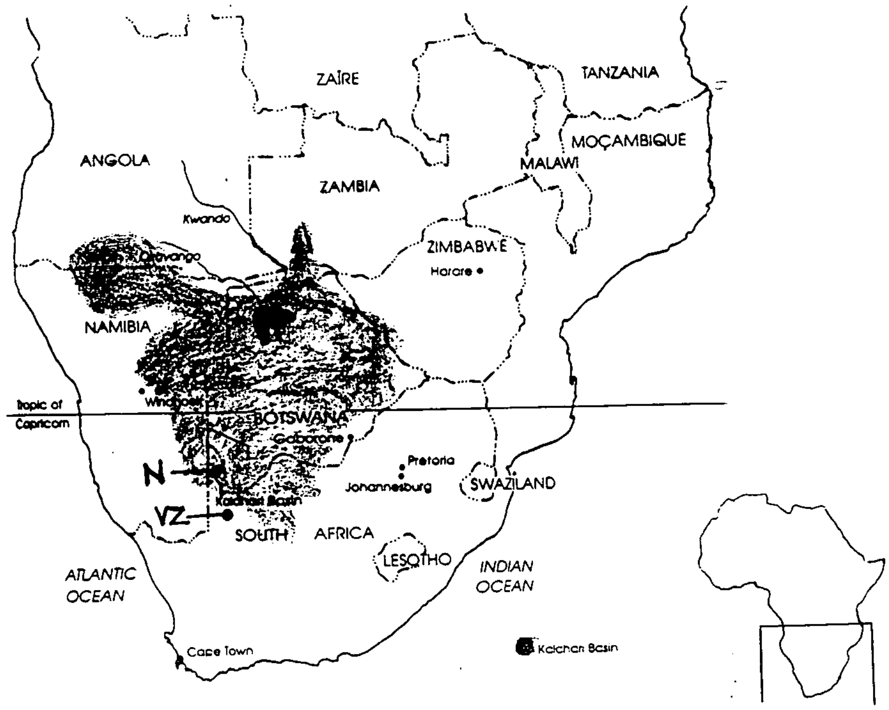

Figure 3.1 Map of southern Africa with the position of study sites Nossob and Van Zyls marked.

Many features of the ecology of the two sites are similar, for example average annual rainfall at Nossob is 240mm/year and at Van Zyls 217mm (Clutton-Brock et al. in press c). The main important difference between the two sites is predator density which is substantially higher at Nossob than Van Zyls (Clutton-Brock et al, in press c). The Nossob site is in a national park and has one of the highest reported raptor densities in the world (Gaynor pers. comm.). This is significant as raptors are the primary predators of meerkats (Clutton-Brock et al. 1998). The Van Zyls site is on ranchland where food webs have been disturbed by hunting and other human activities, and consequently the number of predators, including raptors, is lower. Mortality rates in meerkats are 1.7 times higher at Nossob and the population is almost twice as dense at Van Zyls with 1.69 individuals per km^2 compared with 0.95 individuals per km^2 at Nossob (Clutton-Brock et al. in press c).

The overall aim of the meerkat project is to estimate inclusive fitness pay-offs of different life history and behavioural strategies in order to understand the evolution of cooperative behaviour in this species. To achieve this a combination of detailed monitoring of cooperative behaviours has been carried out and genetic relationships have been inferred (from the analyses described in this thesis). Where feasible, experimental approaches are also being used to investigate cooperation. In order to use entire groups as independent data points in analyses, an emphasis has been placed on maximising the number of groups under study. Spreading effort over two study sites helps identify effects on behaviour associated with population density and predation pressure which differ between them.

2.3 The meerkat

2.3.1 General biology

Meerkats (*Suricata suricatta,* family Herpestidae) or suricates, as they are also called, are desert-adapted carnivores which range over arid regions of southern Africa. They live in stable groups occupying a territory of 2-5 km^2 containing anything between 2 and 15 sleeping burrows between which they alternate. Typically, groups are composed of a dominant pair, equal numbers of subordinate males and females and dependent young, comprising 5-20 animals per group (Clutton-Brock et al. in press a-c). Adults of both sexes weigh approximately 750g. The dominant pair are easily distinguished as they are rarely threatened or displaced by other group members, receive more grooming than other animals and mark territory boundaries ten times more frequently than the other group members (Clutton-Brock et al. in prep.).

Breeding can take place throughout the year but most often takes place in the months December to April when rainfall is highest. Usually a single female appears to get pregnant and gives birth to up to 3 litters in a year, but when rainfall is low, breeding may cease altogether. Gestation lasts for around 60 days; litter size is 3-5 pups at emergence and it is rare for either sex to breed before the age of 24 months (Clutton-Brock et al. in press c). Pups emerge when they are around three weeks old and move with the group a week after emergence.

Group membership is stable from day-to-day but subordinates of both sexes are observed to disperse from their natal groups. Subordinate adult females are commonly expelled from the group by the dominant female during the later stages of the dominant female's pregnancy (Clutton-Brock et al. in press, a). Subordinate males were not expelled from their natal groups but left voluntarily during their second or third year of life. Around half of all subordinates of both sexes that left their natal groups returned again within three months. Females which did not return either disappeared or founded a new territory. Males that did not return to their natal group either disappeared, founded a new group or immigrated into an established breeding group (Clutton-Brock et al., in press c, Chapters 5 & 6, this thesis).

2.3.2 Cooperation

Meerkat behaviour is characterised by highly developed sociality. Group members cooperate to defend their territory, guard against predators, construct and maintain burrow systems, and care for offspring. By evolving cooperative behaviours meerkat groups are able to survive in an environment where the probability of survival for solitary individuals is negligible. In a period of drought at Nossob, survival of individuals was dependent on the size of group in which they lived. Only groups containing over nine individuals survived (Clutton-Brock et al., in press c).

Meerkats are diurnal, leaving burrows in the morning to forage for insects and insect larvae, although small reptiles and eggs are also taken. In order to find sufficient food, meerkat groups range 1-1.25 km a day from their sleeping burrows (Brotherton pers. comm.). Foraging is carried out mainly by digging for prey. While excavating, they are vulnerable to attack from above by raptors. To be able to forage in relative safety, meerkats have evolved a coordinated system of vigilance for predators in which individuals take it in turns to go on guard. This behaviour incurs substantial costs: guarding may involve keeping watch from a prominent position in high temperatures for several hours while the rest of the group forage and feed. If a predator approaches the individual on guard makes an alarm call which tells the rest of the group to run to the nearest bolthole.

Cooperation is probably the key to reproductive success as well as predator evasion. Among 120 litters followed, there have been no observed instances in which a solitary female successfully raised a litter without assistance from helpers (Brotherton pers. comm.). Subordinates carry out almost all of the tasks associated with raising young - babysitting the litter before and immediately after emergence from the burrow, feeding pups when they travel with the group and protecting them against predators (Clutton-Brock et al. 1998). The amount of help a helper provides varies between individuals and also depends on the number of helpers in the group. In groups with fewer helpers, each helper compensates by providing care more often (Clutton-Brock et al. 1998).

In 13 out of 16 closely monitored breeding attempts all adults apart from the dominant pair contributed to babysitting, and in the remainder only one or two individuals did not contribute (Clutton-Brock et al. 1998). Both breeding adults and subordinates adjusted their contributions to babysitting depending on the number of helpers present in the group. However, there is little evidence to suggest that pup survival over the babysitting period depends on group size (Clutton-Brock et al. 1998). Although individuals share babysitting duties, there are usually one or two individuals that provide most of the babysitting. Babysitting may result in significant weight loss over the breeding period. Top babysitters average 3.8% loss of body weight over the period of babysitting and second-ranked babysitters lose 0.73% of body weight compared with an average weight gain for other non-lactating group members of 0.26% of body weight (n = 24 litters) (Clutton-Brock et al. 1998). Once the pups have started to move with the group they are still highly vulnerable to predation and unable to feed themselves. Subordinate helpers bring food to pups at this time and train them to hunt for themselves. The breeding female's role may be limited to suckling, although subordinate females have also been observed to allosuckle young without visible signs of pregnancy (Clutton-Brock et al. 1998).

2.4 Study groups

Samples of a small piece of skin from the end of the tail were obtained for genotyping (Chapter 3) from as many individuals as possible in groups under observation at both sites. Animals could be trapped, but in habituated groups it was usually possible to obtain samples without trapping. Some groups left the study area, went extinct or were not habituated successfully and this is reflected in lower numbers of litters sampled from the group (Tables 2.1 and 2.2). Only groups from which the majority of animals had been sampled were used in further analyses described in Chapters 4, 5 and 6.

Group name	**Years studied**	**no. litters sampled**	**no. individuals sampled**
Sandile	1996-8	7	32
Breakaway	1993-8	7	31
Camp	1993	1	5
Delta	1994-5	3	18
Haagners	1993	1	3
Jackson	1993-7	8	48
Kwang	1994	1	3
Lookout	1993-4	3	17
North	1995	1	7
Pan	1994	1	3
South	1993-8	11	56
Tess	1996	2	7

Table 2.1 Summary of information on number of litters born per group and number of individuals sampled per group at Nossob.

Group name	**Years studied**	**no. litters sampled**	**no. individuals sampled**
Avatar	1993-5	5	19
Balrog	1993-5	2	3
Centaur	1993-4	2	10
Drie Doring	1993-8	8	31
Elvira	1996-8	5	23
Frisky	1997-8	3	11
Griffin	1994	1	10
Jungle	1995	1	3
Lazuli	1995-8	8	41
Mixed Pickle	1993-5	6	17
Phantom	1993-8	6	21
Red	1993	1	3
Shifty	1993-4	3	7
Taurus	1994	1	4
Vivian	1995-8	8	33
Youngones	1996-8	9	33
Zion	1995-6	2	29

Table 2.2 Summary of information on number of litters born per group and number of individuals sampled per group at Van Zyls.

CHAPTER 3.

MICROSATELLITE PROFILING OF MEERKATS

3.1 Introduction

The addition of molecular techniques to existing ecological, demographic and behavioural methods has generally revealed that the behavioural dynamics of populations are far more complex than first thought (reviewed in Hughes 1998). Genetic data have overturned long-held hypotheses about monogamy and polygamy, for example in studies on dunnocks (*Prunella modularis*) (Davies et al. 1992), African lions (*Panthero leo*) (Packer et al. 1991) and splendid fairy wrens (*Malurus splendens*) (Brooker et al. 1990). Genetic profiling techniques have also allowed assessment of kinship in studies of social groups. This has greatly increased understanding of phenomena such as ant colony sex ratios (Sundstrom 1994) and cooperative breeding in birds (Stacey & Koenig 1990). In this study, genetic profiling using microsatellite markers was used together with ecological and behavioural information to reveal the role of kinship in determining cooperative behaviour in meerkat groups.

3.2 Choice of genetic marker

Microsatellites are single-locus Mendelian markers comprised of tandem repeat regions, usually up to 50 base pairs long, which are widely dispersed throughout the eukaryotic genome (Tautz 1989, Litt & Luty 1989). Due to slippage events during

DNA replication they show high polymorphism in length, which can be viewed by autoradiography after PCR amplified products have been separated on a sequencing gel. Microsatellites have many advantages over other types of molecular markers such as allozymes and multi-locus DNA fingerprinting (Schlotterer & Pemberton 1994, Queller et al. 1993). For instance, they give good yields of product from small amounts of template due to PCR amplification, amplify reliably, often show high heterozygosity and primers identified in one species will often cross-amplify in other related species. Microsatellites were chosen for genetic analysis of meerkat groups because: (1) Only a very small amount of material (tail tip) was obtained per animal. A PCR-driven genetic typing method was therefore essential;

(2) Samples become available from different members of social groups at different times. Due to exact measurement of allele lengths on sequencing gels microsatellite genotyping allows new samples to be screened and compared with previous samples stored on a database, without re-running previous samples and (3) Microsatellites have codominant Mendelian inheritance patterns making parentage analysis and inference of relatedness relatively straightforward.

3.3 Developing polymorphic loci

3.3.1 Testing microsatellite primers from other carnivore species

Cloning and characterising microsatellite loci from a new study organism is laborious and time-consuming (see later). Since several carnivore studies had already isolated microsatellites, I adopted the strategy of searching among existing carnivore loci for polymorphic meerkat loci. Studies in other vertebrates have shown that sequence homology is maintained sufficiently for a proportion of loci to work across species (Moore et al 1991; Slate et al. 1998). Fifty-six microsatellite primer pairs (Appendix I) were tested (using methodology described in Section 3.4 and Appendix II) from domestic cat (*Felix catta*), domestic dog (*Canis familiaris*), grey seal (*Halichoerus grypus*), elephant seal (*Mirounga leonina*) and European badger (*Meles meles*). 5/56 loci tested amplified microsatellites which were polymorphic. One of the five polymorphic loci was originally cloned in dog (AHT130), 3/5 in cat (Fca45, Fca8, Fca77) and 1/5 in grey seal (Hg8.10). The locus Fca77 was not extensively screened as only 3 alleles amplified, of which two were rare. The locus Fca8 amplified inconsistently and was also excluded from further analysis. The higher success rate with cat primers probably reflects phylogenetic relationships within the carnivora: meerkats are most closely related to true cats (Vrana et al. 1994). Three heterospecific loci (AHT130, Hg8.10 and Fca45) were, therefore, used in further analysis. Information on number of alleles amplified and heterozygosity in meerkats is given in Tables 3.2a and 3.2b.

3.3.2 Cloning loci specific to meerkats

Given the small number of loci cloned in other species of carnivore which were usefully polymorphic in meerkats, I cloned microsatellite loci directly from meerkat DNA. Full details of the protocol used can be found in Appendix II, Section 1.

In summary, genomic meerkat DNA (fragment sizes 500 - 700 base pairs) was cloned into the plasmid pGEM (Promega). The ligation efficiency was tested to ensure >90% incorporation of meerkat DNA. This library was transformed into *E. coli* (strain JM107) and screened with end-labelled $(CA)_{15}$. A secondary screen of positive colonies was carried out, in which each colony that gave a signal in the first round was streaked out repeatedly and re-hybridised. The second hybridisation process maximised the yield of microsatellites without also generating a large number of false positives. A radioactive signal from the majority of these replicate streaks gave assurance that a given colony contained a microsatellite. Based on this technique, 80 positive clones were identified and sequenced on an ABI377 automated sequencer of which 9 resulted in reliably amplifying, polymorphic loci suitable for further analysis (Table 3.1). Loci cloned for this study are denoted by the prefix 'Ssu' (*Suricata suricatta*) and repeat structure and primer sequences are given in Appendix IV.

3.4 Screening method

DNA was extracted from 548 meerkat tail tip samples by standard proteinase K digestion, followed by phenol/chloroform extraction and ethanol precipitation (Sambrook et al. 1989). Microsatellite loci were then amplified by PCR following methodology described in section 2 of Appendix II.

3.5 Description of loci

Table 3.1 summarises information on polymorphism and the proportion of loci and individuals genotyped at each study site.

	Nossob	**Van Zyls**
number of individuals	236	312
number of loci	12	12
mean number of loci typed per individual	9.10	8.73
mean proportion of individuals typed per locus	0.760	0.752
mean number of alleles per locus	10.33	10.42
mean observed heterozygosity	0.749	0.772
mean expected heterozygosity	0.787	0.785

Table 3.1 Summary information for loci and individuals genotyped, for Nossob and Van Zyls study sites.

Only a small proportion of sampled individuals at either site are from the adult breeding population, most are sibling or half sibling pups, subdivided into groups. However, observed levels of heterozygosity across loci were consistent with Hardy-Weinberg expectations. Observed and expected levels of heterozygosity were not significantly different at the Nossob study site ($P = 0.194$, Binomial test) or at Van Zyls ($P= 0.387$, Binomial test). Comparison of observed and expected heterozygosity for each locus (Tables 3.2a and 3.2b) showed no substantial or consistent (i.e. in both sites) deficit of heterozygotes, suggesting that if null alleles exist they do so at low frequency. Furthermore, mis-matches within putative mother-offspring pairs were rare (Chapter 4). Full information on allele frequencies at each locus is given in Appendix III.

LOCUS	No. of individuals typed	No. alleles	H OBS.	H EXP
AHT130	154	10	0.643	0.656
FCA 45	210	9	0.657	0.799
HG8.10	167	13	0.743	0.795
Ssu7.1	214	10	0.836	0.808
Ssu8.5	153	15	0.791	0.857
Ssu10.4	202	14	0.891	0.836
Ssu13.9	129	5	0.729	0.722
Ssu13.8	219	12	0.808	0.819
Ssu14.14	187	8	0.679	0.758
Ssu14.18	147	9	0.633	0.796
Ssu12.1	185	9	0.724	0.779
Ssu11.12	184	10	0.859	0.816

Table 3.2a Number of alleles, observed and expected heterozygosity at each locus at the Nossob study site.

LOCUS	**No. of individuals typed**	**No. alleles**	**H OBS.**	**H EXP**
AHT130	219	11	0.699	0.754
FCA 45	279	12	0.814	0.808
HG8.10	259	14	0.761	0.820
Ssu7.1	278	8	0.871	0.780
Ssu8.5	109	16	0.881	0.898
Ssu10.4	268	12	0.836	0.807
Ssu13.9	234	4	0.667	0.663
Ssu13.8	239	10	0.791	0.779
Ssu14.14	253	9	0.783	0.815
Ssu14.18	194	9	0.655	0.689
Ssu12.1	236	11	0.750	0.815
Ssu11.12	248	9	0.754	0.792

Table 3.2b Number of alleles, observed and expected heterozygosity at each locus at the Van Zyls study site.

3.6 Use of microsatellite genotype data

Microsatellite genotype data was used in two ways to infer relationships between individuals: (1) by inferring parentage of specific pups using the computer program CERVUS 1.0 (Marshall et al. 1998) and building pedigrees from parent-offspring relationships or (2) by measuring relatedness using the extent to which individuals share alleles identical by state, using the program KINSHIP 1.1.2 (Queller & Goodnight 1989). My approach to using these two methods of analysis is described in Chapter 4.

CHAPTER 4

PARENTAGE ANALYSIS AND MEASURING RELATEDNESS

4.1 Introduction

It is widely acknowledged that behavioural observation alone is an unreliable method for inferring genetic relatedness in wild populations of animals (reviewed in Hughes 1998). Microsatellites have become the marker of choice in genetic studies of populations in the wild (see Chapter 3). Depending on the questions being investigated with molecular marker data, such as microsatellites, two main types of analyses may be conducted to investigate genetic relationships between individuals: 1) specific hypotheses concerning the relationship between two individuals may be tested (e.g. that one individual is the offspring of an other). By identifying alleles identical by descent, pedigrees can be constructed from assigned parent-offspring relationships and individuals can be categorised by familial relationship, e.g. full siblings distinguished from half siblings. 2) No specific hypothesis of relationship is tested, and relatedness is measured by the extent to which individuals share alleles identical by state. In this context, relatedness is a continuous variable and average relatedness between multiple individuals or whole populations can be quantified, in the absence of pedigree information.

4.1.1 Parentage Analysis

Paternity (or maternity) analysis uses genetic information to identify the parent of an offspring. A number of candidates are assessed by the proportion of loci at which they share an allele in common with offspring. If this procedure identifies a single candidate male matching at all loci, paternity is assigned to that individual. This is the basic methodology underlying exclusionary methods of paternity inference. Exclusionary methods are a useful starting point in attempts to identify the father of an offspring. However, multiple males which are genetically compatible with an offspring may be found, even when multiple polymorphic loci are used in analysis. Statistical techniques based on likelihood can be used to differentiate the most likely father of an offspring from other non-excluded males (Thompson 1975, 1976 and Meagher 1986). A computer program, CERVUS, which infers paternity using methodology based on likelihood statistical techniques, has recently been developed by Marshall et al. (1998). CERVUS provides a method by which marker data can be used to identify parent-offspring pairs while addressing many problems encountered in parentage analysis in natural populations.

The main advantages that CERVUS has over previous approaches are the function by which confidence levels are assigned to paternities by simulation, the ability to incorporate a typing error rate into the calculation and the ability to take into account the proportion of candidates which are unsampled (Marshall et al. 1998) (see section 4.2.3.2). In the exclusionary approach to paternity inference, a mismatch at a single locus is often taken to be sufficient evidence to reject a candidate parent. This may not be justifiable in many cases given the possibility of lab typing errors, mutation (Queller et al. 1993) and null alleles (Pemberton et al. 1995) resulting in mismatches between offspring and a true parent. With exclusion, the more loci that are typed, and the higher the polymorphism, the greater the chance that at least one locus will cause an erroneous exclusion of the true parent. By allowing for errors, mismatches need not lead to a candidate being excluded. A disadvantage of incorporating errors is that it dampens the effect of allele frequency on the likelihood assigned to parent-offspring mismatches. A candidate parent which shares a rare allele with an offspring will only be considered a little more likely than a candidate parent which shares a common allele with an offspring. In other words, incorporating errors may lead to a loss of statistical power to differentiate candidate parents.

4.1.2 Measuring relatedness

Relatedness (r) is a measure of the proportion of genes identical by descent between individuals, so between a pair of full sibs r = 0.5, for a pair of half sibs r = 0.25 and so on. Measuring relatedness by the extent to which individuals share alleles at molecular marker loci (or band-sharing), can provide information about the relationship between individuals for which no pedigree history is known. However, measures of band-sharing do not provide information about the nature of the relationship between individuals, for example, a parent-offspring pair will be indistinguishable from a full sib pair as both have r values of 0.5. A widely used measure is simply the correlation of the frequency of an allele in a potential actor with that in a potential beneficiary - Wright's correlation coefficient . r is symmetric and varies from -1 to +1 as a statistical correlation. Further subtleties are involved, however, when measuring inclusive fitness with Hamilton's inequality which states that an animal should provide benefit for another individual if rb-c > 0 (Chapter 1). Genetic similarity can be caused by factors besides the sharing of a common ancestor (e.g., if a population is inbred) and it is this similarity and not common ancestry that is often most relevant in evolutionary terms.

Grafen (1991) defines 'the relatedness of a potential actor *A* to the potential recipient *R* [as] the extent to which *A* helping *R* is like *A* helping itself.' In other words, the important measure of genetic similarity when considering the 'r' in Hamilton's inequality, is the genetic similarity between two individuals relative to that between random individuals in the population as a whole. Queller and Goodnight (1989) have proposed a method of estimating Grafen's 'identity by descent' relatedness measure from single-locus genotypic data. Programs RELATEDNESS and KINSHIP have been written which calculate estimates for *R values* using the equation:

$$R = [p_{i(-j)} - P_{(-i)}] / [p_{ij} - P_{(-i)}],$$

where $p_{i(-j)}$ is the frequency of a given allele amongst all individuals in group i other than individual j, p_{ij} is the frequency of the allele in individual j in group i, and $P_{(-i)}$ is an estimate of the population allele frequency,.corrected by excluding the entire group in question from the calculation of the population allele frequency. In order to combine information from several multiallelic loci, the numerator and the denominator are first summed over all alleles, loci, individuals in each group, and groups.

This method has several advantages over other methods as it allows information from multiple loci with multiple alleles to be amalgamated to provide a single estimate. Also, crucial to the study social interactions, estimates can be made for the relatedness between as few as two individuals (Pamilo 1989).

4.2 Methods I: parentage analysis

4.2.1 Group composition at conception

Each meerkat group contained a dominant pair and a variety of other members. Mean group composition at Nossob and Van Zyls at the time of conception is shown in Figure 4.1. Mean group size at Nossob was 10 individuals and at Van Zyls, 7, but groups containing up to 25 individuals have been observed. Apart from the dominant pair, group members can be described as subordinate males, subordinate females, which are sexually mature, and juvenile males and females, less than one year of age, which are not sexually mature.

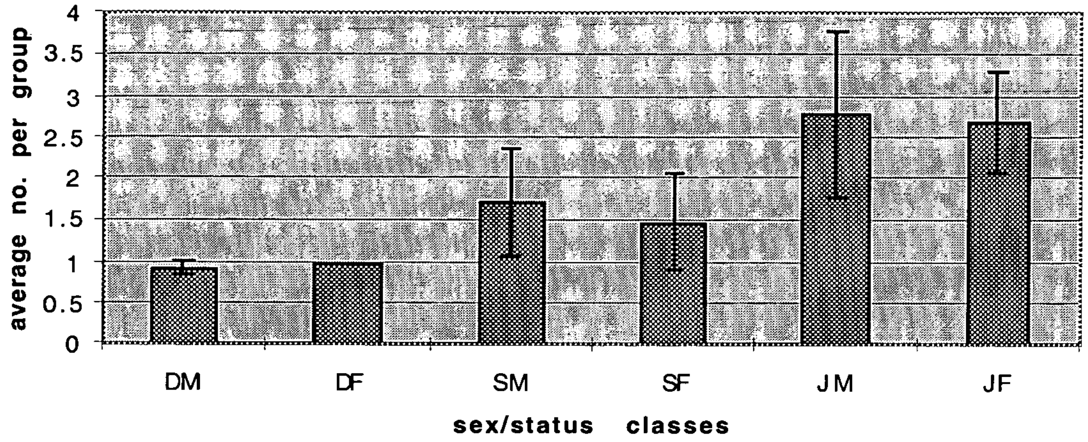

Fig 4.1a Average group composition at Nossob (+/- standard errors). Sex/status classes are abbreviated as follows: DM = dominant male, DF = dominant female, SM = subordinate male, SF = subordinate female, JM = juvenile male (under 1 year of age), JF = juvenile female (under 1 year of age), n = 5 groups.

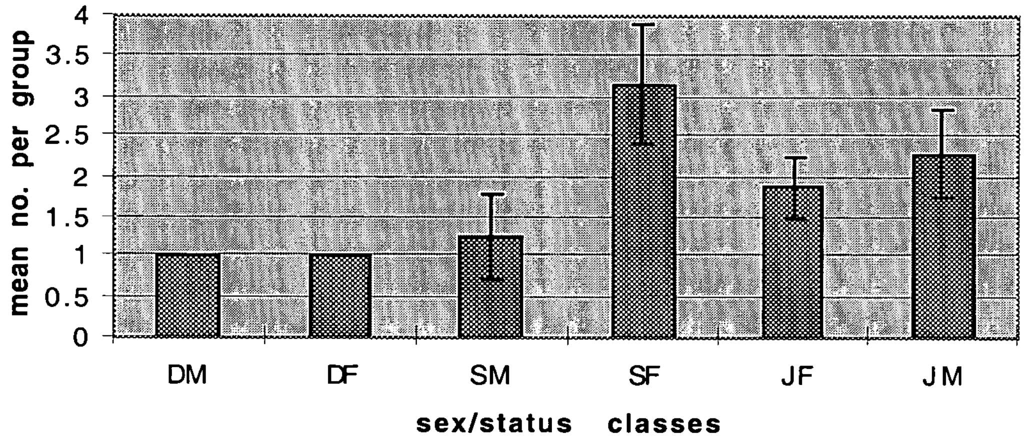

Fig 4.1b Average group composition at Van Zyls (+/- standard errors). Sex/status classes are abbreviated as follows: DM = dominant male, DF = dominant female, SM = subordinate male, SF = subordinate female, JM = juvenile male (under 1 year of age), JF = juvenile female (under 1 year of age), n = 8 groups.

4.2.2 Field cues to parentage

4.2.2.1 Maternity

Pregnancy lasts for 60 days after which time the female gives birth in the burrow (Clutton-Brock et al. in press a). Maternity in meerkats can be detected with some degree of certainty as the abdomens of females are visibly distended in the second half of pregnancy. There is rarely more than one pregnant female observed in a group at one time, making it possible to infer maternity for individual pups. In such cases the pregnant female was treated as the only candidate mother. In a few cases, where groups were not under regular observation in the field, the mother of a litter was uncertain and all mature females in the group were included in a list of candidate mothers.

4.2.2.2 Paternity

Observational estimates of paternity are much harder to make with confidence. Mating takes place underground where it can not be observed, and in contrast to females, there are no outward signs of a successful conception. It is possible for observers to identify the dominant male as the most likely candidate, given the high probability that he has priority of access to females. However, other males in the group cannot be ruled out as candidate fathers and so were included. Furthermore, solitary males, which are rarely trapped and sampled, have been observed to enter the territories of groups where there are oestrous females and so were included in the list of candidate fathers. A potential cost of following habituated groups is that frequency of extra-group paternities may be underestimated. Solitary males may not have come from a habituated group, and may therefore be unwilling to approach habituated groups when human observers are present). Unless these males have been sampled as part of another group, fathers from outside the group in which a litter was born will be unidentified. Extra-group paternity of litters may still be detected, however, if all males present in the group are sampled and have very low (i.e. negative) likelihoods of being the father.

4.2.3 Assigning parentage with microsatellites

Samples were collected from both study sites, Nossob and Van Zyls over a period of 4 years. A total of 66 litters from 9 groups were sampled from Van Zyls and 44 litters from 5 groups from Nossob. In total, parentage analysis was carried out for 220 pups (Van Zyls) and 166 pups (Nossob) from these litters. All sampled individuals were typed at a mean of 9.10 loci at Nossob and 8.73 loci (out of a possible 12) at Van Zyls (Table 3.1). Only those typed at more than 6 loci out of a possible 12 were included in the following analyses. For each pup, parentage was assigned using CERVUS 1.0.

CERVUS (Marshall et al. 1998) assigns parentage by first calculating a log-likelihood ratio (LOD score) for each candidate-offspring pair. Δ, the statistic used, is defined as the difference between the log-likelihood ratio (LOD) scores of the most likely candidate and the second most likely candidate parent of an offspring. Parentage is assigned with a particular level of confidence when the Δ score exceeds a critical value, (critical Δ) derived by simulation. Δ is affected by factors other than the extent to which genotypes match between offspring and putative parents, as it is a difference and not a measure of compatibility. A parent will not be assigned if, for example, there are two or more well-matched candidates. This is because the LOD score for the most likely candidate will not be sufficiently greater than the LOD score for the second most likely candidate. In this case, even when no parent has been assigned, it is probable that the true parent of the litter has been sampled and belongs to one of a few particularly likely candidates.

4.2.3.1 Finding critical Δ - simulations

It is not possible to derive confidence levels for LOD scores analytically (Meagher 1986) and so instead, in CERVUS, confidence is estimated empirically by simulation as described by Marshall et al. (1998). The simulation uses allele frequency data from the actual loci being analysed and several parameters associated with the population under study. The following values were chosen as conservative estimates for each of the parameters and are described in more detail below: number of candidate parents = 10; mean proportion of loci typed = 0.760 (Nossob), 0.752 (Van Zyls); proportion of candidates sampled = 0.6; error rate = 0.02; no. of cycles = 10,000. Population allele frequencies appropriate for each site were used (see Appendix III).

Number of candidate males (set at 10): critical Δ is relatively insensitive to this parameter (Marshall et al. 1998). The number of candidate males was set at values between 1 and 30 males in 1,000 cycles of the simulation to assess the effect on critical Δ. Between values of 10 and 30 the effect on critical Δ was negligible but dropped sharply between 10 and 1. To reduce the frequency of false positives this parameter was set at 10. The number of candidate males for any litter was rarely as high as 10 and is unlikely to exceed this number even when unsampled males are taken into account.

Loci typed (set at 0.760 for Nossob, 0.752 for Van Zyls): the average number of loci typed was calculated directly from the genotype data.

Proportion of males sampled (set at 0.6): the probability that a genetic match represents an actual parent-offspring relationship depends on whether there are other matching males in the population which are unsampled. CERVUS is one of the first paternity inference packages to account for this problem. In the meerkat population, there are an unknown number of roving males in the population which are rarely caught for sampling as they do not frequent the known burrow systems. Also, the size of the study sites is small compared to the distance over which meerkats have been observed to travel, and the meerkat population surrounding the study sites is of an unknown size (Clutton-Brock pers. comm.). Males from outside the study population are less likely to be observed as they are not habituated and will, therefore, be unlikely to approach a group when an observer is present. However, solitary meerkats are likely to suffer significantly higher predation when not living in a group. It may be assumed, therefore, that the number of unsampled candidate males from outside a group is low. An upper boundary for this parameter would be found by measuring the proportion of unsampled candidates within groups. However, this varies widely between groups. All candidates are sampled in some groups at the time of conception of a litter but more often the number of unsampled candidates (even within a group) is unknown. Given the difficulty in estimating a value for this parameter that reflects its true value in nature, several simulations were carried out in order to observe how critical Δ responded to values between 0 and 1. As expected, this parameter had a marked effect on the critical Δ levels and from simulation a proportion of 0.6 was selected as a conservative estimate, to avoid false assignment of parentage to relatives.

Error rate (set at 2%):

The error rate can be estimated from mother-offspring mismatches and it was found to be around 1%. However, mismatches between mothers and offspring are likely to be lower than that in the data set as a whole because it is possible to focus on re-checking of these mismatches, using information from observation in the field (see Chapter 3). The error rate for this analysis was set at 2% as a conservative estimate.

Number of cycles (set at 10,000): In order to obtain a reliable estimate of critical Δ it is necessary to run a sufficiently high number of simulation cycles. 1,000 cycles gave reasonably consistent estimates for testing the effects of different parameters. To ensure accuracy of confidence levels in the actual parentage analysis, however, 10,000 cycles were carried out to obtain critical Δ values.

Allele frequencies (measured from within site): If an offspring and a candidate parent share an allele which is rare in the rest of the population, they will have a higher critical Δ score than an offspring and candidate parent that share a common allele. In the latter case, there is a higher chance that they share the allele by chance, without it actually being identical by descent. Problems can arise, however, in deciding which animals to include in the population from which allele frequencies are measured, both spatially and temporally.

When a population is subdivided spatially, when animals in different groups rarely or never interbreed. Allele frequencies differ between groups so population allele frequencies do not accurately describe the genetic environment in which a particular parentage analysis is carried out. However, an immigrant may have a rare allele in the group in which parentage is being assessed, but common in the population as a whole. Therefore, measuring allele frequencies within groups would artificially reduce the frequencies of alleles from other groups. Luckily, critical Δ is not sensitive to changes in allele frequencies. Given that meerkats migrate between groups with an unknown frequency, it was decided that allele frequencies would be measured across all groups within each site, but that separate simulations would be conducted for each site.

A second, similar problem arises as allele frequencies are dynamic, changing as groups die out or as animals with novel alleles enter the population and become breeders. Therefore, the allele frequencies measured from all animals ever sampled during the study will not accurately represent the allele frequencies found in the population at the time of any given conception. Despite this problem, allele frequencies were estimated from five years worth of samples included together, given the difficulty in accurately measuring allele frequencies at specific time points and small sample sizes of sampled individuals at the time of conception of early litters.

4.2.3.2 Assigning parents

Maternity was analysed first as mothers were usually the easiest to assign. In most cases pregnancy was observed in the field and the mother was classed by observers in the field as 'certain'. Errors may have arisen, however, in sampling and/or labelling, or if there were cryptic pregnancy of other females in the group. To identify such errors, the likelihood that 'certain' mothers were the true mothers of a litter was measured using CERVUS. Mothers were assigned to pups with either 80% or 95% confidence depending on the Δ scores.

Identifying which allele in the pups' genotypes are maternally inherited increases the probability of identifying the father. If a mother has already been identified it is possible to use CERVUS to calculate critical Δ scores for candidate fathers from paternally inherited alleles only. Fathers were assigned to pups with either 80% or 95% confidence depending on the critical Δ scores. The simulation calculates separate Δ scores for parentage analysis with and without genetic data from the opposite-sex parent.

4.2.3.3 Identifying litters with mixed maternity and paternity

When a candidate matches some pups but not others in the same litter there is a possibility of mixed parentage in a litter. Mixed maternity is rarely observed in the field as there is rarely more than one pregnant female in the group at one time. If more than one female was observed to be pregnant simultaneously, both females were considered as candidate mother for all emerging pups in CERVUS maternity analysis.

When a candidate male was assigned to some but not all pups in a litter, this was not taken as sufficient evidence for mixed paternity. If other males are present at the time of conception which are related to the father, they may also be assigned high LOD scores by virtue of the fact that they are related to the father. There is an increased chance that the true father may not always have a LOD score sufficiently higher than the second most likely male to exceed critical Δ. In such circumstances, it is still likely that he is the only father of the whole litter. To avoid overestimating the frequency of mixed paternity, two criteria were used to distinguish cases where paternity was assigned to more than one male correctly, from cases where paternity was assigned to more than one male by chance: (1) when more than one male was found to match one or more of the pups in the litter with >80% confidence, or (2) when a male was found to match some of the pups in a litter with >80% confidence but found to have a negative LOD score for the remaining pups in that litter. (A negative LOD score indicates that a candidate is less likely to be the true father than a randomly-selected male). The second criterion is designed to detect mixed paternity even when only one of the candidates is sampled.

4.3 Results I: Parentage analysis

4.3.1 Simulation results

The number of meerkat parentage tests predicted to be resolved using the program CERVUS was estimated by simulation. Table 4.1 shows the proportion of simulated parentage tests (of 10,000) in which a candidate fulfilled the required criterion (i.e., was awarded parentage). Simulations were carried out for parentage inference with one parent sampled and neither parent sampled with relaxed (80% confidence) and strict (95% confidence) criteria. Critical Δ was derived by simulation separately for the two field sites (Table 4.1).

	one parent known		**neither parent known**	
	80%	**95%**	**80%**	**95%**
NOSSOB				
critical Δ proportion pups assigned	0.85 0.66	2.11 0.42	0.9 0.53	2.01 0.23
VAN ZYLS				
critical Δ proportion pups assigned	0.74 0.69	2.01 0.45	0.93 0.51	1.91 0.25

Table 4.1: Simulation results (n = 10,000) showing critical Δ and the estimated proportion of pups assigned at either 80% or 95% confidence for Nossob and Van Zyls.

4.3.2 Outcome of maternity analysis

166 pups (44 litters) from Nossob and 220 pups (66 litters) from Van Zyls were typed at six or more loci out of a maximum possible 12 and classed as 'typed'. Of the typed pups, 147 (89% of those typed) and 217 (99% of those typed) were analysed for maternity, at Nossob and Van Zyls respectively. Maternity analysis was not carried out in cases where none of the candidate mothers were not classed as 'typed'. Maternity was analysed before paternity for each pup and so in each case, critical Δ was taken from simulations assuming no known parent. A summary of the success rates are shown in Table 4.2 along with success rates predicted by simulation.

	neither parent known	
NOSSOB (n=147 pups)	**80%**	**95%**
observed no. pups assigned % of total pups	116 79%	80 54%
expected no. pups assigned % of total pups	78 53%	34 23%
VAN ZYLS (n=217 pups)		
observed no. pups assigned % of total pups	191 88%	126 58%
expected no. pups assigned % of total pups	111 51%	54 25%

Table 4.2 The results of maternity inference using CERVUS for pups born at Nossob and Van Zyls. The critical values of delta used to assign parentage at different levels of confidence are given in Table 4.1. Maternity was analysed for each pup without paternal genotype data. The number of maternities obtained with 80% and 95% confidence (observed) are listed above the number of maternities predicted from success rates shown in Table 4.1 (expected).

The proportion of pups for which maternity was resolved with 95% confidence was markedly higher than that predicted by simulation despite attempts to obtain conservative critical Δ scores. This is probably due to the fact that the most likely candidate mother can reliably be identified by observation in the field. The average number of candidates per litter was 1.35 at Nossob and 1.42 at Van Zyls. In simulation the number of candidates was set at 10 to obtain a conservative critical Δ. Furthermore, more than 60% of the candidates were sampled.

4.3.3 Outcome of paternity analysis

166 pups (44 litters) from Nossob and 220 pups (66 litters) from Van Zyls were typed at six or more loci out of a maximum possible 12 and classed as 'typed'. Of the typed pups, 160 (96% of those typed) and 213 (97% of those typed) were analysed for paternity, at Nossob and Van Zyls respectively. In those cases where paternity analysis was not carried out, all of the candidate fathers were either unsampled or not 'typed'. Critical Δ was taken from simulations assuming no known parent or one parent already known depending on whether a mother had been assigned previously (Section 4.3.2). A summary of the success rates is shown in Table 4.3 along with success rates predicted by simulation.

	one parent known (n=121)		neither parent known (n=39)	
NOSSOB	80%	95%	80%	95%
observed no. pups assigned % of total pups	105 87%	92 76%	30 77%	24 62%
expected no. pups assigned % of total pups	80 66%	51 42%	21 53%	9 23%
VAN ZYLS		(n=197)		(n=16)
observed no. pups assigned % of total pups	37 63%	86 44%	6 63%	4 25%
expected no. pups assigned % of total pups	47 69%	89 45%	4 51%	4 25%

Table 4.3 The results of paternity inference using CERVUS for pups born at Nossob and Van Zyls. The critical values of Δ used to assign paternity at different levels of confidence are given in Table 4.1. Paternity was analysed for each pup taking into account the maternal genotype data if maternity had been assigned. The number of paternities obtained with 80% and 95% confidence (observed) are listed above the number of maternities predicted from success rates shown in Table 4.1 (expected).

As in the case of the maternity analysis (Table 4.2) success rates in paternity analysis tended to be greater than those predicted by simulation (Table 4.3). However, in the case of paternity analysis this effect is only marked for pups analysed from Nossob. This may be due to the fact that the number of candidate fathers is small relative to that at Van Zyls and there were fewer cases were paternity analysis involved related candidates. The average number of candidate fathers per litter was 1.87 at Nossob and 2.74 at Van Zyls. There is less overlap between territories at Nossob compared with Van Zyls and consequently fewer candidate fathers from outside the group which may not be identified (Brotherton pers. comm.).

4.3.4 Overall success of parentage analysis

4.3.4.1 Success of parentage analysis in terms of pups

There were only a few pups (8% at Nossob, 5% at Van Zyls), for which neither parent was assigned for pups using CERVUS analysis with 80% confidence (Table 4.4). Both mother and father were identified for 60% of pups at Nossob and 53% of pups at Van Zyls.

NOSSOB	both parents assigned	mother only assigned	father only assigned	neither parent assigned
	100	17	35	13
% of total pups analysed	60%	10%	21%	8%
VAN ZYLS				
no. of pups	116	75	17	12
% of total pups analysed	53%	34%	8%	5%

Table 4.4 Summary of number of pups for whom parentage was resolved using CERVUS. The number of pups in each category (depending on how many parents were assigned) is shown together with the percentage of total pups for which analysis was carried out (n=147 and 217 for pups analysed for maternity; n= 160 and 213 for pups analysed for paternity, at Nossob and Van Zyls respectively).

In some cases the only candidate parent was not sampled, this accounts for most of the pups for which a mother was unassigned at Nossob (Table 4.4). Even when parents were sampled there is a proportion of pups for which parentage analysis was performed and mothers and/or fathers were not assigned. Failure to assign parentage occurred either because all candidates in the group were excluded, or because no single candidate was sufficiently more likely to be the true parent than all other candidate parents. In the former case, when a paternity was not assigned and all adult males in the group have been typed, it was assumed that the father was from outside the group. When a number of candidate parents within the group matched well, but the Δ score for the most likely candidate did not exceed critical Δ, it was more likely that the parent came from among the candidate parents within the group.

4.3.4.2 Success of parentage analysis in terms of litters

Pups for which maternity and/or paternity were not assigned were distributed evenly between litters. Parentage was assigned for at least one pup in every litter sampled (Table 4.5).

	maternity and paternity resolved	maternity partially resolved	paternity partially resolved
NOSSOB	n=35 litters		
no. litters % of litters	10 29%	19 54%	21 60%
VAN ZYLS	n=61 litters		
no. litters % of litters	11 18%	25 46%	51 84%

Table 4.5 The number and percentage of litters for which maternity and paternity have been resolved by CERVUS analysis separately at each study site. (Not included in this analysis are 9 additional Nossob litters and 5 additional Van Zyls litters from which not all littermates were typed.) See section 4.2.3.4 for criteria used to infer mixed maternity and paternity.

4.3.5 Mixed maternity and paternity

A proportion of litters were confirmed to have mixed maternity or paternity by CERVUS analysis using the criteria described above (Table 4.6).

	mixed maternity	**mixed paternity**
NOSSOB	n=35 litters	
no. of litters % of litters	0 0%	2 6%
VAN ZYLS	n=61 litters	
no. of litters % of litters	4 7%	14 23%

Table 4.6 Number and percentage of litters for which mixed maternity and paternity was assigned by CERVUS analysis. (Not included in this analysis are 9 additional Nossob litters and 5 additional Van Zyls litters from which not all littermates were typed.) See section 4.2.3.4 for criteria used to infer mixed maternity and paternity.

No confirmed instance of mixed maternity was observed in litters from Nossob although it was shown to occur with low frequency at Van Zyls. Mixed paternity occurred in almost 1 in every 4 litters sampled from Van Zyls. For eleven of the litters with mixed paternity the status of both fathers was known. In 6/11 litters the dominant shared paternity with a subordinate, in 3/11 litters the dominant shared paternity with a male from outside the group and in 2/11 litters paternity was shared between two subordinates. All 4 litters at Van Zyls with mixed maternity involved the dominant and a subordinate.

4.4 Methods II: measuring relatedness

Relatedness data was generated in the form of matrices constructed for each group using the computer program KINSHIP 1.1.2 (Queller & Goodnight 1989). Matrices contained all individuals ever born in a group, seen with a group, or known to have been in contact with a group from parentage analysis (sections 4.2 and 4.3). The latter were usually males from neighbouring groups which had been assigned paternity of pups in a matrix. KINSHIP makes no specifications about the number of loci required to give an accurate relatedness measure. Indeed it is possible to generate an estimate from comparison of genotypes at one locus in a pair. For the purposes of this study all individuals which had been scored at fewer than 6 loci were excluded from calculations as in CERVUS analysis.

The relatedness calculation corrects for bias in allele frequency towards that found in the group in which relatedness is being measured. To do this, KINSHIP calculates Jackknife statistics by dropping out one group at a time and performing repeat calculations on the reduced data set (Queller & Goodnight 1989). This presented a problem when analysing the meerkat data because both males and females were sometimes involved in more than one group over their lifetime. Each individual could only be included once in every run of the calculations. In order to include these individuals in the matrices for every group to which they ever belonged, separate runs of the relatedness calculations were carried out for each group so that multiple group membership could be entered for a single individual.

The r values derived from KINSHIP were compared with those expected from parentage analysis using CERVUS as follows: The relationships between members of each group were categorised by CERVUS into mother-offspring, father-offspring, full sibs, half sibs or unrelated. The relatedness coefficients calculated by KINSHIP for each category were then averaged over groups for comparison. Care was taken to maximise sample sizes whilst avoiding pseudoreplicating pairs of relatives. To achieve this, the average relatedness between a mother and offspring was calculated using a single mother as a data point. Relatedness between each mother and all pups which had been attributed to her, were averaged to obtain each mother-offspring data point. The same was done to calculate average relatedness between male parents and offspring. Relatedness between full sibs was measured by taking an average over all litters for which a single mother and father had been assigned parentage by CERVUS. Each data point was obtained by taking the average relatedness between littermates. Sample sizes of mixed litters were too small to take means in litters to calculate average relatedness between half sibs. Also, in the case of mixed paternity shared by related males it was often unclear which pups belonged to each male parent and, therefore, which littermates were full sibs and which were half sibs. In order to measure the average relatedness between half sibs, therefore, an average was taken between all half sibs in a group which were born in different litters, i.e. which shared the parent of one sex but had different parents of the other sex. Relatedness between non-relatives was calculated for individuals within groups not identified as related by CERVUS, and averaged across groups

4.5 Results II: measuring relatedness

Relatedness coefficients derived from the analysis using KINSHIP were in broad agreement with those predicted from CERVUS analysis. The extent to which KINSHIP and CERVUS gave consistent results depended on the category of relationship within which the two methods were compared (Table 4.7). r values between offspring and parents of both sexes were significantly lower using KINSHIP than the 0.5 predicted from CERVUS analysis. This pattern was more marked in father-offspring relationships than mother-offspring relationships. Relatedness between full sib littermates was not significantly different from the 0.5 predicted by CERVUS analysis.

Half sibs were assigned r values significantly lower than 0.25, however. Thus, KINSHIP did discriminate successfully between full sibs and half sibs, average r values for both categories were significantly different from one another. Non-relatives were predicted to have a relatedness value of 0. KINSHIP measures relatedness based on 0 being the average relatedness between a pair of random individuals in the population from which allele frequencies are measured. Pairs which were inferred to be non-relatives by CERVUS had relatedness values not significantly different from that between a random pair in the population.

presumed relationship from CERVUS analysis	**expected r (CERVUS)**	**n**	**observed r (KINSHIP)**	**t**	**d.f.**	**significance**
mother-offspring	0.5	26	0.44	4.39	25	P<0.01
father-offspring	0.5	19	0.42	4.56	18	P<0.01
full sibling	0.5	34	0.49	0.22	33	NS
half sibling	0.25	5	0.22	3.71	4	P<0.05
unrelated	0	6	-0.01	0.55	5	NS

Table 4.6 Results of t-tests comparing relatedness measurements using KINSHIP with expected relationship inferred from CERVUS analysis. Mean relatedness coefficients (r) calculated by KINSHIP are shown for each category of relationship. For mother-offspring and father-offspring measurements n = number of parents, full sibs n = number of unmixed litters, half sibs n = number of groups, non-relatives n = number of groups.

4.6 Discussion

4.6.1 Parentage analysis

Analysing microsatellite data with CERVUS in order to assign parentage, proved a successful method for identifying which candidates most likely to be true parents. Of all pups analysed for parentage at Nossob, 92% were assigned maternity, paternity or both, while at Van Zyls 95% of pups were assigned maternity, paternity or both (Table 4.4). In some cases, the most likely parent had mismatches with a pup and would not have been assigned using exclusionary methods of parentage assignment. High success rates in finding the most likely parent of an offspring, however, does not necessarily imply a high success rate at finding the *true* parent of an offspring. The only way to test the accuracy of parentage assignment is to measure the frequency of erroneous assignments of parentage where the true parent (usually the mother) is known for certain from observation. This is an effective method of testing accuracy of parentage analysis in cases where a mother can be observed to give birth to and closely associate with offspring, for example in red deer. In meerkats, however, females give birth underground and all group members associate thereafter with the litter produced. It is possible to use observation of a single pregnant female in a group as a cue to identify a 'certain' mother (Section 4.2.2.1) although this may not be a completely reliable cue.

In every case where a female had been observed to be pregnant in the field, LOD scores calculated by CERVUS analysis identified her as the most-likely mother, even when she had a few mismatches with pups in the litter. This suggests that the number of erroneous assignments of maternity by CERVUS was low. Errors would be expected to occur with higher frequency, however, in paternity assignment. There was generally less chance of having sampled the true father, given the possibility that the true father came from outside the group and a greater number of candidates to compare on average. CERVUS was least successful when resolving paternity between relatives. This was a problem particularly when resolving paternity between three full sib brothers which all bred as immigrants in three groups at Van Zyls.

4.6.2 Relatedness

KINSHIP r values successfully distinguished categories of relatedness which had been inferred by CERVUS analysis. For instance, half sibs had a significantly lower average r-value than that between full sibs. Measurements of relatedness between non-relatives were not significantly different from 0, the relatedness between two individuals chosen at random from the population (Table 4.7). However, r values measured by KINSHIP were sometimes significantly different from r values predicted by CERVUS pedigrees. Whether a significant difference was found or not depended on which category was being compared (Table 4.7). There was no significant difference between r-vales calculated between full sibs and 0.5, but in all other categories compared - mother-offspring, father-offspring and half sibs - there was a significant downward bias in r values calculated by KINSHIP.

These results do not necessarily suggest that KINSHIP is calculating r values incorrectly. It is more likely that CERVUS has assigned parentage incorrectly in a proportion of cases. Parentage assignments by CERVUS are expected to be a mixed group of which 80% or 95% are correctly assigned (mean $r = 0.5$), and a remaining

20% and 5% which are incorrectly assigned (mean r = 0, or >0, if false assignments tend to be to relatives). This explanation is supported by the fact that relatedness between littermates was not significantly different from 0.5. This category alone does not depend closely on correct assignment of parentage as littermates are still likely to be full sibs even when one parent has been assigned incorrectly. Furthermore, the average relatedness between fathers and offspring was a little lower than the average between mothers and offspring (Table 4.7) suggesting that fathers are assigned incorrectly more often than mothers.

CHAPTER 5.

DISTRIBUTION OF REPRODUCTION AMONG GROUP MEMBERS

5.1 Introduction

Groups of cooperative breeders are commonly despotic, with some individuals, usually a male and a female, being dominant over other group members. A dominant individual is characterised by its ability to out-compete subordinates for resources, such as food and reproductive opportunities. Subordinates are thought to receive limited opportunities to reproduce, but often invest most in the production of the dominants' offspring. In meerkats, subordinates carry out practically all of the behaviours associated with rearing offspring in a group. To understand why subordinate meerkats help raise offspring it is crucial to understand the fitness consequences of doing so. Fitness from helping could be acquired in three ways (1) directly through breeding, (2) indirectly by helping to enhance the reproductive success of kin and (3) through enhancement of future reproductive success. This chapter investigates the first of these mechanisms, direct fitness benefits, by establishing how reproduction is distributed among members of meerkat groups. If subordinates do achieve a substantial amount of reproduction then they will gain direct fitness benefits by helping raise their own offspring. Social status in cooperative breeders has been shown to reliably predict the amount of direct fitness an individual acquires in mammals (Creel & Waser 1991), birds (Brown 1987) and insects (Queller & Strassmann 1989). Despite the fact that dominants appear to be monopolising reproduction it may be possible that subordinates, particularly males, help because they are obtaining occasional opportunities to mate. For instance, in dunnocks (*Prunella modularis*), females mate sneakily with a second male in order to elicit help from him when her clutch is born (Davies 1992). In cooperatively breeding stripe-backed wrens (*Campylorynchus nuchalis*), subordinate males were thought to be non-reproductive but were revealed to sometimes share paternity with the dominant by genetic analysis of parentage (Rabenold et al. 1990). The following chapter will attempt to establish whether helping enhances the reproductive success of kin or enhances future reproductive success.

5.1.2 Aims

The general aim of this chapter is to determine how reproduction is distributed among members of meerkat groups in two separate populations (Nossob and Van Zyls). The first specific aim is to determine how reproduction is divided between the dominant pair and subordinates in a group. Establishing how direct fitness is distributed among group members will determine which group members benefit from investment in rearing offspring.

The second specific aim is to determine the effect of relatedness to opposite sex breeder on breeding success. Relatedness to the opposite sex breeder present in a group during a breeding period (at the conception of a litter) will be compared for breeders and non-breeders. This will test the prediction, made by all skew models, that unrelated subordinates will be more likely to breed than subordinates who are related to dominant breeders.

5.2 Methods

5.2.1 Effect of dominance on reproductive success

To measure the extent of reproductive skew, breeders and non breeders in each group (n=6 groups at Nossob, n=10 groups at Van Zyls) were identified using CERVUS as described in Chapter 4. Not all litters for which genetic data had been compiled were included in the analysis: 34/44 were used from Nossob and 55/66 litters at Van Zyls. Litters were omitted from the analysis in cases where data on dominance status was poor or when few animals in the group at the time had been sampled or genotyped. The dominance status of each individual present in a group at the time of birth of the selected litters was established in the field through observation of behavioural cues such as displacement, grooming and marking behaviours indicative of dominance status (Chapter 2). Each pup for which parentage could be assigned was then placed into one of the following categories: offspring of dominant male, offspring of dominant female, offspring of subordinate male, offspring of subordinate female or offspring of outside male (a male from outside the group into which the litter was subsequently born).

Proportion data, such as proportion of pups sired by dominants, usually have non-normally distributed error variance and unequal sample sizes. To avoid these problems while retaining maximum power, all proportion data were analysed with a general linear model analysis of deviance, assuming binomial errors, and a logit link function in the GLIM statistical package (Crawley 1993). Importantly, this form of analysis weights each data point according to its sample size (e.g. number of pups in the group) and so controls for the fact that different numbers of pups were sampled from different groups, and that the error variance is greater with small samples. In addition, because it takes into account variation in the error variance (due to mean and sample size) it gives asymmetric standard error estimates. Significance testing was carried out with χ^2-tests.

5.2.2 Effect of dispersal strategy on reproductive success

Relatedness between dominants and subordinates was first inferred from dispersal strategies of group members. Individuals may come to be group members in different ways, they may be founding members - having originally established the group in a territory of its own, they may be natal to a group - having been born into it, or they may have immigrated into it from another group. Individuals were categorised, therefore, as founders, natals, immigrants or unknown, in the absence of information. Estimating relatedness from dispersal strategies has the advantage of using the same information available to the meerkats themselves and the information they may use to choose mating partners that are unlikely to be close kin. However, this assumes that meerkats have no kin recognition mechanism beyond being able to identify familiar individuals. To investigate the effect of dispersal on the probability of attaining dominance, the numbers of dominant males and females which were founders, natal or immigrant to the group in which they bred was compared.

5.2.3 Effect of relatedness to the opposite sex breeder on reproductive success

As well as investigating patterns of dispersal associated with breeding success the effect of relatedness to the opposite sex breeder during a breeding period was measured directly from microsatellite data. The 'opposite sex breeder' was identified as any individual of the opposite sex responsible for a breeding attempt, and so could include subordinates and individuals from outside the group as well as dominants. In investigating dispersal strategies (above) immigrants were assumed to be unrelated to the group into which they immigrate. This may be unjustified, however, given the fact that males are known to move between and breed in more than one group. Pedigree construction using parentage analysis by CERVUS (Chapter 4) may not be successful in identifying common ancestors of animals in different groups. Relatedness was measured using the program KINSHIP based on allele sharing as described in Chapter 4, as an alternative to establishing the pedigree.

Relatedness to the opposite sex breeder was compared between successful and unsuccessful breeders, whether dominant or subordinate. Sufficient information on age and group membership at the time of conception of litters was only available for groups at Van Zyls so the results presented are from that site only. For 46 litters information on adults present at birth and conception was compiled. This was used to identify the opposite sex breeder present in a group during a breeding period for each adult (n=96). Each adult was categorised according to sex, dominance status and breeding status for each litter. When the same individual was present in a group during the birth of more than one litter, an average relatedness to opposite sex breeders was calculated to avoid pseudoreplication.

5.3 Results

5.3.1 How does dominance affect reproductive success?

5.3.1.1 Parentage of pups

Reproductive success was strongly dependent on dominance (Figure 5.1). The mean and standard error in number of pups assigned to each dominance category was measured across groups (n = 6 groups at Nossob, n = 10 groups at Van Zyls). The dominant female was estimated to be the mother of all 111 pups for which maternity had been assigned at Nossob, and of 92.44 +/- 3.48% (mean +/- standard error) of 176 pups at Van Zyls. Dominant males were estimated to be the father of 84.35 +/- 12.53% of 115 pups for which paternity was assigned at Nossob, and 62.09 +/- 6.18% of 153 pups at Van Zyls.

Subordinate males were more successful than subordinate females in both sites. Reproductive skew was significantly higher among females than males at Nossob (χ^2=21.73, 1d.f., P<0.001) and at Van Zyls (χ^2=29.43, 1d.f., P<0.001). At least 2.61 +/- 0.02% (Nossob) and 17.00 +/- 6.79% (Van Zyls) of paternities were assigned to males which did not belong to the group in which the litter was born (Figure 5.1). The number of paternities assigned to males from outside the group was significantly higher at Van Zyls than at Nossob ($\chi^2 = 19.32$, 1d.f., $P<0.001$).

The level of reproductive skew differed between sites with dominants of both sexes monopolising reproduction to a greater extent at Nossob (males: $\chi^2=18.16$, 1 d.f., $P<0.001$; females: $\chi^2=19.52$, 1 d.f., $P<0.001$).

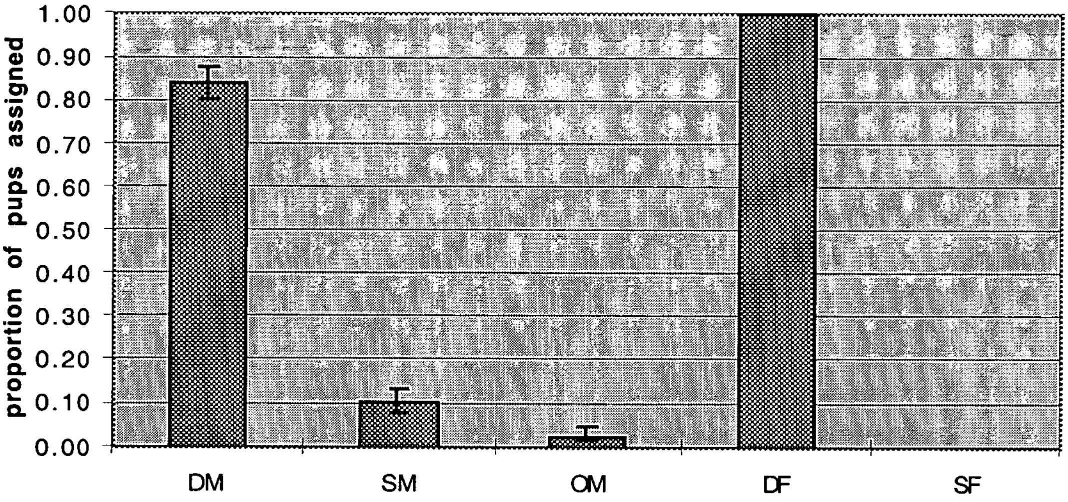

Figure 5.1a Mean proportion (+/- standard error) of maternity and paternity assigned to different status classes at Nossob. Abbreviations as follows: DM = dominant male, DF = dominant female, SM = subordinate male, SF = subordinate female, OM = outsider male (male from outside group in which litter was born), n = 6 groups (34 litters, 130 pups).

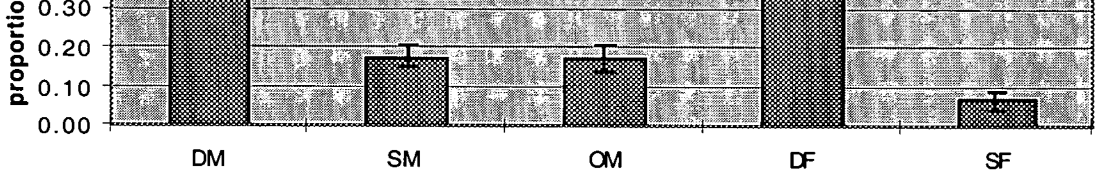

Figure 5.1b Mean proportion (+/- standard error) of maternity and paternity assigned to different status classes at Van Zyls. Abbreviations are as follows: DM = dominant male, DF = dominant female, SM = subordinate male, SF = subordinate female, OM = outsider male (male from outside group in which litter was born), n = 10 groups (55 litters, 199 pups).

5.3.1.2 Parentage of litters

Only the dominant pair bred in 86% (25/29) of Nossob litters where both parents were identified and of known dominance status (Table 5.1a). All Nossob litters were born to the dominant female and the dominant male bred in 90% (26/29) of litters. In 90% (26/29) of the dominant female's litters, the dominant male fathered at least some of the pups born.

Dominants bred in a smaller proportion of litters at Van Zyls than at Nossob and there was a greater proportion of litters with mixed paternity (7% (2/29) at Nossob, compared with 24% (12/46) at Van Zyls). In 54% (25/46) of Nossob litters where both parents were identified and of known dominance status, only the dominant pair bred (Table 5.1b). The dominant female bred in 87% (40/46) of all litters at Van Zyls (mixed and unmixed) while the dominant male bred in 74% (34/46) of litters. In 78% (31/40) of litters in which the dominant female bred, the dominant male fathered at least some of the pups born. Of litters in which the dominant male bred, the dominant female was assigned maternity in 91% (31/34) of cases. In litters where subordinate males bred the dominant female was almost always the mother (92% of litters (12/13). 50% (3/6) of litters in which subordinate females bred were fathered by the dominant male.

	status of mother	status of father(s)	N	prop. of unmixed n = 27	prop. of mixed n = 2	prop. of total n = 29
unmixed litters n = 27	D	D	25	0.93		0.86
	S	D	0	0		0
	D	S	2	0.07		0.07
	S	S	0	0		0
	D	O	0	0		0
	S	O	0	0		0
mixed litters n=2	D	D/S	1	0.04	0.50	0.03
	D	D/0	0	0	0	0
	D	S/S	0	0	0	0
	D	S/O	1	0.04	0.50	0.03
	S	D/S	0	0	0	0
	S	D/0	0	(	0	0
	S	S/S	0	0	0	0
	S	S/O	0	0	0	0

Table 5.1a Dominance status of both sexes of parents of 29 Nossob litters as a proportion of unmixed, mixed and total number of litters analysed. D = dominant, S = subordinate, O = male from outside group in which the litter was born.

	status of mothers	status of fathers	N	prop. of unmixed n = 35	prop. of mixed n=11	prop. of total n=46
unmixed litters n = 35	D	D	25	0.71		0.54
	S	D	1	0.03		0.02
	D	S	6	0.17		0.13
	S	S	0	0		0
	D	O	0	0		0
	S	O	3	0.09		0.07
mixed litters n = 11	D	D/S	4	0.11	0.36	0.09
	D	D/0	2	0.06	0.18	0.04
	D	S/S	2	0.06	0.18	0.04
	D	S/O	1	0.03	0.09	0.02
	S	D/S	1	0.03	0.09	0.02
	S	D/0	1	0.03	0.09	0.02
	S	S/S	0	0	0	0
	S	S/O	0	0	0	0

Table 5.1b Dominance status of both sexes of parents of 46 Van Zyls litters as a proportion of unmixed, mixed and total number of litters analysed. D = dominant, S = subordinate, O = male from outside group in which the litter was born.

5.3.2 How do dispersal strategies affect reproductive success?

Unfortunately, there is currently limited information on the immigration status and age of many of the candidates analysed. Many of the litters included in the analysis were born at a time when the natal group had not been followed for long enough to know the history of its adult members. Despite small sample sizes patterns have emerged as summarised in Tables 5.2a and 5.2b. (1) There were no known cases, at either site, of males breeding in their natal groups. (2) Of males which bred, all did so either in groups into which they immigrated or, at Van Zyls, which they had founded. The absence of cases of males breeding in a group which they had founded at Nossob is probably due to the fact that only one group was observed from the time of formation: At Nossob 11 out of 18 male breeders had an unknown origin in the group in which they bred. (3) Unlike males, females did breed in their natal groups (26% at Nossob (n =61) and 28% (n=60) at Van Zyls). Only two instances of females immigrating into an established group have been observed, both at Nossob and in neither case did the female go on to breed. (4) Females which managed to found new groups stood a relatively high chance of breeding, relative to females who remained in natal groups. Of the few cases identified, 1/2 (50%) bred at Nossob and 7/10 (70%) bred at Van Zyls. The difference between sexes was statistically significant - a higher proportion of males bred as immigrants than females at Nossob ($\chi^2 = 30.55$, 1 d.f., $P<0.001$) and at Van Zyls ($\chi^2 = 31.34$, 1 d.f., $P<0.001$).

	founder	natal	immigrant	unknown
female breeders	1	16	0	8
female non-breeders	1	45	2	3
male breeders	0	0	8	11
male non-breeders	0	43	0	2

Table 5.2a Numbers of adult individuals categorised according to dispersal strategies (founder = established group in new territory, natal = residing in group of birth, immigrant = immigrated into group from another group, unknown = origin in group unknown) and breeding status (breeders = adults for which parentage was assigned by CERVUS analysis) (Nossob, n = 76 females, n = 64 males).

	founder	natal	immigrant	unknown
female breeders	7	17	0	8
female non-breeders	3	43	0	4
male breeders	6	0	8	2
male non-breeders	1	67	4	1

Table 5.2b Numbers of adult individuals categorised according to dispersal strategies (founder = established group in new territory, natal = residing in group of birth, immigrant = immigrated into group from another group, unknown = origin in group unknown) and breeding status (breeders = adults for which parentage was assigned by CERVUS analysis) (Van Zyls, n = 102 females, n = 89 males).

Dispersal strategies appear to have important implications for the probability of attaining dominance for males and females (Table 5.3). Only information for individuals from Van Zyls is currently available. The same patterns hold in a larger data set which also includes individuals for which there is no genetic data available (Clutton-Brock et al. in press, d).

	founder	natal	immigrant	unknown
Dom. Female	4	3	0	8
Dom. Male	5	0	6	2

Table 5.3 Dispersal status (founder = established group in new territory, natal = residing in group of birth, immigrant = immigrated into group from another group, unknown = origin in group unknown) of all dominants in breeding groups where parentage analysed at Van Zyls. Males, n= 9, Females, n=10.

In all known cases, males attained dominance outside their natal group, either by immigrating into established groups or by forming a new group with females. Females, on the other hand, have never been observed to immigrate successfully into an established group at Van Zyls. (The two known cases of females immigrating into an established group occurred at Nossob where neither bred.) For females, there is insufficient data to draw conclusions about the relative probability of attaining dominance either in a natal group or in a newly founded group. It is also difficult to obtain reliable information on the success rate of females attempting to found new groups as once females disappear from a group it is hard to keep track of their movements.

5.3.3 How does relatedness to opposite sex breeder affect reproductive success?

With the exception of non-breeding males, all categories had low relatedness to the opposite sex breeder on average (not significantly different from 0, P>0.05) (Table 5.4). Dominant females were always unrelated to the opposite sex breeder (range = -0.25 to 0.151): in 3 cases where the dominant female didn't breed the opposite sex breeder was a male with which she had bred previously and/or subsequently. In contrast, relatedness to the opposite sex breeder was occasionally high in other categories (range in subordinate females = -0.10 to 0.43, dominant males = -0.39 to 0.58, subordinate males = -0.17 to 0.75).

	dominance status	breeding status	n	mean relatedness to OSB	standard error
FEMALES	dominant	breeder	12	-0.018	0.041
		non-breeder	3	-0.033	0.055
	subordinate	breeder	6	0.102	0.071
		non-breeder	30	0.106	0.208
MALES	dominant	breeder	7	-0.039	0.048
		non-breeder	9	0.037	0.112
	subordinate	breeder	5	0.001	0.080
		non-breeder	25	0.356*	0.054

Table 5.4 Mean and standard error of relatedness to opposite sex breeder (OSB) present in group during a breeding period (46 litters) for 96 adults at Van Zyls with dominance status and breeding status. (* significantly different from 0, d.f. = 24, $P<0.01$)

Relatedness to the opposite sex breeder was compared within each sex and dominance class between breeders and non-breeders by ANOVA (Table 5.5). There was no significant difference between dominants which bred and dominants which did not breed in relatedness to opposite sex breeders ($F_{(1,12)}=0.38$, $P>0.05$ in females and $F_{(1.14)}=0.25$, $P>0.05$ in males). Given that dominants were almost always unrelated to the opposite sex breeder this was an expected result (Table 5.4). The availability of unrelated opposite sex breeders had no effect on the probability of reproductive success for subordinate females ($F_{(1,34)}=0.0002$, $P>0.05$). In 4/6 litters born to subordinate females, a male from outside the group was assigned paternity or all males in the group were excluded. Relatedness to male breeders was low in cases where subordinate females bred (Table 5.4) but also low in some cases where they did not breed.

In contrast, subordinate male breeders were significantly less related to the female breeder than non-breeders ($F_{(1,27)}$ =9.73, P<0.01). This difference between the sexes in subordinate breeding was not due to greater availability of unrelated opposite sex breeders for males. In fact, on average, subordinate females were significantly less related to opposite sex breeders than were subordinate males ($F_{(1,63)}$ =12.31, P<0.01).

dominance/sex class	**n**	**F**	**P-value**
dom. females	14	0.38	>0.05
sub. females	36	0.0002	>0.05
dom. males	18	0.25	>0.05
sub. males	29	9.73	<0.01

Table 5.5 Results of ANOVA to test for a difference in the relatedness to the opposite sex breeder of individuals who bred and did not breed for different sex/dominance categories.

5.4 Discussion

5.4.1 Distribution of direct fitness between dominants and subordinates

Reproductive success was shown to be strongly dependent on dominance, with the dominant pair monopolising reproduction in 86% (25/29) of litters at Nossob and 54% (25/46) of litters at Van Zyls (Tables 5.1 a and 5.1b).

Reproductive skew is significantly higher in females than in males. Among Nossob females, for instance, only the dominant female has been observed to breed successfully. Subordinate males may be more successful than subordinate females for several reasons. In order to breed successfully, subordinate females are required to mate, gestate, give birth and raise young in the same territory as the dominant, avoiding aggressive interference (Clutton -Brock et al., in press a). Males only have to mate, and they can do this either in their natal territory or in neighbouring territories. Furthermore, males need not gain sole access to females as males can share the paternity of a single litter. There are, therefore, more opportunities for dominants to suppress female reproduction than male reproduction.

The difference in reproductive skew between Nossob and Van Zyls provides a clue as to what factors are important for the breeding success of subordinates. At Nossob, where reproductive skew in favour of the dominant pair is higher, groups are widely spaced and risk of predation is higher (Chapter 2, Clutton-Brock et al., in press d). Group composition in terms of dispersal strategy is comparable between sites (Tables 5.2a and 5.2b) although the frequency of matings with roving males is lower at Nossob than at Van Zyls, suggesting that there are fewer roving, solitary males in the Nossob population. Nonetheless with only two sites for comparison, it is not possible to draw firm conclusions.

5.4.2 Dispersal strategies

Dispersal strategies appear to be important in predicting the probability that an individual will attain dominance and hence the main breeder status. Sample sizes of dominants with known histories are small, but results suggest that successful males and females adopt different strategies to maximise fitness (Table 4.6). Males have rarely been observed to attain dominance in their natal group (Clutton-Brock et al., in press c) whereas females have been observed to become dominant in their natal territory on the death of the dominant or by leaving their natal group and founding a new territory (Table 5.3).

The patterns that emerge from the small sample size of observations suggest that dispersal strategies are also important in determining breeding success of subordinates. Males, the dispersing sex, *only* achieve reproductive success as subordinates after dispersing from the natal group (Table 5.2a and 5.2b). Females never bred while subordinate at Nossob, while subordinate females at Van Zyls did so only in natal groups or those they had co-founded (Tables 5.2 a, 5.2b, 5.3).

Observation suggests that although males and possibly females increase direct fitness by leaving their natal groups, males do so willingly while females are evicted by the dominant female despite their efforts to return to their natal territory (Clutton -Brock et al., in press a). This would be expected given that males are able to increase their direct fitness without founding a new territory. Females must establish a territory and be joined by others in order to breed successfully, and while solitary, stand a high chance of being predated or being attacked by other meerkats. By leaving their natal group, subordinates of both sexes may also lose indirect fitness benefits from helping raise kin, but while females may breed eventually without dispersing, males must disperse and so costs of losing indirect fitness may be outweighed for males.

5.4.3 Effect of relatedness

Variation in relatedness to opposite sex breeder, generated by male-biased dispersal between groups, appears to affect the probability of breeding both as a dominant and as a subordinate.

All dominant male breeders were immigrants or founders, none that were included in the genetic analysis were known to have inherited dominant breeding position in their natal groups (although this has been observed twice out of a larger sample set in the field (Clutton-Brock et al., in press c)). Dominant female breeders were founders or inherited breeding position in their natal group. Consistent with these observations, relatedness between dominant breeders in a pair was universally low (Table 5.4). The few cases in which a dominant was not the parent of a litter were not associated with high relatedness between the dominant pair (Table 5.5) and all involved pairs which did breed together previously or subsequently.

Subordinate males never bred in their natal groups. Consistent with this, subordinate male breeding was associated with low relatedness to the dominant breeding female in the group. Only six cases of subordinate female breeding were observed. Subordinate females were likely to be in their natal group and this possibly related to the dominant breeding male. In this small sample of cases, subordinate female breeding was not associated with variation in relatedness to the dominant breeding male, but interestingly, in four out of six litters, a male from outside the group was assigned paternity, or all males in the group were excluded as the father, implying inbreeding was avoided in those cases.

These results show that direct fitness does not provide an incentive to remain and help in the natal group for males or females. This is in contrast to another cooperatively breeding species - the stripe-backed wren, in which subordinate males share paternity with the dominant male in their natal group (Rabenold 1990). Subordinate male meerkats do obtain direct fitness from helping in groups into which they have immigrated. Subordinate females rarely breed, but when they do, may do so with unrelated males. This suggests that, unlike males, females are reproductively constrained mainly through suppression by the dominant female, in keeping with behavioural observation (Clutton -Brock et al., in press a). By 'tolerating' suppression, however, subordinate females may increase their chance of breeding in the future by inheriting dominance

5.4.4 Skew models

The results presented in this chapter suggest that the mechanisms which determine the breeding success of subordinates differs between sexes. Both sexes preferentially mate with unrelated individuals and their reproductive success is, therefore, determined to an extent by inbreeding avoidance. However, subordinate males are able to take advantage of the availability of unrelated mates whereas subordinate females are not, suggesting that suppression is more important for females than males as predicted in conflict models of reproductive skew (Clutton -Brock 1998).

All models predict patterns of reproductive skew in the direction observed in males (Figure 2). Unrelated males obtain greater breeding success than related males as explained by conflict models and by concession models, because immigrants obtain no indirect fitness from reproductive constraint. The difference between sites is predicted by concession models because dispersal costs are probably higher at Nossob where predators are more numerous (Chapter 2, Clutton-Brock et al., in press d) so subordinates will require lower fitness incentives to stay and help. Furthermore, in the case of subordinate males, breeding patterns are explained simply by the availability of unrelated mating partners.

One possible way to distinguish between mechanisms would be to compare skew in different sized groups (Clutton -Brock pers. comm.) This was not attempted here due to lack of data on the effect of helpers on group reproductive output. Conflict models predict an increase in subordinate reproduction with group size because dominants have more subordinates to suppress and may be less successful at suppression. Concession models predict a reduction in subordinate reproduction as group size increases as any one individual becomes less valuable as a helper and so a dominant will have less incentive to retain them in the group. However, meerkats live in ecologically unpredictable habitat where group extinction depends on group size (Chapter 2, Clutton-Brock et al., in press d). In this situation it may always pay dominants to maximise group size as a buffer against catastrophe, even under the concession model.

CHAPTER 6

HOW INDIRECT FITNESS IS DISTRIBUTED BETWEEN GROUP MEMBERS AND THE IMPORTANCE OF FUTURE FITNESS

6.1 Introduction

The different ways by which costly helping behaviours may provide fitness benefits were discussed in Chapter 5. In summary they are: (1) directly through breeding, (2) indirectly by helping to enhance the reproductive success of kin and (3) through enhancement of future reproductive success. Analyses in Chapter 5 establish that subordinate helpers are rarely or never the parents of the pups in which they invest, leaving this as an unsatisfactory explanation for helping behaviour.

6.1.1 Relatedness and indirect fitness benefits from helping

6.1.1.1 Current Indirect fitness

The amount of indirect fitness a helper acquires by investing in the production of non-descendent offspring depends on how closely related they are to those offspring. If kin selection were the only mechanism by which helping behaviour is adaptive, then the extent to which an individual helps would be expected to depend on actual or perceived relatedness to breeders, depending on the ability to recognise kin. The extent to which relatedness influences helping behaviour in cooperatively breeding species is unclear. Helpers preferentially chose to help closest genetic relatives in white-fronted bee-eaters (Emlen & Wrege 1988) but not in the splendid fairy wren

(Dunn et al. 1995), the pied kingfisher (Reyer 1984) or *Polistes* wasps (Hughes et al. 1993), (for review of effect of kinship on helping behaviour see Keller 1997).

6.1.1.2 Future effects on fitness from helping

Helping may enhance acquisition of fitness in the future in two ways: directly through increasing the chance of reproductive success in the future (Creel 1990b, Lucas et al. 1987); and indirectly through reducing investment required by breeding relatives in current offspring, and thereby increasing their residual reproductive value (Mumme et al. 1989).

A trait that many cooperative species share is longevity (Rowley & Russell 1990). This means that failing to reproduce in the first years of sexual maturity may not have an adverse affect on lifetime reproductive success. In fact, reproductive restraint may increase lifetime reproductive success by increasing reproductive value in subsequent years. Older individuals may have superior foraging skills, territory quality and /or experience raising young (Brown 1987, Woolfenden & Fitzpatrick 1984) which make them more effective breeders than younger individuals. In several species, helping has been documented in non-related helpers as a mechanism by which they may 'pay-to-stay' in a group. By investing in costly behaviours such as helping subordinates may increase reproductive success of dominants who, in turn, tolerate their presence in a group. Subordinates may then increase their chance of inheriting dominance and breeding position in the future.

Survival correlates positively with group size in many cooperative breeders. Meerkat groups containing less than nine individuals went extinct during a drought when all larger groups survived (Clutton-Brock et al. in press, c). Subordinates may be selected to maximise group size through helping by increasing offspring survival. Subordinates themselves will then stand a higher chance of surviving until they have a chance to inherit dominance. Furthermore, when subordinates inherit dominance there will be more helpers present to help raise their pups.

Helping can also enhance future acquisition of indirect fitness. Helping can increase the future reproductive potential of related breeders by reducing current expenditure on breeding. Mumme et al. (1989) reanalysed data on the effects of helping for three species of cooperatively breeding birds - the Florida scrub jay, the pied kingfisher and the splendid fairy wren. The results of the study suggested that the effects of helping on indirect fitness in the future may be substantial, comprising 29-49% of total indirect fitness acquired by helpers. Mumme et al. (1989) suggest that in assessing this effect, it is more useful to view aid-giving behaviour as directed towards breeders rather than their offspring. Helpers may also enhance future survival and/or fecundity of related breeders by raising offspring that become helpers for subsequent litters. This effect has been demonstrated in pied kingfishers (Reyer 1984) and stripe-backed wrens (Rabenold 1985).

Future effects on fitness described above are potentially important in that they clarify how behaviour that affects unrelated group members may be subject to selection. Helpers may be under selection to help even in the absence of any current direct or indirect fitness benefits. These future effects are, therefore, extremely important to consider when attempting to understand the distribution of fitness in cooperatively breeding systems.

6.1.2 Aims

Given the low direct fitness most subordinates acquire from helping behaviour, the first aim of this chapter is to establish whether helpers commonly acquire indirect fitness by helping. To achieve this, the average relatedness of each group member to pups produced during their presence in a group was measured.

Secondly, this chapter aims to investigate the probability that individuals enhance future reproductive success by helping. This is especially important in understanding how helping behaviour has evolved in subordinates which acquire no current direct or indirect fitness from helping. The probability of obtaining dominance and /or breeding as subordinate was investigated for individuals shown to receive little or no direct fitness benefit from helping (Chapter 5) or indirect fitness from helping (this Chapter).

6.2 Methods

6.2.1. Indirect fitness benefits

To establish whether subordinate meerkat helpers obtained indirect fitness by investing in rearing pups, it was necessary to measure relatedness between helpers and offspring. Lists of helpers present in a group at the time of birth of a litter were only available for groups at Van Zyls (n= 46 litters, n= 9 groups) and so all analyses presented in this chapter are of litters from Van Zyls only.

Each individual in the study was genotyped at 6-12 microsatellite loci as described in Chapter 3. This data was then used to construct relatedness matrices for each group using the computer program KINSHIP, as described in Chapter 4. The relatedness matrices contain relatedness coefficients between every individual ever seen in a group's territory, or genetically related to members of a group. Lists of all individuals present in a group at the time of birth of a litter were compiled from data collected in the field by observers. For each litter the mean relatedness of each individual to pups born was calculated using information from the relatedness matrices constructed by KINSHIP. Parents of the litter were identified using CERVUS as described in Chapter 4 and individuals in the group were categorised into breeders and non-breeders accordingly. Individuals were then further categorised into one of the following categories depending on dispersal strategy: founder (having originally established the group in a territory), natal (having been born into it), immigrant (having immigrated into the group from another group or unknown.

When individuals were present in a group for the birth of more than one litter, an average was taken of mean relatedness to pups across all litters. Some animals were in the 'breeder' category for some litters and the 'non-breeder' category for others. In these circumstances averages were taken separately for litters in which an animal bred from those in which an animal did not breed. Average relatedness to pups in a litter was then averaged across individuals in each category. By averaging within groups, each relatedness data point per breeding/dispersing category was independent and pseudoreplication was avoided (Hurlbert 1984).

The relatedness value for each category was then tested against the expectation that non-breeding immigrants were unrelated to pups ($r = 0$) for which they provide care and that non-breeding natal animals were related to pups at the full or half sibship level ($r=0.5$ or 0.25 respectively).

6.2.2 Future fitness benefits

Factors which might be expected to affect fitness acquired in the future as a result of helping include the effect of help on the recipients reproductive success, the probability of dispersing successfully, the probability of attaining dominance and reproductive success after attaining dominance. Data on the first two of these - effect of help on recipients success and the probability of dispersing successfully - are not presented in this thesis. The third and fourth of these - the probability of attaining dominance and reproductive success after attaining dominance - were investigated in the previous Chapter 5 (Table 5.3 and Figures 5.1a and 5.1b respectively) in the context of factors responsible for determining direct fitness. This information was used as far as possible to investigate future fitness effects as follows.

Indirect fitness acquired by non-breeding subordinate helpers was measured according to dispersal strategy in the previous section. This information was used to identify groups of individuals which obtained little or no direct or indirect fitness from helping. A crucial point is whether these individuals increase future reproductive success by helping. This was tested by comparing the probability that individuals which acquired little or no current fitness benefits had a higher probability of inheriting dominance in the future compared with those that did acquire current fitness benefits. In the absence of detailed information on the amount of helping each individual performed, group membership was taken as indication of help-giving behaviour. All subordinates provide some help and dominants provide little or no help apart from suckling by females (Chapter 2, Clutton-Brock et al. 1998).

6.3 Results

6.3.1. Do subordinates acquire indirect fitness from helping?

The amount of indirect fitness acquired from helping depends on whether helpers are natal, or have dispersed into the group into which the pups they care for are born. As expected, non-breeding immigrants (all immigrants were males) had low relatedness to pups, with relatedness coefficients not significantly different from 0 (t=4.29, d.f. = 2, NS) (Table 6.1). Before dispersal males were related to pups at a level not significantly different from 0.25, i.e. that between half sibs (t = 0.91, d.f. = 7, NS) but significantly different from that between full sibs (t=7.71, d.f. = 7, P<0.01). Unless founding males breed, they should be unrelated to the pups that are born into a group. Relatedness between non-breeding founding males and pups was not significantly different from 0 (t = 1.46, d.f. = 1, NS).

	BREEDING MALES				NON BREEDING MALES			
group	founder	natal	immigrant	unknown	founder	natal	immigrant	unknown
Avatar				0.37		0.33		0.08
D-doring			0.19			0.24	0.03	
Elvira	0.42					0.26	0.08	0.02
Frisky					0.03			
Lazuli	0.30				0.01	0.16		
M-Pickle				0.48		0.36		0.43
Phantom				0.20		0.28		0.22
Vivian			0.38			0.19	0.07	
Youngones	0.43					0.40		
N individuals per category	4	0	3	3	5	48	5	7
N groups	3	0	2	3	2	8	3	4
MEAN	**0.39**		**0.29**	**0.35**	**0.02**	**0.28**	**0.06**	**0.19**
SE	**0.04**		**0.09**	**0.08**	**0.01**	**0.03**	**0.01**	**0.09**

Table 6.1 Means and standard errors of relatedness between pups and male breeders/helpers of different dispersal category at Van Zyls (n= maximum of 9 groups). Means and standard errors of relatedness are given for each category of animals according to dispersal status in the group into which the litter was born. (founder = established group in new territory, natal = residing in group of birth, immigrant = immigrated into group from another group, unknown = origin in group unknown)

Natal, non-breeding females were related to pups at a level not significantly different from 0.25, i.e. that expected between half sibs ($t = 2.23$, d.f. = 7, NS) but significantly different from that between full sibs ($t = 9.7$, d.f. = 7, $P<0.01$). Non-breeding founders were on average unrelated to pups (not significantly more related than 0, $t = 1.68$, d.f. = 4, NS) in a group, unless they were the mother of a successful subordinate breeder as in the case of the group Vivian and Youngones. Often, founding females were the sisters of females that attained the dominant breeding position but never bred, as in groups Elvira, Frisky and Lazuli. Breeding males had lower mean relatedness to pups than breeding females but this difference was not significant ($F_{(1,20)} = 3.80$, NS).

	BREEDING FEMALES				NON-BREEDING FEMALES			
group	founder	natal	immigrant	unknown	founder	natal	immigrant	unknown
Avatar				0.45		0.29		
D-doring		0.39		0.45		0.30		
Elvira	0.49	0.40			-0.01	0.37		0.16
Frisky	0.30				0.00			
Lazuli	0.39	0.32			0.10	0.17		
M-Pickle				0.42		0.31		0.43
Phantom		0.41		0.41		0.30		0.13
Vivian	0.56	0.52			0.42	0.29		
Youngones	0.37				0.15	0.34		
N individuals per category	5	6	0	4	8	44	0	7
N groups	5	5	0	4	5	8	0	3
MEAN	**0.42**	**0.41**		**0.43**	**0.13**	**0.30**		**0.24**
SE	**0.05**	**0.03**		**0.01**	**0.08**	**0.02**		**0.10**

Table 6.2 Means and standard errors of relatedness between pups and female breeders/helpers of different dispersal category at Van Zyls (n= maximum of 9 groups). Means and standard errors of relatedness are given for each category of animals according to dispersal status in the group into which the litter was born. (founder = established group in new territory, natal = residing in group of birth, immigrant = immigrated into group from another group, unknown = origin in group unknown)

Almost all non-breeding helpers acquired indirect fitness through helping (93% of females, n=59; 83% of males, n=58). Founding females had a relatedness to pups not significantly different from 0 (Table 6.2) but in groups Elvira, Frisky and Lazuli were known to be sisters of breeding, dominant co-founders from pedigree construction from CERVUS analysis. They were, therefore, likely to be receiving some indirect fitness benefits from raising nieces and nephews. Males which did not receive any indirect fitness were either founders (n=5) or immigrants (n=5) and were not known to be related in any way to breeders from CERVUS analysis.

Relatedness between pups and non-breeding animals whose dispersal status was not known suggest that this category contained a mixture of natals, founders and immigrants (Table 6.1) in the case of males, and natals and founders in the case of females (Table 6.2).

6.3.2 Future fitness effects

Certain classes of non-breeding individuals receiving little or no indirect fitness benefits from helping were identified. These classes were founders of both sexes and immigrant males. Non-breeding founders of both sexes had an average relatedness to pups which was not significantly different from 0. In the case of females, relatedness to pups was also not significantly different from 0.25 (i.e. that between half sibs). Immigrant males were related by 0.06 ± 0.01(mean ± standard error) to pups (Table 6.3) and so accrue no measurable indirect fitness from investment in rearing offspring in groups to which they emigrate.

	founders		**natals**		**immigrants**		**unknown**	
	n	mean r to pups	n	mean r to pups	n	mean r to pups	n	mean r to pups
female non-breeders	**8**	**0.13** **(± 0.08)**	44	0.30 (± 0.02)	0		7	0.24 (± 0.10)
male non-breeders	**5**	**0.02** **(± 0.01)**	48	0.28 (± 0.03)	**5**	**0.06** **(± 0.01)**	7	0.19 (± 0.09)

Table 6.3 Mean and standard error of relatedness between non-breeders and pups they helped, separated according to dispersal strategy (data from Tables 6.1 and 6.2). Three categories of non-breeders have no measurable indirect fitness gain from helping (shown in bold), founders of both sexes and immigrant males.

Founders and immigrants which were unrelated to pups they helped, were significantly more likely to breed than animals in natal groups which were related to the pups they helped (Table 6.4).

	dispersal status	**number of breeders**	**number of non-breeders**	**G d.f.=1**	**significance**
males **n = 65**	founder/ immigrant	7	10	21.38	P<0.001
	natal	0	48		
females **n = 63**	founder/ immigrant	5	8	4.336	P<0.05
	natal	6	44		

Table 6.4 Results of a G-test to show that founders and immigrants from analysis shown in Tables 6.1 and 6.2 are significantly more likely to breed compared with individuals in natal groups.

There are small sample sizes of dominants about which information on dispersal status is known from observation in the field. The three classes of individuals which, on average, obtained little or no direct or indirect fitness from helping also had a relatively high probability of inheriting dominant breeding position (Table 6.5). Of dominant females, 4 /7 were known to have founded the group in which they bred and 11/11 males either founded or immigrated into the group in which they bred. As predicted, these are the same dispersal classes of individuals which have been demonstrated to receive no current fitness pay-offs from helping. 3/7 females inherited dominance in their natal groups where they were also likely to receive substantial indirect fitness benefits from helping .

	founder	**natal**	**immigrant**	**unknown**
Dom. Female	4	3	0	8
Dom. Male	5	0	6	2

Table 6.5 Dispersal status (founder = established group in new territory, natal = residing in group of birth, immigrant = immigrated into group from another group, unknown = origin in group unknown) of all dominant breeders in groups for which parentage analysed in Chapter 5 for Van Zyls. Females n=15, Males n= 13.

6.4 Discussion

6.4.1 Indirect fitness effects of helping

Given the small proportion of individuals recruiting to the breeding population as dominants, or breeding as subordinates (Figures 5.1a and 5.1b), indirect fitness was expected to be the most important component of inclusive fitness acquired over an average lifetime. This applied in particular to females who generally only breed when dominant (Figures 5.1a and 5.1b).

6.4.1.1 Females

As expected, dispersal strategies strongly influence the way in which an individual acquires fitness. Females rarely disperse from their natal group and generally do so only when evicted by the dominant female, when they may go on to found new groups (Clutton-Brock et al. in press, a). All females in a group are, therefore, either foundresses or descendants of female foundresses. From this, females were predicted to acquire substantial indirect fitness benefits from helping raise offspring of the dominant, and this was shown to be the case (Table 6.2). The average relatedness to offspring was not significantly different from 0.25 which is equivalent to relatedness between half sibs but was significantly different from 0.5 which is equivalent to relatedness between mother and offspring. Dominant females were generally the mother of the helpers in her group. The half sib relatedness to pups was probably due, therefore, to the fact that tenure of male dominance is generally shorter than the length of time that a helper helps in a group. Also, dominant females may mate with males from outside the group within the tenure of a dominant male. Females, therefore, transmit a lower proportion of their genes by helping than they do by breeding. Helping is still the most common strategy, however, probably because of constraints on breeding or because helping is twice as productive. Subordinate females attain very low breeding success as subordinates, probably as a result of suppression by dominants, but they have a significantly greater probability of breeding in a group which they had founded relative to their natal group (Table 6.4). Furthermore, very few females successfully recruit into the small, dominant, breeding population. The exact proportion is unknown, as females that disappear from groups under observation are hard to follow. Females also stand a small chance of inheriting dominance in their natal groups (Table 6.5). By inheriting dominance without dispersing from their natal group they avoid the mortality risks associated with dispersing to found a new group.

6.4.1.2 Males

Males also obtain fitness according to predictions based on dispersal strategy. A small proportion of males have been observed to emigrate successfully out of their natal group. Like females, males stand a higher probability of breeding outside their natal groups (Table 6.4). These immigrant males are successful at breeding when subordinate and at inheriting dominance (Table 6.5), while natal males have never been observed to breed (Tables 5.2a, 5.2b & Table 6.1). An unknown number of males achieve direct fitness by mating with females in a group to which they do not belong by sneaking into their territories. However, the proportion of paternities attributed to males outside the group is generally low (Figure 5.1a and 5.1b). The results presented in this chapter (Tables 6.1 and 6.3) show that indirect fitness benefits acquired by helping in a natal group are still the most important way that the average subordinate male acquires fitness. As with females, the average relatedness to offspring they provide care for is not significantly different from 0.25 which is equivalent to relatedness between half sibs but significantly lower than that between fathers and offspring (0.5). This means that helping is a less effective way of enhancing fitness relative to breeding unless productivity is twice as high from helping.

6.4.2 Future fitness effects of helping

It was not possible from the information available to assess the importance of future effects on fitness from helping. Specifically, the probability of dispersing successfully is not known and little is known about the probability of attaining dominance. This makes it difficult to reach firm conclusions about the effect of helping on future direct fitness. Also, it is not known what effect helping has on the lifetime reproductive success of related breeders. This information is crucial to understanding the effect helping has on future indirect fitness (Mumme et al. 1989).

Results presented in this chapter do suggest, however, that future effects on fitness from helping may play some role. It has been demonstrated that some helpers, although a very small proportion, obtain little or no immediate fitness benefits from helping. These individuals are generally founders of both sexes and immigrant males (Table 6.3). Future fitness effects have been shown to be useful in providing understanding of why helping behaviour may be adaptive in these classes of individuals. Specifically, dominant breeding males originate solely from the founder and immigrant categories while half of dominant breeding females are founders by origin (Table 6.4).

Moreover, to answer the question - why help - it would be necessary to investigate the effect of withholding help. This has not been possible because all subordinates generally provide help (Chapter 2). It is not known, for instance, whether the failure to provide help would result in eviction from the group by dominants. This could be tested experimentally by preventing helpers from providing care. Mulder & Langmore (1993) conducted experiments on cooperatively breeding superb fairy wrens (*Malarus cyaneus*) to demonstrate that helpers removed during the breeding season were attacked on their return to the nest by the dominant male, but not if they were removed outside the breeding season. Interestingly, Clutton-Brock et al (in prep) provide experimental evidence (using genetic data presented in this thesis) to show that relatedness has no effect on the amount of help provided in meerkats. The amount of help provided was shown to depend primarily on the relative nutritional status of potential helpers.

Greater understanding of helping in meerkats can best be achieved by measuring reproductive success over the lifetime of individuals adopting different strategies. This will only be possible when sufficient numbers of animals have been observed throughout their lifetimes in the field. However, results presented here are important in the absence of complete information, in that they demonstrate that the majority of helpers do acquire indirect fitness from helping. Current indirect fitness is probably the most important component of inclusive fitness over lifetime with the exception of the small numbers of individuals which attain dominance and breeding status. Results also suggest that selection to maximise fitness obtained in the future may explain why subordinates help to raise pups to which they have no actual or perceived relatedness. The importance of future effects may also explain the results obtained by Clutton-Brock et al. (in prep.) that the amount of help given does not depend on kinship, but on the ability to provide help.

CHAPTER 7

GENERAL DISCUSSION

7.1 Distribution of fitness in meerkat groups

The reproductive success achieved in meerkat groups depends entirely on the investment of subordinate helpers. There has been no recorded instance of a single breeder successfully raising a litter unaided, at either study site, over five years since observation began (Chapter 2). There are, therefore, strong constraints on breeding independently and breeders depend on the cooperation of helpers in order to raise a litter successfully. Helping individuals greatly enhance the fitness of breeders and incur substantial costs to themselves in terms of weight loss and increased risk of predation (Clutton-Brock et al. 1998). Helpers, however, are not true altruists (defined as an *unselfish* act committed solely for the benefit of another). Despite the high costs incurred by providing care for offspring, helpers can enhance their *own* fitness in a number of possible ways: (1) directly through breeding; (2) indirectly by helping to enhance the reproductive success of kin, and (3) through enhancement of future reproductive success (Chapter 1).

Helpers are always subordinate, with dominants contributing little or no investment in rearing offspring. The first important point that this thesis has established is that reproduction in meerkat groups is highly skewed in favour of dominants which provide no help (Section 5.3.1). Helpers, therefore, rarely acquire significant direct fitness benefits from helping. The failure to provide care is associated with dominance and not breeding status. When subordinates do occasionally obtain breeding success, offspring care is still provided solely by subordinates, including the breeding subordinates (Clutton-Brock et al. 1998).

The average tenure of dominance could not be calculated from the data available, but there is low turnover of dominance status, especially for females (Chapter 2). In the parentage analysis (Chapter 5) the same dominant females, and occasionally the same dominant males, could be observed to breed continuously for 2-3 years. This is a substantial length of time relative to the time it takes for a pup to reach sexual maturity which was estimated to be around one year of age, although no animal was observed to breed under two years of age.

The great majority (> 90%) of subordinate helpers in meerkat groups are natal to that group (Section 6.3.2). This means that subordinate helpers are likely to be providing care for their full or half siblings. In fact, the average relatedness between natal helpers and the offspring to which they provided care, was 0.28 for males and 0.30 for females (Section 6.3.1). These average relatedness values were found to be significantly different from 0.5 (expected between full sibs) but not from 0.25 (expected between half sibs), suggesting that, on average, helpers are investing in the production of half sibs. The second important point established in this thesis is, therefore, that the great majority of subordinate helpers obtain substantial indirect fitness benefits from helping. Each pup a subordinate successfully raises by investing in helping in its natal group carries on average a quarter of its genes which are identical by descent to the helper.

As pointed out above, the average relatedness of subordinates to the pups they invest in (0.28 for males, 0.30 for females) is significantly less than the relatedness expected between parents and offspring (0.5). Therefore, despite acquiring indirect fitness benefits, subordinates acquire less fitness per pup by helping than dominants do by breeding. Given that breeding leads to greater fitness benefits acquired, and given the high costs associated with helping behaviour (which are unlikely to be matched by costs associated with breeding), why don't subordinates breed instead of help?

There are many factors (in common with many other cooperatively breeding species discussed in Chapter 1), such as ecological constraints, which may prevent subordinates from taking the option to breed independently. Consequently, it may pay to help and accrue some fitness indirectly rather than not help and accrue no fitness. As has been discussed, meerkats are obligate communal breeders, being unable to breed successfully outside a group. Dispersing to breed is, therefore, only a viable option if there are opportunities to found a new group with others or join an already established breeding group. Dispersal (and therefore breeding) strategies differ between males and females (Chapters 5 & 6, Clutton-Brock et al., in press c).

Females either disappeared or founded new territories. Males either disappeared, joined females to found a new group or, unlike females, were able to join established breeding groups (Chapter 2, Clutton-Brock et al., in press c).

The helping behaviour of male immigrants is particularly intriguing. The offspring that these male immigrants care for are unlikely to be of close kin, given the low frequency of successful migration between groups. In fact, immigrant males were shown to gain no measurable indirect fitness benefits from helping (Sections 6.3.1 and 6.3.2) in their new groups.

This leads to the third important point established in this thesis which is the potential importance of enhancing fitness acquired in the future through helping. Despite the fact that immigrants are unrelated to the offspring they invest in, they are more likely to breed and gain direct fitness than in their natal groups. In fact, no male was ever observed to breed in its natal group (Tables 5.2a and 5.2b). Furthermore, immigrant males have a substantially increased probability of attaining dominance in a group into which they have immigrated or founded, relative to their natal group. None of the males included in the analyses of this thesis were observed to attain dominance in their natal territory (Table 5.3) and only 2/38 were ever observed to inherit dominance in their natal territory in the field (Clutton-Brock et al. in press c). Crucially, the proportion of dispersers which successfully found or join established groups is not accurately known. However, given the small proportion of immigrants seen in established groups under observation and the small numbers of new groups founded at the study sites, the proportion of subordinates recruiting to the dominant breeding population by dispersal is likely to be small.

Options to breed within a natal group may be limited for a number of reasons. Firstly, subordinates' attempts to breed may be suppressed by the dominant. The extent to which this occurs has been predicted to depend on factors such as relatedness to offspring and ecological constraints on dispersal by concession models of reproductive skew (Chapter 1). Subordinate meerkats in their natal group would be predicted to require little or no reproductive concession from the dominant in order to retain them as helpers according to these predictions. In some species, for example dwarf mongooses (Creel & Creel 1991), subordinates are hormonally deficient in the presence of a dominant and are physiologically unable to breed. In meerkats, however, subordinate females were occasionally observed to produce litters successfully. Whether pups of subordinate females survived, however, was dependent on the timing of the birth in relation to the birth of a dominant female's litter. It is suggested that the dominant female is able to suppress reproduction of a subordinate by infanticide (Clutton-Brock et al., in press a).

Even if suppression by dominants is unimportant, there may be constraints on subordinate breeding due to the availability of unrelated opposite sex breeders, given the expected avoidance of inbreeding (Chapter 1, Section 5.1). The fifth main finding of this thesis was that subordinate breeding in males and females is dependent to different extents on the extent of relatedness to potential opposite sex breeders in a group. All breeding pairs, whether dominant or subordinate, were unrelated (Table 5.4). This suggests that meerkats prefer to mate with non-relatives. This fact is further backed up by the result that relatedness to the breeding (dominant) female determined the likelihood of subordinate males obtaining a mating that led to successful breeding (Chapter 5, Table 5.5).

The work in this thesis emphasises that one of the most important lines for future research will be to carry out long term studies that measure the entire lifetime reproductive success of individuals. This is important because future benefits to helping, such as the increased probability of immigrant males obtaining dominance, can only be determined accurately in this way. In species where this has been done, for example in the dwarf mongoose, it is clear that the optimal strategy can vary between age and sex classes (Creel & Waser 1994). Young animals which disperse may stand substantially lower chances of obtaining fitness directly than they would if they dispersed at a later age, *and* incur costs in terms of loss of indirect fitness by doing so (Chapter 1). This emphasises the importance of determining the consequences of different dispersal strategies for individuals of different ages. Measures of lifetime reproductive success are also necessary in order to obtain a quantitative estimate of the extent to which reproduction is skewed towards dominants (termed the reproductive skew index (Keller & Perrin 1995, Pamilo &

Crozier 1996, Tsuji & Tsuji 1998), which could then be compared with other cooperative breeding species.

7.2 Summary

Reproduction in meerkats is highly skewed towards dominants, who produce the majority of offspring (92-100% in females, and 62-84% in males). Despite producing a small fraction of offspring, it is the subordinates that carry out the majority of offspring care. Offspring care is costly, leading to weight loss and increased risk of predation. The two most important factors favouring such costly helping behaviour by the subordinates appear to be: (1) ecological constraints preventing independent breeding, and (2) the substantial indirect fitness benefits that can be obtained by helping raise closely related individuals (full and half siblings). The major exception to this rule is immigrant males who help raise offspring to which they are not related, hence gaining no indirect fitness benefits. The reason that these males help is likely to be that they do so in order to stay in a group where they stand a chance of obtaining some matings as a subordinate and possibly even dominance (male meerkat nirvana). Taken together the molecular genetics (this thesis) and detailed field studies (Clutton-Brock et al. 1998, In press a-c) on this species provide a clear example of the complex manner in which ecological and genetic factors can interact to favour the evolution of cooperative breeding and helping.

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APPENDIX I

LOCI CLONED IN OTHER CARNIVORES AND TESTED IN MEERKATS

dog loci

AHT106
AHT111
AHT117
AHT119
AHT121
AHT129
AHT122
AHT125
AHT128
AHT140
AHT142
AHT130
AHT109
AHT115
AHT118
AHT120
AHT127
AHT124
AHT126
AHT137
AHT141
AHT141

(total = 22)

Primers kindly donated by N. Holmes, Animal Health Trust, Newmarket. See also: Holmes, N.G., Dickens, H.F., Parker, H.L., Binns, M.M., Mellersh, C.S. and Sampson, J. (1995) Eighteen canine microsatellites. Animal Genetics 26, 132-133

badger loci

BAD3
BAD5
CGBA6
CGBA19
CGBA25
CGBA28
CGBA35
CGBA38
CGBA47
CGBA71
CGBA78
DOG2201
BAD4
BAD6
CGBA15
CGBA23
CGBA26
CGBA33
CGBA37
CGBA40
CGBA53
CGBA75
DOG2200

(total = 21)

Primers kindly donated by T.A. Burke and collaborators, Department of Zoology, Leicester University

Seal loci

B-Hb
CLD
HG8.10
PV11
HG4.20
PV2
HG1.30
PV9
HG3.66
PV10
HG3.70
PV13
HG6.10
PV14
HG6.30
PV15
AA4
PV16
AA6
PV17

(total = 20)

Primers from S.J.Goodman and P.J.Allen, Department of Genetics, Cambridge University (Goodman 1997, Allen et al. 1995).

cat loci

Fca8
Fca23
Fca35
Fca43
Fca45
Fca77
Fca78
Fca90
Fca96
Fca126

(total = 10)

Primers sequences from Menotti-Raymond, M.A. & O'Brien, S.J. (1995).

APPENDIX II
LAB PROTOCOLS

1. CLONING MICROSATELLITES
2. GENOTYPING MICROSATELLITES

1. Cloning of Microsatellites

Protocol adapted from Tautz (1989).

1. High molecular weight genomic DNA was prepared and test-digested with several restriction enzymes, e.g. EcoRI/PstI - BamHI/HindIII - Sau3A - and visualised on an agarose gel with an undigested DNA control to check its quality. If the DNA had been stored for a long time, a sample was loaded which had been incubated without enzyme to check for heat degradation.

2. Bulk digests of the genomic DNA was carried out using Sau3A which gave the highest fragment density between the desired size range of between 500 to 700 bp. Digested DNA was run out on low-melting point agarose gel, and the desired size range was cut out. DNA was prepared for transformation with Promega PCR Magic Preps following manufacturers instructions. Optical density was determined by photospectrometry.

3. Bulk digests of plasmid (pUC18) DNA were carried out with the Bam H1 enzyme and purified with Promega Wizard DNA Clean-up System following manufacturers instructions.

Preparation of the vector

1. Subsamples of the pUC18 digests were taken and diluted to 100 ng/μl and used for the following:
2. 1 μl was test-transformed to check quality of the digest,

3. 1 μl was test-ligated and transformed to check the quality of the ligase.

4. The vector was then phosphatased (alkaline phosphatase) to prevent/ minimise self-religation, and purified with Promega DNA Clean-up system following manufacturers instructions. Optical density was determined by photospectrometry and the sample was diluted to 100 ng/μl.

5. To check the result of the phosphotasing the sample was test-religated for 3 days at 16° C and transformed.

6. A test-religatation with PNK was carried out and transformed to recheck the quality of the vector .

<u>If this proceeded satisfactorily the vector and genomic DNA were ligated as follows:</u>

4. Test-ligations were carried out overnight with vector and genomic DNA in varying proportions and transformed to establish the optimal ratio.

5. The real ligation was then carried out for 3 days at 16° C, 1 ml test-transformed and plated out separately in 10 ml and 100 ml of culture to determine a colony density.

6. Transformations were carried out using twin-pulse electroporation and plated out in appropriate amounts on large (22 x 22 cm) agar plates. After overnight growth, colonies were lifted onto Hybond-N. DNA was crosslinked onto the membrane with a UV crosslinker.

7. $(CA)_{15}$ probe was prepared by labelling repeat with ^{32}P g dATP and PNK. Probe was purified through a Sephadex column and incorporation checked with a Geiger counter to be ~ 80%.

8. Probe was hybridised to filters in a Hybaid hybridisation oven. Hybridisation temperature for $(CA)_{15}$ was 52°C. Filters were washed in SDS solution and exposed to autorad film.

9. Putative positive colonies were picked with a toothpick and streaked onto small round agar plates. The following day, individual colonies were picked from these plates and streaked in small straight lines onto fresh agar plate (in replicate, from each original colony in a large plate). Colonies were grown overnight and lifted onto Hybond-N, hybridised and visualised by autoradiography. True positives came up as strong replicate streaks and contained a microsatellite with > 90% probability. Positives were grown in liquid L-broth culture and DNA was extracted with Promega Wizard Mini-Prep according to manufacturers instructions.

10. DNA extracted from positive clones with a Perkin-Elmer sequencing kit according to manufacturers instructions and sequenced on an ABI377 automated sequencer and primers were then designed using the program PRIMER obtained from the HGMP (Human Genome Mapping Project) website.

2. Genotyping microsatellites

Adapted from protocol described in Bancroft et al. (1995).

Loci were amplified as follows:

Approximately 50 ng genomic DNA was used as template for 2 pmol of each primer in a 10 uL reaction overlaid with 1 drop of mineral oil (reaction conditions: 0.1 mM dATP, dGTP and dTTP; 0.01 mM dCTP; 0.1-0.3ul 1.5 (see Table II) mM $MgCl_2$; 5-10% dimethylsulphoxide (see Table II); <1 uCi alpha-^{32}P cCTP; 1*'PARR' buffer (Cambio); 0.25 units *Taq* polymerase). A Hybaid thermocycler was used with the following 2-stage programme: an initial 95 °C denaturation for 5 mins was followed by 7 cycles of 94 °C denaturation for 30 s, 44- 58°C (see Table II) annealing for 1 min and 72 °C extension for 30 s, and 25 cycles of 89 °C denaturation for 30s, 46-60°C (see Table II) annealing for 1 min and 72 °C extension for 30s.

On completion of the amplification cycles, 8uL of the reaction was added to 4 uL of sequencing loading buffer (95% formamide, 20 mM EDTA, 0.05 % bromophenol blue and 0.05 % xylene cyanol). The reactions were denatured by heating to 94 °C prior to loading 3uL into lanes of a standard denaturing sequencing gel (6% polyacrylamide/ 8M urea/ TBE buffer; 'Sequagel'). As a size marker, a nonrecombinant M13 mp18 sequencing reaction was also loaded. The amplification products were electrophoresed for approximately 2.5 hours. The gel was dried under vacuum at 80 °C and exposed to X-ray film.

locus	1.5 mM $MgCl_2$ (ul in 10ul rxn)	DMSO (ul in 10ul rxn)	annealing temp stage 1	annealing temp stage 2
AHT 130	0.1	1	44°C	46°C
Fca 45	0.3	1	52°C	54°C
Hg 8.10	0.1	1	50°C	52°C
Ssu 7.1	0.3	0.5	56°C	58°C
Ssu 8.5	0.3	0.5	56°C	58°C
Ssu 10.4	0.3	0.5	58°C	60°C
Ssu 13.9	0.3	0.5	54°C	56°C
Ssu 13.8	0.3	0.5	54°C	56°C
Ssu 14.14	0.3	0.5	58°C	60°C
Ssu 14.18	0.3	0.5	52°C	54°C
Ssu 12.1	0.3	0.5	54°C	56°C
Ssu 11.12	0.3	0.5	54°C	56°C

Table II Description of locus-specific reaction conditions where variations from standard reaction conditions described above were adopted.

APPENDIX III
ALLELE FREQUENCIES

1. NOSSOB

****** Locus AHT130 ******

number of alleles: 10

number of individuals typed 154

Allele	Count	Frequency
126	2	0.007
134	1	0.003
136	158	0.513
140	5	0.016
142	82	0.266
144	25	0.081
146	20	0.065
148	1	0.003
150	11	0.036
154	3	0.010

****** Locus Fca45 ******

Number of alleles: 9

Number of individuals typed: 210

Allele	Count	Frequency
156	1	0.002
158	20	0.048
160	37	0.088
162	72	0.171
164	112	0.267
166	40	0.095
168	120	0.286
170	17	0.040
176	1	0.002

****** Locus Hg8.10 ******

Number of alleles: 13

Number of individuals typed: 167

Allele	Count	Frequency
193	2	0.006
198	108	0.323
200	1	0.003
213	10	0.030
215	37	0.111
217	9	0.027
219	86	0.258
221	5	0.015
223	2	0.006
224	45	0.135
225	4	0.012
229	19	0.057
231	6	0.018

****** Locus Ssu7.1 ******

Number of alleles: 10

Number of individuals typed: 214

Allele	Count	Frequency
140	6	0.014
144	31	0.072
146	26	0.061
148	49	0.114
150	27	0.063
152	19	0.044
154	10	0.023
156	122	0.285
158	123	0.287
160	15	0.035

****** Locus Ssu8.5******

Number of alleles: 15

Number of individuals typed: 153

Allele	Count	Frequency
208	23	0.075
212	31	0.101
214	6	0.020
220	53	0.173
222	9	0.029
224	2	0.007
226	21	0.069
228	85	0.278
230	4	0.013
232	1	0.003
234	7	0.023
236	22	0.072
238	31	0.101
240	9	0.029
242	2	0.007

****** Locus Ssu10.4 ******

Number of alleles: 14

Number of individuals typed: 202

Allele	Count	Frequency
96	10	0.025
111	126	0.312
113	34	0.084
115	9	0.022
120	2	0.005
121	32	0.079
122	68	0.168
123	1	0.003
124	17	0.042
126	53	0.131
127	4	0.010
130	23	0.057
131	23	0.057
146	2	0.005

****** Locus Ssu13.9 ******

Number of alleles: 5

Number of individuals typed: 129

Allele	Count	Frequency
128	1	0.004
130	59	0.229
132	69	0.267
134	97	0.376
136	32	0.124

****** Locus Ssu13.8 ******

Number of alleles: 12

Number of individuals typed: 219

Allele	Count	Frequency
135	23	0.053
137	1	0.002
139	80	0.183
141	15	0.034
143	17	0.039
147	22	0.050
149	144	0.329
151	37	0.085
153	67	0.153
155	21	0.048
157	10	0.023
159	1	0.002

****** Locus Ssu14.14 ******

Number of alleles: 8

Number of individuals typed: 187

Allele	Count	Frequency
113	23	0.062
115	100	0.267
117	43	0.115
119	4	0.011
121	30	0.080
123	24	0.064
125	142	0.380
127	8	0.021

****** Locus Ssu14.18 ******

Number of alleles: 9

Number of individuals typed: 147

Allele	Count	Frequency
129	11	0.037
131	34	0.116
135	39	0.133
137	72	0.245
139	95	0.323
141	13	0.044
143	2	0.007
147	26	0.088
149	2	0.007

****** Locus Ssu12.1 ******

Number of alleles: 9

Number of individuals typed: 185

Allele	Count	Frequency
136	33	0.089
142	17	0.046
144	33	0.089
148	37	0.100
150	153	0.414
152	5	0.014
154	44	0.119
156	32	0.087
158	16	0.043

****** Locus Ssu11.12 ******

Number of alleles: 10

Number of individuals typed: 184

Allele	Count	Frequency
119	46	0.125
121	17	0.046
123	124	0.337
125	1	0.003
129	43	0.117
131	33	0.090
133	6	0.016
135	32	0.087
137	58	0.158
141	8	0.022

2.VAN ZYLS

****** Locus AHT130 ******

Number of alleles: 11

Number of individuals typed: 219

Allele	Count	Frequency
124	5	0.011
126	3	0.007
134	6	0.014
136	178	0.406
140	8	0.018
142	104	0.237
144	42	0.096
146	21	0.048
148	50	0.114
150	7	0.016
154	14	0.032

****** Locus Fca45 ******

Number of alleles: 12

Number of individuals typed: 279

Allele	Count	Frequency
150	1	0.002
158	7	0.013
160	34	0.061
162	83	0.149
164	189	0.339
166	73	0.131
168	92	0.165
170	47	0.084
172	9	0.016
174	1	0.002
176	8	0.014
178	14	0.025

****** Locus Hg8.10 ******

Number of alleles: 14

Number of individuals typed: 259

Allele	Count	Frequency
190	10	0.019
193	4	0.008
198	178	0.344
200	29	0.056
213	2	0.004
215	22	0.043
217	69	0.133
219	87	0.168
221	21	0.041
222	15	0.029
223	7	0.014
224	35	0.068
225	38	0.073
226	1	0.002

****** Locus Ssu7.1 ******

Number of alleles: 8

Number of individuals typed: 278

Allele	Count	Frequency
140	28	0.050
146	1	0.002
148	170	0.306
150	120	0.216
152	23	0.041
154	22	0.040
156	145	0.261
158	47	0.085

****** Locus Ssu8.5 ******

Number of alleles: 16

Number of individuals typed: 109

Allele	Count	Frequency
208	25	0.115
212	16	0.073
214	4	0.018
218	15	0.069
220	9	0.041
222	3	0.014
226	37	0.170
228	36	0.165
230	15	0.069
232	8	0.037
234	7	0.032
236	28	0.128
238	5	0.023
240	5	0.023
242	2	0.009
244	3	0.014

****** Locus Ssu10.4 ******

Number of alleles: 12

Number of individuals typed: 268

Allele	Count	Frequency
111	118	0.220
113	38	0.071
121	10	0.019
122	181	0.338
123	24	0.045
124	65	0.121
125		0.024
126	4	0.008
130	11	0.021
131	45	0.084
134	5	0.009
138	22	0.041

****** Locus Ssu13.9 ******

Number of alleles: 4

Number of individuals typed: 234

Allele	Count	Frequency
128	3	0.006
130	119	0.254
132	180	0.385
134	166	0.355

****** Locus Ssu13.8 ******

Number of alleles: 10

Number of individuals typed: 239

Allele	Count	Frequency
135	8	0.017
139	79	0.165
143	2	0.004
147	50	0.105
149	179	0.375
151	12	0.025
153	41	0.086
155	89	0.186
157	17	0.036
159	1	0.002

****** Locus Ssu14.14 ******

Number of alleles: 9

Number of individuals typed: 253

Allele	Count	Frequency
111	1	0.002
113	97	0.192
115	116	0.229
117	40	0.079
119	12	0.024
121	96	0.190
123	20	0.040
125	116	0.229
127	8	0.016

****** Locus Ssu14.18 ******

Number of alleles: 9

Number of individuals typed: 194

Allele	Count	Frequency
129	4	0.010
131	7	0.018
133	2	0.005
135	75	0.193
137	194	0.500
139	27	0.070
145	6	0.016
147	24	0.062
149	49	0.126

****** Locus Ssu11.12 ******

Number of alleles: 9

Number of individuals typed: 248

Allele	Count	Frequency
119	83	0.167
121	47	0.095
123	180	0.363
125	63	0.127
129	74	0.149
131	10	0.020
133	13	0.026
135	20	0.040
137	6	0.012

****** Locus Ssu12.1 ******

Number of alleles: 11

Number of individuals typed: 236

Allele	Count	Frequency
136	29	0.061
138	1	0.002
140	1	0.002
142	46	0.098
144	63	0.134
146	2	0.004
150	159	0.337
152	59	0.125
154	65	0.138
156	40	0.085
158	7	0.015

APPENDIX IV

MEERKAT MICROSATELLITE STRUCTURE AND PRIMER SEQUENCES

Locus Name	Microsatellite Repeat Structure	F/R Primer Sequences (5' to 3')
Ssu7.1	$(TC_{17})T(CA_{14})$	ATCCCTTAATGCATAGGCACAC CCTGCTAGTCTTCTCCGTGC
Ssu8.5	$(CT_{27})(CA_{23})$	AAGTCAGGTGCTTAACTGACTGG TGGAGTCACTCATTTGGTTTTG
Ssu10.4	$(CA_9)TA(CA_{17})$	CATTGGGTGCACACTGTCTC CTCCAGTTCTTTTCCCTGGAG
Ssu11.12	CA_{22}	CTCATTTTCAGGAAATTTTCATCC CCTAGCTTTATTTTTCTCTGTGGC
Ssu12.1	CA_{20}	TGAAGATAGGCTTGCTTTCTCC CCTGGTGACACAAACAATGC
Ssu13.8	$(CA_5)TA(CA_{18})$	AACAGAAGTGCCTGAATGTGC TTTCCTCCACAATGAGTAAGACA
Ssu13.9	CA_{15}	AAGTAGGTAGAAGACATTTCCCCC GGGATGAGAAGAACCACCCT
Ssu14.14	CA_{18}	TGCTGAGAGTTTTCCCAACA CCCGAGGACAGAGACAAAAT
Ssu14.18	CA_{19}	TTGCACTACTCAAAAAGTGATGTC ACAGTCCGCAAGCAAATTG

Table IV Repeat structure of the nine microsatellite loci cloned from meerkats used in analysis with forward and reverse primer sequences.