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The evolutionary genetics of meerkats

(*Suricata suricatta*)

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The University of Edinburgh

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Declaration

I declare that the work described in this thesis has been carried out by myself unless otherwise acknowledged. It is entirely of my own composition, the main text and bibliography do not exceed 70,000 words, and it has not, in whole or part, been submitted for any other degree.

Johanna F. Nielsen

December 2012

Dedication

Till Aaron Ibbotson
den mest förstående och tålmodiga människa jag känner
med all min kärlek

Abstract

Cooperative species have long been the focus of much research due to the 'special difficulty' cooperation poses to the theory of evolution via natural selection. Despite this long history of scientific interest we actually know relatively little about the evolutionary genetics of cooperative mammalian species, especially in the wild. In this study I use long-term data from the Kalahari Meerkat Project to investigate some aspects of the evolutionary genetics of meerkats (*Suricata suricatta*).

First, I reconstructed a genetically-validated pedigree of the Kalahari meerkat population. 1,494 meerkats (83% of the total known population) were genotyped at a panel of 18 highly variable microsatellite markers. This genetic data, in combination with phenotypic information and two different programs, COLONY2 and MASTERBAYES, was used to infer familial relationships. The resulting pedigree spanned seven generations and included 1,614 individuals of which 1,076 had both parents known. I conclude by discussing the particular merits of using COLONY2 to infer familial relationships for social animals such as meerkats.

Second, I investigated the extent of inbreeding and inbreeding depression in early life traits in the Kalahari meerkat population. In the pedigree, 44% of individuals have non-zero ($F>0$) inbreeding coefficients. Although I found more inbreeding in meerkats than initially expected, there were few cases of inbreeding between very close relatives. Nonetheless, even low to moderate inbreeding appeared to result in inbreeding depression for pup mass at emergence, hind-foot length, growth until independence, and juvenile survival. I also found some tentative evidence for a positive effect of the social environment in ameliorating the effects of inbreeding depression.

Third, I conducted a quantitative genetic analysis on mass, skull length, skull width, forearm length, and hind-foot length in up to five key meerkat life stage periods, while accounting for a number of fixed effects, including inbreeding coefficient. By attempting to apportion variance in these traits to a variety of sources I found that birth litter identity often explained much of the variance in morphological traits, although the magnitude of this effect appeared to decline with age. Furthermore, when birth litter was removed from models, the amount of variance explained by additive genetic effects tended to increase.

Finally, I conducted a quantitative genetic analysis on two measures of cooperative care and on adult mass. Fixed effects, including inbreeding and relatedness coefficients, were also examined, which revealed that inbred individuals contribute more to pup-feeding, and that helper-recipient relatedness was negatively associated with baby-sitting. I found low heritable variation for baby-sitting ($h^2 = 0.10$) and pup-feeding ($h^2 = 0.08$), and higher heritable variation for adult body mass ($h^2 = 0.19$). I also estimated the magnitude of non-genetic sources of variation in these traits and provide evidence for positive genetic correlations between baby-sitting and pup-feeding, and baby-sitting and adult mass.

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The supervision of Jinliang and a CASE partnership enabled me to maintain links with the Institute of Zoology (IoZ) in London, which has been beneficial to me in many ways and I would therefore like to thank the Zoological Society of London for providing me with this opportunity. I have relished the time I've spent back at the IoZ and would particularly like to thank **Bill Jordan**, who started as my boss but quickly grew to be a friend and who was always wonderfully supportive and kind - he is missed terribly.

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Papers arising from this thesis

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Nielsen, J. F., English, S., Goodall-Copestake, W. P., Wang, J., Walling, C. A., Bateman, A., Flower, T. P., Sutcliffe, R. L., Samson, J., Thavarajah, N. K., Kruuk, L. E. B., Clutton-Brock, T. H. & Pemberton, J. M. (2012). Inbreeding and inbreeding depression of early life traits in a cooperative mammal. *Molecular Ecology* **21**(11): 2788-2804.

Bateman, A. W., Ozgul, A., **Nielsen J. F**., Coulson, T. & Clutton-Brock, T. H. (2012). Social structure mediates environmental effects on group size regulation in an obligate cooperative breeder, *Suricata suricatta*. *Ecology* (in press).

Leclaire, S., **Nielsen, J. F**., Thavarajah N., Manser, M. & Clutton-Brock, T. H. (2012). Odour-based kin discrimination in the cooperatively breeding meerkat. *Animal Behaviour* (in press).

Madden, J. H., **Nielsen, J. F**. & Clutton-Brock, T. H. (2012). Do networks of social interactions reflect patterns of kinship? *Current Zoology* **58**(2): 319-328.

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Chapter 1

Introduction



The primary goal that arguably drives the vast majority of evolutionary biology is explaining biological diversity. Biological diversity occurs at different levels, including between individuals within a population, and the study of quantitative, or continuous, trait variation between individuals essentially forms the field of quantitative genetics. One of the major aims of quantitative genetics is to quantify the genetic basis of traits as this serves as an essential step in understanding their evolutionary potential. This thesis describes an analysis of sources of variation in, and associations, between a range of key phenotypic traits in a wild population of cooperatively breeding meerkats. In this introductory chapter I briefly summarise the fields of quantitative genetics in wild populations and cooperative breeding. In doing so I highlight the relative lack of information to date on genetic and non-genetic sources of variation in behavior in cooperative mammalian species and the potential value of this thesis in redressing this. I also give more detailed backgrounds on the principles underlying inbreeding, inbreeding depression and quantitative genetic methods of analysis, and describe the utility of genetically-validated pedigrees in addressing questions pertaining to these areas of research. I conclude by summarising my study species and field-based system and outlining the specific structure and aims of this thesis.

1.1 Evolutionary quantitative genetics of wild populations

The study of quantitative genetics in wild populations is currently a burgeoning field of research (Kruuk 2004, Wilson *et al.* 2010). In the past, with some notable exceptions (e.g. Smith & Dhondt 1980, van Noordwijk *et al.* 1980, Grant & Grant 2000, Keller *et al.* 2001), studies of the genetic basis of quantitative traits ('quantitative genetics') have typically focused on captive or laboratory populations, the findings from which have greatly advanced our understanding of many aspects of evolutionary biology, including elucidating the mechanisms and effects of inbreeding (Falconer & Mackay 1996, Charlesworth & Charlesworth 1999), and the quantitative genetic architecture and response to selection of many traits (Falconer & Mackay 1996, Lynch & Walsh 1998). Captive or laboratory-based populations do not, however, necessarily accurately reflect the true conditions experienced by wild populations of organisms. Organisms in the wild tend to experience more temporally and spatially heterogeneous biotic and abiotic environmental conditions and so are likely to be subject to different selective pressures than either captive or laboratory-based organisms (Kruuk & Hadfield 2007). Studying the evolutionary quantitative genetics of wild populations is not, however, without its challenges and the recent growth of research in this field has been facilitated by a number of factors including the availability of long-term, individual-based data from wild populations, molecular advances in pedigree construction, and the application of more sophisticated methods of statistical analysis (Kruuk 2004, Wilson *et al.* 2010).

Long-term, individual-based studies of wild populations are an extremely valuable resource. The investments required to establish and maintain long-term, individual-based studies of wild populations should not be underestimated, but they do produce a wealth of data, and it is due to the great foresight and dedication of certain scientists that there are now a handful of, primarily mammalian and avian, long-term field studies that are ongoing (Clutton-Brock & Sheldon 2010). Long-term data from wild populations are valuable in a number of regards. Amongst other things, long-term data provide greater sample sizes of individuals than point estimates from a population, which reduces the margin of error involved in any subsequent analyses.

Long-term studies also span multiple generations, yielding accurate measures of individuals' life-history traits, such as survival and lifetime reproductive success measures, which are key components of evolution by natural selection (Mousseau & Roff 1987). Individuals from long-term studies can also be measured for a trait more than once over their lifetimes, which can reveal the individual variation, and age-related and/or environmentally influenced changes, for any given trait (Reále *et al.* 1999, Wilson *et al.* 2005, Kruuk & Hadfield 2007). For example, longitudinal studies have been shown to reveal patterns of within-individual age-related declines that are otherwise missed in cross-sectional studies (Bérubé *et al.* 1999, Weladji *et al.* 2006, Nussey *et al.* 2011).

Taking into account the effect of the natural environment is particularly relevant given that one of the general themes to emerge from the long-term studies of wild populations has been an awareness of the fact that biotic and abiotic environmental conditions can have substantial impact on aspects of evolutionary processes (Kruuk & Hill 2008). For example, abiotic environmental conditions have been shown to modify the magnitude of genetic variance expressed (Brommer *et al.* 2008), the strength of natural and sexual selection (Cockburn *et al.* 2008), and the extent of inbreeding and inbreeding depression (Szulkin & Sheldon 2007, Szulkin & Sheldon 2008).

However, while the number of quantitative genetics analyses of wild populations has greatly advanced our understating of many evolutionary processes, previous studies have tended to focus on a relatively narrow taxonomic scope of organisms, with a heavy bias towards ungulates in passerine birds in temperate climates. In particular, there is a lack of research on cooperative-breeding species, which tend to occur in more variable environments (Jetz & Rubenstein 2010, Cockburn & Russell 2011). In this thesis I use data from a long-term study of a wild cooperative mammal, the meerkat (*Suricata suricatta*), to investigate the effect of inbreeding on, and the genetic basis of variation in, key phenotypic traits, including core aspects of cooperative behaviour. Although there is a vast amount of research investigating the behavioural ecology of cooperative species, there have been very few attempts to

investigate the extent and effect of inbreeding, or to quantify the genetic basis of any trait variation, for cooperative species. Determining the extent of inbreeding and the effect of inbreeding depression are key to understanding whether inbreeding is an important consideration in the evolution of inbreeding avoidance mechanisms and/or cooperation. Determining the genetic basis of any variation is also evolutionarily relevant as natural selection can only generate an evolutionary response if a trait is heritable.

1.2 Cooperative breeding

Cooperation is social behaviour in which the action of the cooperator increases the fitness of the receiver, often at the expense of the cooperator's own fitness. Cooperation has always posed a 'special difficulty' to the theory of evolution by natural selection (Darwin 1859) as it goes against Darwin's fundamental principle of the 'survival of the fittest' which implies that selfish and competitive strategies are favoured over evolutionary time (West *et al.* 2006, Foster 2008). Numerous theories have therefore been developed that seek to explain the evolution and maintenance of cooperation (Nowak 2006, West *et al.* 2007, Foster 2008).

One of the most prevailing explanations was originally proposed by W. D. Hamilton (Hamilton 1963, Hamilton 1964b). Hamilton's relatively simple rule states that altruistic behaviours will be favoured by natural selection when:

$$rb > c$$

where r is the genetic relatedness between the altruistic actor and the recipient, b is the reproductive benefit to the recipient, and c is the cost (in terms of lifetime reproduction) for the actor (Hamilton 1963, Hamilton 1964b). Hamilton's 'kin selection' rule therefore deals with the inclusive fitness of an organism by considering the sum of both its direct (i.e. offspring propagated directly) and indirect fitness (i.e. production of non-descendant kin) (Hamilton 1963, Hamilton 1964b). While the sterility and division of work and reproduction in social insects represent some of the best examples of Hamilton's rule (Hamilton 1964b), the importance of kin selection in vertebrate species has not always been conclusively supported (Clutton-Brock 2002, Griffin & West 2002, Griffin & West 2003).

Another mechanism which might explain cooperative behaviour is 'reciprocal altruism', where actors direct cooperative actions towards individuals with the expectation that the recipient will return the investment at a later stage (Trivers 1971). This 'tit-for-tat' mechanism has been shown to be important in human cooperation (Trivers 1971), but the requirement for recognition and memory means that it has been reported in relatively few other species (but see Koenig 1988,

Wilkinson 1988 for examples).

While cooperators may forgo their own reproduction in favour of helping to raise the offspring of other (typically dominant) individuals, group living can also confer direct benefits to all group members (Clutton-Brock 2002). 'Group augmentation' describes how increases in group size in turn increase the capacity of larger groups to catch, produce and/or defend food, detect and/or repel predators, disperse, raise young, and outcompete smaller groups, which thus facilitates the evolution and maintenance of cooperation (Clutton-Brock 2002). In many social animals, the costs of dispersal are high and individuals (especially females) may thus maximise their own fitness by remaining in their natal group. In this way they can benefit from the increased survival offered by group augmentation with the additional possibility of also increasing their chances of breeding by queuing for the dominant position.

Genetic relatedness is an important component of social animal systems. The 'monogamy hypothesis' states that monogamy was the most likely the ancestral state of cooperative breeding (Hughes *et al.* 2008, Cockburn 2010, Cornwallis *et al.* 2010, Lukas & Clutton-Brock 2012). In contemporary mammals, cooperative breeding is almost totally restricted to species where a single female virtually monopolizes reproduction and breeds with a single dominant male (Lukas & Clutton-Brock 2012). Furthermore, recent phylogenetic reconstruction has shown that transitions to cooperative breeding have been restricted to lineages characterized by monogamy (Cornwallis *et al.* 2010, Lukas & Clutton-Brock 2012). This suggests that relatively high levels of kinship between group members are a necessary condition for the evolution of cooperation (Hughes *et al.* 2008, Cockburn 2010, Cornwallis *et al.* 2010, Lukas & Clutton-Brock 2012). The importance of genetic relatedness in cooperation also has interesting potential implications for the effect of inbreeding on social organisms. While the consensus is that the negative consequences of inbreeding are thought to have selected for the evolution of inbreeding avoidance mechanisms, there is also an alternative suggestion that the increased kinship within social groups that results from inbreeding may in fact promote or maintain

cooperative behaviour in line with kin selection theory (Hamilton 1963, West *et al.* 2002, Griffin & West 2003, Rossiter *et al.* 2005).

Cooperation is found throughout the animal kingdom; in mammals, the specific attributes of cooperatively-breeding species include some degree of formation of stable groups of females, reproductive suppression (by dominants towards subordinates), cooperative care, and delayed dispersal from the natal group (Jennions & Macdonald 1994, Solomon & French 1997). Cooperative breeding has received a large amount of general attention from behavioural ecologists but there is also an increasing awareness of the need to consider between-individual variation, and in particular, to determine the genetic basis of this variation. There is thus an increasing interest in quantifying key genetic parameters. In particular, if altruism presents such a puzzle for evolutionary biology, is there genetic variation for altruistic behaviour? Furthermore, inbreeding can potentially have substantial effects on fitness and consequently studies on inbreeding and inbreeding depression have a long history of importance in evolutionary biology. Group-living species with high reproductive skew may potentially have high levels of inbreeding. While quantitative genetic analyses have been applied to a variety of non-cooperative systems, few have applied them to populations of cooperatively breeding organisms. Genetic analyses provide a very important means to understand cooperative behaviour for a number of reasons: they can help to elucidate whether there is heritable variation in cooperative behaviour, and to determine whether high levels of relatedness between potential mates leads either to the evolution of inbreeding avoidance or actually promotes the evolution of cooperation.

In this thesis I apply quantitative genetic analyses to a population of cooperatively-breeding mammals in an attempt to determine the genetic and non-genetic sources of variation in cooperative behaviours and the extent of inbreeding and inbreeding depression in meerkats.

1.3 Pedigree construction

In this thesis I am interested in determining the sources of variance in a variety of meerkat phenotypic traits. As such, I am primarily interested in determining the genetic basis of morphometric and behavioural traits, but also aim to establish to what extent both non-genetic sources of variance and inbreeding affect phenotypes. There are a variety of ways in which to address these questions and so I first outline my reasons for using a genetically-validated pedigree to do so.

Pedigrees are a valuable tool in quantitative genetics. Although many aspects of evolutionary biology can be investigated without them, multigenerational pedigrees are currently viewed as providing one of the most practical and accurate measures of relatedness and individual inbreeding coefficients (Pemberton 2008). Although pedigree, or family tree, construction is a relatively simple concept due to the fact that the process essentially only requires knowledge of the parentage of each individual in the population, accurately determining parentages in wild populations is not always straightforward (Pemberton 2008). Historically, parentage inference has been based on behavioural observations of, for example, birth, pregnancies, suckling, mate or harem-guarding behaviour, reproductive or dominance status, and territory or nest occupancy. However, whereas direct observations of (e.g. mammalian) births give conclusive maternities, accurately determining the paternity of an individual in almost any wild-based population is error prone, especially in systems where males provide no parental care, where extra-pair paternity occurs or where individuals live in groups or territories consisting of more than one reproductively mature male (Charmantier & Réale 2005, Walling *et al.* 2010). Furthermore, in species in which intraspecific brood parasitism occurs, or where opportunities for direct observations are limited (e.g. wild fish (Serbezov *et al.* 2010) and in species where multiple females produce young in a communal borrow or nest (McRae 1996, Griffin *et al.* 2003)), accurate maternity assignment may also be impossible from field data alone. In these cases, parentage inference is best determined using molecular methods, or more specifically, genotypes from polymorphic codominant markers, for example microsatellite loci, which often have many alleles per locus (Griffin *et al.* 2003,

Jones & Ardren 2003, Butler *et al.* 2004, Pemberton 2008, Jones *et al.* 2010). The concept of molecular parentage inference is relatively simple: as individuals inherit 50% of their alleles from each parent, at each of the loci of an offspring there must be one allele in common with each parent (in contrast to full siblings, which also have the same level of allele sharing but which can share no alleles at some loci).

Applying molecular parentage inference methods to wild populations is not without its challenges (Pemberton 2008): reagents, for example microsatellite primers for a number of highly polymorphic microsatellite loci, need to first be developed, and field-based records of candidate parents and tissue samples from both offspring and candidate parents need to be collected. Consequently, although numerous 'behavioural' wild pedigrees exist, in which parentage is based on behavioural observations, there are relatively few genetically-validated pedigrees for wild populations, for example red deer (*Cervus elaphus*) (Pemberton *et al.* 1999, Walling *et al.* 2010), song sparrows (*Melospiza melodia*) (Sardell *et al.* 2010), Soay (*Ovis aries*) (Overall *et al.* 2005) and bighorn (*Ovis canadensis*) (Coltman *et al.* 2005) sheep, and house sparrows (*Passer domesticus*) (Ockendon *et al.* 2009). Furthermore, due to the added challenges involved in genetically determining parentages in cooperative species, where intra-group relatedness is typically high (Thompson 1976, Thompson & Meagher 1987), there are currently very few genetically-validated pedigrees for wild populations of cooperatively breeding avian species (to my knowledge, Superb Fairywrens (*Malurus cyaneus*) (Double & Cockburn 2003), Seychelles warblers (*Acrocephalus sechellensis*) (Hadfield *et al.* 2006), and long-tailed tits (*Aegithalos caudatus*) (Sharp *et al.* 2008)). Genetically-validated pedigrees for cooperative mammals are currently lacking.

There are a number of statistical methods that can be used to infer parentage from microsatellite markers (Jones & Ardren 2003). While most previous studies have inferred parentages using a single program, research has revealed the benefits of using more than one program and a combinatorial approach to parentage inference (Walling *et al.* 2010). In this thesis, I combine parentage inference from two recently developed programmes, MASTERBAYES (Hadfield *et al.* 2006) and COLONY2

(Wang 2004, Wang & Santure 2009). The primary advantages of MASTERBAYES, is the incorporation of phenotypic information in the parentage inference process. This is particularly successful in species where, for example, territory occupancy and behavioural observations are useful predictors of paternity (Walling *et al.* 2010). In cooperative mammals, dominance status is typically a very strong predictor of reproductive success and is thus an example of a phenotypic descriptor that has the potential to inform the parentage inference process (Griffin *et al.* 2003, Hodge *et al.* 2008, Spong *et al.* 2008). Whereas MASTERBAYES incorporates both genetic and phenotypic information and utilises a Bayesian approach, COLONY2 uses solely genetic data and a more traditional likelihood-based method of parentage inference (Wang 2004, Wang & Santure 2009). The major advantage of COLONY2, however, is its ability to simultaneously assign not only parentage, but also full- and half-sibship relationships, which is particularly advantageous in wild populations where complete sampling of candidate parents is often impossible. As I show, the ability of COLONY2 to infer sibling relationships may be particularly beneficial in cooperatively-breeding species that are often characterized by strong reproductive skew, and consequently large full- and half-sibship groups (Griffin *et al.* 2003).

Here, I use both behavioural observations and genotype data from 18 variable microsatellites and both MASTERBAYES and COLONY2 in order to construct a genetically-validated pedigree. Chapter 2 provides a more detailed description of the methods used to construct the pedigree and further discusses the difficulties involved in genetically inferring parentages for a species that lives in groups consisting of highly related individuals. Below, I consider the use of this pedigree information to investigate the extent and implications of inbreeding, and the heritable genetic basis of variation in phenotypic traits.

1.4 Inbreeding

Inbreeding has important implications for conservation, agricultural and evolutionary biology research (Falconer & Mackay 1996, Hedrick & Kalinowski 2000, Keller & Waller 2002). The negative effects of inbreeding are of major concern in the management and conservation of endangered species, whereas inbreeding may in fact help to preserve desirable traits in agricultural settings (Falconer & Mackay 1996, Hedrick & Kalinowski 2000). Inbreeding is of evolutionary importance because of its potential impact on individual fitness and thus its ability to explain trait variation. Depending on patterns of migration, the strong reproductive skew that characterises facultatively cooperative-breeding species has the potential to lead to inbreeding. Inbreeding refers to the mating of two genetically related parents, which, due to the shared ancestry of the parents, has the effect of increasing the number of alleles that are identical by descent and thus the levels of homozygosity in their inbred offspring (Charlesworth & Charlesworth 1999) (Fig. 1.1).

Studying inbreeding in wild populations is important for a number of reasons: firstly, wild populations can reveal the levels of inbreeding in naturally occurring, rather than manipulated, populations and, secondly, because environmental conditions in the wild are generally more heterogeneous, the magnitude of inbreeding depression can vary more from that found in either captive or laboratory settings (Keller & Waller 2002).

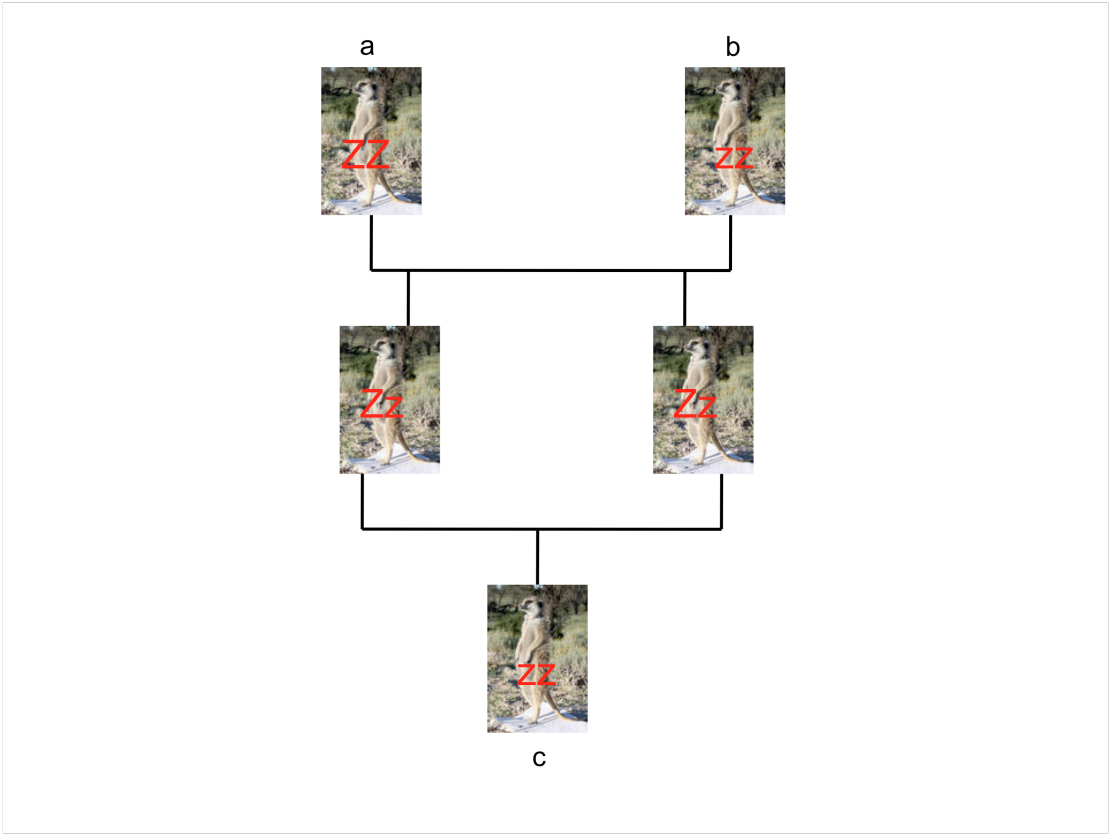

Figure 1.1 Pedigree of a full-sib inbreeding event showing how the inbred individual at the base of the pedigree is homozygous and has two (z) alleles that are identical by descent from individual b.

One of the most accurate methods of determining the level of individual inbreeding is to use a multigenerational pedigree from which inbreeding coefficients can be obtained (Pemberton 2008). The commonly used metric of inbreeding is Wright's coefficient of inbreeding (F), which expresses the probability that two alleles drawn at random are identical by descent (Wright 1921, Lynch & Walsh 1998). Pedigree based inbreeding coefficients are generally calculated via path analysis such that:

$$F=\sum[(0.5)^{n1+n2+1}(1+F_A)]$$

where F is the individual's inbreeding coefficient, F_A is the inbreeding coefficient of the common ancestor, $n1$ is the number of generations from the mother to the common ancestor, $n2$ is the number of generations from the father to the common ancestor, and $\sum$ sums all known individual-specific inbreeding loops (e.g. one loop

between individual a, b and c in fig. 1.1). While pedigree-derived *F*-values are commonly used to estimate individual inbreeding values, it is important to note that this method implicitly assumes that founders and immigrants are unrelated, which is unlikely to be true in most wild populations, and that the estimation of *F* is an expected, statistical value for an unspecified, hypothetical, perfectly Mendelian locus and not a true representation of the actual allelic homozygosity across the genome of an individual (Wright 1921). In the absence of a pedigree, some previous studies of wild populations have used individual, average observed heterozygosity at panels of microsatellite loci as indirect measures of inbreeding (Coltman *et al.* 1999, Slate *et al.* 2000). There is, however, increasing evidence to suggest that marker heterozygosities are not necessarily accurate measures of inbreeding (Balloux *et al.* 2004, Slate *et al.* 2004, Chapman *et al.* 2009, Szulkin *et al.* 2010, Szulkin & David 2011), and that pedigrees with as little as three to five generations can provide more accurate measures of individual levels of inbreeding (Balloux *et al.* 2004).

While the number of studies of inbreeding on wild populations is growing, most research has, until now, focused primarily on non-cooperative species (but see Hoogland 1992, Keane *et al.* 1996, McRae 1996, Brown & Brown 1998, Daniels & Walters 2000, Richardson *et al.* 2004, Spiering *et al.* 2011). There are, however, particular reasons why studying inbreeding in cooperative species is of interest. As cooperative species generally live in family groups with close relatives of the opposite sex in near proximity and tend to exhibit strong philopatry in at least one of the sexes, the traditional expectation is for strong inbreeding avoidance (Koenig & Haydock 2004). Alternatively, there may be valid reasons for tolerating inbreeding in certain circumstances where for, example, the opportunities for reproduction are low and the costs of producing inbred offspring do not outweigh the costs of discriminating between kin and non-kin (Waser *et al.* 1986, Kokko & Ots 2006). This may be especially relevant in cooperative species where there is also the suggestion that inbreeding may even promote or maintain cooperative behaviour due to the increased levels of intragroup kinship that result from inbreeding (Hamilton 1963, West *et al.* 2002, Griffin & West 2003, Rossiter *et al.* 2005). The ultimate evolutionary effects of inbreeding on cooperative species currently remain relatively

unexplored. In this thesis I use a genetically-validated pedigree to calculate Wright's inbreeding coefficients and describe the extent of inbreeding in a study population of meerkats.

1.5 Inbreeding depression

Inbreeding can lead to negative fitness consequences, or inbreeding depression, which is known to lower an individual's ability to survive and reproduce (and hence potentially a population's viability) and can explain some degree of trait variation (Falconer & Mackay 1996, Hedrick & Kalinowski 2000, Keller & Waller 2002). Inbreeding depression acts via one of two possible mechanisms: the partial recessive or overdominance mechanism (Charlesworth & Charlesworth 1999). In the partial recessive hypothesis, increased allelic homozygosity leads to the expression of deleterious recessive alleles in inbred offspring, whereas in the overdominance, or heterozygote advantage, mechanism inbred individuals have lower fitness because they are homozygous at loci at which heterozygotes are fitter (Charlesworth & Charlesworth 1999). Regardless of the specific mechanism responsible, inbreeding depression has been reported in a wide range of organisms and for a variety of traits (e.g. Keller 1998, Richardson *et al.* 2004, Szulkin *et al.* 2007, Brekke *et al.* 2010, Grueber *et al.* 2010, Walling *et al.* 2011).

While the vast majority of evidence for the negative fitness consequences of inbreeding comes from captive laboratory based studies, there are an increasing number of studies that have specifically investigated the effects of inbreeding on wild populations of organisms (see examples above and reviews by Crnokrak & Roff 1999, Keller & Waller 2002). Although strictly speaking, 'inbreeding depression' refers to a loss of fitness (i.e. in survival and/or reproduction), many studies investigate the effect of inbreeding on traits that are associated with fitness, such as weight and morphological measures (e.g. Coltman *et al.* 1998, Coulson *et al.* 1998, Slate & Pemberton 2002, Walling *et al.* 2011). This is particularly relevant in wild populations where accurate long-term measures of fitness are often difficult to collect. In this thesis I investigate the effect of inbreeding on a range of morphological traits in meerkats.

There is increasing evidence suggesting that the magnitude of inbreeding depression is actually greater in harsher environmental conditions (Fox & Reed 2011). While

research on the effects of the environment on inbreeding depression has primarily focused on abiotic ecological factors (Keller *et al.* 2002, Marr *et al.* 2006, Szulkin & Sheldon 2007, Walling *et al.* 2011), and environmental interactions may not be detected easily unless in extreme conditions, the social environment of cooperative species may also show similar interactive effects. One element of cooperative species is the provisioning of care and food to young (Jennions & Macdonald 1994, Solomon & French 1997). These cooperative behaviours essentially create a 'social environment', the relative importance of which may vary spatially (i.e. between groups) and temporally (i.e. change over the course of a focal individual's life). As a consequence, I hypothesise that the magnitude of inbreeding depression may vary with the social environment, and explore whether the magnitude of inbreeding depression changes depending on the composition of the social environment, and at different meerkat life stages.

To the best of my knowledge, the effect of inbreeding on cooperative behaviour remains unexplored. Contributions to cooperative behaviour are key components of the social environment and are thought to increase the fitness of the recipient offspring (Russell *et al.* 2002). Consequently, investigating the effects of inbreeding on contributions to cooperative behaviour is another potentially interesting line of research. While inbreeding has been shown to negatively affect individuals' morphometric and life-history traits, and to have negative consequences on certain behaviours such as courtship, competition and aggression (Sharp 1984, Aspi 2000, Ala-Honkola *et al.* 2009), inbreeding effects on cooperative care remain unexplored. In the absence of previous research it is difficult to predict the effect of inbreeding on cooperation: on one hand, inbreeding may act in a classical fashion whereby (less fit) inbred individuals contribute less cooperative care than outbred individuals. However, it is also possible that the lower fitness of inbred individuals restricts them to remaining as helpers rather than attempting to compete for reproductive opportunities, thereby creating a positive correlation between inbreeding and contributions to cooperative care. I discuss these alternative options on more detail in Chapter 5, in which I investigate the effect of inbreeding on two types of cooperative care in the meerkat study population.

1.6 Quantitative genetics

Chapters 4 and 5 of this thesis contain quantitative genetic analyses of a range of both behavioural and morphological meerkat phenotypic traits. Quantitative genetics is the study of quantitative, or continuously varying, traits, such as height and weight, or indeed inbreeding effects, which are influenced by genes at many loci as well as by the environment (Falconer & Mackay 1996, Lynch & Walsh 1998). This is in contrast to qualitative traits, such as eye colour or blood type, which divide individuals into distinct phenotypic categories and are typically governed by allelic differences at one or a few loci and have no environmental causation (Falconer & Mackay 1996, Lynch & Walsh 1998). Whereas the simple inheritance of qualitative traits means they display familiar Mendelian ratios, the effects of each individual locus involved in determining quantitative trait differences are assumed to be typically small in relation to the overall variation (Falconer & Mackay 1996, Lynch & Walsh 1998).

Much of the discipline of quantitative genetics focuses on explaining the variance of quantitative traits. For a given trait, the total phenotypic variance (V_P) will be comprised of some degree of genetic (V_G) and environmental (V_E) components (Falconer & Mackay 1996, Lynch & Walsh 1998):

$$V_P = V_G + V_E$$

where the genetic component of variance is the combined effect of all genetic effects and can be further decomposed into additive (V_A), dominance (V_D), and epistatic or interaction (V_I) genetic variance:

$$V_G = V_A + V_D + V_I$$

and where the environmental component of variance (V_E) can be further decomposed into, amongst others, parental, spatial (e.g. birth location), and temporal (e.g. birth year) effects, and residual (V_R) variance, depending on the nature of the study system. Residual variance accounts for the variance that remains unaccounted for by the other terms considered (Falconer & Mackay 1996, Lynch & Walsh 1998). The proportion of total phenotypic variance that is attributed to all genetic contributions, or the broad-sense heritability, is thus defined as (Falconer & Mackay 1996, Lynch

& Walsh 1998):

$$H^2 = V_G/V_P$$

However, because the variances attributed to both dominance and epistatic sources are difficult to estimate in non-experimental settings and are typically assumed to have negligible contributions to the total phenotypic variance, their effects tend to be accounted for in the residual component of variance (Wilson *et al.* 2010). The degree of resemblance, or narrow-sense heritability, is therefore defined as the proportion of total phenotypic variance of a trait that is explained simply by the additive genetic variance (Falconer & Mackay 1996, Lynch & Walsh 1998):

$$h^2 = V_A/V_P$$

Quantitative genetic analyses estimate not only the additive genetic component of variance but also other sources of non-genetic variance. The theoretical basis of quantitative genetics was formally synthesised during the early 20th century (Fisher 1918, Wright 1921, Haldane 1932), and because it has primarily been used to used to evaluate the extent to which traits have a genetic basis, the application of quantitative genetics has since played an important role in the fields of animal and plant breeding (Falconer & Mackay 1996, Lynch & Walsh 1998). Quantifying the genetic basis, or the heritability, of a trait involves estimating the additive genetic variance (see above) and is ultimately of interest as it enables predictions of the magnitude of evolutionary change in response to selection to be made (Falconer & Mackay 1996, Lynch & Walsh 1998, Kruuk 2004). While this is clearly of great interest to plant and animal breeders who seek to increase yields, applying quantitative genetics to wild populations of organisms is of growing interest (Kruuk 2004, Kruuk & Hadfield 2007). In addition to being able to estimate the genetic basis of traits, and therefore theoretically enabling predictions of the response to selection to be made, the approach used in modern quantitative genetic analyses can simultaneously provide estimates of the proportion of trait variance that is determined by non-genetic sources.

Determining the proportion of trait variance that is determined by non-genetic sources is particularly relevant in wild populations that are likely to experience

greater variation in biotic and abiotic environmental conditions than agricultural populations (Kruuk 2004, Kruuk & Hadfield 2007). For example, maternal or common environment effects can themselves be of intrinsic interest (Kruuk & Hadfield 2007). The amounts of variance explained by these sources are calculated in a similar manner to heritability, i.e. as a proportion of the total phenotypic variance (e.g. maternal effect: $m^2 = V_M/V_P$). In cooperative species, helpers can have important long-term fitness effects on the offspring they help to raise and, to some extent, replace the maternal effects that are commonly found to govern the fitness of non-cooperative vertebrates (Jennions & Macdonald 1994, Russell *et al.* 2002). The helping component of cooperative species therefore introduces an interesting element to quantitative genetic analyses as the social environment may account for a considerable proportion of trait variances. Thus, in addition to determining the additive genetic component of a variety of traits, I also investigate the degree to which the social environment explains variation in a range of meerkat traits.

While estimates of additive genetic variance can be calculated in a number of different ways (e.g. via selection experiments, parent-offspring regressions or sibling/twin comparisons) (Falconer & Mackay 1996, Lynch & Walsh 1998), one of the most precise and powerful ways to decompose phenotypic variance into different sources of variance in wild populations is to use a pedigree-based 'animal model' (Lynch & Walsh 1998, Kruuk 2004). Animal models are a type of mixed-effect model that are capable of accounting for both fixed and random terms and are particularly powerful because they exploit information from all types of relationships within complex, incomplete and often unbalanced pedigrees to generate estimates of additive genetic variance based on covariance in phenotype values between relatives (Kruuk 2004, Wilson *et al.* 2010). While the distinction between fixed and random effects is not always clear, fixed effects generally control for the variance explained by terms for which all possible levels are known, whereas random terms allow for the variance remaining, after controlling for the effects of fixed terms, to be partitioned into the specific components considered (Wilson 2008). Using a multivariate version of an animal model, it is also possible to estimate genetic covariances and correlations between traits. Genetic correlations occur when the

same genes, or genes linked on the same chromosome, influence different phenotypic traits meaning that selection on one trait will produce a correlated response in the other (Falconer & Mackay 1996, Lynch & Walsh 1998). In meerkats, mass is a strong predictor of dominance status, and therefore survival and reproductive success (Hodge *et al.* 2008, Spong *et al.* 2008). Thus, one theory is that, if mass is largely explained by additive genetic variance, smaller individuals may be 'genetically predisposed' to remain as subordinate helpers rather than obtain dominance. I thus consider genetic correlations in this thesis primarily in order to determine whether meerkat mass is negatively genetically correlated with greater contributions to cooperative care.

Applying traditional quantitative genetic frameworks to cooperative species introduces some unique challenges. Cooperative species tend to live in groups consisting of close relatives and consequently one potential problem is separating the effects of shared genes from shared environments (Moore *et al.* 1997, Wolf *et al.* 1998, Agrawal *et al.* 2001). While traditional quantitative genetic analyses have tended to partition causal influences of phenotypic expression into (direct) genetic and environment factors (Falconer & Mackay 1996, Lynch & Walsh 1998), there is a growing awareness of the fact that environmental elements can themselves be composed of indirect genetic factors (Moore *et al.* 1997, Wolf *et al.* 1998, Agrawal *et al.* 2001). In essence, indirect genetic effects (IGE) describe the influence of genes expressed by social conspecifics on the phenotype of the focal individual (Moore *et al.* 1997, Wolf *et al.* 1998, Agrawal *et al.* 2001), for example when a mother's genotype affects the maternal care she provides to her offspring. While IGEs have been considered in a variety of systems, they may be particularly relevant in cooperative species where the social environment can be an important component of trait expression, especially with regards to social interactions or cooperative care (Moore et al. 1997, Wolf et al. 1998, Bijma et al. 2007, Bijma & Wade 2008). Accounting for IGEs requires complex models and thus the analyses I present herein are best viewed as initial quantitative genetic analyses and a preliminary attempt to decompose the variation of meerkat traits into genetic and social environmental components.

Quantitative genetic analyses have been conducted on a range of different traits and, as a consequence, one general finding that has emerged is for life-history traits to exhibit lower levels of heritability than morphometric traits. An individual's fitness is determined by its performance across different fitness components, such as survival and breeding success, and natural selection acts more strongly and directly on these traits than any others (Mousseau & Roff 1987). Theory thus predicts that selection should deplete heritable genetic variance for fitness, so heritability estimates should be negatively correlated with a trait's association with fitness (Mousseau & Roff 1987, Kruuk *et al.* 2000). Life-history traits are also likely to be governed by more loci than morphometric traits and/or be explained by other (including residual) variance components (Rowe & Houle 1996). Accordingly, quantitative genetic studies on wild populations have tended to report higher heritability estimates for morphometric than life-history traits (Mousseau & Roff 1987, Kruuk *et al.* 2000).

While most quantitative genetic analyses of wild populations have tended to deal with life-history or morphometric traits, few have focused on cooperative behavioural traits. The genetic basis of some behaviour is already well documented in humans (Bouchard & McGue 2003) and an increasing number of studies on other species are providing evidence of a heritable basis for certain behaviours (see examples in Stirling et al., 2002 & Taylor et al., 2012). This includes evidence for significant heritability for traits such as boldness (Sinn *et al.* 2006), vigilance (Blumstein *et al.* 2010), and aggression and dispersal (Duckworth & Kruuk 2009) in wild populations of both invertebrates and vertebrates. In terms of care-giving behaviour, research has also revealed significant non-zero heritability estimates for the rate at which parents feed offspring in a long-tailed tits (*Aegithalos caudatus*) (MacColl & Hatchwell 2003) and Savannah sparrows (*Passerculus sandwichensis*) (Freeman-Gallant & Rothstein 1999). Although individual variation in cooperative behaviour has a strong condition-dependant element to it (Russell *et al.* 2003b), consistent among individual differences in contributions to cooperation could also have a genetic basis. While two studies have specifically investigated the genetic basis of cooperative behaviour (Charmantier *et al.* 2007, Cesarini *et al.* 2008),

research investigating the relative contribution of different sources of variation in cooperative behaviour for a wild mammalian species has yet to be explored.

Furthermore, whereas life-history traits are single measure traits, summarising either an individual's survival or lifetime reproductive success, morphometric traits deal with an individual's size and shape, which generally change with age (Wilson *et al.* 2005). Biotic and abiotic conditions also change throughout the life of an organism and consequently the selective pressures experienced by organisms may vary in strength and/or direction depending on ontogenic stage. Most traditional quantitative genetic analyses of morphological traits have averaged across ages, and while this provides a 'snapshot' of the genetic and non-genetic components of variance explaining the trait(s) of interest it does not reveal whether, or how, the components of variance change with age and selective pressures. Investigating the age-related changes in the variance components that explain morphometric traits in cooperative species is thus of potential interest and is something I touch on in this thesis.

Finally, there is a growing awareness of the importance of considering the effect of inbreeding in quantitative genetic analyses. One of the possible fixed effects that may be relevant when modelling a quantitative trait in populations experiencing inbreeding depression is an individual's inbreeding coefficient. In a simulation study, Reid and Keller (2010) showed that population structure might result in correlations in F between parents and offspring, and amongst half-siblings, and that – if inbreeding affects trait values – such correlations would inflate estimates of V_A and h^2 estimates if F is not accounted for. In addition to this growing awareness of the need to control for the effects of inbreeding in any quantitative genetic analysis (Reid & Keller 2010), inclusion of inbreeding coefficients as fixed effects in a quantitative genetics framework allows for the simultaneous estimation of inbreeding effects on the trait(s) considered, which are of interest in their own right. I therefore consider inbreeding coefficient as one of the possible variables that may affect the traits considered in this thesis.

1.7 Meerkats (*Suricata suricatta*)

This thesis considers the evolutionary genetics of a cooperatively-breeding mammal, the meerkat (*Suricata suricatta,* Schreber 1776), using data from a long-term study of a population in South Africa (Clutton-Brock *et al.* 1999a, Russell *et al.* 2002). Meerkats are small, diurnal, cooperatively breeding mongooses that inhabit the semi-arid Kalahari region of southern Africa (Doolan & Macdonald 1999). Meerkats live in groups, with each group consisting of a dominant pair and approximately 20 (but up to 50) male and female subordinate helpers, who are generally offspring from the dominants' previous breeding attempts or immigrant males (Clutton-Brock *et al.* 1999b, Doolan & Macdonald 1999). Meerkats are characterised by large dominant-biased skews in survival (Clutton-Brock *et al.* 2006, Sharp & Clutton-Brock 2010) and reproduction (Griffin *et al.* 2003, Spong *et al.* 2008), and large subordinate-biased skews in contributions to cooperative care (Clutton-Brock *et al.* 1998b). For example, dominant males and females monopolize 80-90% of the group's reproductive output (Griffin *et al.* 2003, Spong *et al.* 2008), but contribute comparatively little to the postal-natal raising of their pups (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999). While subordinates of both sex are physiologically capable of breeding, reproduction is much less frequent in both male and female subordinates, and subordinate males tend to secure only breeding opportunities by roving between, or immigrating into, different groups (Griffin *et al.* 2003, Young *et al.* 2007). Correspondingly, the strong female philopatry that characterises meerkat groups tends to restrict subordinate females to breeding with rover or immigrant subordinate males who tend to be less related than their natal group conspecifics (Griffin *et al.* 2003). Pups born to subordinate females are generally born into 'mixed litters' composed of other (subordinate or dominant) pups of the same age (M. Bell, *pers. comm.*), but are more commonly prevented from breeding by acts of aggression, eviction and infanticide by the dominant female (Clutton-Brock *et al.* 1998a).

Meerkats participate in a number of cooperative activities. The key contributions to cooperative care are baby-sitting, where helpers remain at the burrow with pups

while the rest of the group is away foraging (Clutton-Brock *et al.* 2000), and pup-feeding where helpers give a proportion of their food items to pups while foraging (Clutton-Brock *et al.* 2001a). Both baby-sitting and pup-feeding are accompanied by appreciable energetic costs to the helper: baby-sitters forgo feeding for up to 24 hours, which leads to significant weight loss (Clutton-Brock *et al.* 1998b, Clutton-Brock *et al.* 2000), and pup-feeders sacrifice their own forage items in favour of provisioning them to pups (Brotherton *et al.* 2001). Subordinate females can also spontaneously lactate to the dominant female's newborn pups – a behaviour termed allolactation (Scantlebury *et al.* 2002) – although, as suckling seldom occurs outside the burrow, little is known about the actual extent and duration of allolactation in meerkats. Both sexes also participate in cooperative 'raised guarding', in which individuals take turns acting as sentinels and watching out for predators whilst the other group members forage (Clutton-Brock *et al.* 1999c), and social digging where group members create and maintain the group's burrows and bolt holes (Clutton-Brock *et al.* 2004). Whilst raised guarding benefits the group as a whole, it is not strictly an altruistic behaviour as guarders have been shown to gain immediate benefits themselves (Clutton-Brock *et al.* 1999c). For example, sentinels are the often the first to take action to avoid threats from predators, and subordinate males are thought to gain information on neighbouring meerkat groups which may inform their decisions regarding roving and/or emigration (Clutton-Brock *et al.* 1999c).

There are a number of key life stages of a meerkat (Clutton-Brock *et al.* 1998a, Doolan & Macdonald 1999) that will be referred to throughout this thesis and so are documented here:

- Pup (<90 days): from birth through emergence from the natal burrow at approximately three weeks of age, and up until they reach nutritional independence at approximately three months of age. During these first three months, pups are primarily cared for by subordinates (aged ≥6 months) who baby-sit and allolactate them at the natal burrow (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999, Scantlebury *et al.* 2002), and who feed and teach them once they begin foraging with the group (Clutton-Brock *et al.* 2001b, Thornton & McAuliffe 2006).

- Juvenile (90 - 180 days): the three-month period from independence until six months of age during which time individuals forage solely for themselves and begin to contribute to cooperative behaviours.
- Sub-adult (180 – 365 days): from approximately six months of age meerkats steadily increase their contributions to cooperative care.
- Adult (>365 days): between eight and 11 months meerkats reach reproductive maturity and are considered adults at one year of age (Clutton-Brock *et al.* 2002). Competition for dominance acquisition and retention is strong: generally neither males nor females obtain dominant positions until after reaching adulthood and dominants are typically older and weigh more than their subordinate conspecifics (Hodge *et al.* 2008, Spong *et al.* 2008).

1.8 The Kalahari Meerkat Project

Almost all of the meerkat findings outlined above have resulted from research on the population of meerkats that form the Kalahari Meerkat Project. This long-term study was established in 1993 by Professor Tim Clutton-Brock and the University of Cambridge with the primary objective of studying the causes and consequences of cooperative behaviours in meerkats (Clutton-Brock *et al.* 1998a, Clutton-Brock *et al.* 1999b). The study site is located in the southern Kalahari Desert at the Kuruman River Reserve, Vanzylsrus, South Africa (26°58'S, 21°49'E) and spans a 3,500 ha site of uncultivated former ranch land (Fig. 1.2).



Figure 1.2 The Kalahari Meerkat Project study site (Vanzylsrus). Map courtesy of The Kalahari Meerkat Project.

All data in this study come from the long-term Kalahari Meerkat Project database which currently includes over 2,000 individuals that have been habituated to close observation (<1 m) and are identified with unique codes and subcutaneous transponder chips (Clutton-Brock *et al.* 2001a). At any one time at least eight

researchers at the Kalahari Meerkat Project collect behavioural and life-history data from up to 12 different meerkat groups up to three times a day and on average three to four times per week. Tissue samples are obtained from tail clippings, either collected from pups upon emergence from the natal burrow or from recently habituated, anaesthetised adults (Griffin *et al.* 2003, Spong *et al.* 2008).

Most meerkat maternities can be confidentially assigned based on behavioural data: consistent weight gain and visible abdominal distension over the 70-day gestation period is followed by abrupt weight loss post-parturition (Clutton-Brock *et al.* 1999b). Occasionally, however, the dominant female and one or more subordinate female(s) give birth to 'mixed litters' at similar times in the same burrow. As pups only emerge from the natal burrow approximately three weeks after birth, the maternity of these pups cannot be assigned based exclusively on behavioural data. As a result, the maternity of 'mixed litters' can only be confidently assigned using genetic data. Similarly, although dominant males monopolize the vast majority of reproduction, subordinate or roving males can also sire whole litters or part of mixed paternity litters (Griffin *et al.* 2001, Young *et al.* 2007) and thus accurate paternities can only be assigned genetically. The collection of further specific data required for the analyses herein are described in detail in the following chapters.

1.9 Scope of this thesis

This thesis details a study of the evolutionary quantitative genetics of the wild Kalahari Meerkat Project population. The general aims of this study are to construct a genetically-validated pedigree for the population in order to investigate the levels of inbreeding, extent of inbreeding depression, and levels of additive genetic variance and other components of variance for a number of meerkat morphometric and behavioural traits. An additional aim is to consider whether the magnitude of inbreeding depression and/or the components of variance in some traits change at different life stages. More specifically, in the following chapters I:

- Chapter 2: provide further details of the genetic markers and parentage assignment methods used to construct a pedigree for the study population, and of the resulting pedigree.
- Chapter 3: describe the extent of inbreeding in the population and investigate whether inbreeding affects a number of early life morphometric and survival traits.
- Chapter 4: investigate the sources of variation, including additive genetic variation, in a range of morphometric traits measured at different life stages. I also investigate whether these traits are influenced by inbreeding.
- Chapter 5: investigate the sources of variation, including additive genetic variation, in contributions to baby-sitting and pup-feeding and whether these cooperative behaviours are influenced by inbreeding and relatedness between participants.
- Chapter 6: synthesize the findings of this study, discuss their broader implications, and offer suggestions for future work.

Chapter 2

The Kalahari Meerkat Project pedigree



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JFN, LEBK, JMP and THC-B designed the study; SE provided the growth data; WPG-C optimised the microsatellite markers; JW helped with the pedigree reconstruction; CAW and AB provided help with the statistical analyses; TPF, RLS, JS and NT collected the field data, and THC-B established and continues to maintain the field site. JFN analysed the data and wrote the manuscript.

2.1 Abstract

A pedigree for the Kalahari Meerkat Project population was recovered using an efficient and reliable high throughput microsatellite genotyping protocol, which was designed by assessing and optimising previously isolated microsatellite markers. This 18-loci multiplex kit was used to genotype 1,494 meerkats (96% of the sampled population). Combining this genotypic data with additional phenotypic information, and by using two different programs (MASTERBAYES and COLONY2), familial relationships within the population were inferred. The resulting seven-generation deep pedigree links 1,614 individual meerkats to 1,536 maternities and 1,076 paternities with known identity and at high average individual-level confidences. The multiplex kit described herein summarises and optimises all previous genetic work relating to meerkats and is proven to be an efficient and reliable tool for use in determining familial relationships when implemented in MASTERBAYES and COLONY2. The particular merits of using COLONY2 to infer familial relationships for social animals such as meerkats are discussed.

2.2 Introduction

Pedigrees are a valuable tool for understanding the evolutionary dynamics of wild populations. The use of multigenerational pedigrees has a long history in animal and plant breeding, mainly driven by the desire to improve agricultural yields (Lynch & Walsh 1998). Increasingly, pedigrees of wild populations of organisms are being constructed, but in contrast to the agricultural environment these pedigrees are used to understand naturally occurring processes and/or predict evolutionary change (Pemberton 2008). Pedigrees for wild populations have now been constructed for a variety of different animal species. For predominantly monogamous pair-breeding animals, such as song sparrows (*Melospiza melodia*) (Keller 1998), collared flycatchers (*Ficedula albicollis*) (Kruuk *et al.* 2002), great tits (*Parus major*) (Szulkin & Sheldon 2007), and cooperatively breeding western bluebirds (*Sialia mexicana*) (Charmantier *et al.* 2007), pedigree construction has generally relied on social observational records, or in the case of humans (*Homo sapiens*), historical church records (Lummaa *et al.* 1998). Although these pedigrees by and large reflect the true familial structure of the population, they cannot accurately assign fathers to offspring born to extra pair paternity (EPP) events (Charmantier & Réale 2005). Furthermore, for many polygamous or group-living animals, observational data are often not informative enough to allow for accurate pedigrees to be constructed in this manner. Molecular analysis provides a powerful means to determine familial links for these populations and can correctly resolve parentage of offspring resulting from EPP events (Pemberton 2008). Genetic wild pedigrees have now been constructed for populations of red deer (*Cervus elaphus*) (Marshall *et al.* 1998, Walling *et al.* 2010), Soay sheep (*Ovis aries*) (Pemberton *et al.* 1999, Overall *et al.* 2005), bighorn sheep (*Ovis canadensis*) (Coltman *et al.* 2005) and song sparrows (Sardell *et al.* 2010). These pedigrees continue to contribute greatly to our understanding of a variety of different evolutionary processes in the wild.

The long-term Kalahari Meerkat Project (KMP) provides an outstanding opportunity to explore the evolutionary dynamics of a cooperative mammal. The KMP population of meerkats (*Suricata suricatta*) has been individually monitored since

1993 and research has resulted in a number of different behavioural, ecological and evolutionary research findings (e.g. Clutton-Brock *et al.* 2000, Manser & Avey 2000, Griffin *et al.* 2003, Carlson *et al.* 2006a, Thornton & McAuliffe 2006, Russell *et al.* 2007b, Spong *et al.* 2008). Recovery of a multi-generational pedigree for this population is required in order to develop an even broader field of research on the evolutionary dynamics of meerkats, including further studies on reproductive success and new studies on selective pressures, inbreeding depression and avoidance, the quantitative genetics of traits, and ultimately the evolution of cooperation (Pemberton 2008).

Re-constructing a pedigree requires knowledge of an individual's parents, which can be determined via behavioural observations, genetic data or a combination of the two. As with many other intensively studied populations, maternity of a single meerkat litter can usually be confidently determined via behavioural data (in meerkats consistent weight gain and visible abdominal distension over the 70-day gestation period is followed by abrupt weight loss post-parturition) (Clutton-Brock *et al.* 1999b). However, in contrast to most birds, where maternity of hatchlings is known from the maternal identity of the nest, meerkats communally share burrows and pups only emerge from their natal burrow approximately three weeks after birth. Litter maternity can therefore be uncertain when multiple females occupying a single burrow give birth at similar times and consequently pups from different maternal litters cannot be accurately differentiated once they emerge from the burrow (Clutton-Brock *et al.* 1999b). The candidate mothers of these 'mixed maternity' litters are generally known from pregnancy records (usually the dominant and between one and five subordinate females) and genetic information provides a means to accurately determine the maternity of each pup born to these multiple birth events.

In contrast to maternity, the paternity of pups cannot be determined based on behavioural data, as although dominant males have previously been shown to father the majority of litters in the population, roving and immigrant subordinate males can also gain some reproductive success (Griffin *et al.* 2001, Young *et al.* 2007). Furthermore, multiple males can share paternity of a single maternal litter (Griffin *et*

al. 2001). As a habituated but natural population, paternal links among Kalahari meerkats are most accurately determined using genetic information, such as genotypes from highly polymorphic microsatellite loci (Griffin *et al.* 2003, Jones & Ardren 2003, Butler *et al.* 2004, Pemberton 2008, Jones *et al.* 2010). This genetic information, in combination with behavioural observations, can then be analysed using one or more of the numerous software programs that have been developed to infer familial relationships and ultimately recover pedigrees (Blouin 2003, Jones & Ardren 2003, Butler *et al.* 2004, Berger-Wolf *et al.* 2007, Jones *et al.* 2010).

Determining parental links and ultimately re-constructing the pedigree for a population of social and cooperative mammals, such as meerkats, using genetic markers introduces a number of potential challenges. Typically a meerkat group consists of an unrelated dominant male and female, who are responsible for the majority of the group's breeding, and between two and 50 other subordinate individuals (Clutton-Brock *et al.* 1999a, Griffin *et al.* 2003). As most of the subordinates in the group are offspring of the dominant pair and dominants can produce up to 83 offspring throughout their life (Clutton-Brock *et al.* 1999b), the average relatedness among members of a group is relatively high (Griffin *et al.* 2003). Although the genetic relatedness between full sibling and parent-offspring pairs should in theory be equal ($r = 0.5$) (Lynch & Walsh 1998), with large offspring numbers it is statistically likely that siblings actually share more alleles in common than they do with their parents, an occurrence termed the 'sib paradox' (Thompson 1976, Thompson & Meagher 1987). Furthermore, meerkat groups are characterised by strong female philopatry (Stephens *et al.* 2005) and thus the 'sib paradox' may be especially relevant when determining the maternity of pups born to 'mixed maternity' litters as sisters of high genetic relatedness that remain in their natal group have the potential to complicate the genetically inferred maternities. As this study represents the first attempt to re-construct a pedigree for a population of meerkats it also provides an opportunity to investigate the influence of the 'sib paradox' in social mammals.

Microsatellite markers are highly informative for the parental inference process. Currently (until single nucleotide polymorphisms (SNPs) supersede them) microsatellites are the marker of choice when inferring familial relationships; they are ubiquitous throughout the eukaryotic genome, single-locus, codominantly inherited, have high mutation rates and can thus display high levels of allelic polymorphism and, when used in well-designed protocols, are fast, easy and reliable to score (Parker *et al.* 1998, Pemberton 2008). Microsatellite primers can either be developed by using cloning techniques (Queller *et al.* 1993), or by taking advantage of the fact that microsatellite flanking regions are often conserved and thus primers developed for one taxon may amplify across closely related species (Waldick *et al.* 2003).

Griffin and co-workers (2001) conducted the first molecular work on meerkats; nine loci were cloned directly from meerkat DNA and a further three loci were successfully cross-amplified using existing carnivora primer sets (Griffin *et al.* 2001). Spong *et al.* (2008) subsequently attempted to develop multiplex reactions for these 12 slightly modified primer sets (plus a further two cat loci). A set of 11 primer pairs has also been cloned from a closely related Herpestidae species, the banded mongoose (*Mungos mungo*), and six of these were reported to be variable in meerkats (Waldick *et al.* 2003). By optimising all these primer sets for their specific use in meerkats, an efficient and reliable high-throughput microsatellite multiplex genotyping protocol was designed by W. Goodall-Copestake (2006).

Since the widespread use of microsatellite markers, numerous software programs to infer the familial relationships of populations have been developed (see reviews of Blouin 2003, Jones & Ardren 2003, Jones *et al.* 2010). Likelihood-based, pair-wise, parentage assignment programs that rely solely on genetic data have historically been the most widely used and this method, implemented in the program CERVUS (Marshall *et al.* 1998), was previously used with the Kalahari meerkat data (Griffin *et al.* 2003, Spong *et al.* 2008). Two other programs, MASTERBAYES (Hadfield *et al.* 2006) and COLONY2 (Wang 2004, Wang & Santure 2009), have recently been shown

to recover a greater number of more accurate familial relationships than CERVUS, especially when their outputs are combined (Walling *et al.* 2010).

Rather than using likelihood-based methods and relying solely on genetic information, MASTERBAYES utilises a Bayesian approach to assign parentage and incorporates phenotypic predictors in the parentage inference process (Hadfield *et al.* 2006). As meerkat breeding is characterised by a strong reproductive skew in favour of the dominants of both sexes, with additional breeding mainly resulting from male roving attempts, dominance status and roving activity are likely to be useful phenotypic predictors of parentage (Griffin *et al.* 2003, Young *et al.* 2007, Spong *et al.* 2008).

For most natural, open populations, such as the Kalahari meekats, complete sampling is unrealistic. However, the pronounced reproductive skew, greater longevity of dominants versus subordinates (Sharp & Clutton-Brock 2010) and the documented patterns of migration (Clutton-Brock *et al.* 1999b, Stephens *et al.* 2005, Young *et al.* 2007) create a large number of full- and half-sib groups within meerkat groups (Griffin *et al.* 2003). In addition to assigning paternity and maternity, where possible COLONY2 simultaneously estimates full-sibships and maternal and paternal half-sibships (Wang 2004, Wang & Santure 2009). The recovery of sibships by COLONY2 therefore has the added advantage that sibships due to unsampled parents can be assigned to 'dummy' parents and these unsampled parents may be assigned a known parental identity if there are adequate behavioural observations available. This procedure can thus ameliorate the effects of unsampled breeders on the pedigree construction process. By taking advantage of the different benefits provided by two different parentage inference programs and combining their outputs, a multigenerational pedigree was constructed for the KMP population (Walling *et al.* 2010).

2.3 Materials and methods

2.3.1 Sample collection and DNA extraction

The collection of groups that form the KMP population are monitored regularly and, once habituated to humans, each meerkat is individually identified with hair dye markings (Thornton 2008). Tissue samples were collected from as many of the members of the population as possible between November 1993 and March 2009. The vast majority of samples consisted of a 2-5mm piece of tail-tip obtained from living habituated individuals, although some samples (from deceased individuals) consisted of whole pup paws or other miscellaneous body parts. Tail-tip samples were generally collected when pups first emerged from their natal burrows (at approximately three weeks of age) and were obtained simply by removing the tip of the tail using a pair of scissors and sealing the wound with a small drop of superglue. This procedure did not seem to adversely affect the meerkats: sampled individuals quickly returned to undisturbed behaviour once a sample was taken and none subsequently developed any (visible) tail infections (Spong *et al.* 2008). Some adult samples (e.g. from immigrant or recently habituated adults) were also obtained from anaesthetised meerkats. Once collected, tissue samples were immediately stored in microcentrifuge tubes containing either 100mM EDTA 95% ethanol or 10% DMSO in saturated salt and kept in a -20ºC freezer at the field site before being transported to Europe for DNA extraction and genotyping. The sample collection has resided in various labs since the project's inception including labs in the universities of Edinburgh, Cambridge and Uppsala, but is now held in Edinburgh and is where the I conducted the following molecular work. My lab work was greatly assisted by W. Goodall-Copestake who optimised the meerkat microsatellite maker set, S. Morrissey who helped me extract DNA and genotype a number of the samples, and E. Heap who independently checked all the genotypes.

The meerkat DNA extraction and microsatellite marker optimisation methods reported herein were developed by W. Goodall-Copestake, a research assistant at the University of Edinburgh and the following molecular methods provide a synopsis of his detailed protocols (Goodall-Copestake 2006). Genomic DNA was extracted from

the 2002 to 2009 tissue samples using a DNeasy Blood and Tissue Kit™ (QIAGEN) following slightly optimised manufacturer's instructions (Goodall-Copestake 2006). Samples that were collected prior to 2002 were extracted either using a DNeasy Blood and Tissue Kit™ (Goodall-Copestake 2006), standard chelex (Spong *et al.* 2008) or phenol-chloroform methods (Griffin *et al.* 2003, Spong *et al.* 2008). DNA quantity and quality values were obtained using a Nanodrop ND-100 spectrophotometer (Nanodrop Technologies) and visual assessment of extracts electrophoresed on 1% agarose gels stained with SafeView Nucleic Acid Stain (NBS Biologicals).

2.3.2 Microsatellite marker optimisation

All of the previously isolated meerkat (*n*=9; Griffin *et al.* 2001) and banded mongoose (*n*=11; Waldick *et al.* 2003) primers were selected for multiplex optimisation. In addition, a single dog (*Canis familiaris*), three cat (*Felix catta*) and one grey seal (*Halichoerus grypus*) loci that had previously been successfully amplified in meerkats (Griffin *et al.* 2001, Spong *et al.* 2008) were selected, resulting in an initial set of 25 potential loci. The selection of these microsatellite markers and the subsequent optimisation of them (as described below) were conducted by W. Goodall-Copestake (Goodall-Copestake 2006).

To assess the utility of these primers for multiplex PCR with meerkat DNA, they were tested for amplification using 20-50 ng of freshly extracted DNA from 11 Kalahari meerkats individuals, plus one H_2O sample as a negative control. A gradient of PCR annealing temperatures (45-65ºC), buffer concentrations (0.5X-2.0X), Mg^{++} concentrations (1.0-3.0 mM) and the impact of the additives bovine serum albumin (0.3 µg/µL) and formamide (2%) were assessed for each locus in uniplex PCR reaction conditions (0.3 µM primer, 0.2 mM dNTPs, 0.025 U/µL of BIOTAQ™ polymerase, Bioline). The amplification products were diluted using a range of different H_2O volumes to test for optimal final loading concentrations. 1 µL of optimally diluted PCR product was mixed with 9 µL of loading mix (consisting of 1 mL Hi-Di™ Formamide and 1µL of the internal size standard Applied Biosystems Incorporated (ABI) Genescan LIZ 500 ladder) and run on a capillary ABI 3730 DNA Analyzer. Fragment analysis for all genotypes was performed using the software

GeneMapper^{TM} version 4.0 (ABI). Primer pairs that only weakly amplified target microsatellite loci and/or that generated strong background products within the allele peak range (75-300 bp) were redesigned manually, in order to increase specificity and to complement the other loci in terms of annealing temperature and product size, such that multiplex panels of primers could be generated. Newly designed primers with similar optimal PCR conditions and overlapping base pair size ranges were labeled with different fluorescent labels. These redesigned primer pairs were then assessed using the procedure described above.

Those microsatellite loci that gave good quality amplifications, were sufficiently polymorphic and provided unambiguous scoring were selected for further multiplex-PCR trials. Different combinations of primer sets were tested on the same 11 individuals to achieve the maximum number of loci amplified in a single PCR. For all successful co-amplifications, primer concentrations were refined in order to have similar strength fluorescent profiles in the resulting electropherograms. Final multiplex PCR reactions consisted of the following; 20-50 ng of DNA, 1.5x NH_4 buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.07-0.41 µM of each primer (one of each pair fluorescently labeled with either ABI 6-FAM, VIC, NED or PET (ABI)), 0.0.25 U/µL of *Taq* polymerase and double processed tissue culture distilled H_2O (Sigma) to bring the volume up to 10 µL. The microsatellite cycling protocols involved an initial activation step of 95ºC for 3 minutes, a three step cycling consisting of a denaturing step of 94ºC for 30 seconds, annealing at 58 or 60ºC for 1 minute, ramping at 0.3ºC/second to an extension step of 72ºC for 1 minute. This cycle was repeated 29 times and was followed by a final extension of 60ºC for 15 minutes. Multiplex PCR products were diluted between 1:7 and 1:16 in H_2O and 1uL of this diluted product was mixed with 9 µL of loading mix before being run in a capillary ABI 3730 DNA Analyzer. Loci that were not amenable to multiplex PCR amplification were amplified in uniplex following slightly modified reaction and cycling conditions: $MgCl_2$ concentration increased to 2.5 mM, initial denaturation reduced to 1 minute and annealing temperatures variably reduced. These uniplex products were then diluted and mixed together to form a co-loaded set prior to being run in a capillary ABI 3730 DNA Analyzer.

On the basis of their good amplification, unambiguous allele scoring and sufficiently different allele size ranges, 18 of the 25 potential microsatellite loci chosen from the literature were used in the final multi–plex and co-loaded reactions. These included all nine of the meerkat, five of the total 11 banded mongoose and four additional carnivora primers. 13 of these loci sequences were modified from the original and are listed in Table 2.1 together with their general characteristics, multi–plex and co-loaded panel sets, final primer and $MgCl_2$ concentrations and product dilution factors used. In total, 13 of the loci were amplified in two tetraplex (*Ssu1* and *Ssu2*) and one pentaplex (*Mm1*) multiplex PCR reactions. The other five primer pairs (*FCA77, Ssu14.18, AHT130. FCA045* and *HG810*) were amplified in individual reactions before being combined in a single co-loaded set (*OB*) prior to peak size detection (Table 2.1).

Table 2.1 Characteristics of the meerkat microsatellite kit summarised from Goodall-Copestake (2006). Panel = multiplex (*Ssu1, Ssu2* & *Mm1*) and co-loaded (*OB*) loci sets; references = [1]Griffin *et al.* (2001), [2]Waldick *et al.* (2003), [3]Menottii-Raymond & O'Brien (1995), [4]Holmes *et al.* (1995), [5]Allen *et al.* (1995); *Tm* = annealing temperature; PCR Product dilution factor = multiplex reactions are diluted in H_2O whereas co-loaded reactions are diluted with each other.

Panel	Locus (& modifications from original)	GenBank accession no.	Origin & reference	5'-3' primer sequences (incl. 5' fluorescent label)	*Tm* (°C)	Repeat motif (5'-3')	%GC	Primer conc. (µM)	$MgCl_2$ (mM)	PCR product dilution factor
Ssu1	Ssu11.12 (-6bp)	AF271118	Meerkat[1]	F: (FAM)-CAGGAAATTTTCATCCTGGTAG	58	(GT)	41	0.07	1.50	1:11
	–	–	–	R: CTAGCTTTATTTTTCTCTGTGGC	–	–	39	–	–	–
	Ssu12.1 (+27bp)	AF271119	Meerkat[1]	F: (NED)-TTGTTTAAGCCACCCAGTCTG	58	(AC)	48	0.09	1.50	–
	–	–	–	R: CATTAAACCAGGACTGTTGCC	–	–	48	–	–	–
	Ssu13.8 (+86bp)	AF271120	Meerkat[1]	F: (PET)-AGAACAGAAGTGCCTGAATGTG	58	(TC)(AC)	45	0.41	1.50	–
	–	–	–	R: GTCGTCATATCCTCCTCT	–	–	50	–	–	–
	Ssu13.9 (+135bp)	AF271121	Meerkat[1]	F: (FAM)-AACACACTTGAGGAATCTGACTC	58	(TG)	43	0.17	1.50	–
	–	–	–	R: TTGGATGCTTAACCGAGCTAC	–	–	48	–	–	–
Ssu2	Ssu14.14 (-33bp)	AF271122	Meerkat[1]	F: (NED)-ATTTGCTGAGAGTTTTCCCAAC	60	(AC)	41	0.21	1.50	1:7
	–	–	–	R: TTGCTTATACCAAGGAGCATTG	–	–	41	–	–	–
	Ssu10.4 (+7bp)	AF271117	Meerkat[1]	F: TTCTTTTCCCTGGAGGTAATG	60	(GT)	43	0.26	1.50	–
	–	–	–	R: (PET)-TAGCAGCAAATAGATTCATTGGG	–	–	39	–	–	–
	Ssu7.1 (+19bp)	AF271115	Meerkat[1]	F: (FAM)-ATCCCTTAATGCATAGGCACAC	60	(TC)(AC)	45	0.23	1.50	–
	–	–	–	R: CTGCTACTGTTTTCAAATATGC	–	–	36	–	–	–
	Ssu8.5	AF271116	Meerkat[1]	F: (VIC)-AAGTCAGGTGCTTAACTGACTGG	60	(CT)(CA)	48	0.20	1.50	–
	–	–	–	R: TGGAGTCACTCATTTGGTTTTG	–	–	41	–	–	–

Panel	Locus (& modifications from original)	GenBank accession no.	Origin & reference	5'-3' primer sequences (incl. 5' fluorescent label)	*Tm* (°C)	Repeat motif (5'-3')	%GC	Primer conc. (µM)	$MgCl_2$ (mM)	PCR product dilution factor
Mm1	MmAAAC (+5bp)*	AY142703	Banded mongoose[2]	F: TTTGCACTGACAACATGGAGC	58	(AAAC)	48	0.11	1.50	1:10
	–	AY142702	–	R: (FAM)-TAAACCAGACTAGAAAGTGGAGC	–	–	43	–	–	–
	Mm18.1 (+1bp)	AY155580	Banded mongoose[2]	F: (VIC)-GTTTGATTATATTGTATACCTGAAGCAC	58	(AC)	32	0.11	1.50	–
	–	–	–	R: CTATTTTCTCAGTATAGCAGAAGGTG	–	–	38	–	–	–
	Mm7.5 (+14bp)	AY142694	Banded mongoose[2]	F: (NED)-AGGCAGGAAATGAGATGCAGA	58	(GT)	48	0.16	1.50	–
	–	–	–	R: AGTTGCTAGATACATGACTCAGG	–	–	43	–	–	–
	Mm2.10 (+20bp)	AY142696	Banded mongoose[2]	F: (PET)-AAACTTACTGAGCTTCTCGTGTG	58	(GT)	43	0.15	1.50	–
	–	–	–	R: AAGTCCTCTCCTGCCAGATG	–	–	55	–	–	–
	Mm18.2 (+50bp)	AY142698	Banded mongoose[2]	F: (FAM)-TAGGATATGGAGGAATTGTTGCTG	58	(TG)	42	0.40	1.50	–
	–	–	–	R: CTTCTTAGAAATGTAGACTGTCATCC	–	–	38	–	–	–
OB	FCA77	AF130506	Cat[3]	F: (NED)-GGCACCTATAACTACCAGTGTGA	54	(TG)	48	0.40	2.50	1:16
	–	–	–	R: ATCTCTGGGGAAATAAATTTTGG	–	–	35	–	–	–
	Ssu14.18 (-1bp)	AF271123	Meerkat[1]	F: (VIC)-TTGCACTACTCAAAAAGTGATGTC	49	(CA)	38	0.40	2.50	1:8
	–	–	–	R: ACAGTCCGCAAGCAAATTGG	–	–	50	–	–	–
	AHT130	–	Dog[4]	F: (PET)-CCTCTCCTGGTAAGTGCTGC	51	–	60	0.40	2.50	1:3.2
	–	–	–	R: TGGAACACTGGTCCCCAG	–	–	61	–	–	–
	FCA045	AF130489	Cat[3]	F: TGAAGAAAAGAATCAGGCTGTG	49	(AC)	41	0.40	2.50	1:4
	–	–	–	R: (FAM)-GTATGAGCATCTCTGTGTTCGTG	–	–	48	–	–	–
	HG810	G02093	Grey seal[5]	F: AATTCTGAAGCAGCCCAAG	49	(AC)	47	0.40	2.50	1:4
	–	–	–	R:(VIC)-GAATTCTTTTCTAGCATAGGTTG	–	–	35	–	–	–

*The two *MmAAAC* GenBank sequences appear to refer to the same cloned fragment.

2.3.3 Genotyping and population genetics

Griffin *et al.* (2001) genotyped 228 KMP individuals using autoradiography techniques and the subsequent study by Spong *et al.* (2008) increased the population coverage to 792 individuals using an Applied Biosystems Incorporated (ABI) 377XL sequencer and GenoTyper analysis software. The current optimised meerkat genotyping protocol of Goodall-Copestake (2006) uses an ABI 3730 sequencer and the GeneMapper (v.4.0) analysis software as a detection platform. Unfortunately, as microsatellite alleles sizes can change between detection platforms (Pemberton 2008), the allele sizes of genotypes generated on the previous detection platforms were incompatible with the allele sizes more recently generated using an ABI 3730 sequencer and GeneMapper. As no consistent 'translation metric' could be developed to merge the data generated from the three platforms it was decided to re-genotype all available samples using the most recent detection platform (Goodall-Copestake 2006) in order for them to be compatible with the genotype profiles of newly collected samples.

All samples were genotyped following Goodall-Copestake (2006) (see 2.3.2). In order to ensure consistent genotyping, all troublesome genotypes with an ambiguous allele or failed amplification were re-genotyped in single PCR reactions and all individual genotypes were scored and independently checked at least twice with the help of a research technician, E. Heap.

The mean number of alleles per locus, allele frequencies, expected (H_E) and observed (H_O) heterozygosities, deviations from Hardy-Weinberg (H-W) equilibrium, the probability of excluding a parent at a locus when lacking information on either of the parents ($NE\text{-}1p$) and null allele frequency estimates were calculated in CERVUS v.3.0.3 (Marshall *et al.* 1998). Per locus genotyping error rates were manually calculated from the proportion of mismatching genotypes for any samples that were genotyped two or more times. These values provide an upper estimate of per locus error rates as generally only samples with ambiguous genotypes were re-genotyped and thus the repeat dataset consisted mainly of troublesome samples. Half of this error rate was attributed to allelic dropout (class I error) and

the other half to all other kinds of stochastic typing errors (class II error) (see Wang 2004 for a summary of typing error classes).

2.3.4 Parentage inference

Familial relationships within the Kalahari meerkat population were inferred using field observations (of birth dates and other life-history events), individual genotypes and two freely downloadable programs; MASTERBAYES v.2.47 (http://cran.r-project.org/) (Hadfield *et al.* 2006), which was implemented in R v.2.10.1 (R Development Core Team 2009) and COLONY2 v.2.0.1.1 (http://www.zsl.org/science/research/software/colony,1154,AR.html) (Wang 2004, Wang & Santure 2009). All individuals genotyped at any number of loci were included in the pedigree recovery analyses as both programs can make use of incomplete information.

2.3.4.1 Offspring Model

The KMP population was analysed as two separate datasets; the majority of individuals were born into the population during the study period and thus had known birth dates and full life-history data available for them. These individuals were analysed in the 'Offspring Model' by both MASTERBAYES and COLONY2. The remaining meerkats were either founding members of the studied groups or had immigrated into them from surrounding unstudied groups as adults. As their early life-history data was unknown, the likely birth year for these meerkats could only be estimated and no candidate mothers or fathers could accurately be determined for them. These individuals were therefore analysed in a separate 'Founders and Immigrants Model' (see 2.3.4.2).

MASTERBAYES

In order to optimise the computing time required to run the MASTERBAYES model, analyses were run on a dual Xeon 3GHz Linux computer with 1Gb RAM. The final annotated R code (R Development Core Team 2009) used to run the MASTERBAYES model (summarised below) is found in Appendix 1.

MASTERBAYES genotypic data

A comma-separated value (.csv) file of the entire genotype dataset was provided to MASTERBAYES for inclusion in the analysis. Estimated genotyping error rates were used to set per locus error rates individually and were apportioned evenly between class I and II causes of error.

MASTERBAYES phenotypic data

All individuals in the KMP population were included in the MASTERBAYES Offspring Model with each individual attributed an identifier as to whether or not it was an offspring (1), and thus required parental assignment, or a potential parent (0) in any given month throughout the entire duration of the project. Individuals that were born into the population were identified as offspring in the month of their birth and were thus accessible for parentage assignment. For each subsequent month up until their death an individual was coded with a 0 to allow them to be available in subsequent parentage assignments, although individuals less than six months of age were restricted from being assigned as parents (see below). Founders or individuals that had immigrated into the population were never identified as an offspring in this model and were thus not assigned parentage (although they were available as potential parents). Phenotypic information including the sex (Female/Male/unkown) and group which an individual was a member of (*groupref*; -1 (unknown) to 68) was also provided for each month it was known to be alive. Additionally, monthly records of an individual's subordinate/dominance status (*dominant*; 0/1), the gestational status of females (i.e. whether she was recorded as either being pregnant or had given birth) (*gavebirth*; 0/1), or whether a male was known to have roved (*roved*; 0/1) were provided. All the above phenotypic data was summarised in a comma-separated value (.csv) file and provided to MASTERBAYES for inclusion in the analysis

MASTERBAYES program parameters and settings

The following restrictions were applied in the MASTERBAYES analysis in order to limit candidate mothers to females that: i.) were at least six months of age (to prevent 'sib paradox' effects of true litter mates being mis-assigned parentage) and ii.)

members of the same natal group as the pup(s) in the three months leading up to (and including) a pup's birth. Further phenotypic variables provided were whether a female: iii.) was dominant and iv.) had given birth or was observed as being pregnant in the three months leading up to (and including) a pup's birth. There is likely to be a relationship between these data and the pedigree structure and they are thus predicted to increase the statistical power in the pedigree reconstruction.

Dominance status and roving activity are likely to be significant predictors of male breeding success and these were therefore included as phenotypic data in the MASTERBAYES model: i.) as meerkats become sexually mature at around one year of age (but seldom breed before they are at least two years old) (Spong *et al.* 2008) candidate males were restricted to those older than six months of age at the time of a pup's birth (to prevent 'sib paradox' effects of true litter mates being mis-assigned parentage), and whether a male ii.) was dominant, iii.) had been recorded as roving and iv.) what group(s) it was recorded as member of in the three months leading up to (and including) a pup's birth (i.e. spanning the pup's date of conception) (Clutton-Brock *et al.* 1999b) were also included in the model.

The Markov chain Monte Carlo (MCMC) estimation chain was run for 130,000 iterations, with a burn-in of 30,000 iterations and a thinning interval of 50 iterations. No further prior distributions were specified and default improper uniform priors were used (Hadfield *et al.* 2006). Samples of the posterior probability distributions for the pedigree and phenotypic predictors of parentage (*dominant*, *groupref, roved* and *gavebirth*) were returned. Confidence in an individual assignment was calculated as the proportion of iterations for which an individual was assigned parentage of a particular offspring. A categorical pedigree was generated from this model in which only assignments with at least 80% individual-level confidence were considered in the final combination of program results.

COLONY2

In order to run the COLONY2 offspring dataset in a single analysis and to optimise the computing time required to do so, the COLONY2 Offspring Model was run on a

server with 2 Zeon X5667 Quad-core CPUs, running Windows 2003. Although all data and parameter settings were provided to COLONY2 via a Graphical User Interface (GUI), a text (.txt) file of the COLONY2.dat parameters used to run this model (summarised below) is found in Appendix 2.

COLONY2 data

Unlike MASTERBAYES, COLONY2 does not use any phenotypic descriptors in the parentage assignment process and thus only genotyped offspring and putative parents were included in the COLONY2 model; a .txt file of the entire genotype dataset was provided in the *'Offspring Genotype'* input tab. In order for COLONY2 to make full use of the genetic data by using information contained in sibships, behavioural (known) maternities of single litters were assumed correct and specified as such in the *'Known MaternalSibs'* input tab. Candidate mothers of pups born to 'mixed-maternity' litters were not given in the *'Excluded Maternity'* input tab in COLONY2, whereas all other female genotypes were.

As all paternities are unknown, no paternal sibships were specified in the *'Known PaternalSibs'* input tab. Unlike for MASTERBAYES, where only individuals older than six months were available as potential parents to avoid 'sib paradox' effects, preliminary analyses in COLONY2 did not appear to erroneously assign siblings as parents. Thus, COLONY2 candidate fathers were defined as any known male alive in any group of the population during a two week 'conception window' (between 63 and 77 days prior to the birth of the pup) (Clutton-Brock *et al.* 1999b). Each pup born during the study period therefore had between 2 and 642 (mean = 100) known candidate fathers. Similarly for pups born to 'mixed-maternity' litters, for each offspring all genotyped males except for their candidate fathers were given in the *'Excluded Paternity'* settings of COLONY2.

COLONY2 program parameters and settings

The probability of the true mother and father being in the female and male candidate lists was set at 0.75 in both the *'Female Genotypes'* and *'Male Genotypes'* input tab and no maternal or paternal sibships were excluded (*'Exc. MaternalSibs'* and *'Exc.*

PaternalSibs' input). Both 'Female' and 'Male Mating Systems' were defined as polygamous, whereas default settings were used in all the other *'Parameters'* input tab options. As for the MASTERBAYES model, a .txt file of the estimated genotyping error rates were used to set per locus error rates individually and were apportioned evenly between class I and II causes of error. The allele frequencies calculated in CERVUS v.3.0.3 (Marshall *et al.* 1998) were provided as a .txt file in the *'Markers'* input tab. Only parentage assignments with at least 80% individual-level confidence were considered in the final combination of program results.

Combining program results

Program results

Upon completion of analysis, MASTERBAYES returns a single parentage list giving the most likely maternity and paternity (and the associated probabilities) for each offspring based on the male and female genotypic and phenotypic data provided. COLONY2 also returns a list of the most likely *Maternity* and *Paternity* (and the associated individual-level probabilities) for each offspring based on the genotyped candidate parents and siblings provided. Additionally, COLONY2 also provided a Best Maximum Likelihood Configuration (*Best(ML) Config*) for each offspring. This output lists the most likely maternity and paternity of each individual but furthermore summarises the information gained from any recovered sibships in order to report any offspring assigned to unsampled 'dummy' parents. From extensive simulation studies, the *Best(ML) Config* has been shown to give the best parentage inference (Wang 2004, Wang & Santure 2009) and these parentage inference results were thus used in conjunction with the >80% MASTERBAYES parentages results to determine final parental assignments. In order to obtain threshold probabilities associated with any genotyped parents inferred by the *Best(ML) Config*, COLONY2 *Maternity* and *Paternity* results (>80%) were also referred to so that only consensus COLONY2 *Best(ML) Config* parentages assigned with at least 80% individual-level confidence were used. As individual-level confidences were not available for unsampled 'dummy' parents from the *Best(ML) Config* inferences, these parental links were only accepted if they met a number of further criteria (see Tables 2.2 & 2.3 and associated rules).

General rules

One of the distinguishing differences in the data provided to the two programs is that all behavioural (known) maternities are accepted as true in COLONY2 whereas all mothers are treated as unknown in MASTERBAYES. All behavioural maternities were also assumed correct in the final pedigree although any discrepancies between the behavioural and MASTERBAYES inferred maternities (and the MASTERBAYES and COLONY2 paternities) were investigated further as they have the power to identify potential sampling, labeling or genotyping errors.

When the parentage was unknown and there were discrepancies in the parents assigned to pups by the two programs, COLONY2 assignments were used, but only if the same parent was assigned in both the *Best(ML) Config* and the *Maternity* and *Paternity* results (>80%). Preference for COLONY2 inferences was based on the fact that COLONY2 inferred more parents than MASTERBAYES and accurately determined more correct maternities in trial runs where maternal identity was known but this information was not provided to either program. Furthermore, when certain genotyped fathers were excluded from trial COLONY2 runs, the inferred genotype of the unsampled 'dummy' fathers assigned to the sibship in question matched the genotype of the assigned (true) father in subsequent analyses when all genotyped candidate fathers were included.

MASTERBAYES assignments were, however, additionally informative in the few instances where it appeared that COLONY2 had either split true half-sib groups, assigning some the correct genotyped parent and some as half-sibs to an unsampled 'dummy' parent, or taken complete half-sib groups but failed to confidently assign the correct genotyped parent (Walling *et al.* 2010). In these cases MASTERBAYES assignments, used in conjunction with detailed comparisons of the true and the COLONY2 inferred 'dummy' parent genotypes and re-analysis of these sib ships, resulted in the final assignment of the sampled parent. The parentage assignments from the two programs were compared and summarised following Walling *et al.* 2010; matching assignments between the programs were split into those where both programs assigned the same parent, assigned no parent or the parents assigned were

different.

Maternity rules

The following specific rules were used when combining the maternity inferences from the MASTERBAYES and COLONY2 Offspring Models (an example of each rule is given in Table 2.2). If an offspring did not have a behavioural (known) maternity (or had more than one candidate mother) and:

I. neither MASTERBAYES or the COLONY2 *Best(ML)* results recovered a maternity then **no mother was assigned**
II. only MASTERBAYES recovered a maternity then **no mother was assigned**
III. only the COLONY2 *Best(ML) Config* results recovered a maternity then the **COLONY2 mother was assigned**
IV. the MASTERBAYES and COLONY2 *Best(ML) Config* results recovered the same maternity then the **MASTERBAYES/COLONY2 mother was assigned**
V. the MASTERBAYES and COLONY2 *Best(ML) Config* results conflicted then the **COLONY2 mother was assigned**

When the true mother was not sampled or was ungenotyped COLONY2 could not directly assign it to its offspring. In these cases and where individuals were recovered in a sibship by the COLONY2 *Best(ML) Config* results a 'dummy' maternal identity was assigned. If an offspring had more than one candidate mother and the other candidate(s) were genotyped (and therefore assignable) the 'dummy' maternal identity was attributed the ungenotyped candidate mother identity in the following cases. When:

VI. MASTERBAYES did not recover a maternity but the COLONY2 *Best(ML) Config* results assigned a 'dummy' maternal identity then the **ungenotyped candidate mother was assigned**
VII. MASTERBAYES recovered the ungenotyped mother (based on the phenotypic descriptors) and the COLONY2 *Best(ML) Config* assigned a 'dummy' maternal identity then the **ungenotyped candidate mother was assigned**
VIII. MASTERBAYES did not recover the unsampled female as mother but the COLONY2 *Best(ML) Config* assigned a 'dummy' maternal identity then the

ungenotyped candidate mother was assigned

Furthermore:

IX. if an offspring had more than one ungenotyped candidate mother and thus it was not obvious who the dummy' maternal identity could be attributed to then **no mother was assigned**

X. if an offspring did not have a behavioural maternity (or any candidate mothers) but was recovered in a full-sibship by the COLONY2 *Best(ML) Config* results then a **'dummy' maternal identity was assigned**

In all other cases where the behavioural mother of an offspring was known this maternal identity took precedence over all other inferences. When:

I. neither the MASTERBAYES or COLONY2 *Best(ML) Config* results recovered a maternity then the **behavioural mother was assigned**

II. MASTERBAYES recovered a different mother than the behavioural mother then the **behavioural mother was assigned**

III. only MASTERBAYES recovered the behavioural mother then the **MASTERBAYES/behavioural mother was assigned**

IV. MASTERBAYES did not recover the behavioural mother but the COLONY2 *Best(ML) Config* results did then the **COLONY2/behavioural mother was assigned**

V. MASTERBAYES and the COLONY2 *Best(ML) Config* results both recovered the behavioural mother then the **MASTERBAYES/ COLONY2/behavioural mother was assigned**

VI. MASTERBAYES recovered a different mother but the COLONY2 *Best(ML) Config* results recovered the behavioural maternity then the **COLONY2/behavioural mother was assigned**

VII. the behavioural mother was ungenotyped but correctly recovered by MASTERBAYES and all her offspring were recovered together in a full-sibship by the COLONY2 *Best(ML) Config* results. The COLONY2 *Best(ML) Config* 'dummy' was thus attributed the ungenotyped behavioural maternal identity and the **MASTERBAYES/behavioural mother was assigned**

VIII. the behavioural mother was ungenotyped and not recovered by MASTERBAYES but all her offspring were recovered together in a full-sib ship by the COLONY2 *Best(ML) Config* results. The COLONY2 *Best(ML) Config* 'dummy' was thus attributed the ungenotyped behavioural maternal identity and the **behavioural mother was assigned**

IX. the behavioural mother was ungenotyped but all her offspring were recovered together in a full-sib ship by the COLONY2 *Best(ML) Config* results. The COLONY2 *Best(ML) Config* 'dummy' was attributed the ungenotyped behavioural maternal identity in preference over MASTERBAYES, which recovered a different mother than the behavioural mother and thus the **behavioural mother was assigned**

Table 2.2 Maternity acceptance rules employed when combining the outputs from MASTERBAYES and COLONY2. A, B and C represent different mothers; – = unknown/unassigned mother; NG = not genotyped; Dummy ID = an unsampled 'dummy' maternal identity assigned to a sibship by the COLONY2 *Best(ML) Config* results. Criteria in bold represent cases where there is a discrepancy in the inferences between the two programs and/or the field data.

Maternity acceptance criteria	Behavioural maternity	MASTERBAYES maternity	COLONY2 *Best(ML) Config* maternity	Final assigned maternity
I.	–/A or B	–	–	–
II.	–/A or B	A or C	–	–
III.	–/A or B	–	A	A
IV.	–/A or B	A	A	A
V.	**–/A or B**	**B or C**	**A**	**A**
VI.	A(NG) or B	–	Dummy ID	A
VII.	A(NG) or B	A	Dummy ID	A
VIII.	**A(NG) or B**	**B or C**	**Dummy ID**	**A**
IX.	A(NG), B(NG) or C	– or A	Dummy ID	–
X.	–	–	Dummy ID	Dummy ID
XI.	A	–	–	–
XII.	A	B	–	A
XIII.	A	A	–	A
XIV.	A	–	A	A
XV.	A	A	A	A
XVI.	**A**	**B**	**A**	**A**
XVII.	A(NG)	A	Dummy ID	A
XVIII.	A(NG)	–	Dummy ID	A
XIX.	**A(NG)**	**B**	**Dummy ID**	**A**

Paternity rules

The following specific rules were used when combing the paternity inferences from the MASTERBAYES and COLONY2 Offspring Models (an example of each rule is given in Table 2.3):

I. when neither MASTERBAYES or the COLONY2 *Best(ML)* results recovered a paternity then **no father was assigned**
II. when only MASTERBAYES recovered a paternity then **no father was assigned**
III. when only the COLONY2 *Best(ML) Config* results recovered a paternity then the **COLONY2 father was assigned**
IV. when the MASTERBAYES and COLONY2 *Best(ML) Config* results recovered the same paternity then the **MASTERBAYES/COLONY2 father was assigned**
V. when the MASTERBAYES and COLONY2 *Best(ML) Config* results conflicted then the **COLONY2 father was assigned**

When the true father was not sampled or was ungenotyped COLONY2 could not directly assign it to its offspring. In these cases and where individuals were recovered in a sibship by the COLONY2 *Best(ML) Config* results a 'dummy' paternal identity was assigned. In some cases these 'dummy' paternal identities could be attributed to a known unsampled or ungenotyped candidate father by further investigation into the life histories of the candidate males. The steps in this process are outlined as follows:

1.) only offspring with at least one other maternal sibling in the sibship were considered and so **no father was assigned** to singleton paternal half-sibs
2.) If all members of the sibship were born to a group that had an unsampled or ungenotyped dominant male at the time of their conception than that **dominant male was assigned**
3.) If 2.) did not apply then the per individual candidate father lists were further restricted from all males known to be alive in any group during an offspring's conception window to all ungenotyped males either resident in, or known to have roved to, the offspring's natal group during the conception window. An ungenotyped **resident or roving male was assigned** if it was the only candidate available for all

members of the sibship

4.) If more than one ungenotyped male met the criteria of 3.) then the **'dummy' paternal identity was retained**

In summary, offspring recovered into sibships by the COLONY2 *Best(ML) Config* analysis could either be:

VI. **assigned an ungenotyped father** (based on criteria above) if MASTERBAYES did not recover a paternity

VII. **assigned an ungenotyped father** (based on the criteria above) if MASTERBAYES also recovered the same paternity

VIII. **assigned an ungenotyped father** (based on the criteria above) if MASTERBAYES recovered a different paternity

IX. **assigned a 'dummy' paternal identity** (based on the criteria above) if MASTERBAYES did not recover a paternity

X. **assigned a 'dummy' paternal identity** (based on the criteria above) if MASTERBAYES recovered a different paternity

XI. **not assigned a father** if they consisted of only singleton paternal half-sibs, even if MASTERBAYES recovered a paternity

Table 2.3 Paternity acceptance rules employed when combining the outputs from MASTERBAYES and COLONY2. A and B represent different fathers; – = unknown/unassigned father; NG = not genotyped; Dummy ID = an unsampled 'dummy' paternal identity assigned to a sibship by the COLONY2 *Best(ML) Config* results. Criteria in bold represent cases where there is a discrepancy in the inferences between the two programs and/or the field data.

Paternity acceptance criteria	MASTERBAYES paternity	COLONY2 *Best(ML) Config* paternity	Final assigned paternity
I.	–	–	–
II.	A	–	–
III.	–	A	A
IV.	A	A	A
V.	**B**	**A**	**A**
VI.	–	Dummy ID	A
VII.	A	Dummy ID	A
VIII.	**B**	**Dummy ID**	**A**
IX.	–	Dummy ID	Dummy ID
X.	**A**	**Dummy ID**	**Dummy ID**
XI.	**–/A**	**Dummy ID**	**–**

2.3.4.2 Founders and immigrants model

As the early life-history data that is required to construct candidate parent lists is unavailable for founders of or immigrants to the population, a second COLONY2 model was run in an attempt to see if any sibships could be recovered among these individuals without attempting to infer parentage. All genotyped individuals with no known (but estimated) birth dates were included.

COLONY2 data, program parameters and settings

The genotypes of the founders and immigrants were provided in the *'Offspring Genotype'* input tab and the same *'Parameters'* and *'Markers'* settings were used as for the COLONY2 Offspring Model but no further data was provided in the remaining input tabs.

Sibship acceptance and parental assignment rules

The *Best(ML) Config* results not only recover any sibships among individuals but also provide the most likely genotype of each 'dummy' parent to the sibships. It is possible that these 'dummy' parental identities could subsequently be attributed to an

identified individual by searching for a matching genotype in the entire genetic dataset. The Identity Analysis function of CERVUS v.3.0.3 (Marshall *et al.* 1998) was thus used to check whether any of the inferred 'dummy' parent genotypes of the founders and immigrants matched any of the genotypes in the entire genotypic dataset. The sexes were not tested separately and the minimum number of matching loci set to 15 with a maximum of three allelic mismatches between the genotypes. Additional analyses with variable numbers of matching loci and mismatching alleles did not significantly modify results.

As meerkats live in highly related groups and all founders and immigrants were included in the same analysis it is possible that the true parent was itself a founder or immigrant and was erroneously included in the sibship (see Thompson 1976 and Thompson & Meagher 1987 for a description of the 'sib paradox'). The resulting 'dummy' parents would therefore not accurately reflect the genotype of the true parents and consequently could not be found using the CERVUS Identity Analysis function. When it seemed possible that this applied (i.e. when a dominant or an individual with a greater than two year estimated age difference to it's other 'siblings' was included in the sibship) then a third COLONY2 analysis was conducted. In this analysis the genotype of the potential parent was given in the *'Female Genotypes'* and/or *'Male Genotypes'* input tab and the probability of the true mother and/or father being in the candidate lists was set at 0.50. All other sibling genotypes were provided in the *'Offspring Genotype'* input tab and the same *'Parameters'* and *'Markers'* settings were used as for the COLONY2 Offspring Model. Furthermore, the individual allele sizes between the likely parent and the other members of the sibship were compared by eye with at least 15 alleles having to match directly to that of the offspring with the remaining three loci only mismatching by one to two base pairs (depending on the size of the microsatellite repeat motif). This was to allow for the fact the most founders and immigrants were sampled early on in the project and are more likely to contain genotyping errors. Where more than one individual could potentially be the true parent (i.e. two individuals in the sibship had the same estimated age, which was at least two years greater than the other 'siblings') and either was as likely genetically to be the true parent then no parent was assigned.

2.4 Results

2.4.1 Sample collection and DNA extraction

Between November 1993 and March 2009 1,809 individuals have been studied as part of the KMP and 86% of these have been sampled for tissue (*n*=1,563; 584 females, 668 males and 311 unsexed) (Table 2.4). Genomic DNA extracted using a DNeasy Blood and Tissue Kit^{TM} (QIAGEN) yielded consistently good quality DNA (average 260/280 nm ratio=1.91) with average quantities of 30 ng/µL. Older DNA samples (pre-2002) that had been extracted using standard chelex or phenol-chloroform methods yielded similar quantities of DNA (44 ng/µL) but with slightly reduced quality values (average 260/280 nm ratio=2.05).

Table 2.4 Kalahari meerkat sample summary. Offspring = individuals born into the population; founders/immigrants = individuals that were either recorded as group founders or subsequently immigrated into the study population; total no. = total number of meerkats in the study population; sampled no. = number of individuals in the population that have had a tissue sample collected; genotyped no. = number of sampled individuals that have been genotyped; UNK = unsexed. Values in brackets indicate the number of individuals in each category that have behavioural (known) maternities.

Model	Total no.	Sex	Sampled no.	Sex	Genotyped no.	Sex
Offspring	1583	♀=564	1428	♀=538	1367	♀=513
	–	♂=617	–	♂=595	–	♂=564
	–	UNK=402	–	UNK=295	–	UNK=290
Founders/	226	♀=66	135	♀=46	127	♀=44
immigrants	–	♂=138	–	♂=73	–	♂=72
	–	UNK=22	–	UNK=16	–	UNK=11
Total	1809	♀=630 (476)	1563	♀=584 (457)	1494	♀=557 (433)
	(1381)	♂=755 (549)	(1252)	♂=668 (528)	(1195)	♂=636 (500)
	–	UNK=424 (356)	–	UNK=311 (267)	–	UNK=301 (262)

2.4.2 Genotyping and population genetics

A total of 1,494 individuals (96% of the sampled population; 557 females, 636 males and 301 unsexed) were genotyped at one or more locus (Table 2.4). On average 17 loci were genotyped per individual: 1,234 individuals (83% of the genotyped population) had complete genotypes for all 18 microsatellite loci and 1,454 (97% of

the genotyped population) had at least nine loci genotyped, demonstrating the efficiency of this multiplex kit for large-scale genotyping analyses (Table 2.3 & Fig. 2.1). Samples extracted using a DNeasy Blood and Tissue Kit™ (mainly post-2002 samples) yielded a greater number of individuals with more complete genetic profiles (Fig. 2.1). Older DNA samples (pre-2002) that had been extracted using either standard chelex or phenol-chloroform methods were more difficult to score when genotyping which resulted in less complete genetic data for these individuals (Fig. 2.1).

Individuals presented moderate levels of variability at the 18 microsatellite loci, with number of alleles per locus ranging from three (*MmAAAC*) to 15 (*Ssu8.5*), and a mean number of 9 alleles per locus (Table 2.5). Mean expected heterozygosity (H_E) and observed heterozygosity (H_O) was 0.700 and 0.697 respectively (Table 2.5). The combined inclusion probability of excluding a parent across all loci when lacking information on either of the parents (NE_1p) was 0.0005 (Table 2.5). Prior to Bonferroni correction, 13 loci showed deviations from H-W equilibrium with nine of these exhibiting significant heterozygote excess, although these deviations (H_E - H_O) were generally less than 0.020 (Table 2.5). The remaining four loci (*Ssu13.8*, *Mm7.5*, *Ssu14.18* and *HG810*) exhibit significant heterozygote deficit indicating the possible presence of null alleles, particularly *Ssu13.8*, which had a null allele estimated frequency of 0.0717 and, subsequently, a high estimated genotyping error rate of 8.00% (Table 2.5). Per locus genotyping error rates varied between 1.33% (*Mm18.2*) and 8.10% (*Ssu13.9*) with an average error rate of 3.43% (Table 2.5). As outlined in the methods, these error rate estimates are likely to be maxima, making parentage analyses conservative.

Table 2.5 Kalahari meerkat microsatellite marker and population genetics summary statistics. Population genetic values were obtained from an analysis of the entire genotype dataset (1,494 individuals) within CERVUS 3.0.3 (Marshall *et al.* 1998). Per locus genotyping error rates were calculated manually from repeat genotypes. H_E = expected heterozygosity; H_O = observed heterozygosity; H_E - H_O = deviation from Hardy-Weinberg equilibrium; NE_{-1P} = non-exclusion probability of excluding a parent when lacking information on either of the parents; estimated null allele frequency = inferred frequency of null alleles.

Locus	No. of individuals	Allele size range (bp)	No. of alleles	H_E	H_O	H_E - H_O	NE_{-1P}	Estimated null allele frequency	Estimated genotyping error rate
Ssu11.12	1465	109-127	9	0.779	0.791	-0.012***	0.583	-0.009	0.038
Ssu12.1	1442	161-183	11	0.820	0.829	-0.009***	0.526	-0.006	0.031
Ssu13.8	1449	213-239	11	0.720	0.620	0.100***	0.679	+0.072	0.080
Ssu13.9	1444	255-281	8	0.664	0.668	-0.004	0.771	-0.004	0.081
Ssu14.14	1468	72-88	9	0.798	0.817	-0.019***	0.572	-0.014	0.051
Ssu10.4	1454	120-147	11	0.774	0.783	-0.009*	0.609	-0.006	0.034
Ssu7.1	1464	156-177	9	0.811	0.817	-0.006***	0.549	-0.003	0.028
Ssu8.5	1434	205-240	15	0.890	0.902	-0.012***	0.363	-0.007	0.019
MmAAAC	1464	113-121	3	0.581	0.574	0.007	0.831	+0.006	0.028
Mm18.1	1460	140-164	4	0.263	0.267	-0.004	0.965	-0.010	0.022
Mm7.5	1445	180-223	12	0.638	0.619	0.019***	0.758	+0.014	0.060
Mm2.10	1455	170-194	6	0.678	0.693	-0.015	0.734	-0.013	0.022
Mm18.2	1438	251-275	8	0.731	0.751	-0.020**	0.685	-0.016	0.013
FCA77	1479	110-127	4	0.432	0.414	0.018	0.907	+0.020	0.018
Ssu14.18	1395	122-148	11	0.710	0.680	0.030***	0.694	+0.012	0.031
AHT130	1375	136-158	11	0.750	0.769	-0.019***	0.631	-0.016	0.017
FCA045	1390	159-181	10	0.792	0.796	-0.004***	0.576	-0.003	0.025
HG810	1429	193-233	13	0.763	0.754	0.009***	0.610	+0.001	0.021
Average	1442	–	9	0.700	0.697	0.003	–	–	0.034

*level of significance to which a locus deviates from Hardy-Weinberg equilibrium prior to Bonferroni correction: * = 0.05; ** = 0.01; *** = 0.005.

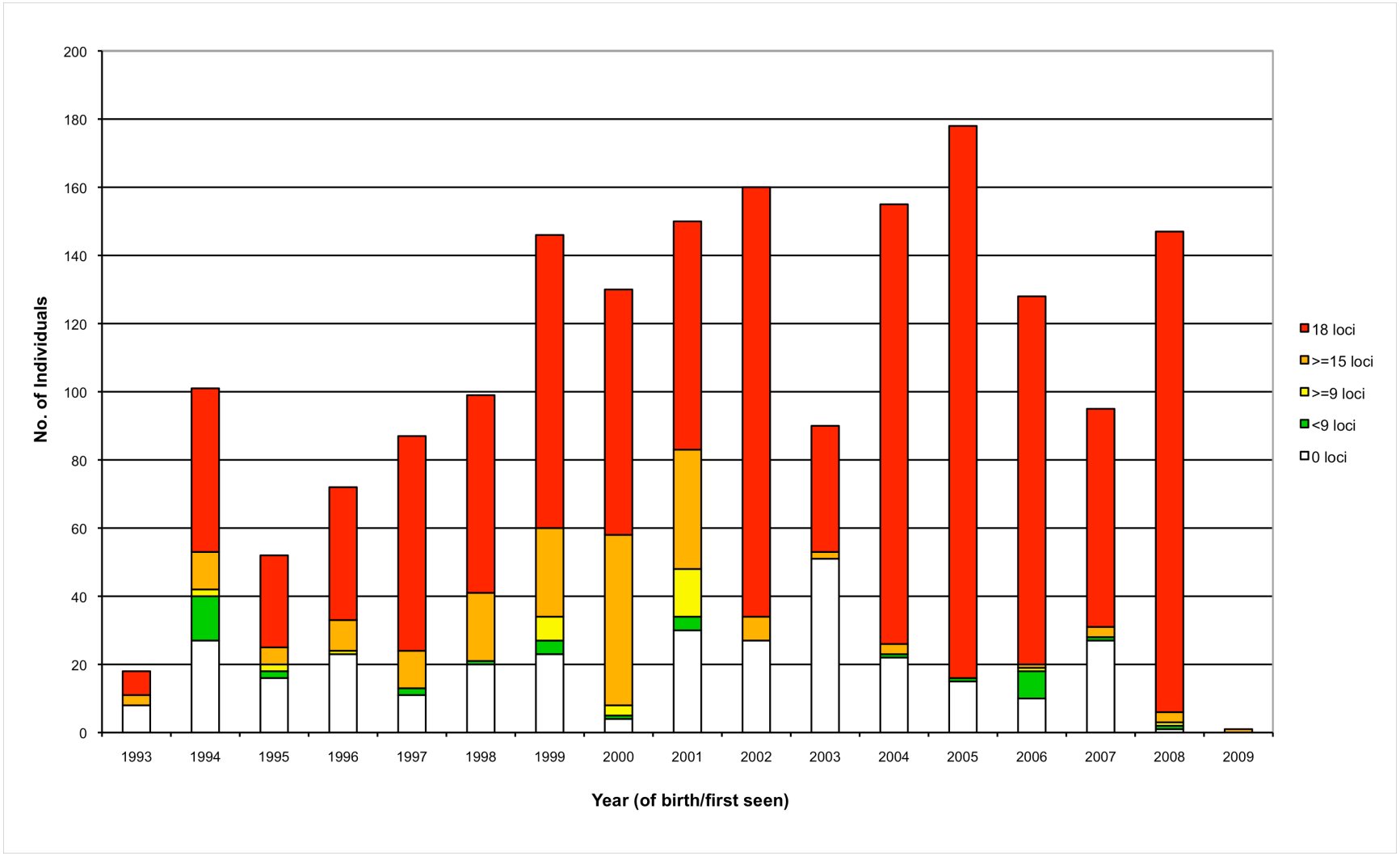


Figure 2.1 Summary of the total number of individuals in the population (1,809 individuals) and loci genotyped per year of birth (if born into the population) or year first seen (if a founder or immigrant). Most of the individuals with no loci genotyped (0 loci) were not sampled or were unavailable to this study.

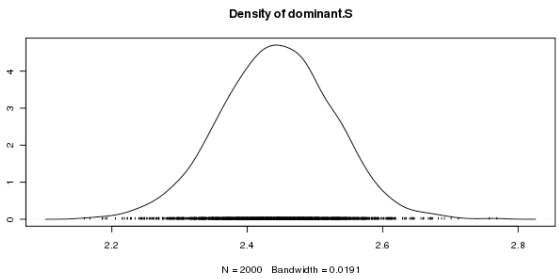
2.4.3 Parentage inference

2.4.3.1 Offspring model

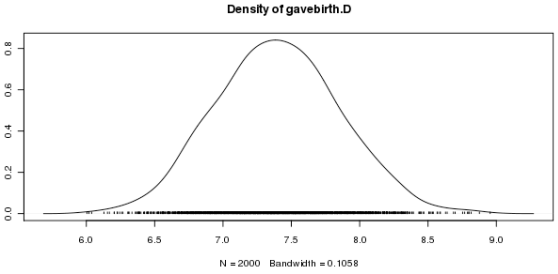
MASTERBAYES phenotypic predictors of parentage

Samples of the posterior probability distributions for all the phenotypic descriptors showed that although the dominance status (*dominant*) of both males and females in the three months leading up to (and including) a pup's birth was a significant predictor of parentage, the dominance status of males was a stronger predictor than females; dominant males were 9.78 to 13.46 times more likely to sire offspring than subordinate males, whereas dominant females were between 3.90 and 5.70 times more likely to mother offspring than subordinate females (Fig. 2.2*a.* & *b.*). Accordingly, group membership (*groupref*) was also a strong phenotypic predictor of paternal success; males that were members of the same group as the offspring in the three months leading up to (and including) a pup's birth were 29.67 to 40.85 times more likely to sire them than non-natal group males (Fig. 2.2*c.*). Consequently, the activity of male roving (*roving*) was a weak phenotypic predictor as males who had been recorded as having roved in the three months leading up to (and including) a pup's birth were as likely (0.62 - 1.11 times) to sire offspring than those that had not roved (Fig. 2.2*d.*). These results are substantiated by the fact that there were estimated to have been many more unsampled fathers than mothers (Fig. 2.3*a.* & *b.*). By far the strongest female phenotypic predictor of maternity was gestational status (*gavebirth*) (i.e. whether a female gave birth or was recorded as being pregnant in the three months leading up to (and including) a pup's birth); these females were 665.14 to 4188.09 times more likely to be assigned maternity than females that were neither recorded as having given birth or as being pregnant in the same time period (Fig. 2.2*e.*).

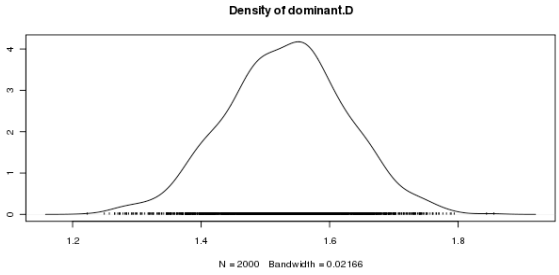
a.)



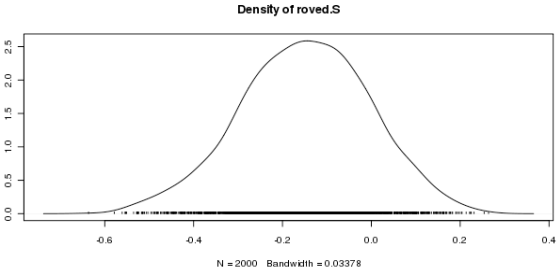
d.)



b.)



e.)



c.)

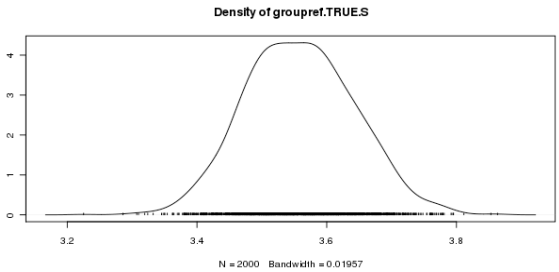
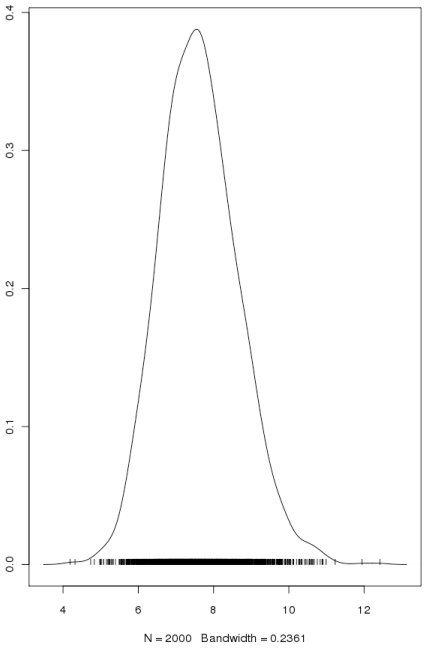


Figure 2.2 Samples of the posterior probability distributions for all MASTERBAYES phenotypic predictors of parentage. *a.*) male and *b.*) female dominance status (*dominant*); *c.*) male group membership (*groupref*) and *d.*) roving behaviour (*roved*), and *e.*) female gestational status (*gavebirth*). S = sire; D = dam.

a.)



b.)

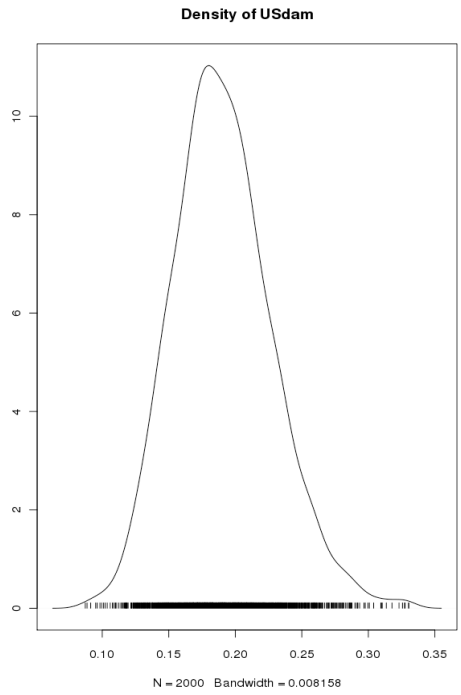


Figure 2.3 Samples of the posterior probability distributions of the MASTERBAYES estimated number of unsampled *a.*) fathers and *b.*) mothers. S = sire; D = dam.

MASTERBAYES parentages

1,381 (87%) of the total 1,583 offspring population of meerkats have behavioural (known) maternities and MASTERBAYES assigned a total of 1,043 of these. 71% (*n*=979) of these were assigned correctly and 90 of these inferences were based purely on the phenotypic descriptors (i.e. the offspring were ungenotyped). A further 4.6% (*n*=64) of the offspring with behavioural maternities were assigned a different mother by MASTERBAYES. As 60 of these had been genotyped (and were thus included in the COLONY2 model) they contributed significantly to the number of assignment discrepancies between the two programs (Table 2.6). In addition, MASTERBAYES assigned maternity to 60% (*n*=122) of the remaining 202 offspring either born to 'mixed-maternity' litters or for which maternity was unknown for other reasons (Table 2.7). Two of these maternal inferences were based purely on the phenotypic descriptors.

MASTERBAYES assigned 875 paternities to genotyped offspring (55% of the offspring population) but was unable to recover any paternities for ungenotyped offspring for which only phenotypic data were available (Table 2.7). 69 paternal inferences also conflicted with final COLONY2 inferences.

COLONY2 parentages

1,195 (87%) of the total 1,367 genotyped offspring have behavioural (known) maternities and 1,181 of these maternities were assumed correct by the COLONY2 offspring model and were thus assigned accordingly (Table 2.7). The remaining fourteen genotyped offspring had ungenotyped but known mothers. These offspring were recovered in sibships with their known maternal siblings and assigned a 'dummy' mother in the COLONY2 *Best(ML) Config* results, which was eventually attributed to the known, behavioural mother in the final pedigree (Table 2.7). In addition, COLONY2 assigned maternity to 84% (*n*=144) of the remaining 172 genotyped offspring with unknown mothers. A further 14 offspring lacking known mothers were recovered in *Best(ML) Config* sibships and assigned unsampled 'dummy' mothers, 11 of which were eventually attributed to an ungenotyped female as the other candidate mothers for the offspring had been genotyped (and were thus assignable if one of them was the true mother) (Table 2.7). The remaining three offspring retained the unsampled 'dummy' maternal ID as no suitable candidate mothers were available.

The COLONY2 Offspring Model assigned 882 paternities to identified males (Table 2.7). A further 395 offspring were recovered in *Best(ML) Config* sibships and assigned unsampled 'dummy' fathers, 66 of which were eventually attributed to an ungenotyped male following the criteria outlined in the methods. In a further 128 of these cases it appeared that COLONY2 had either split true half-sib groups and assigned some as half-sibs to an unsampled 'dummy' father, or created correct half-sib groups but failed to confidently assign the correct genotyped father. After detailed comparison of the true and the COLONY2 inferred 'dummy' parent genotypes and re-analysis of these sibships, the 'dummy' fathers of these offspring were also eventually attributed to an identified male. In total 1,277 offspring were

assigned a paternity, 1,076 of which were to an identified male. The remaining 201 offspring retained the unsampled 'dummy' paternal identity as no suitable candidate fathers were available (Table 2.7).

Table 2.6 Comparison of the parentage inference performance of MASTERBAYES and COLONY2. The programs were compared following Walling *et al.* 2010: matching assignments between the programs are split into those where both programs assigned the same parent (Match assigned) or where both programs assigned no parent (Match unassigned). Mismatches are split into cases where MASTERBAYES assigned a parent and COLONY2 did not (MASTERBAYES assigned), where COLONY2 assigned a parent but MASTERBAYES did not (COLONY2 assigned) and cases where both assigned a parent but they did not match (Assignments mismatch). Comparisons include COLONY2 *Best(ML) Config* 'dummy' parentages which had been attributed to an identified candidate mother/father.

Parent	Match assigned	Match unassigned	MASTERBAYES assigned	COLONY2 assigned	Assignments mismatch
Maternities	993	118	111	301	60
Paternities	788	285	21	420	69

Table 2.7 Comparison of the accepted maternity and paternity assignments made by MASTERBAYES and COLONY2. MASTERBAYESG = only genotyped individuals included in the MASTERBAYES Offspring Model (comparable to COLONY2); MASTERBAYESP = in addition to genotyped individuals this model also includes non-genotyped individuals; COLONY2 = includes only parentages assigned to genotyped parents; COLONY2FULL = includes parentages for sibships with (identified and unidentified) unsampled parents as estimated from the *Best(ML) Config* dummy' Offspring Model assignments; COLONY2F/I = results from the Founders and Immigrants Model; total = final pedigree summary including outputs from both programs (an additional 78 maternities determined purely on field data are not included); known maternities = behaviourally observed; unknown maternities = lacking behavioural maternities.

Model	No. of meerkats in the model	No. of meerkats with unknown/known maternity	No. of unknown/known maternities assigned	Average confidence of unknown/known maternal assignments	No. of paternities assigned	Average confidence of paternal assignments
MASTERBAYESG	1367	172/1195	120/953	0.876/0.843	875	0.980
MASTERBAYESP	1583	202/1381	122/1043	0.963/0.969	875	0.980
COLONY2	1367	172/1195	144/1181	0.973/0.998	882	0.997
COLONY2FULL	1367	172/1195	158/1195	0.973/0.998	1277	0.997[†]
COLONY2F/I	127	127/0	75/0	NA	75/0	NA
Total	1710	329/1381	233 (155[*])/1290	–	1352 (1076[*])	–

[*]'Dummy' parents removed.
[†]'As 'dummy' parents do not have confidence probabilities the COLONY2 and COLONY2FULL average probabilities are the same.

Combining program results

Maternal and paternal discrepancies

Prior to combining the program results, all 64 of the behavioural versus MASTERBAYES inferred maternal discrepancies were individually investigated in order to ascertain the probable cause of these differences. 25 offspring were assigned their sister versus the behavioural mother. The true mother of these siblings had large total reproductive outputs ranging from between 23 to 86 pups. A further 31 were mis-assigned their maternal aunt or grandmother. The majority of the MASTERBAYES inferred mothers of both forms of mis-assignment were members of the same group as the offspring at the time of their birth and had been recorded as being pregnant (with different pups) in the three months prior to their birth. The remaining nine cases of MASTERBAYES maternal mis-assignments were to more distantly related females that were members of the same group as the offspring at the time of their birth, some of which had also been recorded as being pregnant (with different pups) in the three months prior to their birth.

All 69 MASTERBAYES versus COLONY2 inferred maternal discrepancies were individually investigated in order to ascertain the probable cause of these differences. In most cases the MASTERBAYES paternity was assigned to a brother that had remained in it's natal group.

Maternities

The vast majority of accepted maternities were assigned by both field observations (and thus assumed true in COLONY2) and correctly recovered by MASTERBAYES (*n*=881; Table 2.8 XV.). A further 93 and 245 offspring with behavioural mothers were also assumed correct by COLONY2 and accurately recovered by MASTERBAYES respectively (Table 2.8 XIII. and XIV.). Only 40 offspring with no known mothers were not assigned a maternity either because neither program recovered a maternity (*n*=28; Table 2.8 I.), there was more than one possible ungenotyped candidate female available for the sibship (*n*=10; Table 2.8 IX.), or only MASTERBAYES inferred a mother (n=2; Table 2.8 II.). The maternities of the remaining offspring were assigned primarily by COLONY2.

Paternities

The vast majority of accepted paternities were assigned by both COLONY2 and MASTERBAYES (*n*=679; Table 2.8 IV.). A further 177 offspring were assigned to an identified male or assigned a 'dummy' paternal identity by COLONY2 (Table 2.8 III. and IX.). 306 offspring were not assigned a paternity either because neither program recovered a paternity (*n*=230; Table 2.8 I.), the COLONY2 *Best(ML) Config* sibships consisted of only singleton paternal halfsibs (*n*=74; Table 2.9 XI.), or only MASTERBAYES inferred a father (n=2; Table 2.8 II.). The paternities of the remaining 244 offspring were assigned mainly by COLONY2.

Table 2.8 Summary table of the number of cases of each parentage rule. Values in bold represent cases where there was a discrepancy in the inferences between the two programs and/or the field data. In these cases either the final parentage was left unassigned or preference was given to the field or COLONY2 inferred data. More detailed descriptions of each criterion are given in the main text.

Parentage acceptance criteria example	No. of maternities	No. of paternities
I.	28	230
II.	2	2
III.	41	177
IV.	99	679
V.	**4**	**26**
VI.	5	65
VII.	4	109
VIII.	**6**	**20**
IX.	10	177
X.	3	24
XI.	91	74
XII.	**9**	–
XIII.	93	–
XIV.	245	–
XV.	881	–
XVI.	**55**	–
XVII.	4	–
XVIII.	2	–
XIX.	**1**	–

2.4.3.2 Founders and immigrants model

56% (*n*=127) of the total 226 founders and immigrants were genotyped and could thus be included in the Founders and Immigrants Model. Of these 75 were recovered in 19 maternal and 18 paternal sibships with the number of siblings included ranging from between two to eleven individuals. Both parents of one of the sibships were attributed an identified meerkat via the CERVUS Identity Analysis function. Only one other 'dummy' mother was attributed a know identity after re-analysis, whereas the parents of the remaining sibships retained their 'dummy' parent identity (Table 2.8).

All the inferred parentages were visualized in a pedigree using both PEDANTICS (http://wildevolution.biology.ed.ac.uk/awilson/pedantix.html) (Morrissey & Wilson

2010, see Fig. 2.4) and PEDIGREE VIEWER (http://www-personal.une.edu.au/~bkinghor/pedigree.htm) (Kinghorn 1994).

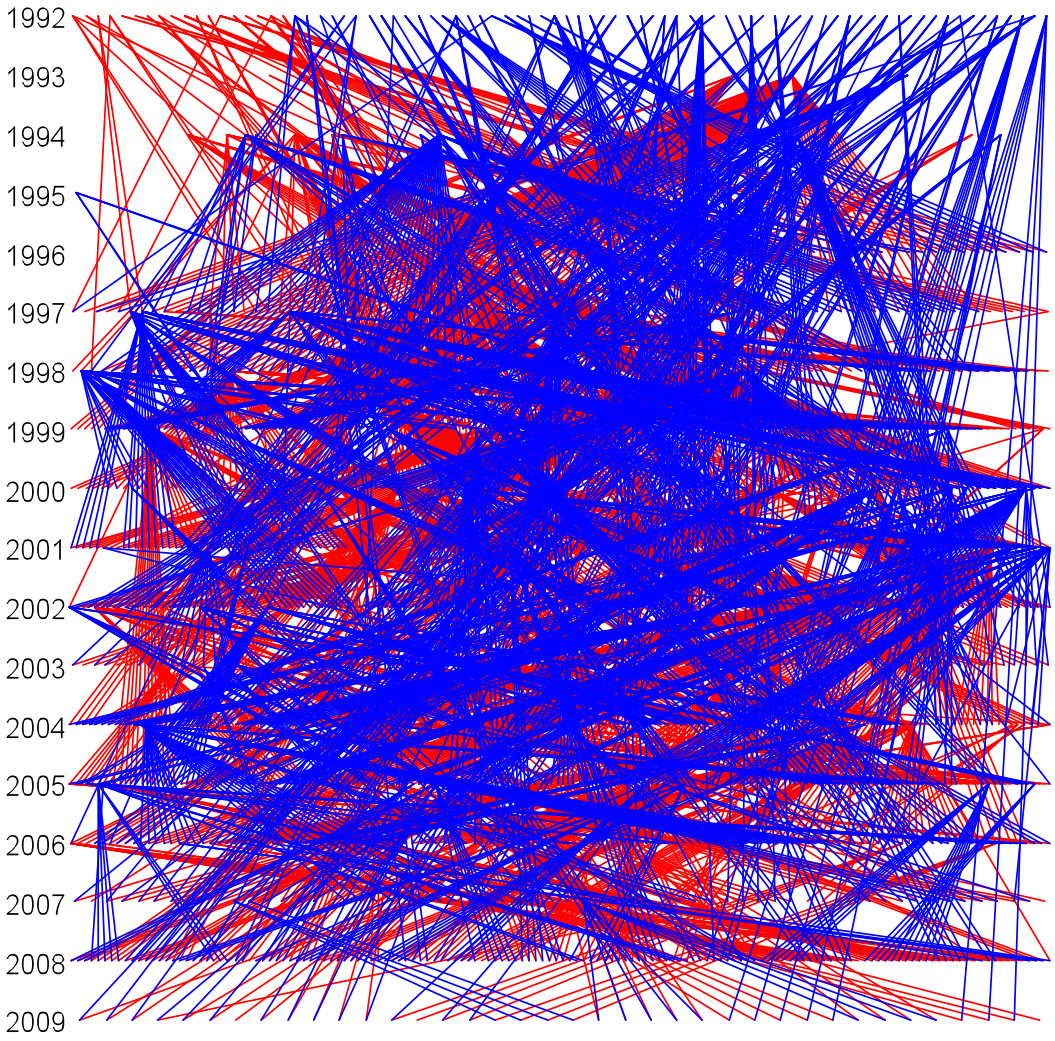


Figure 2.4 The Kalahari meerkat pedigree reconstructed in PEDANTICS (Morrissey & Wilson 2010). Red and blue lines connect offspring to their mothers and fathers respectively.

2.5 Discussion

2.5.1 Microsatellite marker performance

The results of this study indicate that the microsatellite kit described herein is an efficient and reliable tool for the high-throughput genotyping of meerkats. Individuals presented moderate levels of variability at the 18 microsatellite loci, and although the majority of loci did show deviations from H-W equilibrium the difference between the observed and expected heterozygosities was generally very small. Most of these loci exhibited heterozygote excess, which is consistent with findings from other large populations of kin groups exhibiting inbreeding avoidance and strong reproductive skew (Chesser 1991, Dobson *et al.* 1998, Spong *et al.* 2008). Meerkats are characterized by strong female philopatry, which is thought to have evolved in part to avoid consanguineous matings and to increase the prevalence of outcrossing via the emigration of unrelated breeding males into other groups, thereby increasing the observed heterozygosity.

13 of the total 18 loci screened were not in H-W equilibrium. Four loci showed patterns consistent with the presence of null alleles, particularly *Ssu13.8*, which had an estimated null allele frequency greater than 0.05. This locus is characterised by a cluster of 11 alleles, each varying in size by two base pairs and it is possible that, due to preferential amplification of smaller alleles, the larger allele of certain heterozygotes was not scored. This is reflected in the fact that the estimated error rate (8%) for this locus was particularly high. Although deviations from H-W equilibrium in this direction are not desirable when the end point use of a genetic dataset is parentage assignment, having eleven easily scored loci in the majority of individuals outweighs this disadvantage.

The fact that the subsequent pedigree analyses recovered most known mother-offspring relationships with very high probabilities indicates that these loci generally adhere to patterns of Mendelian of inheritance. Furthermore, the extremely small (0.0005) combined non-exclusion probability of a parent across all loci when lacking

information on either of the parents (NE_{-1p}) indicated that this dataset was highly effective for parentage assignment.

By combining the 18 loci into four multiplex and co-loaded reactions the lab time and financial costs of ongoing genotyping of the KMP population has been significantly reduced. In addition to continuing to be used to gather genetic profiles from this population, the meerkat microsatellite multiplex kit also provides a solid framework from which microsatellite protocols for closely related Herpestidae species (e.g. yellow (*Cynictis penicillata*) and slender (*Galerella sanguinea*) mongoose) can be designed (Veron *et al.* 2004). Furthermore it has the potential to be used in geographically wider population-level genetic research on meerkats.

2.5.2 Parentage inference program performance

Using the meerkat microsatellite multiplex kit the majority (82%) of the Kalahari meerkat population was genotyped. These genetic profiles were used together with a variety of field observations in order to infer familial relationships. By combining outputs from two parentage inference programs, MASTERBAYES and COLONY2, a multigenerational pedigree for the KMP was constructed. Using extensive data from a population of red deer, Walling *et al.* 2010 showed that by making use of all available information, such as using informative phenotypic data (MASTERBAYES) and considering relationships other than just parent–offspring (COLONY2), the number and accuracy of paternity assignments is maximised. Results from this study generally support the combinatorial approach of Walling *et al.* 2010 although certain challenges in dealing with social mammals that live in groups consisting of related individuals (Griffin *et al.* 2001) were encountered. These are covered below.

MASTERBAYES

Although MASTERBAYES assigned a large number of maternities (74%) and paternities (55%) to genotyped offspring, the phenotypic data provided to MASTERBAYES did not appear to be informative enough to allow it to assign an appreciable number of additional parentages to non-genotyped meerkats. For offspring lacking behavioural mothers, this is most likely because they were born as

members of mixed-maternity litters and usually to subordinate females. The candidate mothers of these litters are likely to have had similar phenotypic histories (i.e. the same dominance and gestational status in the three months prior to offspring birth). Consequently the relationship between the phenotypic data provided and the pedigree structure was weak and the statistical power of maternal assignments in these cases could not be increased. Similarly, although the dominance status of males was a strong phenotypic predictor, MASTERBAYES estimated there to be a large number of unsampled males and could thus not confidently assign paternity to an individual based purely on phenotypic descriptors. It is of interest to note that male dominance status was a stronger phenotypic predictor of parentage than female dominance status. I do not, however, think this is in conflict with the results from a previous genetic analysis on the same population that found that 93% of all pups were produced by dominant females, whereas 77% were fathered by dominant males (Griffin *et al.* 2003). Male dominance status was probably a stronger phenotypic predictor of parentage than female dominance status because gestational status (*gavebirth*) was by far the strongest female phenotypic predictor of maternity which necessarily reduced the strength of female dominance status predictor.

One of the distinguishing features between the present use of the two programs is that behavioural mothers were assumed to be correct in COLONY2 whereas, all data being true, they should be accurately recovered by MASTERBAYES. Approximately 5% of all MASTERBAYES assignments differed from the either the behavioural observations (maternities) or COLONY2 inferences (paternities). Detailed investigation of the maternal mismatches in this population seemed to reveal incidences of the 'sib paradox', as approximately a third of the maternities were assigned to sisters born to a highly fecund mother (Thompson 1976, Thompson & Meagher 1987). An extension of the 'sib paradox' could also apply to the instances where an offspring was mis-assigned maternity to either a fecund aunt or grandmother that had remained in the natal group. In the majority of these cases the MASTERBAYES assigned mother had also been reported to be pregnant or to have given birth (to different pups) in the three months prior to the offspring's birth. Given that gestational status was the strongest phenotypic predictor, this could

influence mis-assignment if either the true mother or her offspring were ungenotyped, had a number of genotyping errors and/or the offspring had one or more paternally inherited rare allele(s) (Thompson 1976, Thompson & Meagher 1987). Similarly, MASTERBAYES had 'mis-assigned' paternities to older (juvenile) brothers of offspring that had remained in the natal group. Given that MASTERBAYES 'mis-assigned' a similar number of maternities and that the group membership of males at the time of offspring conception was a strong phenotypic predictor, it is likely that these paternities also suffered from the 'sib paradox'.

One way to make the programs more comparable and avoid maternities being mis-assigned to related females that had remained in their natal group is to weight the prior probability of a behavioural mother being assigned maternity to her pups in MASTERBAYES. This option was investigated; preliminary trial runs of the MASTERBAYES model indicated that behavioural mothers were 2,987 – 3,4002,428 times more likely to be assigned maternity of offspring than any other females and were thus weighted accordingly in the model. This approach was successful in eliminating mis-assignments between behavioural and MASTERBAYES inferred maternities but resulted in a significant reduction in the total number of maternal assignments (n=248 fewer assignments). It also significantly modified the posterior probability distribution of both maternal and paternal phenotypic predictors, for example dominant females were 80,017 – 21,162 times less likely to mother pups than subordinates, which is highly unrealistic. Furthermore, there was a reduction in the total number of paternal assignments (n=18 fewer assignments) made by the maternally weighted model, although there were no conflicts in the paternal assignments made by the two models as both recovered the same fathers. In light of these preliminary findings, the prior probability of behavioural mothers being assigned maternity of her pups was not weighted. The model unweighted by behavioural maternities was considered more informative in that any mismatches between MASTERBAYES and the behavioural observations (maternities) and/or COLONY2 inferences (paternities) could potentially reveal any sampling, labelling or genotyping errors in the data. These could potentially be rectified, thereby decreasing the total pedigree error rate. In this case, including mismatches that could

potentially be explained by the sib paradox, MASTERBAYES indicated a parentage mis-assignment rate of 5% which is in line with other wild pedigrees (Charmantier & Réale 2005).

COLONY2

COLONY2 assigned a large number of maternities (75%) and paternities (56%) to the genotyped offspring, and furthermore provided information that enabled additional individuals to be recovered in sibships and assigned unsampled 'dummy' parents, which further increased the total number of pedigree links. In general COLONY2 appears to be well suited to recovering parentages for social animals with large reproductive skews, such as meerkats, which is not suprising given that it was initially developed for use in bumble bees (*Bombus* spp.) (Wang 2004). COLONY2 did, however, introduce several potential errors by either fully recovering or splitting a large true sibship while also failing to confidently assign the correct genotyped parent. This occurred either because the configuration reconstruction and searching algorithms were not efficient enough for this large dataset or because a higher than expected number of genotyping errors within the sibship precluded the true father from being assigned because it had a low likelihood value (J. Wang 2010, *pers. comm.*). As COLONY2 *Best(ML) Config.* results also returned estimated genotypes of the 'dummy' parents of these sibships, these could be used in combination with the MASTERBAYES assignments to rectify these errors. This provides further support for a combinatorial approach to pedigree reconstruction in natural populations.

2.5.3 Conclusions

Results from this study generally support the combinatorial approach of re-constructing pedigrees (Walling *et al.* 2010) although improvements to the type of phenotypic data provided to MASTERBAYES may increase the concordance of results between the two programs. Recommendations for the future analysis of the meerkat pedigree include:

- Continuing to obtain tissue samples from as many individuals in the population as possible with particular emphasis on dominants and, where possible, roving and immigrant males.

- Run an initial MaserBayes model to investigate any discrepancies between the behavioural and MASTERBAYES inferred maternities to check for any potential sources of sampling, labelling or genotyping error and whether these can be rectified prior to subsequent analysis.
- Provide more restricted data and further limit the phenotypic variables in MASTERBAYES, for example:
    - only indicate whether a specific female had given birth in the month of an offspring's birth rather than whether it had either given birth or had been reported as being pregnant in the three months prior to an offspring's birth.
    - specify the group in which a roving male had been observed during the offspring's conception window (when known) rather than whether it had simply been reported as having roved or not in the three months prior to offspring birth. The aim of this would be to increase the informativeness of the phenotypic data in paternity inference and hopefully prevent natal brothers being 'mis-assigned' paternity due to 1.) the 'sib paradox' and 2.) the fact that male group membership is currently a strong phenotypic predictor.
    - provide data on the exact location of groups to take into account that roving males may be more likely to secure matings with females from neighbouring groups. As groups are transient and can vary their sleeping burrows within a single month, this may require that the phenotypic descriptors be given more frequently than monthly.

Chapter 3

Inbreeding and inbreeding depression of early life traits in a cooperative mammal



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JFN, LEBK and JMP designed the study; SE provided the growth data; WPG-C optimised the microsatellite markers; JW helped with the pedigree reconstruction; CAW and AB provided help with the statistical analyses; TPF, RLS, JS and NT collected the field data, and THC-B established and continues to maintain the field site. JFN analysed the data and wrote the manuscript.

3.1 Abstract

Mating between relatives often results in negative fitness consequences or inbreeding depression. However, the expression of inbreeding in populations of wild cooperative mammals and the effects of environmental, maternal and social factors on inbreeding depression in these systems are currently not well understood. This study uses pedigree-based inbreeding coefficients from a long-term study of meerkats (*Suricata suricatta*) in South Africa to reveal that 44% of the population have detectably non-zero ($F>0$) inbreeding coefficients. 15% of these inbred individuals were the result of moderate inbreeding ($F\geq0.125$), although such inbreeding events almost solely occurred when mating individuals had no prior experience of each other. Inbreeding depression was evident for a range of traits: pup mass at emergence from the natal burrow, hind-foot length, growth until independence and juvenile survival. However, I found no evidence of significant inbreeding depression for skull and forearm length or for pup survival. This research provides a rare investigation into inbreeding in a cooperative mammal, revealing high levels of inbreeding, considerable negative consequences and complex interactions with the social environment.

3.2 Introduction

Inbreeding, or the mating of genetically related parents, typically causes a decline in offspring fitness, known as inbreeding depression (Lynch & Walsh 1998). Extensive research on captive and laboratory populations has highlighted the importance of inbreeding depression for many aspects of our understanding of ecology, conservation and evolution. For example, the negative fitness consequences of inbreeding depression can place vulnerable populations at risk of extinction and ultimately act as selective evolutionary pressures on organisms' behaviours and life strategies (Keller & Waller 2002). Despite an accumulated knowledge of inbreeding depression effects on captive and laboratory populations we know much less about the occurrence and effects of inbreeding in wild populations (Crnokrak & Roff 1999, Keller & Waller 2002). Studying inbreeding in wild populations that experience different environmental conditions is important, as the effects of inbreeding in wild populations may not be accurately represented in stable laboratory or captive settings (Keller & Waller 2002, Pemberton 2008).

Although a growing body of work is now revealing inbreeding and inbreeding depression in wild animal systems (Keller & Waller 2002), comparatively few studies have focused on cooperative species (but see Hoogland 1992, Keane *et al.* 1996, McRae 1996, Brown & Brown 1998, Daniels & Walters 2000, Richardson *et al.* 2004, Spiering *et al.* 2011). As cooperative species tend to live in family groups with close relatives of the opposite sex in near proximity the potential for inbreeding is high (Koenig & Haydock 2004). Furthermore, there are unusually strong selection pressures favouring philopatry in cooperative species (Komdeur 1992, Kokko & Ekman 2002, Solomon 2003), which raises the questions of whether individuals remain and breed in their natal group and whether regular inbreeding has purged the population of deleterious recessive alleles. Alternatively, individuals may increase their fitness by dispersing to avoid inbreeding and its negative fitness consequences. The well-documented negative effects of inbreeding may act as strong selective pressures against consanguineous matings and, consequently, cooperative species may have evolved particularly strong incest avoidance mechanisms (Pusey & Wolf

1996, Koenig & Haydock 2004). Although there is evidence to suggest that the further individuals move, the less likely they are to breed with close relatives (Schiegg *et al.* 2006, Szulkin & Sheldon 2008) and evidence for kin recognition has been found in some species (Pusey & Wolf 1996), little inbreeding research has focused specifically on cooperative mammals where social living may in fact generate the greatest opportunities for inbreeding to occur.

Inbreeding may have negative fitness consequences, but it may also be tolerated in certain circumstances where the costs of avoiding mating with kin are greater than the benefits gained by avoidance (Waser *et al.* 1986, Kokko & Ots 2006). For some individuals (e.g. subordinates) consanguineous matings may represent their only opportunity for reproduction and thus the costs of discriminating between mates based on the degree of relatedness between potential mates may not outweigh the benefits of reproducing. Inbreeding may also be tolerated when the costs of incestuous matings for offspring viability are small relative to the inclusive fitness benefits of the increased representation of copies of an individual's genes in subsequent generations (Waser *et al.* 1986, Kokko & Ots 2006). There is also a suggestion that the increased kinship within social groups that results from inbreeding may in fact promote or maintain cooperative behaviours in line with kin selection theory (Hamilton 1963, West *et al.* 2002, Griffin & West 2003, Rossiter *et al.* 2005). Determining accurate levels of inbreeding and inbreeding depression in cooperative species will serve as a first step towards testing these ideas.

Several studies have demonstrated how the effects of inbreeding depression vary with environmental heterogeneity. More specifically, the magnitude of inbreeding depression has been shown to increase under more severe environmental conditions in both experimental systems (summarised in Fox & Reed 2011) and wild populations (Keller *et al.* 1994, Keller *et al.* 2002, Marr *et al.* 2006). These findings suggest an interesting line of research into the social environments of cooperative mammals where (subordinate) members of a social group contribute to the post-natal rearing of offspring other than their own, often more so than the (dominant) parents themselves (Clutton-Brock 2002, Clutton-Brock *et al.* 2004). Subordinate helpers

can have important long-term fitness effects on the offspring they help to raise and, to some extent, replace the maternal effects that are commonly found to govern the fitness of non-cooperative vertebrates (Jennions & Macdonald 1994, Russell *et al.* 2002). Helpers have even been shown to compensate for reductions in maternal investment, in turn influencing the decisions mothers make, conditional on helper availability (Clutton-Brock *et al.* 2001b, Russell *et al.* 2007a). Consequently, in cooperative systems, helpers may modify the social environment experienced by an inbred animal to such an extent that they alleviate the effects of inbreeding depression, in a similar way that environmental heterogenetiy has been shown to mediate inbreeding depression in other systems (see above).

To date the most precise and reliable estimates of individual inbreeding coefficients (*F*) appear to be those derived from accurate genetically-validated multigenerational pedigrees (Pemberton 2008, Taylor *et al.* 2010). However, reconstructing pedigrees for wild populations can be difficult (Pemberton 2008). Genetic parentage assignment can be particularly problematic in cooperative species as individuals usually live in groups of close relatives who share alleles and consequently the power of parentage analysis can suffer from low allelic diversity (McRae & Amos 1999, Richardson *et al.* 2004, Nichols *et al.* 2010). Thus, although several studies have reported the occurrence of inbreeding (Keane *et al.* 1996) and inbreeding depression (McRae 1996, Brown & Brown 1998) in populations of cooperatively breeding vertebrates, few have yet determined the overall frequency of inbreeding and the effects of inbreeding depression in wild mammalian populations using genetically-validated, pedigree-derived inbreeding coefficients.

The long-term Kalahari Meerkat Project (Clutton-Brock *et al.* 1999a, Russell *et al.* 2002) provides a rare opportunity to investigate inbreeding and inbreeding depression in a cooperatively breeding mammal. Meerkats (*Suricata suricatta,* Schreber 1776) are small, desert-adapted mongooses that live in groups of 2-50 (Doolan & Macdonald 1999). These groups contain a dominant pair, which is responsible for 80-90% of the group's reproductive output (Griffin *et al.* 2003, Spong *et al.* 2008). The remainder of the group are male and female subordinates

that assist in raising the dominants' pups by babysitting them at the natal burrow and by feeding and teaching them to forage once they emerge, at approximately three weeks of age (Clutton-Brock *et al.* 2001b, Thornton & McAuliffe 2006). Although subordinates of both sex are physiologically capable of breeding, they appear to avoid mating with (related) group members (Griffin *et al.* 2003). Furthermore, subordinate females are generally prevented from breeding by acts of aggression, eviction and/or infanticide from the dominant female (Clutton-Brock *et al.* 1998a). Subordinate females can, however, spontaneously lactate to the dominant female's newborn pups – a behaviour termed allolactation (Scantlebury *et al.* 2002). Suckling seldom occurs outside the burrow and thus little is known about the extent and duration of allolactation in meerkats, however, allolactating females can be clearly identified by enlarged nipples and surrounding flattened areas of fur that indicate suckling (Scantlebury *et al.* 2002). Meerkat pups generally reach nutritional independence at 90 days and start contributing significantly to cooperative behaviours within the group as sub-adults at 180 days (Clutton-Brock *et al.* 2001b). Previous genetic analyses of breeding success in the same population concluded that the male-biased dispersal pattern of meerkats acts, in part, as an inbreeding avoidance mechanism (Griffin *et al.* 2003, Spong *et al.* 2008). At the time of this previous work, there were insufficient generations available to construct a pedigree for the study population, but in the years since, continued data collection has enabled a genetic pedigree to be constructed and hence a pedigree-based investigation of inbreeding to be undertaken.

Strictly speaking, 'inbreeding depression' refers to a loss of fitness, whereas in this chapter I investigate inbreeding depression in a series of early life traits. Fitness describes the ability of organisms to both survive and reproduce but accurate long-term measures of fitness are often difficult to collect from wild populations. Although some studies have investigated the effects of inbreeding on fitness measures directly (e.g. Keller 1998, Kruuk *et al.* 2002, Szulkin & Sheldon 2007), many have also investigated the effect of inbreeding on traits that are strongly associated with fitness, such as weight and morphological measures (e.g. Coltman *et al.* 1998, Coulson *et al.* 1998, Slate & Pemberton 2002, Walling *et al.* 2011). In

meerkats, offspring weight has positive consequences for both survival and acquiring dominance (Russell *et al.* 2007b, Hodge *et al.* 2008). Due to the large reproductive skew favouring dominants (Sharp & Clutton-Brock 2010), total fitness is closely associated with dominance acquisition and thus offspring weight is likely to be a trait that is strongly associated with fitness in meerkats. The extent to which offspring morphological traits and growth rates are associated with individual fitness in meerkats is less clear although studies in other species have found that both of these variables tend to be subject to strong directional selection due to the reproductive and survival advantages associated with large adult body size (Dmitriew 2011). Juvenile survival is an important fitness component in meerkats since survival of this stage is lower than at any other, yet meerkats rarely breed before they are one year old (Clutton-Brock *et al.* 1999b). Consequently, early-life meerkat weights, morphological measures, growth and survival are likely to be traits that are strongly associated with total fitness and thus appropriate measures in which to investigate the consequences of inbreeding on.

I present here tests for inbreeding depression in a range of early life traits in meerkats. Because these may be subject to maternal effects, and therefore dependent on the phenotype of the mother, I tested for associations between traits and the inbreeding coefficient of both the pup and the mother. The objectives of this research were three-fold: i) to document the extent and nature of inbreeding events in meerkats; ii) to quantify inbreeding depression in a range of early life morphometric and survival traits; and iii) to determine whether, and how, cooperative care affects the expression of inbreeding depression.

3.3 Materials and methods

3.3.1 Study population

This study uses 16 years of data from the Kalahari Meerkat Project, which was established at the Kuruman River Reserve, South Africa (26º5'S, 21º49'E) in 1993. Further details of the habitat and climate of the study system are provided elsewhere (Clutton-Brock *et al.* 1999a, Russell *et al.* 2002). All meerkats in the study population are individually recognisable and habituated to close observation (<1 m), and each group is visited up to two times a day. In addition to detailed behavioural observations for specific individuals, any changes in group composition and individual key life-history events are recorded, including the dates and details of births, deaths, dispersal, eviction, pregnancy, litter emergence and change of dominance events (Sharp & Clutton-Brock 2010). Meerkats emerge from their natal burrow at approximately three weeks of age and are defined as 'pups' until they reach nutritional independence at approximately 90 days. Thereafter they are termed 'juvenile' (91-180 days), 'sub-adult' (181-365 days) and 'adult' (>365 days) (Clutton-Brock *et al.* 1998a, Doolan & Macdonald 1999). The majority of meerkats in the study population are weighed upon emergence (emergence mass) from the natal burrow and up to three times daily thereafter, using a top-pan balance (±1 g) (Clutton-Brock *et al.* 2003). Vernier callipers (±1 mm) are used to measure skull length from the tip of the nose to the base of the skull, forearm length from the proximal end of the radius to the base of the carpals, and hind-foot length from the proximal end of the metatarsal to the distal end of the phalanges (excluding the claws). For DNA analysis 2-5 mm tissue sample from the tail tip of each pup is also taken at emergence (Spong *et al.* 2008). Tissue samples from recently habituated or immigrant adults were taken from anaesthetised or dead animals so that approximately 86% (n=1,563) of the total recorded population has now been sampled for tissue. Full details of the DNA extraction and genotyping methods are provided in Chapter 2.

3.3.2 Pedigree and inbreeding coefficients

The pedigree for the study population was reconstructed using a combination of microsatellite data, phenotypic descriptors and two parentage inference programs: COLONY2 (Wang 2004, Wang & Santure 2009) and MASTERBAYES (Hadfield *et al.* 2006) (see Chapter 2 for detailed pedigree construction methods). In brief, for COLONY2, all behavioural maternities of single litters were assumed correct and specified as such. For pups born to mixed maternity litters, candidate mothers were identified by consistent weight gain and visible abdominal distension over the 70-day gestation period followed by abrupt weight loss post-parturition (Clutton-Brock *et al.* 1999b). Candidate fathers included any known male alive in any group during a two week conception window (between 63 and 77 days prior to pup birth) (Clutton-Brock *et al.* 1999b). The probability of the true parents being in the candidate lists was set at 0.75, both sexes were defined as polygamous and allele frequencies and genotyping error rates were provided (see Chapter 2). Otherwise, default settings were used for all other program parameters. For MASTERBAYES, phenotypic descriptors including the sex, dominance status and the group of which an individual was a member of were provided for each month it was known to be alive. Additionally, monthly records of each female's gestational status (i.e. whether she was recorded as either being pregnant or had given birth) and whether a male was known to have roved were also provided as phenotypic descriptors. There is likely to be a relationship between these phenotypic descriptors and the pedigree structure and using them should increase the statistical power in parentage inference (Hadfield *et al.* 2006). Candidate mothers and fathers were restricted to individuals that were at least six months of age (to prevent sib paradox effects of true litter mates being mis-assigned parentage) (Thompson 1976, Thompson & Meagher 1987). Candidate mothers were also restricted to members of the same natal group as the pup(s) in the three months leading up to (and including) a pup's birth. The Markov chain Monte Carlo (MCMC) estimation chain was run for 130,000 iterations, with a burn-in of 30,000 iterations and a thinning interval of 50 iterations. No further prior distributions were specified and default improper uniform priors were used (Hadfield *et al.* 2006). For both programs, only assignments with at least 80% individual confidence were considered in the final combination of program results, although the

vast majority of these had individual confidence values above 95% (average=97.57%) and matched between the two programs (see Chapter 2). The pedigree was visualised and Wright's inbreeding coefficients (*F*) for pups and mothers calculated using PEDIGREE VIEWER (http://www-personal.une.edu.au/~bkinghor/pedigree.htm) (Kinghorn 1994). Pair-wise relatedness coefficients were obtained using the R software package PEDANTICS (http://wildevolution.biology.ed.ac.uk/awilson/pedantix.html) (Morrissey & Wilson 2010). The resulting pedigree spans seven generations: between 1993 and early 2009 1,583 pups were born into the population and of these 1,539 and 1,277 have a resolved maternity and paternity, respectively. In the absence of parent-offspring matings, accurate *F*-values could only be determined for individuals with at least one grandparent assigned. Furthermore, the traits of interest and candidate parents were not available for the most recent pups. Consequently, I restricted these inbreeding depression analyses to only those pups born between 2000 and 2007 that had known *F*-values (n=963), although all pedigree information back to 1993 was used for the estimation of inbreeding coefficients. I follow Marshall *et al.* (2002)'s terminology of defining 'low' inbreeding as $F<0.125$, 'moderate' as $0.25>F\geq0.125$ and 'high' as $F\geq0.25$.

3.3.3 Study traits

3.3.3.1 Response variables

I examined the effect of mother's and pup's inbreeding coefficient on a number of early life traits including:

- Emergence mass (EM). To obtain a single estimate per individual, an average of all morning mass records obtained within seven days of the pup first being weighed upon emergence was calculated (mean number of mass records per pup=3.120 ± 1.570 S.D.).
- Skeletal traits (skull (SL), forearm (FL) and hind-foot lengths (HL)). Skeletal traits were usually only measured once (upon emergence) and the earliest record was used if they were measured more than once. Records of the dates on which pups were recorded to have left their natal burrows with the group (at approximately four weeks of age) were used to restrict both the mass and

skeletal records to only those that were obtained prior to a pup commencing foraging in order to avoid these measures being influenced by adult helper provisioning (Russell *et al.* 2002).

- Growth until independence (GI). Growth until independence (designated to occur at 90 days) was each individual's idealised growth rate at which it approaches a theoretical body mass. It was measured as the constant (*k*) from a monomolecular curve (Gaillard *et al.* 1997) fitted for each individual through their mass records between age at emergence and 90 days (English *et al.* 2012). The curve describes the relationship between mass and age (*t*) as $Mt=A*(1-e^{-k(t-t0)})$, with age-axis intercept (*t0*) and asymptotic mass (*A*) fixed at approximate population mean levels (*t0*=0 and *A*=730) (English *et al.* 2012). The growth until independence parameter (*k*) was scaled by a factor of 100 in the results table for ease of interpretation.
- Pup (PS) and juvenile (JS) survival. Pup survival was the period of time from emergence (at approximately three weeks) to independence (designated to occur at 90 days), and juvenile survival was from independence to sub-adult age (designated to occur at 180 days) (Table 3.1). For both survival periods the terminal event was pup death. Individuals that remained alive after each survival period were included as censored data points whereas individuals known or assumed with high confidence to have died during the specific survival period remained uncensored (Table 3.1). Individuals that had emigrated out of the study population, were euthanized or were killed in road accidents were excluded in order to avoid upwardly biasing the results.

Table 3.1 Summary statistics for traits: emergence mass (EM); skull (SL), forearm (FL) and hind-foot (HL) lengths; growth until independence (GI); pup survival (PS) and juvenile survival (JS). Sample sizes vary due to missing data for some variables. The growth until independence parameter (*k*) has been scaled by a factor of 100.

	n	Mean ± S.D	Min.	Max.
EM (g)	422	113.229 ± 26.258	47	182.8
SL (mm)	219	47.543 ± 2.345	36.9	52.4
FL (mm)	171	30.549 ± 2.247	23.4	39.2
HL (mm)	219	37.619 ± 3.124	28.5	49.5
GI (*k*)*	523	0.650 ± 0.124	0.2	1.2
PS	561	†0=501, 1=60‡	–	–
JS	497	†0=465, 1=32‡	–	–

*There are fewer records for emergence mass and the skeletal measures as only records obtained prior to a pup foraging were used whereas all mass records were used to calculate growth until independence.
†Mean, minimum and maximum values are not available for proportional hazards models.
‡0=individual was censored (i.e. survived or was removed from the population), 1=died during the specified time window.

3.3.3.2 Explanatory variables

I tested for inbreeding depression in both offspring performance and maternal effects by inclusion of pup and the mother inbreeding coefficients (*F*) respectively as explanatory variables in the models. I also included a number of other pup-level fixed effects in my initial full models: pup age and pup age^2 at measurement (relevant for emergence mass and skeletal traits: mean=26, range=13-39.5 days (EM); mean=23, range=15-31 days (skeletal traits)) were included to control for variation in the age at which pups were measured, as well as pup sex and litter size (mean=3.5, range=1-7). Previous research on the population has found that non-pregnant dominant females are generally heavier than subordinates (Hodge *et al.* 2008), that there is reproductive senescence in females (Sharp & Clutton-Brock 2010) and that mother's mass at conception is an important determinant of pup mass at weaning (Russell *et al.* 2003a). Accordingly I also included mother's status (dominant/subordinate), mother's age and mother's age^2 at pup birth, and mother's conception mass as fixed effects in my initial full models. As typical meerkat gestation is approximately 70 days (Clutton-Brock *et al.* 1998a), the conception mass of mothers was taken as the average of all morning mass records 60-80 days prior to pup birth (mean number of mass records per mother=8). As a social, cooperatively

breeding mammal, several traits have also been shown to be highly influenced by group-level factors (Clutton-Brock *et al.* 1999a, Clutton-Brock *et al.* 2001b, Russell *et al.* 2002, Scantlebury *et al.* 2002, Russell *et al.* 2003a) and consequently I included group size (total number of helpers aged ≥6 months in the group at the time of pup birth, range=1-36) and lactator number (total number of lactators in the group including the mother, range=1-8) as fixed effects in my initial full models. Lactator number was calculated as the total number of females (including the mother) in the group that showed evidence of suckling at any point in the 26 days (average age at emergence mass weighing) after pup birth. Pup year of birth (range=2000-2007) was also fitted as a fixed effect factor in all models to control for environmental variation between years. Finally, as meerkats rarely breed in mid-winter but can breed throughout the year (Clutton-Brock *et al.* 1999b) and because both seasonality and rainfall influence growth in meerkats (Russell *et al.* 2002, English *et al.* 2012), the season in which the pup was born (two-level factor of cold-dry: May-September; hot-wet: October-April) and the total accumulated rainfall in the three months prior to pup birth were also included as fixed effects in my initial full models. Rainfall is thought to have a delayed positive effect on invertebrate abundance and thus rainfall in the months prior to pup birth may reflect the relative amount of food available to adult helpers at pup birth. Total accumulated rainfall in the three months prior to pup birth was extracted for the study site (26°58'S, 21°49E) from the National Aeronautics and Space Administration online Rainfall Analysis Tool (http://disc2.nascom.nasa.gov/Giovanni/tovas/rain.GPCP.shtml) (Bateman *et al.* 2011). In order to account for repeated measures at various levels, mother (max across traits=158), birth group (max across traits=32) and litter (max across traits=435) identifiers were included as random effects in the emergence weight, skeletal traits and growth models whereas only the litter identifier was included as a random effect in the survival models due to coxme package constraints. Litter was chosen as the random effect for this analysis as it is a unique identifier specific to each litter and contains information on both birth group and mother.

3.3.4 Statistical analyses

The emergence mass, skull, forearm and hind-foot lengths and growth until independence residuals were all normally distributed; these traits were therefore analysed using linear mixed models with normal errors in the lmer package in R (R Development Core Team 2009). The survival measures (PS and JS) were analysed in the R coxme model package, a Cox proportional hazards mixed effects model which is capable of handling a random and multiple fixed effects (R Development Core Team 2009). Initial full models contained all the explanatory variables above and were simplified using a stepwise approach in which the least significant terms (based on Wald statistics in lmer and comparison of AIC model value deviances in coxme) were removed incrementally until only significant ($p<0.05$) terms remained or removal of a term decreased the model fit (Crawley 2007). In order to avoid comparing models with varying samples sizes (due to missing explanatory variables) models were compared using a subset of data which contained complete records for all explanatory variables and thus if non-significant terms were removed from the models, sample sizes generally increased. The robustness of the final models and the extent to which they were affected by multicollinearity was tested by removing any strongly correlated pair-wise variables. As the coxme package reports the difference between the model in question (AIC_{model}) and the null model (AIC_n), final minimal survival models were simplified where $-(AIC_{null}-AIC_{model})$ was minimised.

In models where pup *F* was significant, two-way interactions between pup *F* and all other remaining fixed effects were tested. Pearson's correlations were estimated between all pairs of normally distributed response variables and sequential Bonferroni corrections on final minimum model *p*-values were also performed (Rice 1989). Sample sizes varied across the different response variables due to missing data and summary statistics for each trait are presented in table 3.1.

3.4 Results

3.4.1 Occurrence of inbreeding

A large proportion of the population was inbred to some extent. Of the 1,583 pups born into the population between 1993 and 2009, 474 of 1073 (44%) of the individuals for whom inbreeding coefficients (F) could be calculated had F greater than zero (average F=0.078) (Fig. 3.1). The remaining population had F=0 (n=599) or F=NA (n=510). All inbred individuals had at least three known grandparents and 97% had all four grandparents known (n=461) (Fig. 3.1). 91% of known litters (average litter size=3.5) had the same parents and so identical F; in the remainder (n=38), there was mixed paternity and F varied between litter-mates.

Most cases of inbreeding were the result of fairly distant relatives mating. Although there were no instances of 'high' inbreeding at $F \geq 0.25$ (resulting from full sibling or parent-offspring matings) (Marshall *et al.* 2002), the distribution of non-zero inbreeding coefficients shows evidence of 'moderate' inbreeding at $0.25 > F \geq 0.125$ (71 individuals in 28 litters). Moderately inbred pups resulted from either half-sibling (n=13), grandparent-offspring (n=15) or full avuncular (aunt-nephew (n=12) or uncle-niece (n=31) matings) (Fig. 3.1). The distribution also reveals that 403 individuals (>85% of the inbred individuals) had 'low' inbreeding coefficients of F<0.125. For example, 127 individuals (in 42 litters) were inbred at F=0.0625 which resulted from first cousin, great grandparent-offspring or half avuncular matings (Fig. 3.1).

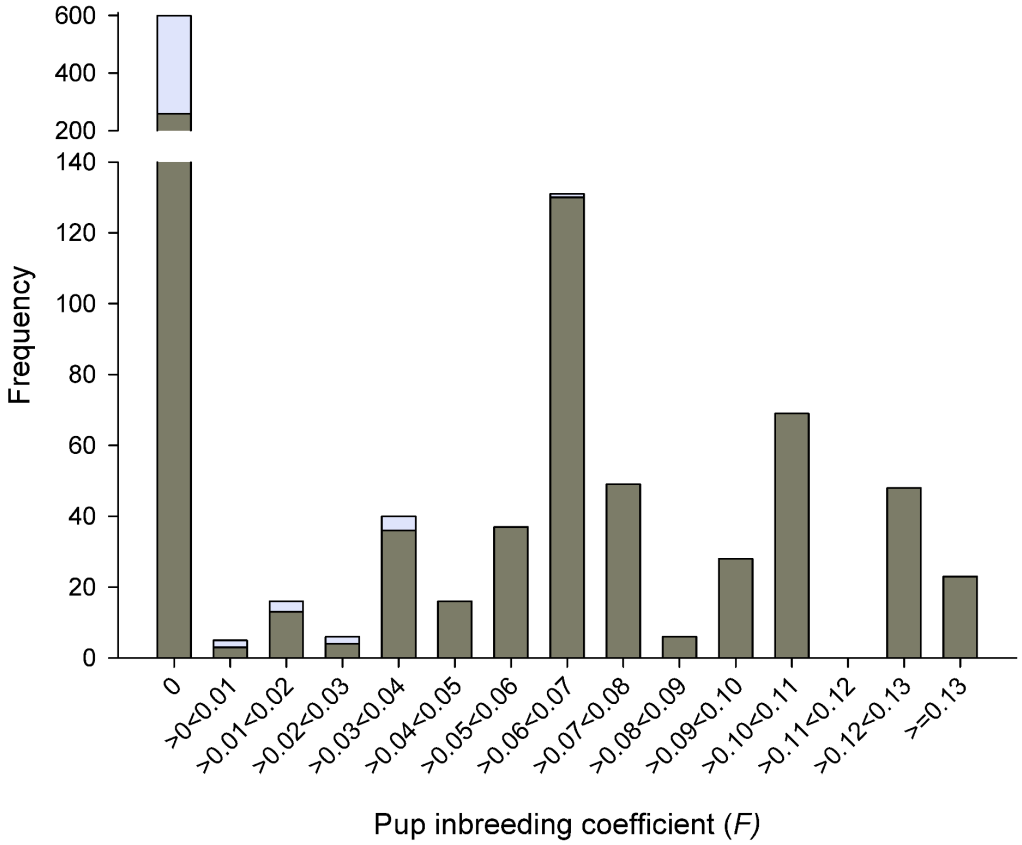


Figure 3.1 The distribution of pup inbreeding coefficients (*F*) (*n*=1,583; *F*=0=599; *F*>0=474). Dark and light grey shading indicates individuals that have all four (*n*=719) or only three (*n*=354) known grandparents respectively. An additional 510 individuals had *F*=NA.

Due to the depth of the pedigree and the inclusion of ancestral inbreeding events, many of the *F* values in the population result from an accumulation of multiple inbreeding events in which distant relatives have mated across multiple generations (see example in Fig. 3.2).

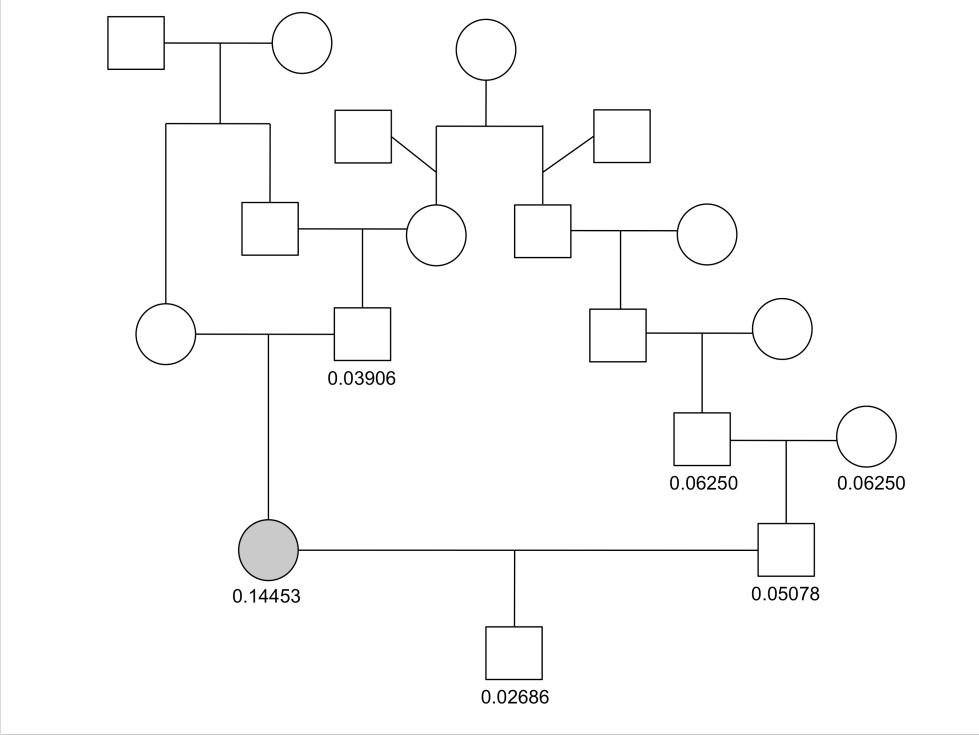


Figure 3.2 Pedigree illustrating an example of an accumulation of multiple inbreeding events in which relatives mated across multiple generations. Circles and squares indicate females and males respectively. *F*-values are given below the shapes and are zero or unknown if absent. The individual at the base of the pedigree is inbred primarily via a half second cousin once removed mating loop, but its *F*-value is inflated by the fact that both its parents and three of its grandparents are also inbred. Similarly, the female in grey is the product of an avuncular mating, which would normally result in *F*=0.125 but is inflated here due to the fact her father is also inbred.

58.01% of all breeding pairs (including dominant pairs and other mated pairs) were related at R>0. Inbred matings predominantly occurred between individuals who had no early-life exposure to each other: for all but eight pups (3 litters) with $F \geq 0.125$ the parents were either born in different groups or one had left the same natal group prior to the other parent's birth. The three inbred litters produced by pairs that had spent time together in the same natal group resulted from a group split: a female became the dominant in a splinter group prior to two of her natal half brothers also immigrating into the group and mating with her.

Subordinate female and male breeders were more likely to produce inbred pups than dominants. 40% (349/867) of the pups born to dominant females had $F>0$ whereas 55% (86/156) of the pups born to subordinate females had $F>0$. Similar patterns were apparent for males: 33% (213/652) of the pups born to dominant males had $F>0$ whereas 56% (182/327) of the pups born to subordinate males had $F>0$. The z-ratios between these two independent proportions were both significant ($p<0.001$) (Table 3.2).

Table 3.2 Summary table of the inbreeding status of pups born to dominant and subordinate parents. Only pups with parents of known status and pups for whom *F* could be calculated for were considered.

		Dominant	Subordinate	*p*
Female	$F=0$	518 (60%)	70 (45%)	0.0005
	$F>0$	349 (40%)	86 (55%)	0.0005
Male	$F=0$	439 (67%)	145 (44%)	<0.0002
	$F>0$	213 (33%)	182 (56%)	<0.0002

3.4.2 Analyses of traits

Strong positive correlations were detected between all pairs of normally distributed traits (Table 3.3). I found no associations between mother's *F* and offspring traits in any models, so I only present models testing for associations with pup's *F*. Any reported negative effects of pup *F* remained after removal of either one of the strongly correlated variables.

Table 3.3 Pearson's correlations between normally distributed traits: EM, SL, FL, HL and GI. Numbers above the diagonal give the correlation value (*r*) whereas numbers below give sample size (*n*). Sample sizes vary due to missing data for some variables. All correlations are significant at $p<0.001$

	EM	SL	FL	HL	GI
EM	–	0.640	0.725	0.640	0.643
SL	211	–	0.672	0.687	0.400
FL	165	171	–	0.738	0.489
HL	211	219	171	–	0.481
GI	401	202	156	202	–

3.4.2.1 Emergence mass

Emergence mass showed strong inbreeding depression, with inbred pups weighing less than those with $F=0$ (parameter estimate=-156.30 g ± 47.24 S.E.; Table 3.4; Figs. 3.3*a* & 3.4*a*). For an individual with $F=0.078$ (the mean F of the population) this parameter estimate translates into an approximately 10.9% reduction in emergence mass compared with individuals with $F=0$. The negative effect of F remained when individuals with $F>0.13$ were removed (Fig. 3.3*a* dashed line). Lactator number had a significantly positive effect on pup emergence mass: inbred pups with three or more lactators emerged at equal or greater mass than $F=0$ pups suckled only by their mother (lactator number=1) (Fig. 3.4*a*). Additionally, there was a significant interaction between pup F and lactator number (Table 3.4) in which emergence mass increased with increasing lactator number for both outbred and inbred pups with four or fewer lactators, but decreased for outbred pups born to groups with five or more lactators. This interaction remained even when moderately inbred ($F\geq0.125$) individuals were excluded from the model but disappeared when one exceptionally large subordinate breeding event (in which five subordinate females simultaneously gave birth to a total of 11 inbred pups in a group with eight lactators (F range=0.03906-0.10156)) was removed. Removal of this breeding event also removed the main effect of pup F. There were also positive and negative effects of pup age and pup age^2 on emergence weight respectively, and interactions between

pup *F* and pup age, pup *F* and pup age^2 (Table 3.4) such that the magnitude of the age effects on emergence weight were reduced for pups weighed at older ages.

3.4.2.2 Skeletal traits (skull, forearm and hind-foot length)

Hind-foot length showed some evidence of inbreeding depression whereas skull length and forearm length did not (hind-foot length parameter estimate=-17.55 mm ± 6.72 S.E.) (Table 3.4 & Fig. 3.3*b*; see Supporting Information of Nielsen *et al.* (2012) for skull and forearm length results). For an individual with F=0.078 this parameter estimate translates into an approximately 3.7% reduction in hind-foot length compared with individuals with F=0 and the negative effect of F remained when individuals with F>0.13 were removed. (Fig. 3.3*b* dashed line). There were no interactions between pup F and any of the remaining variables in the final hind-foot length minimal model.

3.4.2.3 Growth until independence

Growth until independence showed strong inbreeding depression (parameter estimate=-7.57 ± 2.089 S.E.) (Table 3.4 & Fig. 3.3*c*). For an individual with F=0.078 this parameter estimate translates into an approximately 9.3% reduction in growth compared with individuals with F=0 and the negative effect of F remained when F>0.13 values were removed (Fig. 3.3*c* dashed line). Plotted raw data show that on average F=0 pups grew faster than F>0 pups (Fig. 3.4*b*). There was also a negative interaction between pup F and lactator number (parameter estimate=-3.64 ± 1.14 S.E.) (Table 3.4). In contrast to emergence mass, growth until independence was approximately constant regardless of lactator number for outbred pups (F=0) whereas increasing lactator number was negatively associated with growth in inbred pups (F>0) (Fig. 3.4*b*), but as with emergence mass, the effects of inbreeding depression were stronger with large numbers of lactators. This interaction remained even when moderately inbred (F≥0.125) individuals were excluded from the model but disappeared when one exceptionally large subordinate breeding event was removed. In contrast to emergence mass, the main effect of F remained even when this breeding event was excluded from analysis.

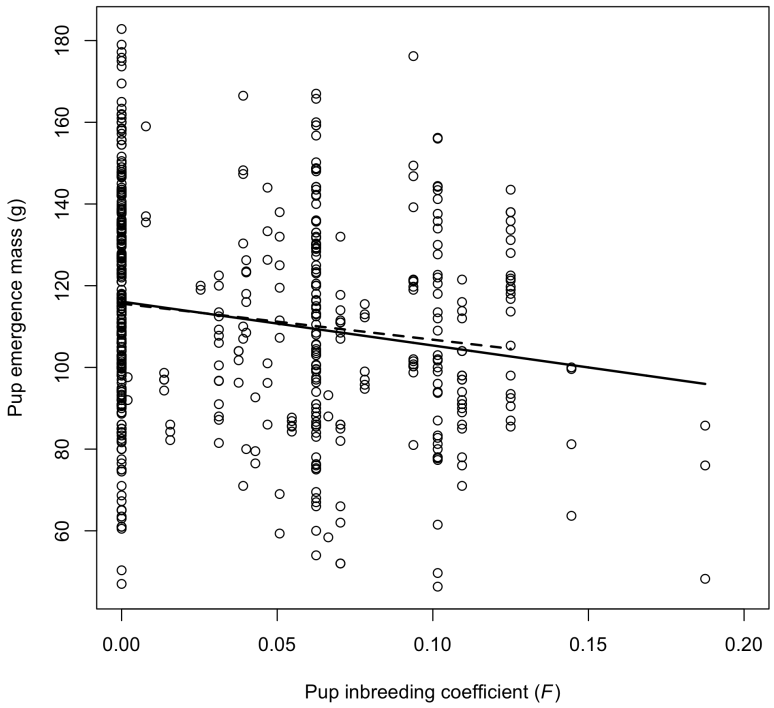
Table 3.4 Summary of final minimal models of emergence mass, hind-foot length and growth until independence. Final minimal models were achieved by sequentially dropping the least significant terms based on Wald statistics until only significant (p>0.05) terms remained. NS terms are not significant and therefore removed. Total deviance explained by all fixed effects=3.067% (emergence mass), 4.291% (hind-foot length) and 1.195% (growth until independence). The percentage deviance of this that was attributable to F is the change in deviance between the final minimal model and a model with F removed, expressed as a proportion of the total deviance explained by all fixed effects in the final minimal model=8.1% (emergence mass), 15.1% (hind-foot length) and 17.9% (growth until independence). The growth until independence parameter (k) has been scaled by a factor of 100.

	Emergence mass (g)			Hind-foot length (mm)			Growth until independence (*k*)		
Fixed effects[*]	Estimate ± SE	F_{df}	*p*-value	Effect ± SE	F_{df}	*p*-value	Effect ± SE	F_{df}	*p*-value
Pup *F*	-156.30 ± 47.420	$9.273_{1,524}$	0.004[‡]	-17.550 ± 6.716	$7.031_{1,261}$	0.019[‡]	-7.57 ± 2.089	$12.298_{1,625}$	0.002[‡]
Pup age	7.904 ± 1.736	$40.989_{1,524}$	<0.001[‡]	0.360 ± 0.124	$4.620_{1,261}$	0.010[‡]	–	–	–
Pup age^2	-0.118 ± 0.033	$11.949_{1,524}$	0.002[‡]	-0.001 ± <0.001	7.109	0.015[‡]	–	–	–
Litter size	-4.083 ± 1.686	$0.265_{1,524}$	0.028	NS	–	–	-0.211 ± 0.067	$0.625_{1,625}$	0.005[‡]
Mother age	-0.006 ± 0.003	$0.734_{1,524}$	0.048[‡]	-0.001 ± <0.001	$4.682_{1,261}$	0.006[‡]	$-3.424*10^{-4} \pm 1.153*10^{-4}$	$1.927_{1,625}$	0.008[‡]
Mother mass	0.097 ± 0.031	$9.534_{1,524}$	0.007[‡]	0.012 ± 0.005	$7.276_{1,261}$	0.016[‡]	$4.804*10^{-3} \pm 1.363*10^{-3}$	$12.399_{1,625}$	0.002[‡]
Helper no.	-1.145 ± 0.327	$1.913_{1,524}$	0.003[‡]	NS	–	–	NS	–	–
Lactator no.	4.188 ±1.126	$11.848_{1,524}$	0.002[‡]	NS	–	–	-0.100 ± 0.046	$3.558_{1,625}$	0.042[‡]
YOB	–	$3.084_{8,524}$	–	–	$5.098_{5,261}$	–	–	$6.549_{8,625}$	–
2000	13.560 ± 7.512	–	0.090	0.162 ± 1.281	–	0.901	-0.064 ± 0.326	–	0.847
2001	20.220 ± 8.261	–	0.026	–	–	–	-0.238 ± 0.327	–	0.476
2002	7.852 ± 8.316	–	0.359	–	–	–	-0.196 ± 0.310	–	0.534
2003	10.230 ± 9.433	–	0.294	–	–	–	-0.099 ± 0.429	–	0.819
2004	9.247 ± 7.312	–	0.224	1.708 ± 1.229	–	0.184	-0.069 ± 0.318	–	0.832
2005	5.583 ± 7.412	–	0.462	-0.839 ± 1.135	–	0.470	-0.582 ± 0.324	–	0.090
2006	6.376 ± 8.590	–	0.469	0.210 ± 1.235	–	0.867	-0.721 ± 0.363	–	0.063
2007	-8.209 ± 12.790	–	0.530	-1.479 ± 1.871	–	0.441	-1.922 ± 0.592	–	0.004[‡]
Season	–	–	–	-1.707 ± 0.613	$3.737_{1,261}$	0.001[‡]	–	–	–
Rainfall	NS	–	–	0.009 ± 0.004	$2.037_{1,261}$	0.004[‡]	$0.036 \pm 7.476*10^{-4}$	$14.496_{1,625}$	<0.001[‡]
F * pup age	-13.544 ± 6.245	$4.672_{1,524}$	0.046	–	–	–	NS	–	–
F * pup age^2	-0.321 ± 0.122	$6.945_{1,524}$	0.018	–	–	–	NS	–	–
F * lactator no.	-131.000 ± 25.270	$26.857_{1,524}$	<0.001[‡]	–	–	–	-3.638 ± 1.135	$10.319_{1,625}$	0.005[‡]

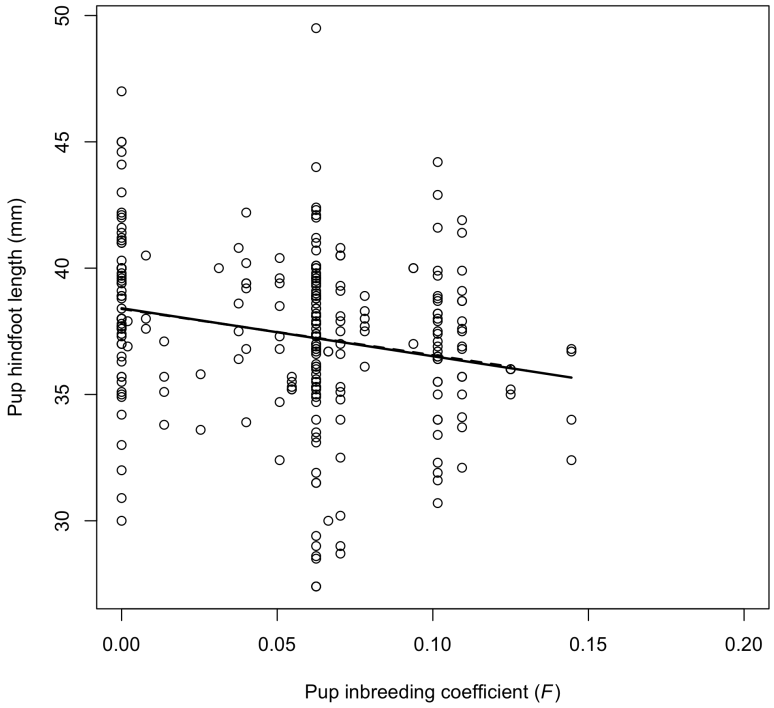
Random effects	Variance	%	Variance	%	Variance	%
Mother ID	<0.001	<0.001	$5.974*10^{-15}$	<0.001	$8.313*10^{-14}$	<0.001
Litter ID	373.240	66.333	4.686	57.845	$7.840*10^{-4}$	58.289
Birth group ID	96.514	17.153	0.000	0.000	$2.029*10^{-4}$	15.1082
Residual	92.925	16.515	3.415	42.155	$3.582*10^{-4}$	26.629

[*]NS variables not fitted in the final minimal model: pup sex; mother *F*; mother status; mother age^2 and helper number^2.
[†]Reference level for pup year of birth is 1999.
[‡] Significant after sequential Bonferroni correction.

a.)


Pup emergence mass (g)
60
80
100
120
140
160
180
0.00
0.05
0.10
0.15
0.20
Pup inbreeding coefficient (*F*)

b.)


Pup hindfoot length (mm)
30
35
40
45
50
0.00
0.05
0.10
0.15
0.20
Pup inbreeding coefficient (*F*)

c.)

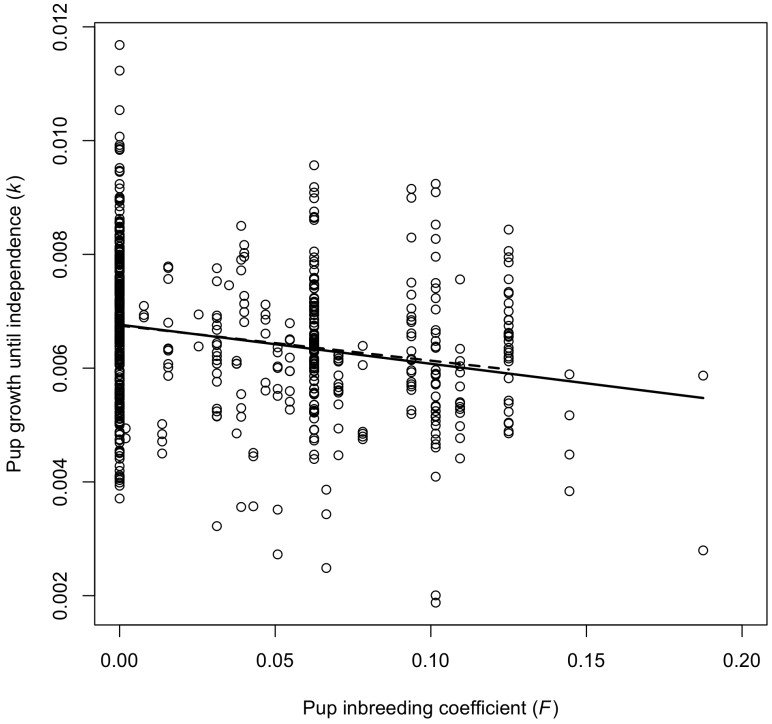
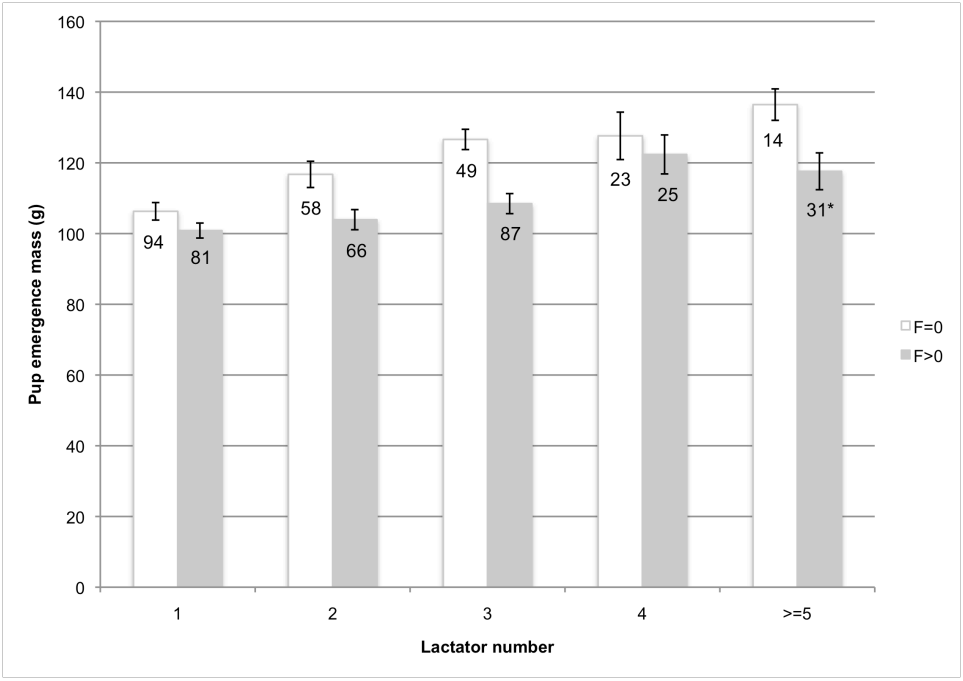


Figure 3.3 The effect of pup inbreeding coefficient (*F*) on *a.*) emergence mass (g), *b.*) hind-foot length (mm), and *c.*) growth until independence (*k*). Circles show individual pups and solid lines show effects predicted by the models. Dashed lines show that the negative trends remain even when *F*>0.13 values are excluded.

3.4.2.4 Survival

Pup survival (from emergence to 90 days) did not show any evidence of inbreeding depression due to the fact that all the pups with high *F* (*n*=37) in the data set survived this period of time (Table 3.5 & Fig. 3.5*a*). Juvenile survival (from 90 to 180 days) showed evidence of inbreeding depression: Pup *F* was positively associated with hazard (parameter estimate=11.573 ± 4.405 S.E.) such that, on average, individuals with *F*=0.078 were 2.46 (exp(11.573*0.078)) times more likely to die than *F*=0 individuals (Table 3.5 & Fig. 3.5*b*). Although I did not specifically test for effects of inbreeding on pup survival from birth to emergence, a strong negative correlation between pup *F* and litter size at emergence was detected (see Supporting Information of Nielsen *et al.* (2012)) suggesting that inbred pups suffer a higher rate of early neonatal mortality within the burrow.

a.)



b.)

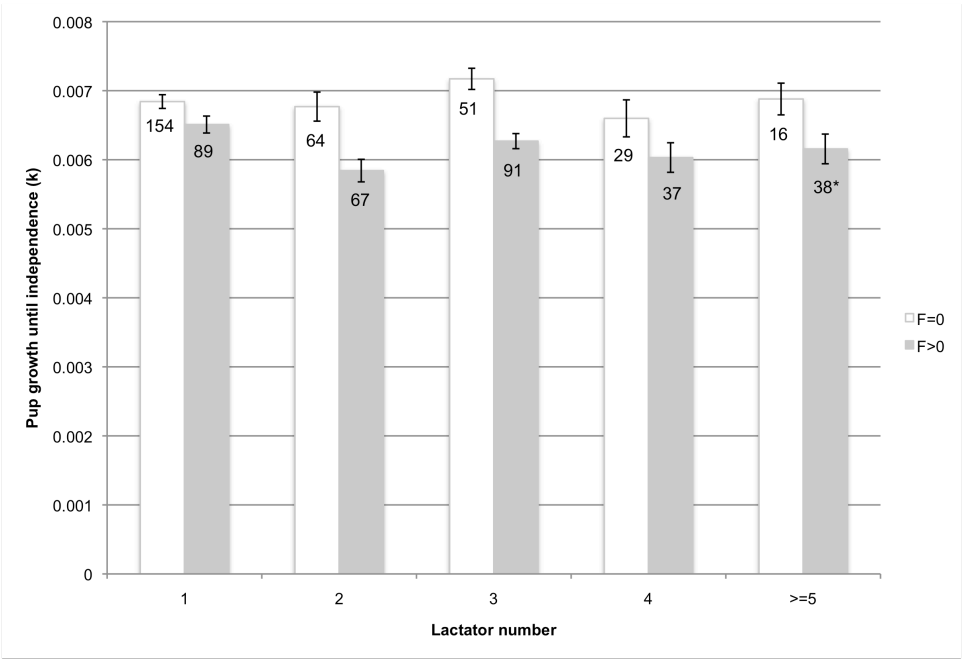
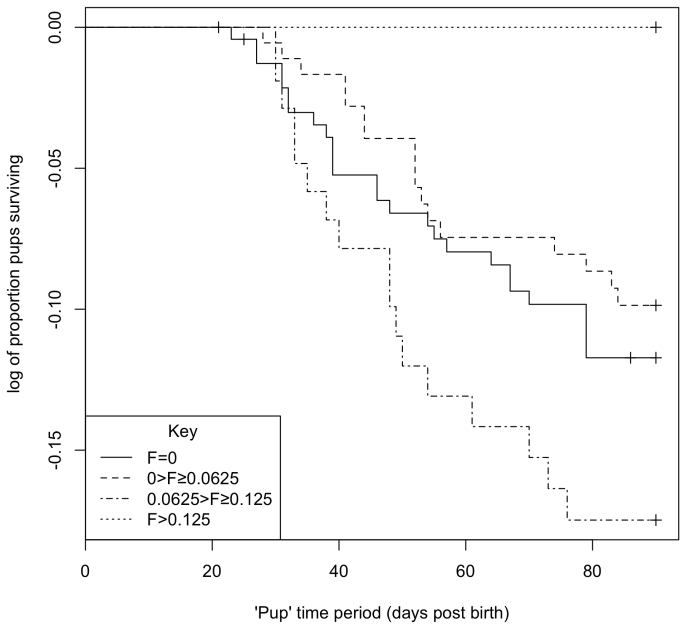


Figure 3.4 The effect of lactator number on inbreeding depression in pup *a.*) emergence mass and *b.*) growth until independence. Sample sizes (number of pups) are given at the top of each bar. Increasing numbers of lactators increased emergence mass for both inbred and outbred pups with ≤4 lactators whereas the mass of inbred pups was depressed with ≥5 lactators. Inbred pups tended to grow more slowly with increasing numbers of lactators. Columns with asterisks include one large subordinate breeding event of 11 pups born to 5 different mothers and 8 lactators, which was removed in a subsequent analysis.

Table 3.5 Results of Cox proportional hazard survival analyses for pup survival (from emergence to 90 days; *n*=561) and juvenile survival (from 90 to 180 days; *n*=497). The final minimal models were simplified where -(AIC_{null}-AIC_{model}) was minimised. ΔAIC gives the difference in AIC values between the final minimal models and a model including the term in question and the *p*-values give the significance of the term prior to its removal. The estimate of effects (and accompanying standard errors) refer to the effects on the proportional hazard rate and variables in bold are retained in the final minimal model. Litter ID was fitted as a random effect.

	Pup survival			Juvenile survival		
Fixed effects	ΔAIC	Effect ± SE	*p*-value	ΔAIC	Effect ± SE	*p*-value
Pup *F*	1.46	-2.905 ± 4.618	0.530	**0**	**11.573 ± 4.405**	**0.009**
Sex	2.21	0.232 ± 0.293	0.430	2.24	0.195 ± 0.371	0.600
Litter size	0	-0.207 ± 0.171	0.220	**0**	**0.263 ± 0.202**	**0.190**
Mother status	0	.0.625 ± 0.003	0.300	1.81	-0.240 ± 0.803	0.770
Mother age	0.74	<0.001 ± <0.001	0.490	1.77	<0.001 ± <0.001	0.610
Mother mass	**0**	**-0.006 ± 0.003**	**0.038**	0.10	0.004 ± 0.004	0.300
Helper no.	1.32	0.001 ± 0.026	0.960	**0**	**-0.064 ± 0.032**	**0.046**
Lactator no.	1.01	0.057 ± 0.110	0.600	0.86	-0.238 ± 0.176	0.180
YOB	0	–	–	**0**	–	–
Season	1.28	-0.104 ± 0.426	0.810	1.84	0.108 ± 0.571	0.850
Rainfall	1.02	0.001 ± 0.003	0.630	0.68	0.003 ± 0.004	0.450

a.)



b.)

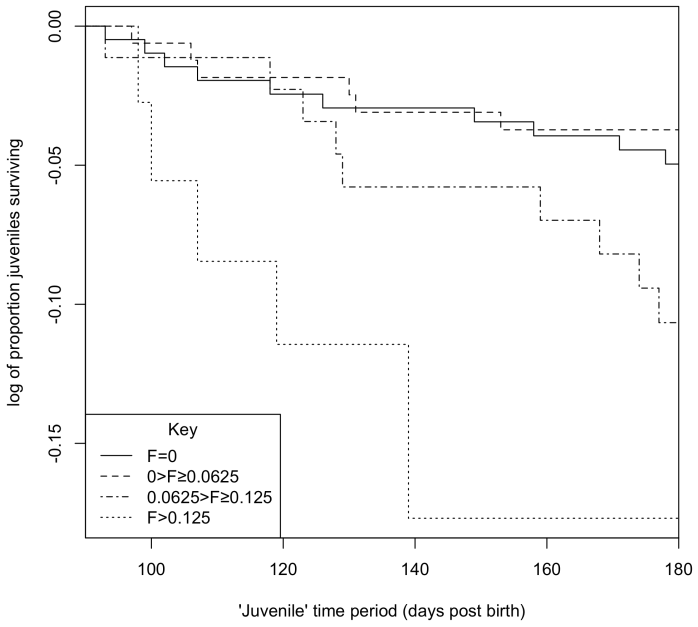


Figure 3.5 Survival curves for *a.*) pup survival (emergence - 90 days) and *b.*) juvenile survival (90 - 180 days) for different *F*-value categories. All moderately inbred pups (*F*>0.125; *n*=37) survived the pup period, whereas the survival of outbred and slighty inbred (*F*<0.125) pups was similar. The hazard rate for inbred individuals was greater in the juvenile time period: survival for *F*=0 or pups with low *F* values were similar, with decreasing survival rates for moderately and highly inbred pups**.**

3.4.2.5 Role of other variables

The other fitted variables had a variety of effects depending on the trait analysed. Pup age had a significant positive effect and pup age^2 had a significant negative effect on emergence mass and hind-foot length (Table 3.4). Litter size either had a significant negative effect (emergence mass & growth until independence) or no effect (hind-foot length and survival) (Tables 3.4 & 3.5). Mother's age and mass had consistently negative and positive effects respectively on the emergence mass and the skeletal traits and no significant effect on juvenile survival (Tables 3.4 & 3.5). Mother's mass did, however, have a positive effect on survival from emergence to independence, i.e. it was negatively associated with hazard rate such that for each gram increase in a mother's mass (for pups of the same age) the risk of death was reduced to 0.994 ± 0.038 of the baseline hazard (Table 3.5). Helper number had a variable effect: it had a negative effect on emergence mass, no effect on hind-foot length and growth until independence, and a positive effect on juvenile survival, i.e. it was negatively associated with hazard rate such that for each unit increase in the number of helpers (for pups of the same age) the risk of death was reduced to 0.938 ± 0.032 of the baseline hazard (Table 3.5*)*. Lactator number also had a variable effect: as reported above it positively influenced emergence mass, but it negatively affected growth until independence and had no effect on hind-foot length and the survival periods. Where significant, rainfall and season had positive (growth until independence & hind-foot length) and negative (hind-foot length) effects respectively and had no effect on survival over either stage investigated. None of the other variables (pup sex, mother *F*, mother status, mother age^2 and helper number^2) had a significant effect on any of the traits.

Litter explained the overwhelming majority of the variance in emergence mass (66%), hind-foot length (58%) and growth until independence (58%) whereas maternal effects were negligible (<0.001%) (Table 3.4). Birth group explained some of the variance in emergence mass (17%) and growth until independence (15%) but not hind-foot length (0%), and unexplained residual variance varied from 17% (emergence mass) to 42% (hind-foot length).

3.5 Discussion

I found that matings between relatively distant relatives resulted in approximately 44% of the study population of meerkats being inbred to some extent. Furthermore I found negative consequences of inbreeding for a range of morphometric and survival traits, which appear to be driven by pup rather than mother's inbreeding coefficients. I also found some evidence that social factors affect the expression of inbreeding depression. I discuss each of these in turn below.

3.5.1 Occurrence of inbreeding

The extent of inbreeding in the study population is generally much higher than that reported in other wild vertebrate systems (e.g. Keller & Waller 2002, Richardson *et al.* 2004, Schiegg *et al.* 2006, Szulkin & Sheldon 2007, Rioux-Paquette *et al.* 2010b, Walling *et al.* 2011). Furthermore, the fact that more than half of the breeding pairs in the study population are related, and that subordinates are statistically more likely than dominants to produce inbred offspring, indicates that inbreeding is widespread rather than being simply the result of a few highly productive, but inbreeding, dominant pairs. The extent and magnitude of inbreeding I report herein is likely to be an underestimate due to the fact that classical pedigree construction assumes that population founders and immigrants are unrelated and non-inbred, and because of the incompleteness of the reconstructed pedigree. Although the two study systems are very different, my results are somewhat comparable to those found using a social pedigree of a population of song sparrows where 44% of all matings were between known relatives (Keller 1998). Keller (1998) concluded that the high level of inbreeding in song sparrows was in part due to that fact that the Mandarte population of song sparrows lives on an isolated island where only 3% of the breeding birds were immigrants, creating a scenario where (related) residents are more likely to mate with each other. In contrast, a very different population scenario governs the Kalahari meerkat population studied herein: although individuals live in distinct groups which are characterised by strong reproductive skews in favour of dominants, it is an open population and there are no distinct geographical barriers preventing individuals from outside the study population migrating into it (Clutton-Brock *et al.*

2002, Stephens *et al.* 2005). An additional similarity to the Mandarte song sparrow population (Keller 1998) is that the majority of meerkat consanguineous matings were between distantly related kin, rather than close relatives, which resulted in low to moderate *F*-values (average F=0.078). Perhaps more pertinent to meerkats, the levels of inbreeding I report here are most comparable to levels reported in a cooperative mammal population of black-tailed prairie dogs (*Cynomys ludovicianus*) in which 26% of all copulations were between kin ($0.2500 > r \geq 0.0078$) (Hoogland 1992). A study on a population of dwarf mongooses (*Helogale parvula*) also reported widespread moderate inbreeding and that 14% of offspring resulted from matings between close relatives (Keane *et al.* 1996). It is worth noting that although Hoogland (1992) tested for (but did not find evidence of) inbreeding depression, neither the Hoogland (1992) nor the Keane *et al.* (1996) data was supported by genetics.

3.5.2 Evidence for inbreeding depression

I found evidence for negative consequences of inbreeding, driven by pup's *F* rather than mother's *F*, for a range of early life traits. Mating between relatively distant meerkat kin and their ancestors appeared sufficient to cause inbreeding depression as the negative effects of inbreeding remained even when high values of *F* (F>0.13) were removed, suggesting that inbreeding depression was not driven by extreme inbreeding events. Similarly, a recent study found that inbreeding in red deer was not dominated by close inbreeding events and that inbreeding depression was not solely driven by individuals with high *F*-values (Walling *et al.* 2011).

Inbreeding depression was evident for emergence mass, hind-foot length and growth until independence. Previous studies in wild mammalian populations have also reported inbreeding depression in early weight measures (e.g. Coltman *et al.* 1998, Coulson *et al.* 1998, Slate & Pemberton 2002, Walling *et al.* 2011). I also found evidence for inbreeding depression in hind-foot length. Although the other two skeletal measures (skull length and forearm length) showed no evidence for inbreeding depression, all the skeletal measures had small sample sizes and may suffer more from measurement error and consequently my analyses here probably

suffered from a lack of power. As the skeletal measures were highly correlated, I predict that larger samples sizes would reveal similar patterns of inbreeding depression in these traits. To the best of my knowledge this is the first study that has investigated the effects of inbreeding on early life growth rates in a wild population of mammals. As weight has been shown to have positive consequences for both fitness and dominance in meerkats (Russell *et al.* 2002, Hodge *et al.* 2008) it follows that a low growth rate due to inbreeding is likely to be disadvantageous in terms of fitness.

There was evidence of inbreeding depression on juvenile survival, but none for pup survival, suggesting that the strong inbreeding depression on emergence mass, hindfooot length and growth until independence did not translate into survival costs until after pups reached independence. Upon reaching independence, helpers discontinue provisioning pups and the juveniles must provide for themselves, although they may continue to receive benefits from group living such as greater protection from predators and neighbouring meerkat groups (Clutton-Brock *et al.* 1999a). Reaching independence may therefore represent a 'stressful' environment in which the consequences of inbreeding depression are more pronounced. Previous investigations of environmental effects on inbreeding depression have demonstrated a general pattern of increased inbreeding depression under more stressful environments (Keller *et al.* 2002, Marr *et al.* 2006, Szulkin & Sheldon 2007, Fox & Reed 2011). My results provide some evidence that the stress imposed by a change in social environment (the discontinuation of helper provisioning for post-independence meerkats) is analogous to the ecologically stressful environments reported in other animal populations. Put another way, it is possible that helpers 'buffer' pups from the effects of inbreeding prior to independence.

It is worth noting that in all these analyses, data were only collected from pups that emerged from their natal burrow. It is therefore possible that pups that suffered from such severe inbreeding depression that they died prior to emergence were excluded. Although the study system prevents us from explicitly testing whether entire litters failed to emerge, the fact that litter size was negatively associated with pup *F*

suggests that inbred pups may be more likely to suffer a higher rate of early neonatal mortality within the burrow and provides further evidence for inbreeding depression in this system.

3.5.3 Interactions between inbreeding depression and other traits

The general positive effect of lactator number on emergence mass provides some evidence that the social environment (allolactators) can, to some extent, buffer (but not completely compensate) inbred pups from the negative effects of inbreeding. This is consistent with the findings of Russell *et al.* (2002) who found that subordinate helping positively influences the weight, survival and daily weight gain of pups. The negative interaction of pup *F* with emergence weight and growth until independence, in which increased lactator numbers had increasingly negative effects on more inbred pups, is more difficult to interpret. As I found that inbred pups were more likely to be born to subordinate females and because subordinates are more likely to give birth into 'mixed litters' composed of other (subordinate or dominant) pups of the same age (M. Bell, *pers. comm.*), I speculate that the negative interaction with allolactation may reflect greater competition between pups and the relative failure of inbred pups to 'win' allolactator's milk. Re-analysis of the data following the removal of one exceptionally large subordinate breeding event in a group with many lactators suggests that mixed litter, subordinate inbreeding may indeed be driving much of the pup *F* x lactator number interactions. Given that I have found that subordinates are more likely than dominants to produce inbred pups, but that dominants are responsible for the majority of the total breeding in the population, I believe it is not appropriate to simply interpret the results with this mixed litter excluded. An alternative possibility is that my measure of lactator number is an overestimate of true allolaction provisioning in this instance.

3.5.4 Effects of other variables

Maternal, social and environmental variables influenced the variance of traits in different ways. All pre-independence traits showing evidence for inbreeding depression were positively affected by mother's conception mass and negatively affected by mother's age, indicating either that maternal condition is an important

early life factor in this cooperatively breeding species (Russell & Lummaa 2009), that conception mass is heritable and/or that it is positively genetically correlated with the traits presented here. Social factors had opposing influences during this period: helper number was either non-significant or had a negative effect whereas the number of lactators had largely positive effects. Previous research in the population has found that social factors, such as subordinate helping, positively influences the weight and survival of pups and concluded that, to some extent, they replace the importance of maternal factors (Clutton-Brock *et al.* 2002). By including lactator number as a separate variable in my analyses I was able to apportion some of this positive effect of helping to a specific helper type during the pre-foraging period, although this variable had complex interacting effects (see above). A previous study on the same population found that pup daily weight gain was positively influenced by the number of helpers *per pup* and rainfall (which positively influences invertebrate abundance), and negatively influenced by daytime temperature (Clutton-Brock *et al.* 2002). Although I found that accumulated rainfall in the last three months was positively associated with pup growth-rate, I found no effect of *total* helper number and a weak negative effect of lactator number, though it is worth noting that Russell *et al.* 2002 used a slightly different index of helper availability than I have here. During the juvenile period helper number decreased the hazard risk which is in line with previous research that demonstrated a positive effect of increased group size (Russell *et al.* 2002). Maternal effects explained very little of the variance in my models so it is perhaps not surprising that mother's *F* did not have an important effect on pup traits, but given the highly social nature of meerkats and that previous research has demonstrated positive effects of helper number, one interesting line of research would be to test whether there is an association between *F* and the amount of care contributed by helpers.

Since the traits investigated herein are proxies for total fitness (see Introduction), future work should investigate the effect of inbreeding on reproductive success and/or on later-life survival periods. Due to the substantial rate of mortality and reproductive skew in meerkats, these analyses require rather larger sample sizes than is currently available.

3.5.5 Evolution of inbreeding avoidance

Inbreeding depression effects are likely to generate strong selective pressures for inbreeding avoidance behaviours (Waser *et al.* 1986). Given that I have shown that low-moderate inbreeding leads to substantial negative consequences, why has a more reliable inbreeding avoidance mechanism not evolved in meerkats? One possibility is that moderate inbreeding is tolerated because the benefits of inbreeding outweigh the costs of inbreeding depression (Waser *et al.* 1986, Kokko & Ots 2006). My results show that subordinates are more likely than dominants to produce inbred pups. In meerkats, where the vast majority of reproductive events are monopolised by the dominant pair, subordinate females are primarily limited to mating with immigrant or roving males, who are typically also subordinates (Griffin *et al.* 2003). The high costs of dispersal (Young *et al.* 2005) places relatives in close proximity to each other and consanguineous matings may represent the only opportunities for reproduction that subordinates will gain. As any subordinate mating opportunity is better than none, there may not be strong selection on discriminating the fine-scale relatedness of mates.

I speculate that experience of relatives early in life may be the main source of information on which meerkats base inbreeding avoidance, as I found that much of the inbreeding in the population came about via matings among reasonably distant kin and that the vast majority of matings resulting in $F>0.125$ were between relatives from different natal groups. Similarly, Hoogland (1992) reported that prairie dogs appear to avoid extreme inbreeding as only 5% of all copulations involved a sexual partner of $r\geq0.25$. My findings support the hypothesis that male-biased dispersal and lack of intra-group matings between natal members may act as an effective way of avoiding mating between closely related meerkat kin (Griffin *et al.* 2003). The existence of mechanisms for avoiding matings between less closely related kin (e.g. cousins) is less convincing. Sex-biased dispersal may have the effect of reducing the specialisation required to recognise kin in order to avoid inbreeding by limiting the rule of kin discrimination to those members an individual is familiar with i.e. natal or current group members (Koenig & Haydock 2004). But as patterns of meerkat

dispersal and male roving can be complicated, it is possible that kin born to neighbouring groups can immigrate or rove into a group and mate with relatives that they have had no previous contact with and thus do not 'recognise' as kin. In this way inbreeding occurs, which I have shown leads to inbreeding depression. Testing whether meerkats are indeed avoiding mating with kin and, if so, further determining the mechanisms they utilise to recognise relatives will be an important future line of research (Jordan *et al.* 2007, Mares *et al.* 2011).

Chapter 4

Evolutionary genetics of morphological traits across life stages in the cooperatively breeding meerkat (*Suricata suricatta*)



4.1 Abstract

In many organisms, morphological traits show ontogenetic change and are associated with fitness. Similarly, the magnitudes of variance components and inbreeding effects may also show age-related variation. The genetic and social components of variance in, and the effects of inbreeding on, mass and four skeletal traits were estimated for up to five different life stages in a study population of meerkats. Heritabilities varied for each trait and life stage, and there was some evidence to suggest that the amount of variance accounted for by additive genetic effects increased with age. Birth litter accounted for large amounts of variance in all traits and there was a tendency for the amount of variance accounted for by this term to decline with age. The detrimental effects of inbreeding were present in a number of traits and life stages although there was little evidence to suggest that the magnitude of these effects changed with post-independence age. This study provides an initial decomposition of variation in meerkat morphological traits into genetic and social environment components, and is one of the first to estimate both variance components and inbreeding effects over the life of a cooperatively breeding mammal. In cooperative species, in which individuals typically live in groups with close relatives, accurately separating social or common environment effects from additive genetic effects may present a particular challenge due to the potential confounding of variance component terms.

4.2 Introduction

Determining the genetic and non-genetic sources of variance for traits in wild populations is a recent but growing field of research. Individual differences in phenotypic traits are a key component of evolution as they 'afford materials for natural selection to act on and accumulate' (Darwin 1859). Natural selection can, however, only generate an evolutionary response in traits if they are heritable and consequently one of the major aims of evolutionary biology, and, more specifically, quantitative genetics, is to evaluate the extent to which phenotypic variation has a genetic basis (Falconer & Mackay 1996, Kruuk 2004). However, phenotypic traits can also be influenced by non-genetic sources of variation. These sources of variation are interesting in their own right, especially in wild populations that experience greater variation in biotic and abiotic environmental conditions than either lab or captive populations (Kruuk & Hadfield 2007). Consequently, a growing number of studies are now applying quantitative genetic techniques to wild populations in order to explore the evolutionary genetics of populations in natural settings (e.g. Kruuk *et al.* 2000, Sheldon *et al.* 2003, Teplitsky *et al.* 2009 , McFarlane *et al.* 2010, Serbezov *et al.* 2010, Charmantier *et al.* 2011, Taylor *et al.* 2012).

Trait variance can be driven by several different factors, both genetic and non-genetic (Kruuk 2004, Kruuk & Hadfield 2007). The heritability (h^2) of a phenotypic trait is typically calculated as the proportion of total phenotypic variance (V_P) that is explained by additive genetic variance (V_A) ($h^2 = V_A/V_P$) (Falconer & Mackay 1996, Lynch & Walsh 1998). Although traditionally the primary goal of quantitative genetics has been to estimate the heritability of quantitative traits, shared environmental conditions can also generate similarities among relatives and rather than being a source of error, as they have conventionally been viewed, the sources and magnitudes of similarities not due to direct genetic effects can themselves be of intrinsic interest (Kruuk & Hadfield 2007). For example, parental, and more specifically, maternal effects, which generally reflect the pre- and post-natal influences (e.g. in provisioning) of a mother on her young (Falconer & Mackay

1996), have been found to account for between 5 and 28% of the phenotypic variance in life-history (Kruuk *et al.* 2000, Laugen *et al.* 2005), behavioural (Taylor *et al.* 2012) and morphological traits (Mousseau & Roff 1987, Kruuk *et al.* 2000, Serbezov *et al.* 2010). In cooperatively reared species, where offspring generally receive care from individuals that are not their parents, the social environment is known, to some degree, to replace the maternal contributions typical of non-cooperative species (Jennions & Macdonald 1994, Russell *et al.* 2002). Consequently, in cooperative species I predict that contributions from the social environment to phenotypic trait variance may be large and may reduce, or even replace, the maternal effect estimates that are reported in non-cooperative species. However, since they live in highly related family groups, disentangling genetic from shared environment effects in cooperative species may prove challenging in a classical quantitative genetic frame-work. In this study, I present an initial quantitative genetic analysis of a number of morphological traits for a wild population of meerkats by decomposing trait variation into genetic and social environment components.

Morphological traits show ontogenetic change and consequently the magnitudes of variance components may also show age-related variation. Biotic and abiotic conditions, including the relative importance of the social environment, also change throughout the life of a cooperative mammal: offspring typically receive care from group members in early life and then forage for themselves upon reaching independence, before contributing care to subsequent offspring in later life. Consequently, pups may be in a better nutritional state when they are supported by helpers than when they are foraging independently and this broad change, from being a recipient to a provider of cooperative care, may be associated with changes in the expression of genetic variance and/or the relative importance of the social environment in explaining variation in phenotypic traits. Although making predictions for changes across age and/or condition is notoriously difficult (Hoffmann & Merilä 1999, Wilson *et al.* 2005, Wilson *et al.* 2006), conducting quantitative genetic analyses on different life stages affords greater resolution than single age analyses and has the potential to investigate whether and how the relative

importance of different variance components changes with age. This contrasts with a more traditional approach of just averaging across all ages, which may lead to different conclusions being reached. Most existing morphological trait heritability estimates for wild populations have only been calculated for non-cooperative species and few have investigated the changes in variance components over different life stages (e.g. Kruuk *et al.* 2000, Milner *et al.* 2000, Poissant *et al.* 2008). In this study I analysed repeated measures of a number of morphological traits at a variety of life stages.

Phenotypic traits may also be shaped by non-additive as well as additive genetic effects (Falconer & Mackay 1996, Lynch & Walsh 1998). One such effect is the mating of genetically related parents, or inbreeding, can lead to inbreeding depression, which constitutes a reduction in fitness in the resulting offspring and may be largely due to the expression of deleterious alleles in the homozygous state (Charlesworth & Charlesworth 1999). Inbreeding depression in a variety of traits has now been reported for a number of wild animal and plant populations (Crnokrak & Roff 1999, Keller & Waller 2002), although evidence in cooperative species remains relatively sparse (Chapter 3 of this thesis, Brown & Brown 1998, Richardson *et al.* 2004, Spiering *et al.* 2011). Furthermore, although the effects of inbreeding in early life stages is now well established (Keller & Waller 2002), few studies have investigated the effects of inbreeding depression on later life stages (though see Grueber *et al.* 2010). Focusing research simply on inbreeding in early life stages has the potential to underestimate its lifetime effects if, for example, parents or carers compensate for any reductions in the fitness of inbred offspring by increasing their rates of provisioning during early life. As a result, the true magnitude of inbreeding depression may only be revealed in later life once inbred individuals reach independence and must provide for themselves (Grueber *et al.* 2010). Furthermore, in populations where inbreeding depression is appreciable, and in situations where population structure can lead to a situation in which parents and offspring have similar inbreeding coefficients, failure to account for inbreeding can actually inflate estimates of additive genetic variance and heritabilities (Reid & Keller 2010). In this study, I therefore account for any effects of inbreeding in my

quantitative genetic analyses of meerkat morphological traits and in doing so investigate the effects of inbreeding at a variety of life stages.

Meerkats (*Suricata suricatta,* Schreber 1776) are cooperatively breeding mongooses that are found throughout the semi-arid Kalahari region of southern Africa (Doolan & Macdonald 1999). All of the following meerkat findings have been generated from the 19-year long Kalahari Meerkat Project, which was established by Professor Clutton-Brock and the University of Cambridge (Clutton-Brock *et al.* 1999a, Russell *et al.* 2002). Groups typically consist of 2-50 individuals and are composed of a dominant pair and a number of male and female subordinate helpers that are either immigrant males or, more commonly, offspring from the dominant pair's previous breeding attempts (Clutton-Brock *et al.* 1999b, Doolan & Macdonald 1999).

There are a number of key life stages of a meerkat: individuals are generally considered 'pups' from birth, through emergence from the natal burrow at approximately three weeks of age, and up until they reach nutritional independence at approximately three months of age, after which point they are termed 'juveniles'. During the first three months of their lives, pups are primarily cared for by subordinates who baby-sit and suckle (allolactate) them at the natal burrow (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999, Scantlebury *et al.* 2002), and who feed and teach them once they begin foraging with the group (Clutton-Brock *et al.* 2001b, Thornton & McAuliffe 2006). At approximately six months of age meerkats begin to contribute significantly to cooperative care and are thus considered 'sub-adults' up until the point at which they become 'adults' (at approximately one year) (Clutton-Brock *et al.* 1998a, Doolan & Macdonald 1999). As growth rates in meerkats vary (English *et al.* 2012), asymptotic mass is difficult to ascertain but most meerkats appear to reach their final mass at approximately 18 months of age (S. English, *pers. comm.*).

Meerkat populations are characterised by large dominant-biased skews in reproduction (Griffin *et al.* 2003, Spong *et al.* 2008), contributions to cooperative care (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999), and survival (Sharp &

Clutton-Brock 2010). Competition for dominance acquisition and retention is therefore strong: size appears to confer fitness advantages as dominant meerkats generally weigh more than subordinates (Hodge *et al.* 2008) and tend to direct acts of aggression and eviction towards older subordinate females (Kutsukake & Clutton-Brock 2006). As part of my thesis, I generated a genetically-validated pedigree for the study population (Chapter 2). In addition to revealing inbreeding and inbreeding depression in a number of early life traits, including emergence mass, hind-foot length, growth until independence and juvenile survival (Chapter 3), this pedigree now provides the possibility for quantitative genetic analyses on the population to be conducted.

The aim of this study was to investigate the relative roles of genetic and social environment effects on, and to obtain heritability estimates for, mass and four skeletal traits with the prediction that, due to meerkats' cooperative behaviour, the social environment would contribute a large proportion of the total phenotypic variance. Furthermore, I predicted that the magnitudes of both the additive genetic and social environment effects might change across different life stages. In addition, I also investigated the genetic and social correlations between the traits with the expectation that, since the traits were all measures of size, they would be strongly correlated with each other via all routes. Finally, I considered the effect of inbreeding on these traits and whether the magnitude of any detected inbreeding depression changed with age, with the prediction that inbreeding depression would increase with age as individuals became less dependent on care from con-specifics and competition among group members increased.

4.3 Materials and methods

4.3.1 Study population

All data presented herein come from the on-going Kalahari Meerkat Project, Kuruman River Reserve, South Africa (26º5'S, 21º49'E), which was initially established by Professor T. H. Clutton-Brock and the University of Cambridge in 1993. The study site continues to be maintained by T. H. Clutton-Brock (University of Cambridge) and Professor M. B. Manser (University of Zurich). Detailed descriptions of the study area and system are provided elsewhere (Clutton-Brock *et al.* 1999a, Russell *et al.* 2002). In brief, resident meerkats are habituated to close observation (<1 m) and individually identified using unique combinations of hair dye marks (Thornton 2008). Each group is visited up to two times a day, at least three times a week, in order for trait measurements, including mass, to be recorded, and for individual key life-history events, such as changes in group composition and dominance, to be noted (Sharp & Clutton-Brock 2010). Upon emergence from the natal burrow (at approximately three weeks of age), a 2-5 mm tissue sample from the tail tip of each pup is taken for genetic analysis (Griffin *et al.* 2001, Spong *et al.* 2008). Other individuals, such as founders and immigrants, are either sampled post-mortem or under anaesthetic. All sampled individuals have been genotyped at up to 18 variable microsatellite loci so that genetic data are now available for 86% of the total recorded population (Chapter 2).

4.3.2 Pedigree and inbreeding coefficients

A combination of phenotypic descriptors, microsatellite data and two parentage inference programs, COLONY2 (Wang 2004, Wang & Santure 2009) and MASTERBAYES (Hadfield *et al.* 2006), were used to reconstruct the pedigree of the study population. Detailed descriptions of the molecular and pedigree construction methods are provided elsewhere (Chapter 2). In summary, in cases where a single female gave birth, observed pregnancy (i.e. changes in mothers' pre- and post-birth weight) was used to assign maternity. All paternities and mixed maternities (where multiple females gave birth at similar times and maternities were thus impossible to determine by observation) were genetically-validated. There was a high level of

confidence (average individual parentage assignment confidence=95.57%) and a high number of matching parents between the COLONY2 and MASTERBAYES inferences indicating minimal parentage assignment errors (see Chapter 2 for further details). Furthermore, because parentage mis-assignments have been shown to reduce estimates of the covariance between relatives (and thus heritability), any erroneous parentage identifications are expected to result in underestimates rather than overestimates of heritabilities (Charmantier & Réale 2005). The full pedigree utilised herein spans a maximum of eight generations and contains 2,147 individuals, of which 1,531 have both parents assigned and 411 have only maternity assigned (includes 'dummy' parents (Chapter 2); Table 4.1). Pups were born to 175 unique mothers (average number of pups per mother=11 ± 16.26 S.D.) and 180 unique fathers (average number of pups per father=9 ± 10.41 S.D.). The strong reproductive skew operating in the system has resulted in maximum maternal and paternal sib-ship sizes of 83 and 54 pups respectively. Pedigree statistics were obtained using PEDANTICS (Morrissey & Wilson 2010) which revealed that, across all life stages, the pedigree is phenotypically informative for a maximum of 1,689 individuals (Table 4.1). Table 4.1 gives further pedigree statistics on phenotypically informative individuals that have either been measured, or are linked to individuals that have been measured, for the traits of interest.

Table 4.1 Summary statistics for the entire Kalahari Meerkat Pedigree ('full' pedigree) utilised herein and as produced by the PEDSTATSUMMARY function in the R-package PEDANTICS. The 'pruned' pedigrees for mass, skull length, skull width, forearm length, and hind-foot length consist of only those individuals that are informative with respect to the specified phenotypic traits (i.e. the individual has been measured for the trait or links individuals which have been measured for the trait). Data are for all life stages combined.

Statistic	No. of links in full pedigree	No. of links in pruned pedigrees				
		Mass	Skull length	Skull width	Forearm length	Hind-foot length
Records	2147	1333	1684	1684	1161	1689
Maternities*	1942	1274	1614	1614	1113	1619
Paternities*	1531	988	1276	1273	865	1278
Full sibs	11165	6055	9047	9045	5864	9049
Maternal sib links	32822	17047	29204	29203	16561	29210
Maternal half sib links	21655	10995	20157	20158	10696	20161
Paternal sib links	15441	7995	12323	12300	7389	12338
Paternal half sib links	4276	1940	3276	3255	1525	3289
Maternal grandmothers	1701	1231	1569	1568	1081	1573
Maternal grandfathers	1624	1173	1421	1420	1016	1425
Paternal grandmothers	1009	728	901	897	650	902
Paternal grandfathers	861	609	728	724	522	729
Maximum pedigree depth (generations)	8	7	7	7	7	7
Founders	205	59	70	70	48	70
Mean maternal sibship size	11.10	9.23	12.51	12.51	10.60	12.36
Mean paternal sibship size	5.51	7.16	8.45	8.43	7.21	8.46

*Maternities and paternities include 'dummy' parents as assigned by COLONY2 (see Chapter 2 for further details).

Wright's inbreeding coefficients (*F*) were calculated for each individual using PEDIGREEVIEWER (http://www-personal.une.edu.au/~bkinghor/pedigree.htm) (Kinghorn 1994), under the assumption that all founders and immigrants were unrelated. An *F*-value for an individual was only calculated when at least two grandparents had been identified (Chapter 3). These restrictions meant that 19% of the total population were not assigned a *F*-value. Since the pedigree is incomplete and limited in depth, there will be error in the *F*-values, but this is predicted to act in a conservative manner.

4.3.3 Study traits

4.3.3.1 Response variables

Mass

Meerkat pups were weighed upon emergence from their natal burrows (at approximately three weeks of age), and up to three times a day thereafter (morning, midday and evening) for as long as they remained present in the population, using a top-pan balance (±1 g) (Clutton-Brock *et al.* 2003). I investigated the sources of variance in, and effect of inbreeding on, mass in five different age windows, which were chosen to reflect different life stages (Table 4.2):

- upon *emergence* from the natal burrow (16 - 26 days; n = 685 individuals);
- upon reaching *independence* (85 - 95 days; n = 1,146 individuals);
- at the end of the *juvenile* period (175 - 185 days; n = 1,083 individuals);
- at the end of the *sub-adult* period (360 - 370 days; n = 943 individuals);
- upon reaching their final mass as an *adult* (543 - 553 days; n = 785 individuals).

Table 4.2 Means and samples sizes for the mass and skeletal traits at each life stage. Full data is described here although for some traits/life stages reduced datasets were subsequently analysed due to missing fixed effect data for some records.

Model	No. records	No. dominants/ no. individuals	Mean no. records per individual	Trait mean ± S.D. (range)	Age mean ± S.D. in days (range)
Mass (g)					
Emergence	1395	0/685	2.04	111.07 ± 24.41 (41 – 182)	23.58 ± 2.27 (16 – 26)
Independence	5438	0/1146	4.75	311.39 ± 47.53 (109 – 494)	89.90 ± 3.12 (85 – 95)
Juvenile	5256	0/1083	4.85	451.45 ± 68.57 (190 – 647)	179.99 ± 3.19 (175 – 185)
Sub-adult	4155	3/943	4.41	583.44 ± 73.77 (229 – 860)	365.12 ± 3.16 (360 – 370)
Adult	3493	11/785	4.45	651.46 ± 80.84 (384 – 969)	548.00 ± 3.15 (543 – 553)
Skull length (mm)					
Pup	901	0/892	1.01	48.70 ± 3.45 (36.9 – 75)	27.36 ± 6.57 (13 – 89)
Juvenile/sub-adult	625	0/436	1.44	72.77 ± 3.57 (58.4 – 83.4)	228.90 ± 79.30 (92 – 365)
Adult	1149	85/488	2.35	77.51 ± 2.36 (70.7 – 84.2)	847.69 ± 536.73 (366 – 3184)
Skull width (mm)					
Pup	895	0/886	1.01	28.52 ± 2.06 (22 – 39)	27.36 ± 6.55 (13 – 89)
Juvenile/sub-adult	624	0/436	1.43	38.91 ± 2.90 (29 – 46.6)	228.99 ± 79.34 (92 – 365)
Adult	1154	86/490	2.36	44.62 ± 2.86 (31.8 – 52.3)	848.41 ± 538.37 (366 – 3184)
Forearm length (mm)					
Pup	768	0/760	1.01	31.53 ± 3.28 (21.5 – 58.9)	27.00 ± 4.62 (17 – 89)
Juvenile/sub-adult	455	0/319	1.43	58.54 ± 4.39 (43.9 – 68.6)	225.22 ± 82.42 (92 – 365)
Adult	730	65/330	2.21	64.45 ± 2.69 (54.4 – 71.3)	883.62 ± 611.64 (366 – 3184)
Hind-foot length (mm)					
Pup	901	0/892	1.01	38.55 ± 4.30 (20 – 70.1)	26.87 ± 5.28 (0 – 89)*
Juvenile/sub-adult	620	0/428	1.45	66.44 ± 3.20 (54 – 79.9)	229.91 ± 79.79 (92 – 365)
Adult	1161	87/491	2.36	68.20 ± 3.01 (57 – 80)	846.50 ± 538.09 (366 – 3184)

*Individuals measured at age zero had died prior to emergence from the natal burrow either as a result of infanticide or unknown cause.

In order to limit the effect of differences in daily foraging success I only used morning mass measures, but in order to make full use of the extensive mass data I considered all measures of morning mass within the time windows defined above (average number of records per individual = 4.1) (Table 4.2). All the mass datasets therefore included repeated measures.

Skeletal traits

Vernier callipers (±1 mm) were used to measure the following skeletal traits at pup emergence and at irregular intervals thereafter, generally whenever individuals were captured for other purposes:

- *skull length*: measured from the tip of the nose to the base of the skull;
- *skull width:* the distance between the zygomatic arches;
- *forearm length*: measured from the proximal end of the radius to the base of the carpals;
- *hind-foot length*: measured from the proximal end of the metatarsal to the distal end of the phalanges (excluding claws).

Due to data limitations and the irregular intervals at which measurements were taken, I investigated the sources of variance in, and effect of inbreeding on, the four skeletal traits in three slightly different time windows from the mass measurements (Table 4.2):

- *pup* stage (0 - 90 days; n = 760-892 individuals for the different traits);
- *juvenile/sub-adult* stage (91 - 365 days; n = 319 - 436 individuals for the different traits);
- *adult* (>365 days; n = 330 - 491 individuals for the different traits).

Had I considered the same time periods for the skeletal data as the mass records I would have severely limited my data (i.e. number of skull length records at emergence (16 - 26 days) = 467; independence (85 - 95 days) = 7; juvenile (175 - 185 days) = 144; sub-adult (360 - 370 days) = 57; and adult (543 - 553 days) = 39). Only approximately 1% of individuals in the skeletal pup life stages had been measured twice within each time window, whereas the juvenile/sub-adult and adult datasets contain repeated measures (average number of records per individual = 1.88) (Table 4.2).

Analyses therefore involved a total of 17 datasets: five life-history stages for the mass measurements, and three for each of the four skeletal traits.

4.3.3.2 Explanatory variables

Fixed effect terms

Extensive research on this study population has revealed a number of variables known to influence traits such as mass at emergence and independence, and growth rates (Chapter 3 of this thesis, Russell *et al.* 2002, Hodge *et al.* 2008, English *et al.* 2012). Consequently, I included several of these variables as fixed effects in each of my models. These were: *sex* (male/female/unknown; as meerkats in this population are sexed based on morphology, unknown sex refers to young individuals who either died prior to the full development of their genitals or have yet to be sexed (5%)), *age* variation within each of the time windows (days), and the *dominance status* (dominant/subordinate) of adults at the time of measurement (and sub-adults in the mass dataset). Four individuals died at birth (most likely due to infanticide) but were measured for hind-foot length once dead and were thus included in analysis. As both season and rainfall are known to influence growth in meerkats (English *et al.* 2012), I also included the *season* (cold-dry, May - September or hot-wet, October - April) and total accumulated *rainfall* (mm) in the previous nine months prior to trait measurement as fixed effects. Total accumulated rainfall was extracted for the study site (26°58'S, 21°49'E) from the National Aeronautics and Space Administration online Rainfall Analysis Tool (http://disc2.nascom.nasa.gov/Giovanni/tovas/rain.GPCP.shtml) (Bateman *et al.* 2011). Finally, in order to test for the effect of inbreeding at the various life stages, individual *inbreeding coefficients* (*F*) were included as a fixed effect in all models. No additional fixed effects (e.g. maternal condition variables or group size) were fitted in order to retain as many data points as possible and to more accurately apportion variance to group-related components.

Random effect terms

I considered a number of different random effect terms in my models in order to investigate the genetic and non-genetic sources of variation in the study traits, to avoid erroneously inflating the additive genetic variance, and to account for repeated measures, and the non-independence of data. Random terms were additive genetic (*A*), *birth litter* (*BL*), *maternal identity* (*M*; to account for maternal effects), *birth group* (*BG*), and *permanent environment* (*PE*).

I initially considered the possibility that the *current group* (*CG*) of which an individual was member when it was measured might also account for trait variation (e.g. due to differences in food availability). However, very few individuals actually moved into different groups (e.g. number of individuals in the mass datasets which were measured in a different group from their birth group = 0.00% (emergence); 0.87% (independence); 1.02% (juvenile); 2.23% (sub-adult); and 3.06% (adult)). Furthermore, preliminary analyses on a subset of combined mass data that included only the individuals that did move into different groups showed little difference between models excluding ($V_A + V_{BL} + V_M + V_{BG}$) versus including current measurement group ($V_A + V_{BL} + V_M + V_{BG} + V_{CG}$) (ΔDIC between the models=0.22). Consequently I did not include a *CG* random term in any of my models as it would be largely confounded with *BG*.

4.3.4 Statistical analyses

The most powerful and accurate way to estimate additive genetic variance in data from wild populations is to use an 'animal model', due to the fact that it utilises all relatedness links in the pedigree and can deal with the complex, incomplete and unbalanced data which are typical of studies of wild populations (Kruuk 2004, Wilson *et al.* 2010). Animal models are a form of mixed model which includes both fixed and random effects and I estimated the variance components of the traits at each of the life stages using an animal model approach implemented in the Markov Chain Monte Carlo for Generalized Linear Mixed Models package (MCMCGLMM) (Hadfield 2009) in R (R Development Core Team). One of the strengths of MCMCGLMM is its ability to deal with non-normally distributed traits and although

the traits I investigated here are expected to be normally distributed, I chose to analyse them using MCMCGLMM in order to determine how correlated the random terms were with each other and to be consistent with the analyses of Chapter 5.

Fixed effects

I first determined the significance of the fixed effects in models with only *A* and *PE* random effects fitted and retained only the fixed terms that were significant in subsequent analyses (note that the pup skeletal models did not include the *PE* term as almost all individuals in these datasets were only measured once). Highest Probability Density Intervals (HPDI) are considered to be the Bayesian equivalent of confidence intervals (Wilson *et al.* 2010) and thus fixed effect terms were deemed significant when the posterior distribution of the 95% HPDI did not overlap zero.

Random effects

I investigated the variance components of each trait and life stage using three different types of models, which varied in terms of the random effects considered: 1.) *full models* (FM), which included all random effect terms; 2.) most *parsimonious models* (PM), which included only those random terms that were deemed significant based on the procedure outlined below. Because of the potential difficulty in separating additive genetic and birth litter effects, I then also specifically investigated the effect of removing birth litter from the model, and so fitted a third type of model: 3.) *reduced models* (FM_{-BL}), which included all random terms except for *BL*. I chose this comparative approach in order to 1.) investigate the relative effect of each random term; 2.) exclude any non-significant random terms; and 3.) investigate the degree to which the *BL* term was confounded with the *A* term (or other variance components).

Model selection

In order to identify the most parsimonious models (PMs), I determined the level of support for individual variance components by comparing Deviance Information Criteria (DIC) values between the full models (FMs) and models where different random effect terms were excluded. The DIC is the Bayesian equivalent of the

Akaike Information Criterion (AIC), in which smaller values indicate a more parsimonious model fit (Taylor *et al.* 2012). However, unlike AIC, where models with less than two AIC values difference between them are considered analogous, randomization tests have shown that a ΔDIC of five is a more suitable threshold value to use in the model selection process (S. Drobniak, *pers. comm.*).

The PMs for 13 out of the total 17 datasets included only random terms for *A*, *BL* and *PE* (see Results). In four datasets, the FM had the lowest DIC but as three of these were within five DIC values of models that included only the terms for *A*, *BL* and *PE*, the models did not differ significantly from each other. For this reason, and for consistent comparison among traits and life stages, I refer to the PMs as those that include only *A*, *BL* and *PE*. The one exception to this is the adult skull length FM, which had a DIC value that was more than five DIC values less than the model with only *A*, *BL* and *PE* effects included (see Results). Note that the pup skeletal models did not include the *PE* term as almost all individuals in these datasets were only measured once: PMs for these models therefore contain only *A* and *BL* effects.

Variance component analysis

Variance components, conditional on the significant fixed effects, were estimated from univariate models as the mode of the posterior distribution, with accompanying HPDI intervals (necessarily bounded above zero). For each trait at each life stage I estimated variance components from the FM, PM and FM_{-BL} models, which took the form:

$$\text{FM: } \boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Z_1}\mathbf{a} + \boldsymbol{Z_2}\mathbf{bl} + \boldsymbol{Z_3}\mathbf{m} + \boldsymbol{Z_4}\mathbf{bg} + \boldsymbol{Z_5}\mathbf{pe}^{1} + \boldsymbol{e}$$

$$\text{PM: } \boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Z_1}\mathbf{a} + \boldsymbol{Z_2}\mathbf{bl} + \boldsymbol{Z_3}\mathbf{pe}^{1} + \boldsymbol{e}$$

$$\text{FM}_{-BL}\text{: } \boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Z_1}\mathbf{a} + \boldsymbol{Z_2}\mathbf{m} + \boldsymbol{Z_3}\mathbf{bg} + \boldsymbol{Z_4}\mathbf{pe}^{1} + \boldsymbol{e}$$

where:

- $\boldsymbol{y}$ = vector of observed phenotypic values
- $\boldsymbol{b}$ = vector of fixed effects
- $\boldsymbol{a}$ = vector of additive genetic effects

[1]The permanent environment effect was excluded from the models of pup skeletal traits as almost all individuals in these datasets were only measured once (see Table 4.2).

- ***bl*** = vector of birth litter effects
- ***m*** = vector of maternal effects
- ***bg*** = vector of birth group effects
- ***pe*** = vector of permanent environment effects
- ***e*** = vector of residual effects
- **X** and $\mathbf{Z_{1\text{-}5}}$ = corresponding design matrices

For each trait and life stage model the total phenotypic variance (V_P) was thus partitioned as the following:

$$\text{FM: } V_P = V_A + V_{BL} + V_M + V_{BG} + V_{PE}^1 + V_R$$

$$\text{PM: } V_P = V_A + V_{BL} + V_{PE}^1 + V_R$$

$$\text{FM}_{\text{-BL}}\text{: } V_P = V_A + V_M + V_{BG} + V_{PE}^1 + V_R$$

where:

- V_P=total phenotypic variance
- V_A=additive genetic effect variance
- V_{BL}=birth litter effect variance
- V_M=maternal effect variance
- V_{BG}=birth group effect variance
- V_{PE}=permanent environment effect variance
- V_R=residual effect variance

Narrow sense heritabilities and the proportion of variance attributed to the other effects were then calculated (conditional on the fixed effects) for each MCMC sample by dividing the relevant variance component by the total phenotypic variance (e.g. heritability $h^2=V_A/V_P$).

As the levels of other components of variance differ between traits and life stages, I also calculated the coefficient of genetic variance ($CV_A=100*\sqrt{(V_A)}/x$), which scales the additive genetic variance by the trait mean (x) rather than the total phenotypic variance. The proportion of variance in each trait and life stage that was due to variation among rather than within individuals, or the repeatability (r^2), was estimated as follows:

$$\text{FM: } r^2=(V_A + V_{BL} + V_M + V_{BG} + V_{PE}^1)/V_P$$

$$\text{PM: } r^2=(V_A + V_{BL} + V_{PE}^1)/V_P$$

$$\text{FM}_{\text{-BL}}\text{: } r^2=(V_A + V_M + V_{BG} + V_{PE}^1)/V_P$$

Correlations between traits and random terms

In order to investigate the presence of any genetic and non-genetic covariances and correlations among the five traits, I ran three multivariate, repeated measure models corresponding to the three main meerkat life stages: pup, juvenile/sub-adult and adult. To this end, for mass, the emergence (16-26 days) and independence (85-95 days) repeated measures were combined and analysed together with the skeletal traits (<91 days) in a pup model, and the juvenile (175-185 days) and sub-adult (360-370 days) mass measures were combined and analysed with the skeletal traits (91-365 days) in a juvenile/sub-adult model. All significant fixed effect terms were included but coded so they were specific only to the trait and life stage in which they were found to be relevant (see Table 4.3), and the random terms of *A*, *BL* and *PE* were considered in each model. Correlations (*r*) were calculated as $\text{COV}_{a,b}/\sqrt{(V_a * V_b)}$ (where *a* and *b* correspond to the traits of interest) and both the covariances and correlations were deemed statistically significant when their 95% HDPI around the posterior distributions excluded zero. Differences in the data and random terms considered mean that the multivariate models are not directly analogous to the univariate models.

Finally, I investigated whether the random terms were potentially confounded using MCMCGLMM (Hadfield 2009). In order to reduce the number of models considered, I combined all repeated measure life stage data for each trait and determined correlation coefficients between the covariance matrices of each pair of random terms.

Program settings

Parameter expansion speeds up the mixing and rate of convergence of Markov chains (Hadfield 2009) and thus, in all models, MCMCGLMM parameter expanded priors were used in order to avoid poor mixing:

univariate models: V=1, nu=1, alpha.mu=0, alpha.V=1000

multivariate model: V=diag(3), n=2, alpha.mu=rep(0,3), alpha.V=diag(3)*1000)

All models were preceded by a burn-in of 15,000 (univariate) or 30,000 (multivariate) iterations, run for 65,000 (univariate) or 130,000 (multivariate) iterations, with estimates of every 50th (univariate) or 100th (multivariate) iteration stored and convergence of the MCMC sampling was assessed by visual inspection of the variance component chains. Note that as residual multivariate variance structures cannot have parameter expansion in MCMCGLMM, these are not presented.

4.4 Results

I analysed the sources of variance in each trait and life stage (n=17 total datasets) and found that in all cases the most parsimonious models (PMs) contained both *A* and *BL* (details below). As the other terms (*M* and *BG*) were non-significant in all but one model (adult skull width), I focus primarily on presenting results from the PMs. However, I also refer to FM and FM_{-BL} results as they provide insight into the degree to which the birth litter (*BL*) term was confounded with the other variance components. In this Results section, I present analyses of each trait in turn; I then consider results for the different fixed and random effects across traits in the Discussion.

Table 4.3 Parameter estimates of the fixed terms for the mass and skeletal traits in each of the life stage specific, univariate, repeated measure animal models. Models included both additive genetic (V_A) and, where necessary, permanent environment (V_{PE}) random effect terms. Statistics are reported for restricted datasets, where any records with missing fixed term values were excluded (*n*=total number of records), and were generated by the R-package MCMCGLMM (Hadfield 2009). Fixed effect terms were deemed significant (indicated in bold) when the posterior distribution of the 95% Highest Probability Density Intervals (HPDI; in parentheses) did not overlap zero. Significant fixed terms were retained in subsequent models for which variance components are shown in tables 4.4-4.9. Mass: emergence (E) = day 16-26; independence (I) = day 85-95; juvenile (J) = day 175-185; sub-adult (S) = day 360-370; and adult (A) = day 543-553. Skeletal traits: pup (P) = <90 days; juvenile/sub-adult (J) = day 91-365; and adult (A) = >365 days.

Trait	*n*	Sex (male)	Age	Status (dominant)[†]	*F*	Season (hot-wet)	Rainfall
Mass							
E	1259	-1.46 (-4.27 – 2.16)	**2.52 (2.34 – 2.63)**	—	-11.06 (-54.15 – 47.99)	0.37 (-3.11 – 2.76)	0.02 (-0.01 – 0.03)
I	5068	1.28 (-4.41 – 5.49)	**1.83 (1.76 – 1.91)**	—	**-244.04 (-329.47 – -161.26)**	1.78 (-1.45 – 3.85)	**0.04 (0.02 – 0.06)**
J	4881	6.92 (-1.06 – 12.93)	**0.92 (0.82 – 1.02)**	—	**-335.93 (-450.79 – -208.93)**	**8.20 (4.53 – 10.71)**	**0.21 (0.19 – 0.25)**
S	3794	**13.62 (4.40 – 21.21)**	**0.35 (0.22 – 0.52)**	**162.62 (77.34 – 233.17)**	**-461.26 (-563.16 – -301.72)**	**13.36 (8.66 – 18.42)**	**0.10 (0.06 – 0.13)**
A	3227	4.97 (-4.47 – 15.02)	**0.27 (0.13 – 0.47)**	**74.72 (37.61 – 117.86)**	**-525.44 (-653.59 – -370.57)**	**18.30 (12.52 – 25.18)**	**0.18 (0.14 – 0.23)**
Skull length							
P[*]	864	0.30 (-0.05 – 0.59)	**0.36 (0.34 – 0.40)**	—	2.77 (-3.49 – 8.07)	**0.99 (0.48 – 1.38)**	**4.11[‡] (1.89[‡] – 6.56[‡])**
J	552	-0.33 (-0.76 – 0.27)	**0.03 (29.92[‡] – 0.04)**	—	**-6.39 (-15.88 – -0.34)**	**-0.78 (-1.10 – -0.29)**	0.70[‡] (-1.78[‡] – 2.75[‡])
A	986	**0.59 (0.25 – 0.99)**	**0.83[‡] (0.63[‡] – 1.24[‡])**	**0.39 (0.02 – 0.73)**	**-9.16 (-14.95 – -4.67)**	0.21 (-0.08 – 0.40)	**1.77[‡] (0.73[‡] – 3.20[‡])**
Skull width							
P[*]	858	0.05 (-0.16 – 0.30)	**0.12 (0.10 – 0.15)**	—	**6.06 (1.26 – 10.52)**	**0.74 (0.43 – 1.12)**	**1.64[‡] (0.32[‡] – 3.91[‡])**
J	551	-0.15 (-0.59 – 0.20)	**21.31[‡] (19.64[‡] – 23.64[‡])**	—	-3.43 (-9.96 – 2.62)	0.38 (-1.31[‡] – 0.69)	**5.33[‡] (3.05[‡] – 6.88[‡])**
A	991	0.14 (-0.38 – 0.42)	**2.34[‡] (2.01[‡] – 2.70[‡])**	**0.23 (0.15 – 1.01)**	**-15.22 (-21.84 – -10.75)**	**0.50 (0.21 – 0.74)**	**3.17[‡] (1.80[‡] – 4.45[‡])**
Forearm length							
P[*]	750	-0.12 (-0.46 – 0.32)	**0.35 (0.31 – 0.40)**	—	5.35 (-5.25 – 10.35)	0.38 (-0.25 – 0.93)	-0.28[‡] (-2.79[‡] – 3.49[‡])
J	397	-0.21 (-0.72 – 0.42)	**0.04 (40.42[‡] – 0.05)**	—	-8.90 (-17.04 – 1.41)	**-1.04 (-1.49 – -0.45)**	18.90 (-7.05 – 43.63)
A	703	**0.99 (0.37 – 1.30)**	**1.53[‡] (1.06[‡] – 1.95[‡])**	-0.34 (-0.79 – 0.12)	**-6.24 (-13.97 – -1.02)**	0.17 (-0.14 – 0.45)	0.52[‡] (-0.086[‡] – 2.06[‡])
Hind-foot length							
P[*]	869	-0.19 (-0.61 – 0.25)	**0.50 (0.45 – 0.53)**	—	0.37 (-4.93 – 10.80)	**1.06 (0.41 – 1.64)**	**3.37[‡] (0.63[‡] – 6.92[‡])**
J	551	**-0.48 (-1.14 – -0.08)**	**16.90[‡] (14.27[‡] – 19.25[‡])**	—	**-6.23 (-18.49 – -2.12)**	0.06 (-0.34 – 0.47)	-1.29 (-3.97 – 0.88)
A	1000	0.37 (-0.12 – 0.72)	**0.54 (0.13 – 0.87)**	0.49 (-0.01 – 0.87)	**-7.94 (-14.05 – -2.48)**	-7.62 (-0.29 – 0.31)	0.73[‡] (-0.96[‡] – 2.03[‡])

[*]Permanent environment effect (V_{PE}) excluded as almost all individuals in the pup datasets were only measured once (see Table 4.2).

[†]The status term was not fitted in the emergence, independence and juvenile (mass), and pup and juvenile/sub-adult (skeletal) models as all individuals in these datasets were subordinate.

[‡]Scaled by a factor of 1000 for ease of interpretation.

Table 4.4 Significance of the random effect terms derived from the life stage specific, univariate, repeated mass measure MCMCGLMM animal models of body mass. Models were analysed including all relevant fixed effects (FE; see Table 4.3) and significance of the random terms was based on the Deviance Information Criterion (DIC) where smaller DIC values are preferred. Number of records (*n*) is given in parentheses and the random effects are permanent environment (V_{PE}), additive genetic (V_A), birth litter (V_{BL}), maternal (V_M), and birth group (V_{BG}). The full (FM; where all possible random effects were included), most parsimonious (PM and in bold); reduced (FM_{-BL}; excluding birth litter) models are indicated. ΔDIC gives the difference between the full model and the most parsimonious model.

Model		DIC
Emergence (n=1395)*		
FM	$\sim V_{PE} + V_A + V_{BL} + V_M + V_{BG}$	8731.16
	$\sim V_{PE} + V_A + V_{BG}$	8799.87
	$\sim V_{PE} + V_A + V_M$	8798.15
PM	$\sim V_{PE} + V_A + V_{BL}$	**8732.64**
	$\sim V_{PE} + V_{BL}$	8735.34
	$\sim V_{PE} + V_A$	8799.50
FM_{-BL}	$\sim V_{PE} + V_A + V_M + V_{BG}$	8797.93
ΔDIC		-1.48
Independence (n=5068)		
FM	$\sim V_{PE} + V_A + V_{BL} + V_M + V_{BG}$	36836.46
	$\sim V_{PE} + V_A + V_{BG}$	36855.94
	$\sim V_{PE} + V_A + V_M$	36854.95
PM	$\sim V_{PE} + V_A + V_{BL}$	**36835.45**
	$\sim V_{PE} + V_{BL}$	36836.54
	$\sim V_{PE} + V_A$	36863.95
FM_{-BL}	$\sim V_{PE} + V_A + V_M + V_{BG}$	36855.24
ΔDIC		1.01
Juvenile (n=4881)		
FM	$\sim V_{PE} + V_A + V_{BL} + V_M + V_{BG}$	38039.07
	$\sim V_{PE} + V_A + V_{BG}$	38085.86
	$\sim V_{PE} + V_A + V_M$	38081.44
PM	$\sim V_{PE} + V_A + V_{BL}$	**38037.48**
	$\sim V_{PE} + V_{BL}$	38037.84
	$\sim V_{PE} + V_A$	38085.08
FM_{-BL}	$\sim V_{PE} + V_A + V_M + V_{BG}$	38081.99
ΔDIC		1.59
Sub-adult (n=3794)		
FM	$\sim V_{PE} + V_A + V_{BL} + V_M + V_{BG}$	32318.08
	$\sim V_{PE} + V_A + V_{BG}$	32344.56
	$\sim V_{PE} + V_A + V_M$	32343.73
PM	$\sim V_{PE} + V_A + V_{BL}$	**32317.14**
	$\sim V_{PE} + V_{BL}$	32317.93
	$\sim V_{PE} + V_A$	32343.59
FM_{-BL}	$\sim V_{PE} + V_A + V_M + V_{BG}$	32344.47
ΔDIC		0.94
Adult (n=3227)		
FM	$\sim V_{PE} + V_A + V_{BL} + V_M + V_{BG}$	27379.47
	$\sim V_{PE} + V_A + V_{BG}$	27389.65
	$\sim V_{PE} + V_A + V_M$	27387.08
PM	$\sim V_{PE} + V_A + V_{BL}$	**27379.41**
	$\sim V_{PE} + V_{BL}$	27380.52
	$\sim V_{PE} + V_A$	27389.12
FM_{-BL}	$\sim V_{PE} + V_A + V_M + V_{BG}$	27388.41
ΔDIC		0.06

*Sample size is larger than that in Table 4.3 as records that were previously excluded due to missing (non-significant) F-values are now included.

Table 4.5 Significance of the random effect terms derived from the life stage specific, univariate, repeated measure MCMCGLMM animal models of skeletal traits. Models were analysed including all relevant fixed effects (FE; see Table 4.3) and significance of the random terms was based on the Deviance Information Criterion (DIC) where smaller DIC values are preferred. Number of records (*n*) is given in parentheses and the random effects are permanent environment (V_{PE}), additive genetic (V_A), birth litter (V_{BL}), maternal (V_M), and birth group (V_{BG}). The full (FM; where all possible random effects were included), most parsimonious (PM and in bold); and reduced (FM_{-BL}; excluding birth litter) models are indicated. ΔDIC gives the difference between the full model and the most parsimonious model and italics indicate instances where the most parsimonious model is >5 DIC values smaller then the full model.

		DIC			
Model		*Skull length*	*Skull width*	*Forearm length*	*Hind-foot length*
Pup[*]		(*n*=901)[†]	(*n*=858)	(*n*=768)[†]	(*n*=901)[†]
FM	$\sim V_A + V_{BL} + V_M + V_{BG}$	3700.08	2791.96	3330.79	4061.88
	$\sim V_A + V_{BG}$	3985.77	3185.50	3528.98	4389.44
	$\sim V_A + V_M$	3987.35	3149.66	3583.52	4417.57
PM	$\sim V_A + V_{BL}$	**3695.73**	**2741.15**	**3306.12**	**4033.13**
	$\sim V_{BL}$	3708.30	2864.34	3355.06	4172.24
	$\sim V_A$	4017.62	3208.17	3593.13	4463.23
FM_{-BL}	$\sim V_A + V_M + V_{BG}$	3980.58	3149.18	3562.03	4381.37
ΔDIC		4.35	*50.81*	*24.67*	*28.75*
Juvenile/sub-adult		(*n*=552)	(*n*=624)[†]	(*n*=455)[†]	(*n*=551)
FM	$\sim V_{PE} + V_A + V_{BL} + V_M + V_{BG}$	2390.22	2381.38	2054.11	2338.74
	$\sim V_{PE} + V_A + V_{BG}$	2448.66	2554.78	2061.49	2356.50
	$\sim V_{PE} + V_A + V_M$	2448.96	2541.97	2050.04	2355.14
PM	$\sim V_{PE} + V_A + V_{BL}$	**2387.11**	**2382.75**	**2024.47**	**2336.14**
	$\sim V_{PE} + V_{BL}$	2398.70	2384.53	2033.94	2344.82
	$\sim V_{PE} + V_A$	2448.63	2552.52	2064.55	2355.80
FM_{-BL}	$\sim V_{PE} + V_A + V_M + V_{BG}$	2447.90	2542.97	2050.48	2355.55
ΔDIC		3.11	-1.07	*29.64*	2.60
Adult		(*n*=986)	(*n*=991)	(*n*=703)	(*n*=1000)
FM	$\sim V_{PE} + V_A + V_{BL} + V_M + V_{BG}$	3627.53	3916.19	2634.10	4221.65
	$\sim V_{PE} + V_A + V_{BG}$	3650.97	3961.80	2641.53	4235.10
	$\sim V_{PE} + V_A + V_M$	3648.82	3946.79	2642.26	4228.64
PM	$\sim V_{PE} + V_A + V_{BL}$	**3628.03**	**3927.96**	**2633.63**	**4223.57**
	$\sim V_{PE} + V_{BL}$	3650.68	3930.63	2637.37	4253.07
	$\sim V_{PE} + V_A$	3653.06	3973.51	2641.20	4237.87
FM_{-BL}	$\sim V_{PE} + V_A + V_M + V_{BG}$	3648.41	3946.96	2641.01	4228.61
ΔDIC		-0.05	-11.77	0.47	-1.92

[*]Permanent environment effect (V_{PE}) excluded as almost all indiviuals in the pup datasets were only measured once (see Table 4.2).
[†]Sample size is larger than that in Table 4.3 as records that were previously excluded due to missing (non-significant) *F*-values are now included.

4.4.1 Mass

Fixed effects

Despite considering a relatively small window of time (11 days), analysis of the fixed effects in the preliminary models of mass revealed that age was the only variable that was significant across all life stages (Table 4.3). In line with estimates of 'monomolecular' growth rates in meerkats (English *et al.* 2012), the positive effect of age was strongest in the emergence and independence life stages, with the magnitude of the age effect decreasing in older life stages (Table 4.3). Dominants were more likely to weigh more than subordinates in both of the life stages where this variable was relevant (sub-adult and adult), whereas sex was only significant in sub-adults, with males weighing more than females (Table 4.3). I found positive effects of rainfall in all four life stages in which individuals were foraging for themselves (i.e. independence, juvenile, sub-adult and adult), but not in pups (Table 4.3). Juveniles, sub-adults and adults tended to weigh more in hot-wet periods of the year (Table 4.3). I also found evidence of inbreeding depression in mass for all stages except the youngest (Table 4.3 & Fig. 4.1*a*). The magnitude of the inbreeding effect was broadly consistent across all life stages (Fig. 4.1*a*): for an individual with F=0.078 (the average F of the total population (Chapter 3)), the F parameter estimates translate into 6.15% (independence), 5.82% (juvenile), 5.86% (sub-adult), and 6.13% (adult) reductions in mass compared with individuals with F=0 (Table 4.3 & Fig. 4.1*a*).

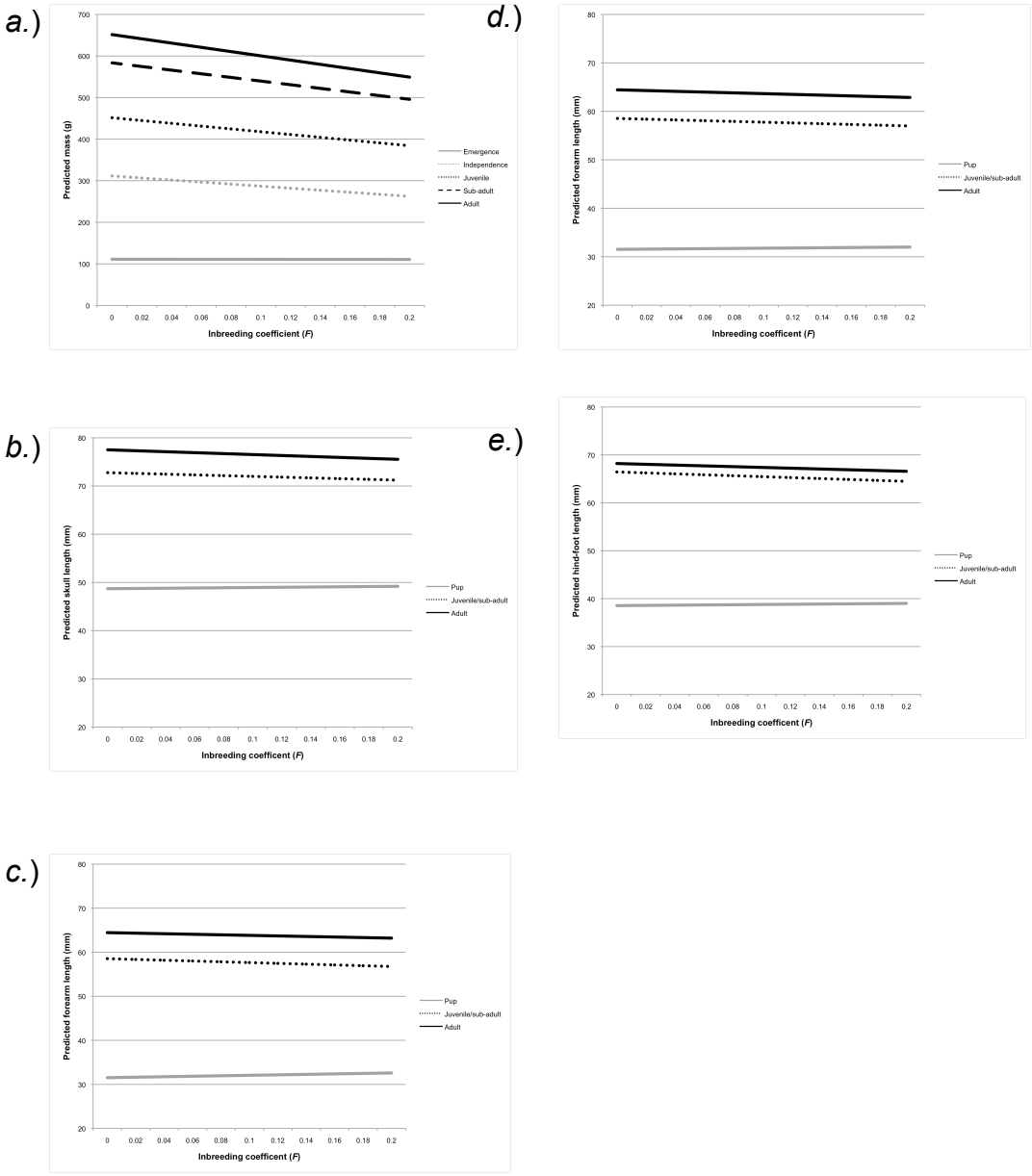


Figure 4.1 The predicted effect of inbreeding coefficient (*F*) on *a.*) mass, *b.*) skull length, *c.*) skull width, *d.*) forearm length, and *e.*) hind-foot length at the various life stages. Data are from initial models (see Table 4.3).

Variance components

Most parsimonious model (PM)

MCMC sampling for the additive genetic chains converged in all PMs (results not shown) and the repeatability of mass across life stages was very high (r^2=0.96 – 0.99), with only approximately 3.60% of the variance in mass remaining unexplained (e^2) (Tables 4.6 & 4.7). Narrow-sense heritabilities (h^2) ranged from 0.18 (emergence) to 0.31 (sub-adult) (Tables 4.6 & 4.7; Figs. 4.2*a* & 4.3*a*). Birth litter accounted for the most variance in mass at all life stages (bl^2=0.44-0.80) although the amount of variance explained by this term tended to decline with age, from 80% for pups to 44% for adults (Tables 4.6 & 4.7; Figs. 4.2*a* & 4.3*b*).

Comparison of full (FM) and reduced (FM_{-BL}) models

The birth litter term appeared to be confounded with additive genetic and maternal effects as the variance accounted for by these two terms generally increased in FM_{-BL} models where birth litter was excluded. Similarly birth group only accounted for detectable levels of variance in the FM_{-BL} emergence model (Tables 4.6 & 4.7; Fig. 4.2*a*).

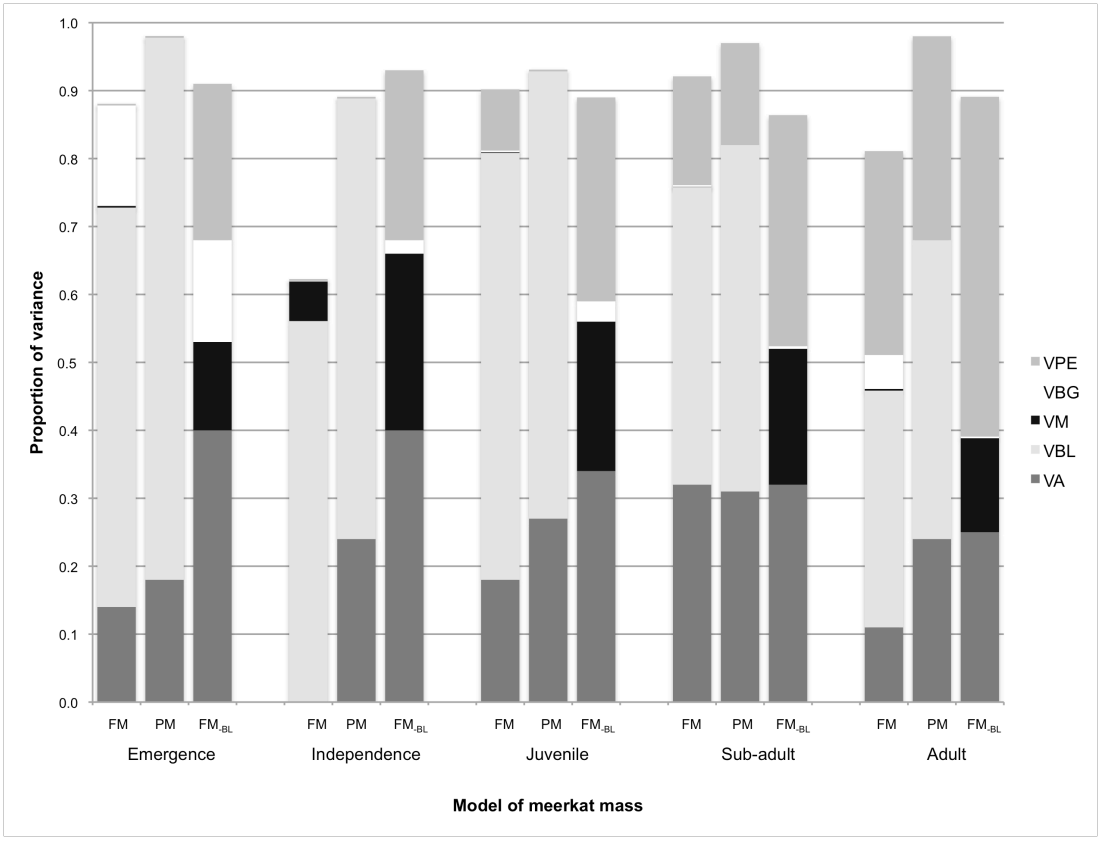
Table 4.6 Variance components of mass at each life stage. Estimates from the full (FM; where all possible random effects were included), most parsimonious (PM and in bold); and reduced (FM_{-BL}; excluding birth litter) models are provided. Models were analysed including all significant fixed effects (see Table 4.3). V_P=total phenotypic, V_A=additive genetic, V_{BL}=birth litter, V_M=maternal, V_{BG}=birth group, V_{PE}=permanent environment, and V_R=residual variance. Values are accompanied by their 95% HPDI (in parentheses) which are bounded above zero.

Trait	Model	V_P	V_A	V_{BL}	V_M	V_{BG}	V_{PE}	V_R
Emergence								
	FM	633.90	102.66	391.55	0.34	119.45	0.18	19.72
		—	(49.68 – 120.02)	(311.22 – 489.36)	(0.68*10^{-4} – 78.58)	(29.67 – 296.16)	(0.04*10^{-4} – 30.85)	(17.91 – 22.00)
	PM	**561.90**	**102.21**	**439.60**	—	—	**0.17**	**19.92**
		—	**(61.60 – 129.27)**	**(365.88 – 575.55)**	—	—	**(0.11*10^{-4} – 27.05)**	**(18.04 – 22.19)**
	FM_{BL}	642.00	265.71	—	113.97	61.33	180.80	20.19
		—	(152.68 – 399.86)	—	(45.50 – 205.41)	(9.17 – 243.46)	(94.89 – 240.93)	(18.17 – 22.11)
Independence								
	FM	1577.29	3.36	1321.82	178.34	2.59	2.72	68.46
		—	(58.01*10^{-4} – 839.19)	(11.04*10^{-4} – 1642.94)	(0.12 – 477.76)	(0.01*10^{-4} – 411.11)	(68.82*10^{-4} – 418.15)	(65.48 – 71.49)
	PM	**1251.65**	**577.20**	**1504.76**	—	—	**1.70**	**67.99**
		—	**(285.97 – 884.34)**	**(1230.32 – 1777.83)**	—	—	**(43.94*10^{-4} – 276.78)**	**(65.59 – 71.66)**
	FM_{BL}	2685.28	1069.04	—	713.70	67.30	766.23	69.01
		—	(716.57 – 1458.95)	—	(361.52 – 1082.11)	(10.99*10^{-4} – 559.25)	(507.34 – 945.68)	(65.54 – 71.55)
Juvenile								
	FM	4139.00	808.30	2882.07	4.30	3.47	324.28	116.58
		—	(335.30 – 1475.89)	(2224.71 – 3272.37)	(4.69*10^{-4} – 475.48)	(27.39*10^{-4} – 705.11)	(26.78 – 638.58)	(111.56 – 121.88)
	PM	**3924.87**	**868.12**	**2935.89**	—	—	**5.05**	**115.81**
		—	**(436.32 – 1633.37)**	**(2388.45 – 3435.13)**	—	—	**(0.29*10^{-4} – 570.20)**	**(0.01 – 121.47)**
	FM_{BL}	4659.56	1601.57	—	1128.58	147.48	1665.72	116.21
		—	(883.61 – 2372.83)	—	(540.51 – 1858.26)	(0.70 – 920.48)	(1203.65 – 2078.23)	(111.39 – 122.05)
Sub-adult								
	FM	4535.62	1298.84	2235.90	4.56	3.73	758.01	234.58
		—	(493.40 – 2476.45)	(1719.79 – 2839.67)	(2.62*10^{-4} – 675.95)	(0.03 – 623.58)	(178.36 – 1263.67)	(222.33 – 247.30)
	PM	**4971.33**	**1587.05**	**2440.37**	—	—	**709.86**	**234.05**
		—	**(774.34 – 2358.60)**	**(1946.99 – 2991.44)**	—	—	**(252.25 – 1109.52)**	**(224.40 – 248.97)**
	FM_{BL}	5442.40	2108.33	—	1076.65	24.37	1994.17	238.88
		—	(907.27 – 3072.56)	—	(434.47 – 1807.84)	(3.55*10^{-4} – 921.84)	(12.26*10^{-4} – 2520.25)	(224.16 – 248.79)
Adult								
	FM	5129.08	640.09	2103.39	7.18	293.22	1860.08	225.12
		—	(180.95 – 1626.28)	(1579.14 – 2841.04)	(0.70*10^{-6} – 850.93)	(4.96*10^{-4} – 1189.68)	(11.70*10^{-4} – 2156.40)	(214.97 – 240.69)
	PM	**5392.44**	**1123.00**	**2497.74**	—	—	**1546.12**	**225.58**
		—	**(568.54 – 2450.93)**	**(1822.72 – 3081.14)**	—	—	**(789.61 – 1890.69)**	**(215.04 – 239.08)**
	FM_{BL}	5268.23	1555.87	—	763.82	8.24	2715.71	224.59
		—	(538.05 – 2591.68)	—	(214.73 – 1491.76)	(48.04*10^{-4} – 1385.37)	(2089.28 – 3423.97)	(215.47 – 240.65)

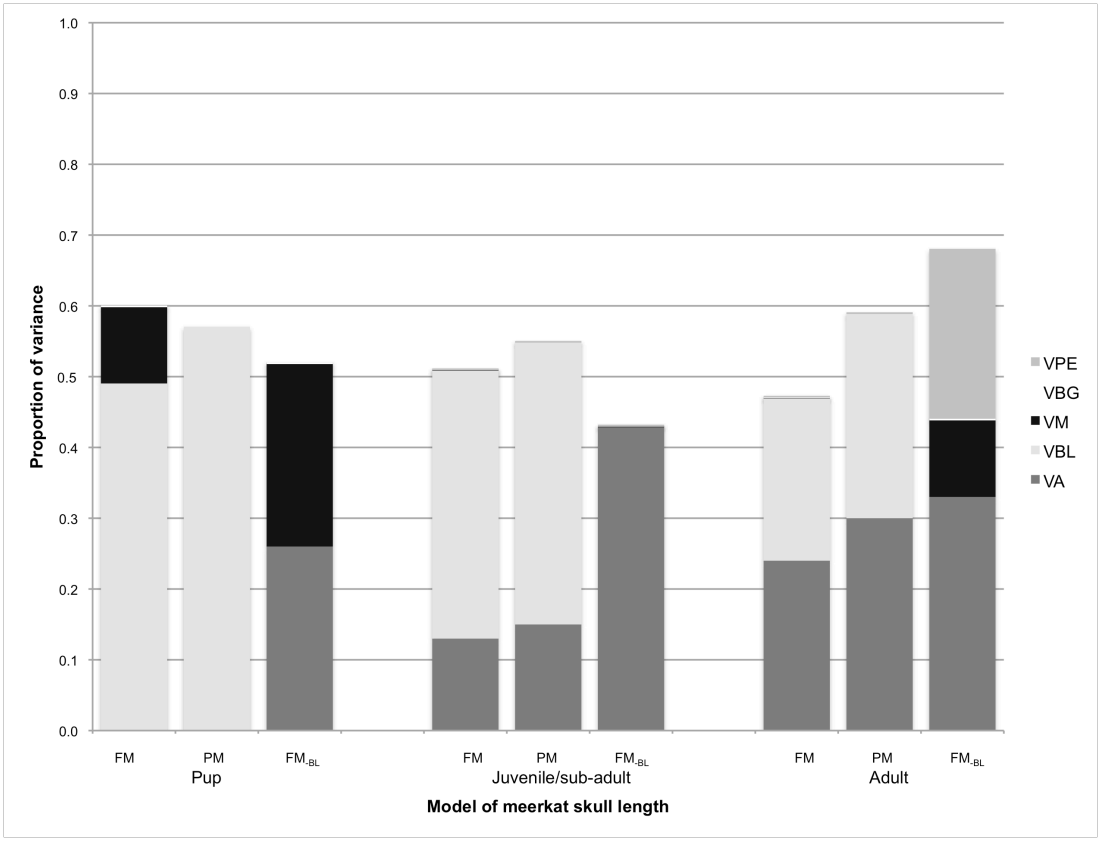
Table 4.7 Heritabilities (h^2) and birth litter (bl^2), maternal (m^2), birth group (bg^2), permanent environment (pe^2), and residual (e^2) effects of mass at each life stage. Estimates from the full (FM; where all possible random effects were included), most parsimonious (PM and in bold), and the reduced models (FM_{-BL}; excluding birth litter) are provided. Models were analysed including all significant fixed effects (see Table 4.3). CV_A=coefficients of additive genetic variance ($CV_A = 100*\sqrt{(V_A)}/x$) and r^2=repeatability. Values are accompanied by their 95% HPDI (in parentheses) which are bounded above zero.

Trait	Model	CV_A	r^2	h^2	bl^2	m^2	bg^2	pe^2	e^2
Emergence									
	FM	9.12	0.97	0.14	0.59	$3.36*10^{-4}$	0.15	$2.92*10^{-4}$	0.03
		—	—	(0.07 – 0.19)	(0.44 – 0.70)	($0.08*10^{-6}$ – 0.11)	(0.07 – 0.37)	($0.54*10^{-8}$ – 0.05)	(0.02 – 0.04)
	PM	**9.10**	**0.97**	**0.18**	**0.80**	—	—	**$3.74*10^{-4}$**	**0.03**
		—	—	**(0.10 – 0.23)**	**(0.73 – 0.84)**	—	—	**($0.02*10^{-6}$ – 0.04)**	**($2.75*10^{-2}$ – 0.04)**
	FM_{BL}	14.68	0.97	0.40	—	0.13	0.15	0.23	0.03
		—	—	(0.24 – 0.59)	—	(0.07 – 0.28)	(0.02 – 0.30)	(0.12 – 0.36)	(0.02 – 0.04)
Independence									
	FM	0.59	0.95	$8.21*10^{-4}$	0.56	0.06	$7.63*10^{-4}$	$10.38*10^{-4}$	0.03
		—	—	($0.03*10^{-4}$ – 0.33)	(0.44 – 0.67)	($0.46*10^{-4}$ – 0.19)	($0.05*10^{-8}$ – 0.16)	($0.03*10^{-4}$ – 0.19)	($2.90*10^{-2}$ – 0.03)
	PM	**7.72**	**0.97**	**0.24**	**0.65**	—	—	**$9.00*10^{-4}$**	**0.03**
		—	—	**(0.13 – 0.38)**	**(0.59 – 0.73)**	—	—	**($0.02*10^{-4}$ – 0.13)**	**($2.61*10^{-2}$ – $3.42*10^{-2}$)**
	FM_{BL}	10.50	0.98	0.40	—	0.26	0.02	0.25	0.02
		—	—	(0.26 – 0.53)	—	(0.15 – 0.36)	($0.44*10^{-6}$ – 0.18)	(0.17 – 0.35)	($1.99*10^{-2}$– 0.03)
Juvenile									
	FM	6.30	0.97	0.18	0.63	$8.35*10^{-4}$	$10.91*10^{-4}$	0.09	0.03
		—	—	(0.08 – 0.31)	(0.52 – 0.71)	($0.10*10^{-6}$ – 0.10)	($0.01*10^{-4}$ – 0.15)	($0.49*10^{-4}$ – 0.14)	(0.02 – $2.95*10^{-2}$)
	PM	**6.53**	**0.97**	**0.27**	**0.66**	—	—	**$9.47*10^{-4}$**	**0.03**
		—		**(0.11 – 0.36)**	**(0.60 – 0.73)**	—	—	**($0.50*10^{-6}$ – 0.14)**	**(0.02 – $3.08*10^{-2}$)**
	FM_{BL}	8.86	0.98	0.34	—	0.22	0.03	0.30	0.02
		—	—	(0.20 – 0.47)	—	(0.13 – 0.35)	($0.04*10^{-4}$ – 0.17)	(0.23 – 0.46)	($1.97*10^{-2}$ – 0.03)
Sub-adult									
	FM	6.18	0.96	0.32	0.44	$6.26*10^{-4}$	$3.96*10^{-4}$	0.16	0.04
		—	—	(0.13 – 0.45)	(0.35 – 0.55)	($0.06*10^{-6}$ – 0.12)	($0.01*10^{-4}$ – 0.11)	(0.03 – 0.26)	($3.90*10^{-2}$ – 0.05)
	PM	**6.83**	**0.95**	**0.31**	**0.51**	—	—	**0.15**	**0.05**
		—	—	**(0.18 – 0.46)**	**(0.43 – 0.57)**	—	—	**(0.04 – 0.24)**	**(0.04 – $5.41*10^{-2}$)**
	FM_{BL}	7.87	0.96	0.32	—	0.20	$39.57*10^{-4}$	0.34	0.04
		—	—	(0.22 – 0.54)	—	(0.10 – 0.31)	($0.06*10^{-6}$ – 0.15)	(0.21 – 0.50)	(0.03 – 0.05)
Adult									
	FM	3.88	0.96	0.11	0.35	$9.32*10^{-4}$	0.05	0.30	0.04
		—	—	(0.03 – 0.28)	(0.28 – 0.48)	($0.01*10^{-8}$ – 0.15)	($0.09*10^{-6}$ – 0.19)	(0.20 – 0.41)	(0.03 – 0.05)
	PM	**5.14**	**0.96**	**0.24**	**0.44**	—	—	**0.30**	**0.04**
		—	—	**(0.11 – 0.42)**	**(0.36 – 0.53)**	—	—	**(0.14 – 0.37)**	**($3.62*10^{-2}$ – 0.05)**
	FM_{BL}	6.05	0.96	0.25	—	0.14	$9.26*10^{-4}$	0.50	0.04
		—	—	(0.11 – 0.45)	—	(0.04 – 0.24)	($0.01*10^{-4}$ – 0.21)	(0.33 – 0.60)	(0.03 – 0.05)

a.)


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion of variance
FM
PM
FM-BL
Emergence
Independence
Juvenile
Sub-adult
Adult
Model of meerkat mass
VPE
VBG
VM
VBL
VA

b.)


1.0
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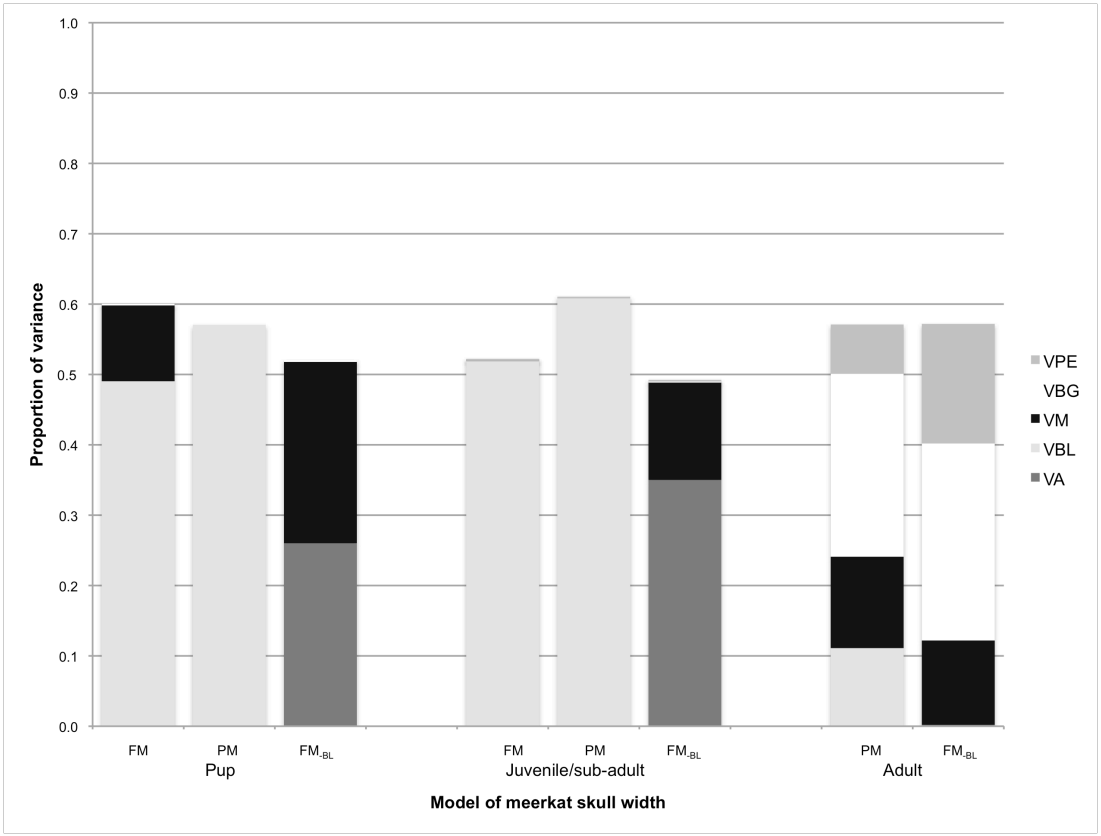
0.5
0.4
0.3
0.2
0.1
0.0
Proportion of variance
FM
PM
FM-BL
Pup
Juvenile/sub-adult
Adult
Model of meerkat skull length
VPE
VBG
VM
VBL
VA

c.)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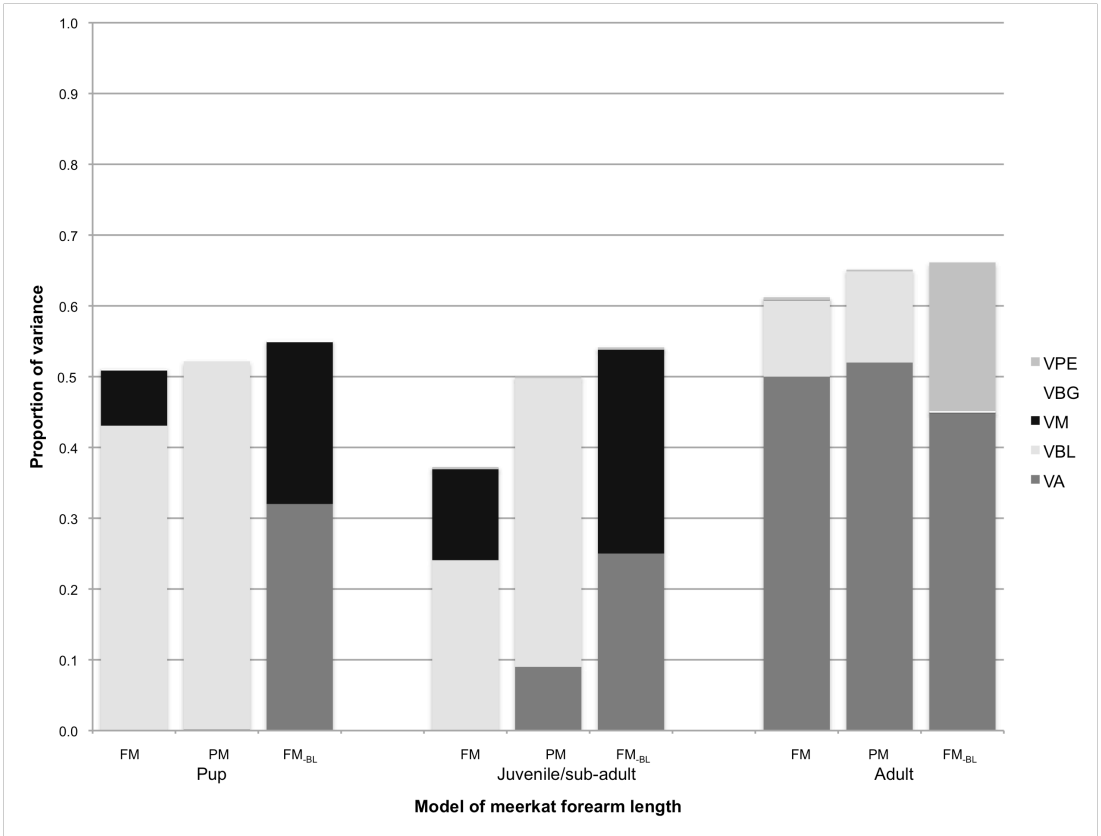
0.0
Proportion of variance
FM
PM
FM-BL
Pup
Juvenile/sub-adult
Adult
Model of meerkat skull width
VPE
VBG
VM
VBL
VA

d.)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0.2
0.1
0.0
Proportion of variance
FM
PM
FM-BL
Pup
Juvenile/sub-adult
Adult
Model of meerkat forearm length
VPE
VBG
VM
VBL
VA

e.)

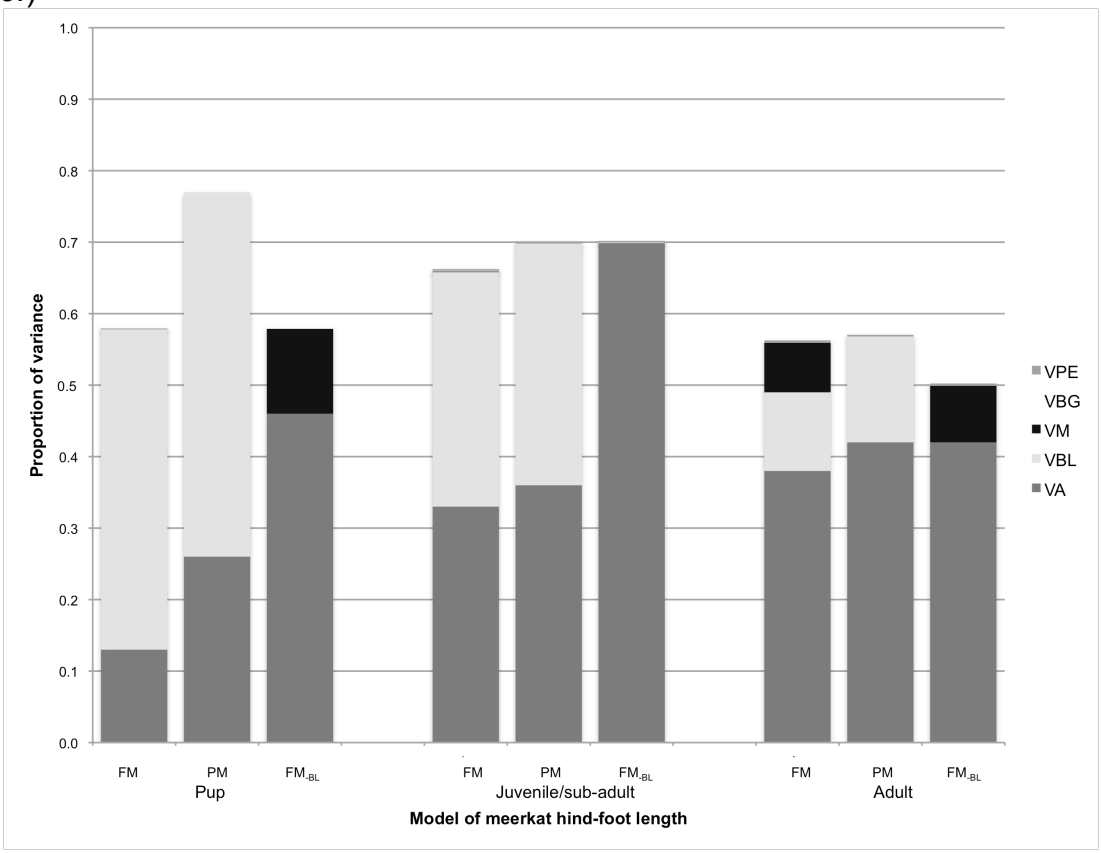
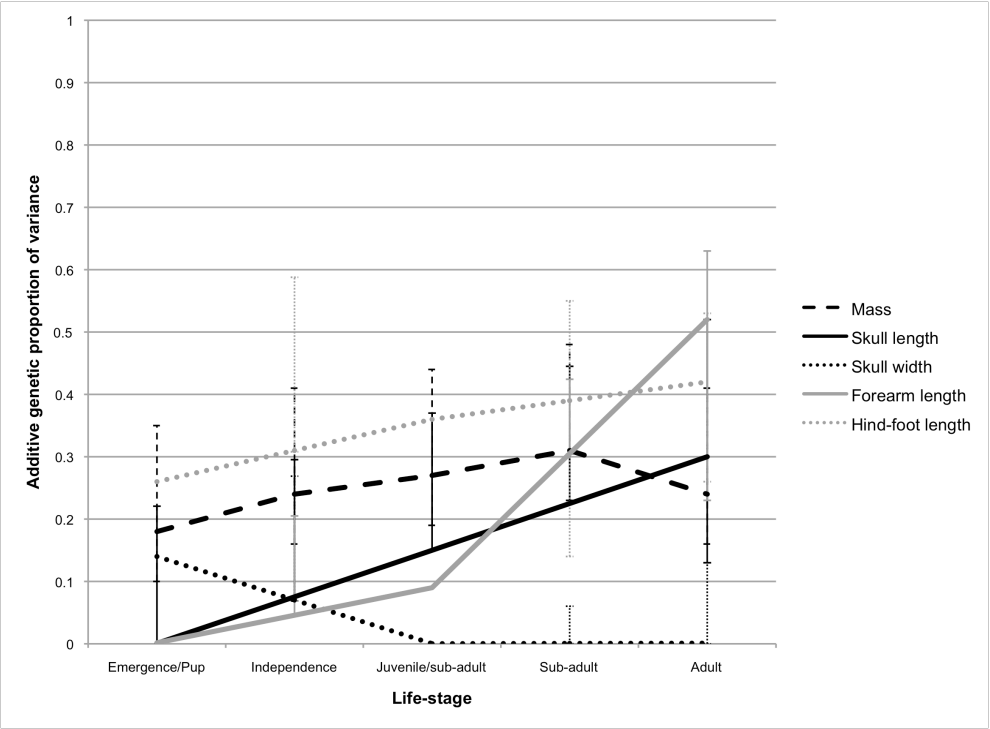


Figure 4.2 Proportion of variance explained by each of the components for *a.*) mass, *b.*) skull length, *c.*) skull width, *d.*) forearm length, and *e.*) hind-foot length at each of the life stages. V_A=additive genetic, V_{BL}=birth litter, V_M=maternal, V_{BG}=birth group, and V_{PE}=permanent environment. Data are from the full (FM; where all possible random effects were included), most parsimonious (PM), and reduced (FM_{-BL}; excluding birth litter) models.

a.)



b.)

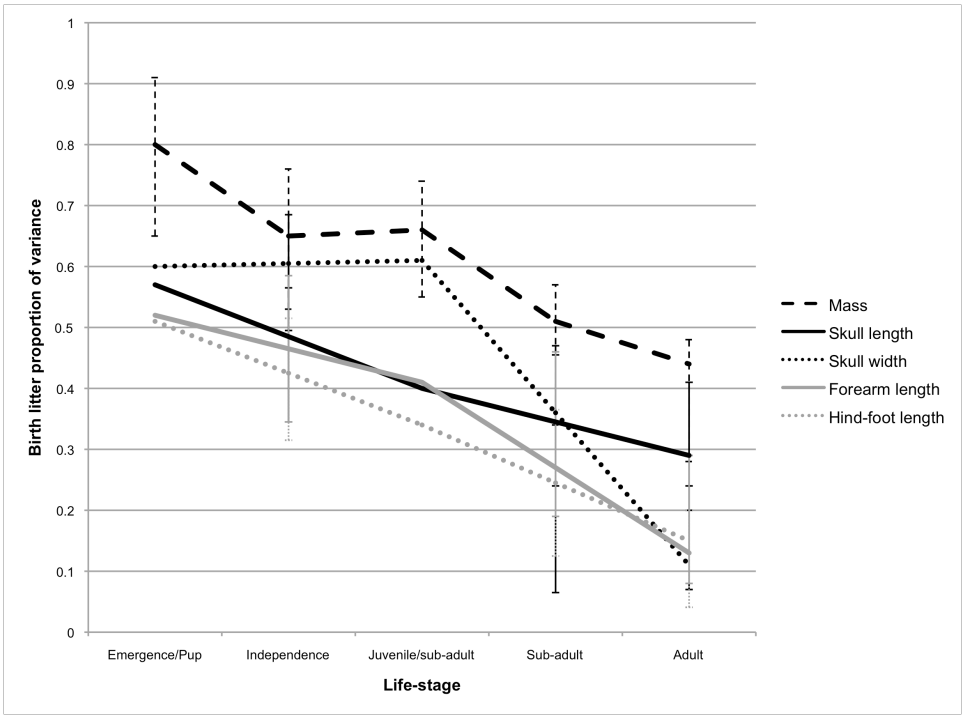


Figure 4.3 Changes in proportion of variance explained by *a.*) additive genetic (V_A) and *b.*) birth litter (V_{BL}) effects for mass and the skeletal traits at each of the life stages. Data are from the most parsimonious models.

4.4.2 Skull length

Fixed effects

As for mass, analysis of the fixed effects in the preliminary models of skull length revealed that age was the only variable that was significant across all life stages, and that adult dominants and males had longer skulls than subordinates or females (Table 4.3). I found positive effects of rainfall on pups and adults, and positive and negative effects of warm-wet seasons on the pup and juvenile/sub-adult life stages respectively (Table 4.3). I also found negative effects of inbreeding coefficient (*F*) in the two life stages when individuals were foraging for themselves (i.e. juvenile/sub-adult and adult) (Table 4.3 & Fig. 4.1*b*). For an individual with *F*=0.078, the *F* parameter estimates translate into reductions in skull length of 0.83% (juvenile/sub-adult) and 0.99% (adult) compared with individuals with *F*=0 (Table 4.3 & Fig. 4.1*b*).

Variance components

Most parsimonious model (PM)

Repeatability of skull length was lower than for mass (r^2=0.59) (Tables 4.8 & 4.9). In the PM, the narrow-sense heritability estimates for skull length appeared to increase with age from non-detectable levels in the pup life stage (MCMC sampling for the additive genetic chain did not converge (results not shown)) to 0.15 in juvenile/sub-adults and 0.30 in adults (Tables 4.8 & 4.9; Figs. 4.2*b* & 4.3*a*). Conversely, the variance in skull length accounted for by birth litter appeared to decline with age (Tables 4.8 & 4.9; Figs. 4.2*b* & 4.3*b*). In comparison to mass, a larger amount of variance in skull length remained unexplained (e^2≈0.38) (Tables 4.8 & 4.9).

Comparison of full (FM) and reduced (FM_{-BL}) models

As for mass, the birth litter term appeared to be confounded with additive genetic and maternal effects as the variance accounted for by these two terms generally increased in FM_{-BL} models where birth litter was excluded, whereas birth group did not explain any of the variance in skull length in the FM_{-BL} models (Tables 4.8 & 4.9; Fig. 4.2*b*).

Table 4.8 Variance components of the skeletal traits at each life stage. Estimates from the full (FM; where all possible random effects were included), most parsimonious (PM and in bold), and reduced (FM_{-BL}; excluding birth litter), models are provided. Models were analysed including all significant fixed effects (see Table 4.3). V_P=total phenotypic, V_A=additive genetic, V_{BL}=birth litter, V_M=maternal, V_{BG}=birth group, V_{PE}=permanent environment, and V_R=residual variance, SL=skull length, SW=skull width, FL=forearm length, and HL=hind-foot length. Values are accompanied by their 95% HPDI (in parentheses) which are bounded above zero.

Trait	Model	V_P	V_A	V_{BL}	V_M	V_{BG}	V_{PE}	V_R
Pup*								
SL	FM	6.76	$10.58*10^{-4}$	3.53	0.49	$29.05*10^{-4}$	—	2.74
		—	($0.33*10^{-6}$ – 0.55)	(2.55 – 4.42)	($3.18*10^{-4}$ – 1.78)	($0.30*10^{-6}$ – 0.54)	—	(2.37 – 3.09)
	PM	**7.06**	**$59.16*10^{-4}$**	**4.22**	—	—	—	**2.83**
		—	**($0.22*10^{-6}$ – 0.83)**	**(3.30 – 5.07)**	—	—	—	**(2.17 – 3.08)**
	FM_{BL}	8.44	2.93	—	2.28	$61.78*10^{-4}$	—	3.22
		—	(1.20 – 4.53)	—	(1.34 – 3.66)	($0.40*10^{-6}$ – 0.71)	—	(2.41 – 4.41)
SW	FM	3.62	$40.30*10^{-4}$	2.12	0.31	$28.81*10^{-4}$	—	1.18
		—	($0.06*10^{-6}$ – 0.90)	(1.58 – 2.56)	($1.07*10^{-4}$ – 0.72)	($0.31*10^{-6}$ – 0.35)	—	(0.75 – 1.37)
	PM	**3.62**	**0.65**	**2.03**	—	—	—	**0.94**
		—	**(0.07 – 1.25)**	**(1.64 – 2.68)**	—	—	—	**(0.58 – 1.26)**
	FM_{BL}	4.20	2.05	—	0.64	$46.58*10^{-4}$	—	1.51
		—	(1.10 – 2.91)	—	(0.26 – 1.20)	($0.05*10^{-8}$ – 0.37)	—	(0.99 – 2.03)
FL	FM	7.59	$60.96*10^{-4}$	4.00	0.02	$76.51*10^{-4}$	—	3.56
		—	($0.03*10^{-6}$ – 1.60)	(2.90 – 5.34)	($2.18*10^{-4}$ – 2.63)	($0.02*10^{-4}$ – 0.93)	—	(2.69 – 4.11)
	PM	**8.05**	**0.01**	**4.75**	—	—	—	**3.29**
		—	**($0.48*10^{-6}$ – 2.49)**	**(3.26 – 5.73)**	—	—	—	**(2.25 – 3.96)**
	FM_{BL}	9.85	2.86	—	2.46	0.01	—	4.52
		—	(0.82 – 5.66)	—	(1.23 – 4.85)	($0.98*10^{-4}$ – 2.03)	—	(3.12 – 5.86)
HL	FM	10.68	1.56	5.71	0.01	0.01	—	3.40
		—	($1.97*10^{-4}$ – 4.16)	(4.39 – 7.20)	($0.03*10^{-4}$ – 1.70)	($0.47*10^{-6}$ – 1.64)	—	(2.19 – 4.73)
	PM	**11.24**	**2.17**	**5.84**	—	—	—	**3.23**
		—	**(0.52 – 4.45)**	**(4.78 – 7.65)**	—	—	—	**(2.07 – 4.41)**
	FM_{BL}	12.85	6.35	—	1.71	0.02	—	4.77
		—	(3.23 – 9.46)	—	(0.44 – 3.23)	($0.73*10^{-4}$ – 2.96)	—	(3.30 – 6.94)
Juvenile/sub-adult								
SL	FM	6.89	0.96	2.63	$80.14*10^{-4}$	$17.93*10^{-4}$	$26.33*10^{-4}$	3.29
		—	($0.13*10^{-4}$ – 1.81)	(1.71 – 4.06)	($0.01*10^{-4}$ – 1.33)	($0.01*10^{-4}$ – 1.00)	($0.13*10^{-4}$ – 0.64)	(2.76 – 3.92)
	PM	**7.26**	**1.04**	**2.98**	—	—	**$24.99*10^{-4}$**	**3.24**
		—	**(0.27 – 2.09)**	**(1.84 – 4.36)**	—	—	**($0.09*10^{-8}$ – 0.59)**	**(2.69 – 3.81)**
	FM_{BL}	6.78	3.27	—	$89.45*10^{-4}$	$94.76*10^{-4}$	$45.67*10^{-4}$	3.49
		—	(1.78 – 4.84)	—	($0.02*10^{-4}$ – 1.44)	($0.02*10^{-6}$ – 1.38)	($0.02*10^{-4}$ – 0.96)	(2.84 – 4.25)
SW	FM	5.04	$17.68*10^{-4}$	2.99	$74.83*10^{-4}$	$46.81*10^{-4}$	$20.17*10^{-4}$	2.03
		—	($0.45*10^{-6}$ – 0.36)	(1.99 – 3.96)	($0.14*10^{-6}$ – 0.84)	($0.08*10^{-6}$ – 0.59)	($0.59*10^{-8}$ – 0.32)	(1.73 – 2.36)
	PM	**5.08**	**$13.59*10^{-4}$**	**3.07**	—	—	**$19.79*10^{-4}$**	**2.01**
		—	**($0.03*10^{-8}$ – 0.40)**	**(2.26 – 4.16)**	—	—	**($0.03*10^{-4}$ – 0.33)**	**(1.72 – 2.33)**
	FM_{BL}	4.74	1.84	—	0.52	$43.26*10^{-4}$	$54.86*10^{-4}$	2.37
		—	(0.60 – 2.97)	—	($1.14*10^{-4}$ – 1.36)	($0.04*10^{-4}$ – 0.64)	($0.09*10^{-4}$ – 0.99)	(2.00 – 3.05)

Trait	Model	V_P	V_A	V_{BL}	V_M	V_{BG}	V_{PE}	V_R
FL	FM	7.49	$34.62*10^{-4}$	2.31	0.93	$96.33*10^{-4}$	$15.61*10^{-4}$	4.24
		—	($0.04*10^{-4}$ – 1.04)	(1.25 – 3.86)	($6.24*10^{-4}$ – 2.91)	($0.79*10^{-6}$ – 0.97)	($0.23*10^{-6}$ – 0.58)	(3.47 – 4.93)
	PM	**7.90**	**0.60**	**3.25**	—	—	**$52.56*10^{-4}$**	**4.04**
		—	**($0.03*10^{-4}$ – 1.72)**	**(1.82 – 4.51)**	—	—	**($0.02*10^{-4}$ – 0.67)**	**(3.10 – 4.53)**
	FM_{-BL}	7.55	1.61	—	1.82	$64.03*10^{-4}$	$57.19*10^{-4}$	4.11
		—	(0.70 – 3.52)	—	(0.80 – 4.51)	($0.01*10^{-8}$ – 1.25)	($0.05*10^{-6}$ – 0.96)	(3.09 – 4.73)
HL	FM	8.66	3.01	2.94	$49.88*10^{-4}$	0.01	$63.49*10^{-4}$	2.69
		—	(0.73 – 4.75)	(1.84 – 4.66)	($0.25*10^{-6}$ – 0.88)	($0.02*10^{-6}$ – 1.12)	($0.02*10^{-4}$ – 1.61)	(2.15 – 3.22)
	PM	**8.85**	**3.11**	**3.17**	—	—	**$41.34*10^{-4}$**	**2.57**
		—	**(1.62 – 4.87)**	**(1.75 – 4.58)**	—	—	**($0.03*10^{-8}$ – 1.06)**	**(2.16 – 3.23)**
	FM_{-BL}	8.78	6.26	—	$62.64*10^{-4}$	$34.66*10^{-4}$	$64.44*10^{-4}$	2.50
		—	(4.80 – 8.69)	—	($0.80*10^{-6}$ – 1.21)	($0.02*10^{-6}$ – 0.83)	($0.07*10^{-6}$ – 1.21)	(2.11 – 3.13)
Adult								
SL	FM	4.74	1.76	1.28	$45.30*10^{-4}$	$17.31*10^{-4}$	$50.45*10^{-4}$	1.69
		—	(0.35 – 2.89)	(0.83 – 2.15)	($0.63*10^{-6}$ – 0.93)	($0.03*10^{-8}$ – 0.52)	($0.21*10^{-8}$ – 1.31)	(1.49 – 1.89)
	PM	**4.84**	**1.51**	**1.64**	—	—	**$52.87*10^{-4}$**	**1.68**
		—	**(0.67 – 2.86)**	**(0.98 – 2.36)**	—	—	**($0.01*10^{-8}$ – 1.11)**	**(1.48 – 1.88)**
	FM_{-BL}	5.71	1.69	—	0.83	$38.87*10^{-4}$	1.48	1.71
		—	(0.58 – 3.35)	—	(0.20 – 1.64)	($0.01*10^{-4}$ – 0.80)	(0.46 – 2.25)	(1.52 – 1.92)
SW†	**FM/PM**	**7.21**	**$76.85*10^{-4}$**	**1.09**	**1.01**	**1.95**	**0.75**	**2.40**
		—	**($0.19*10^{-6}$ – 1.14)**	**(0.59 – 1.81)**	**(0.43 – 2.13)**	**(0.39 – 6.01)**	**(0.08 – 1.16)**	**(2.07 – 2.64)**
	FM_{-BL}	7.29	0.01	—	1.44	2.14	1.27	2.43
		—	($0.19*10^{-4}$ – 1.90)	—	(0.90 – 2.35)	(0.09 – 5.42)	(0.35 – 1.92)	(2.13 – 2.70)
FL	FM	5.78	3.16	0.78	$51.07*10^{-4}$	$43.86*10^{-4}$	$64.46*10^{-4}$	1.82
		—	(1.47 – 4.69)	(0.32 – 1.75)	($5.67*10^{-8}$ – 0.78)	($0.03*10^{-6}$ – 0.85)	($0.11*10^{-4}$ – 1.68)	(1.53 – 2.04)
	PM	**6.18**	**3.43**	**0.96**	—	—	**$62.43*10^{-4}$**	**1.78**
		—	**(1.93 – 4.83)**	**(0.40 – 1.89)**	—	—	**($0.49*10^{-4}$ – 1.48)**	**(1.54 – 2.01)**
	FM_{-BL}	6.64	3.78	—	$66.41*10^{-4}$	$21.71*10^{-4}$	1.07	1.78
		—	(1.53 – 5.55)	—	($1.95*10^{-8}$ – 0.86)	($0.03*10^{-6}$ – 1.00)	($11.65*10^{-4}$ – 2.34)	(1.55 – 2.05)
HL	FM	7.31	2.95	0.83	0.50	0.02	$67.05*10^{-4}$	3.00
		—	(1.86 – 4.42)	(0.11 – 1.53)	($0.61*10^{-4}$ – 1.75)	($0.13*10^{-4}$ – 2.89)	($0.15*10^{-6}$ – 0.71)	(2.74 – 3.39)
	PM	**7.42**	**3.16**	**1.25**	—	—	**$17.99*10^{-4}$**	**3.01**
		—	**(2.49 – 4.62)**	**(0.58 – 1.93)**	—	—	**($0.27*10^{-6}$ – 0.64)**	**(2.72 – 3.41)**
	FM_{-BL}	7.19	3.42	—	0.76	0.01	$68.53*10^{-4}$	2.99
		—	(2.20 – 4.98)	—	(0.04 – 2.06)	($0.21*10^{-4}$ – 2.77)	($1.57*10^{-6}$ – 1.27)	(2.73 – 3.41)

*Permanent environment effect (V_{PE}) excluded as almost all individuals in the pup datasets were only measured once (see Table 4.2).
†The full model has the lowest DIC value (i.e. is the most parsimonious).

Table 4.9 Heritabilities (h^2) and birth litter (bl^2), maternal (m^2), birth group (bg^2), permanent environment (pe^2), and residual (e^2) effects of the skeletal traits at each life stage. Estimates from the full (FM; where all possible random effects were included), most parsimonious (PM and in bold), and reduced (FM_{-BL}; excluding birth litter) models are provided. Models were analysed including all significant fixed effects (see Table 4.3). SL=skull length, SW=skull width, FL=forearm length, HL=hind-foot length, CV_A=coefficients of additive genetic variance ($CV_A=100*\sqrt{(V_A)}/x$), and r^2=repeatability. Values are accompanied by their 95% HPDI (in parentheses) which are bounded above zero.

Trait	Model	CV_A	r^2	h^2	bl^2	m^2	bg^2	pe^2	e^2
Pup*									
SL	FM	0.07	0.63	$3.74*10^{-4}$	0.49	0.11	$1.70*10^{-4}$	—	0.35
		—	—	($0.02*10^{-10}$ – 0.08)	(0.35 – 0.57)	($4.04*10^{-6}$ – 0.22)	($0.14*10^{-6}$ – 0.07)	—	(0.23 – 0.43)
	PM	**0.16**	**0.59**	**$6.80*10^{-4}$**	**0.57**	—	—	—	**0.39**
		—	—	**($3.48*10^{-8}$ – 0.12)**	**(0.52 – 0.66)**	—	—	—	**(0.31 – 0.46)**
	FM_{-BL}	3.51	0.57	0.26	—	0.26	$2.34*10^{-4}$	—	0.40
		—	—	(0.14 – 0.45)	—	(0.16 – 0.36)	($0.69*10^{-10}$ – 0.08)	—	(0.23 – 0.54)
SW	FM	0.22	0.70	$13.11*10^{-4}$	0.52	0.09	$5.38*10^{-4}$	—	0.26
		—	—	($5.80*10^{-8}$ – 0.20)	(0.41 – 0.60)	($1.86*10^{-8}$ – 0.18)	($0.16*10^{-8}$ – 0.09)	—	(0.14 – 0.35)
	PM	**2.83**	**0.70**	**0.14**	**0.60**	—	—	—	**0.24**
		—	—	**(0.02 – 0.31)**	**(0.49 – 0.66)**	—	—	—	**(0.15 – 0.35)**
	FM_{-BL}	5.02	0.60	0.41	—	0.15	$2.98*10^{-4}$	—	0.35
		—	—	(0.27 – 0.61)	—	(0.08 – 0.26)	($0.34*10^{-8}$ – 0.08)	—	(0.19 – 0.50)
FL	FM	0.25	0.60	$7.38*10^{-4}$	0.43	0.08	$3.13*10^{-4}$	—	0.34
		—	—	($0.68*10^{-8}$ – 0.16)	(0.31 – 0.55)	($0.60*10^{-6}$ – 0.26)	($0.45*10^{-10}$ – 0.08)	—	(0.21 – 0.46)
	PM	**0.32**	**0.57**	**$16.26*10^{-4}$**	**0.52**	—	—	—	**0.39**
		—	—	**($5.52*10^{-8}$ – 0.27)**	**(0.42 – 0.61)**	—	—	—	**(0.25 – 0.48)**
	FM_{-BL}	5.36	0.61	0.32	—	0.23	$11.02*10^{-4}$	—	0.35
		—	—	(0.11 – 0.48)	—	(0.12 – 0.36)	($0.33*10^{-6}$ – 0.16)	—	(0.22 – 0.56)
HL	FM	3.24	0.77	0.13	0.45	$8.46*10^{-4}$	$5.46*10^{-4}$	—	0.33
		—	—	($0.15*10^{-4}$ – 0.32)	(0.38 – 0.58)	($0.26*10^{-6}$ – 0.13)	($3.73*10^{-8}$ – 0.12)	—	(0.16 – 0.40)
	PM	**3.82**	**0.73**	**0.26**	**0.51**	—	—	—	**0.28**
		—	—	**(0.06 – 0.37)**	**(0.43 – 0.60)**	—	—	—	**(0.17 – 0.39)**
	FM_{-BL}	6.54	0.67	0.46	—	0.12	$11.67*10^{-4}$	—	0.29
		—	—	(0.26 – 0.60)	—	(0.03 – 0.21)	($0.53*10^{-8}$ – 0.18)	—	(0.13 – 0.49)
Juvenile/sub-adult									
SL	FM	1.35	0.56	0.13	0.38	$5.98*10^{-4}$	$7.26*10^{-4}$	$2.81*10^{-4}$	0.41
		—	—	($0.02*10^{-4}$ – 0.22)	(0.24 – 0.49)	($0.15*10^{-6}$ – 0.15)	($0.12*10^{-6}$ – 0.12)	($0.02*10^{-4}$ – 0.08)	(0.31 – 0.51)
	PM	**1.40**	**0.55**	**0.15**	**0.40**	—	—	**$3.99*10^{-4}$**	**0.45**
		—	—	**(0.04 – 0.27)**	**(0.28 – 0.52)**	—	—	**($0.01*10^{-8}$ – 0.08)**	**(0.34 – 0.52)**
	FM_{-BL}	2.48	0.50	0.43	—	$9.33*10^{-4}$	$8.52*10^{-4}$	$3.87*10^{-4}$	0.44
		—	—	(0.24 – 0.57)	—	($0.26*10^{-6}$ – 0.17)	($0.22*10^{-8}$ – 0.15)	($0.24*10^{-6}$ – 0.12)	(0.33 – 0.57)
SW	FM	0.11	0.59	$4.16*10^{-4}$	0.52	$10.84*10^{-4}$	$3.68*10^{-4}$	$4.22*10^{-4}$	0.36
		—	—	($0.07*10^{-6}$ – 0.06)	(0.40 – 0.63)	($0.03*10^{-6}$ – 0.14)	($0.01*10^{-6}$ – 0.10)	($0.09*10^{-8}$ – 0.06)	(0.29 – 0.45)
	PM	**0.09**	**0.63**	**$2.70*10^{-4}$**	**0.61**	—	—	**$3.11*10^{-4}$**	**0.36**
		—	—	**($0.01*10^{-8}$ – 0.07)**	**(0.50 – 0.66)**	—	—	**($0.45*10^{-6}$ – 0.06)**	**(0.30 – 0.46)**
	FM_{-BL}	3.49	0.52	0.35	—	0.14	$8.82*10^{-4}$	$14.82*10^{-4}$	0.45
		—	—	(0.13 – 0.51)	—	($0.22*10^{-4}$ – 0.23)	($0.78*10^{-6}$ – 0.11)	($0.02*10^{-4}$ – 0.19)	(0.33 – 0.59)

Trait	Model	CV_A	r^2	h^2	bl^2	m^2	bg^2	pe^2	e^2
FL	FM	0.10	0.43	$7.26*10^{-4}$	0.24	0.13	$10.21*10^{-4}$	$4.09*10^{-4}$	0.50
		—	—	($0.45*10^{-6}$ – 0.12)	(0.16 – 0.43)	($0.70*10^{-4}$ – 0.30)	($0.09*10^{-6}$ – 0.11)	($0.02*10^{-6}$ – 0.07)	(0.37 – 0.60)
	PM	**1.32**	**0.50**	**0.09**	**0.41**	—	—	**$2.74*10^{-4}$**	**0.50**
		—	—	**($0.35*10^{-6}$ – 0.22)**	**(0.26 – 0.50)**	—	—	**($0.24*10^{-6}$ – 0.08)**	**(0.37 – 0.59)**
	FM_{-BL}	2.17	0.56	0.25	—	0.29	$6.38*10^{-4}$	$7.26*10^{-4}$	0.43
		—	—	(0.07 – 0.37)	—	(0.11 – 0.43)	($0.16*10^{-10}$ – 0.13)	($0.45*10^{-8}$ – 0.11)	(0.33 – 0.59)
HL	FM	2.61	0.70	0.33	0.33	$5.08*10^{-4}$	$8.43*10^{-4}$	$10.09*10^{-4}$	0.28
		—	—	(0.08 – 0.49)	(0.21 – 0.45)	($0.03*10^{-6}$ – 0.09)	($0.18*10^{-8}$ – 0.12)	($0.16*10^{-6}$ – 0.16)	(0.20 – 0.35)
	PM	**2.65**	**0.71**	**0.36**	**0.34**	—	—	**$4.33*10^{-4}$**	**0.28**
		—	—	**(0.18 – 0.50)**	**(0.22 – 0.46)**	—	—	**($0.33*10^{-10}$ – 0.11)**	**(0.21 – 0.36)**
	FM_{-BL}	3.77	0.74	0.70	—	$7.01*10^{-4}$	$2.18*10^{-4}$	$8.30*10^{-4}$	0.25
		—	—	(0.52 – 0.78)	—	($0.09*10^{-6}$ – 0.11)	($0.20*10^{-8}$ – 0.08)	($0.57*10^{-8}$ – 0.12)	(0.19 – 0.32)
Adult									
SL	FM	1.71	0.61	0.24	0.23	$11.70*10^{-4}$	$5.92*10^{-4}$	$11.63*10^{-4}$	0.30
		—	—	(0.07 – 0.46)	(0.14 – 0.35)	($0.10*10^{-6}$ – 0.15)	($0.65*10^{-10}$ – 0.08)	($0.03*10^{-8}$ – 0.24)	(0.24 – 0.34)
	PM	**1.59**	**0.66**	**0.30**	**0.29**	—	—	**$9.15*10^{-4}$**	**0.31**
		—	—	**(0.15 – 0.49)**	**(0.18 – 0.39)**	—	—	**($0.19*10^{-10}$ – 0.21)**	**(0.25 – 0.35)**
	FM_{-BL}	1.68	0.71	0.33	—	0.11	$6.18*10^{-4}$	0.24	0.28
		—	—	(0.11 – 0.50)	—	(0.03 – 0.24)	($0.17*10^{-6}$ – 0.12)	(0.06 – 0.39)	(0.23 – 0.35)
SW[†]	**FM/PM**	**1.31**	**0.20**	**$10.38*10^{-4}$**	**0.11**	**0.13**	**0.26**	**0.07**	**0.30**
		—	—	**($0.02*10^{-6}$ – 0.13)**	**(0.07 – 0.24)**	**(0.05 – 0.25)**	**(0.08 – 0.55)**	**($0.12*10^{-4}$ – 0.14)**	**(0.18 – 0.38)**
	FM_{-BL}	1.50	0.22	$19.07*10^{-4}$	—	0.12	0.28	0.17	0.30
		—	—	($1.50*10^{-4}$ – 0.24)	—	(0.07 – 0.29)	(0.07 – 0.53)	(0.04 – 0.26)	(0.21 – 0.41)
FL	FM	2.76	0.71	0.50	0.11	$2.38*10^{-4}$	$7.79*10^{-4}$	$10.74*10^{-4}$	0.25
		—	—	(0.21 – 0.61)	(0.05 – 0.25)	($0.75*10^{-8}$ – 0.11)	($0.40*10^{-8}$ – 0.11)	($0.02*10^{-4}$ – 0.25)	(0.20 – 0.31)
	PM	**2.87**	**0.71**	**0.52**	**0.13**	—	—	**$11.24*10^{-4}$**	**0.27**
		—	—	**(0.31 – 0.64)**	**(0.06 – 0.27)**	—	—	**($0.07*10^{-4}$ – 0.23)**	**(0.21 – 0.32)**
	FM_{-BL}	3.02	0.73	0.45	—	$7.74*10^{-4}$	$5.98*10^{-4}$	0.21	0.25
		—	—	(0.25 – 0.69)	—	($0.27*10^{-8}$ – 0.12)	($0.47*10^{-4}$ – 0.13)	($1.50*10^{-4}$ – 0.35)	(0.19 – 0.32)
HL	FM	2.52	0.62	0.38	0.11	0.07	$19.30*10^{-4}$	$6.92*10^{-4}$	0.34
		—	—	(0.22 – 0.49)	($8.43*10^{-4}$ – 0.16)	($0.07*10^{-4}$ – 0.19)	($0.02*10^{-4}$ – 0.27)	($0.02*10^{-6}$ – 0.08)	(0.27 – 0.42)
	PM	**2.61**	**0.59**	**0.42**	**0.15**	—	—	**$4.53*10^{-4}$**	**0.40**
		—	—	**(0.32 – 0.53)**	**(0.07 – 0.23)**	—	—	**($0.03*10^{-6}$ – 0.08)**	**(0.32 – 0.44)**
	FM_{-BL}	2.71	0.60	0.42	—	0.08	$17.36*10^{-4}$	$5.89*10^{-4}$	0.33
		—	—	(0.24 – 0.53)	—	($25.86*10^{-4}$ – 0.22)	($0.02*10^{-4}$ – 0.26)	($0.15*10^{-6}$ – 0.14)	(0.26 – 0.41)

[†]The full model has the lowest DIC value (i.e. is the most parsimonious).

4.4.3 Skull width

Fixed effects

I found that both age and rainfall positively influenced skull width across all life stages, dominance status positively influenced skull width in adults, and hot-wet seasons had positive effects on pup and adult skull widths (Table 4.3). I also found a positive effect of *F* in pups, such that individuals with *F*=0.078 would have skull widths that were approximately 1.59% larger than individuals with *F*=0 (Table 4.3 & Fig. 4.1*c*). Conversely I found a negative effect of *F* in adults, such that individuals with *F*=0.078 would have skull widths that were approximately 0.74% smaller than individuals with *F*=0 (Table 4.3 & Fig. 4.1*c*).

Variance components

Most parsimonious model (PM)

Repeatability of skull width was even lower than for skull length (r^2=0.51) (Tables 4.8 & 4.9). As for mass and skull length, the PMs for the pup and juvenile/sub-adult life stages only included random terms for additive genetic and birth litter (and permanent environment for juvenile/sub-adults), whereas the most parsimonious adult model was the full model, which also included maternal and birth group terms (Table 4.5). The PM MCMC sampling of the additive genetic chains did not converge for any life stages (results not shown), therefore there was no detectable heritability (Tables 4.8 & 4.9; Figs. 4.2*c* & 4.3*a*). Birth litter accounted for all of the variance in pup and juvenile/sub-adult skull width (Tables 4.8 & 4.9; s. 4.2*c* & 4.3*b*), although there was a tendency for the amount of variance this term explained to decrease with age (Tables 4.8 & 4.9; Fig. 4.3*b*). In contrast to mass and skull length, maternal and birth group effects accounted for some of the variance in adult skull width (Tables 4.8 & 4.9; Fig. 4.2*c*). The residual variance was less than for skull length: approximately 30% of the variance in skull width remained unexplained (Tables 4.8 & 4.9).

Comparison of full (FM) and reduced (FM_{-BL}) models

The maternal and additive genetic terms accounted for further variance in pup and juvenile/sub-adult skull widths in the FM_{-BL} models (Tables 4.8 & 4.9; Fig. 4.2*c*).

The additive genetic effect, however, remained undetectable in the adult FM_{-BL} model (Tables 4.8 & 4.9; Fig. 4.2*c*).

4.4.4 Forearm length

Fixed effects

Only age positively influenced forearm length across all life stages, whereas only adult males had significantly longer forearm lengths than females (Table 4.3). I also found negative effects of hot-wet seasons and *F* on juvenile/sub-adult and adult forearm length respectively (Table 4.3 & Fig. 4.1*d*). For adults with F=0.078, the *F* parameter estimate translates into a 0.96% reduction in forearm length compared with adults with F=0 (Table 4.3 & Fig. 4.1*d*).

Variance components

Most parsimonious model (PM)

The average repeatability (r^2) of forearm length for all life stages was 0.59 (Tables 4.8 & 4.9). In the pup and juvenile/sub-adult PMs, birth litter accounted for the vast majority of variance in forearm length (bl^2=0.57 and 0.41 respectively; Tables 4.8 & 4.9; Fig. 4.2*d*), but the amount of variance it explained in adults was considerably less (bl^2=0.13; Tables 4.8 & 4.9; Fig. 4.3*b*). Conversely, there appeared to be a tendency for the narrow-sense heritability estimates to increase with age from non-detectable levels in the pup life stage (MCMC sampling for the additive genetic chain did not converge (results not shown)), to 0.09 in juvenile/sub-adults and 0.52 in adults (Tables 4.8 & 4.9; Figs. 4.2*d* & 4.3*a*). Approximately 39% of the variance in forearm length remained unexplained (Tables 4.8 & 4.9).

Comparison of full (FM) and reduced (FM_{-BL}) models

The additive genetic and maternal effects increased in the pup and juvenile/sub-adult FM_{-BL} models, whereas birth group did not account for any detectable variance in any model (Tables 4.8 & 4.9; Fig. 4.2*d*).

4.4.5 Hind-foot length

Fixed effects

Amongst the fixed effects, only age had a significant influence on hind-foot length across all life stages, and it was only among adults that sex explained variation: females had significantly longer hind-foot lengths than males (Table 4.3). I found positive effects of hot-wet seasons and rainfall on the pup life stage and negative effects of *F* on juvenile/sub-adult and adult hind-foot lengths respectively (Table 4.3 & Fig. 4.1*e*). For individuals with F=0.078, the *F* parameter estimates translate into 1.15% (juvenile/sub-adult) and 0.93% (adult) reductions in hind-foot length compared with individuals with F=0 (Table 4.3 & Fig. 4.1*e*).

Variance components

Most parsimonious model (PM)

Average repeatability (r^2) of hind-foot length across life stages was 0.68 (Tables 4.8 & 4.9) and MCMC sampling for the additive genetic effect chains all converged (results not shown). As for the previous traits, the estimates for narrow-sense heritability increased with age (h^2=0.26 (pup), 0.36 (juvenile/sub-adult), 0.42 (adult)) (Tables 4.8 & 4.9; Figs. 4.2e & 4.3*a*). Conversely, the amount of variance explained by birth litter decreased with age (h^2=0.51 (pup), 0.34 (juvenile/sub-adult), 0.15 (adult)) (Tables 4.8 & 4.9; Figs. 4.2*e* & 4.3*b*). The average amount of unexplained residual variance (e^2) was 0.32 (Tables 4.8 & 4.9).

Comparison of full (FM) and reduced (FM_{-BL}) models

As for skull length and forearm length, birth group did not account for any variance in hind-foot length, even in FM_{-BL} models, whereas maternal effects accounted for some variance in hind-foot length in pup and juvenile/sub-adult FM_{-BL} models (Tables 4.8 & 4.9; Figs. 4.2*e*).

4.4.6 Correlations between traits and random terms

I only found two significant genetic correlations between traits in the pup life stage, but a greater number of significant genetic correlations among traits in the juvenile/sub-adult (nine) and adult (seven) life stages (Table 4.10). All significant

genetic correlations were very strongly positive (r=0.56-1.00) (Table 4.10). In comparison, there were strong positive birth litter correlations between all traits in the pup and juvenile/sub-adult life stages (r=0.36-0.98) and strong positive birth litter correlations between five traits in the adult life stage (r=0.44-0.99) (Table 4.10).

In my per-trait investigations on whether the random terms were potentially confounded, where I combined all life stage data for each trait and determined the extent to which the random terms of additive genetic, birth litter and permanent environment were correlated with each other, I found moderate to high positive correlations between all random terms (Table 4.11). For all traits the strongest correlations were between maternal identity and birth group (average r=0.62), the lowest were between birth litter and birth group (average r=0.29), and the correlation between additive genetic and birth litter terms was approximately 0.34 for all traits (Table 4.11).

Table 4.10 Additive genetic and non-genetic covariances (above diagonal) and correlations (r=$Cov_{a,b}/\sqrt{(V_a * V_b)}$; below diagonal) of meerkat mass and skeletal traits at each life stage. The multivariate, repeated measure models were analysed including additive genetic (V_A), birth litter (V_{BL}), and permanent environment (V_{PE}) as random terms, and all significant trait-specific fixed terms (see Table 4.3). In order to obtain covariances and correlations among all five traits, the emergence and independence mass measures were combined and analysed in the pup model and the juvenile and sub-adult mass measures were combined and analysed in the juvenile/sub-adult model (n=total number of records). The multivariate models are thus not directly analogous to the univariate models. All values are accompanied by their 95% HPDI credible intervals (in parentheses) and are indicated in bold where significant. Note that as residual variance structures cannot have parameter expansion in MCMCGLMM these are not presented.

	Trait				
	Mass	Skull length	Skull width	Forearm length	Hind-foot length
Pup (n=9755)					
Additive genetic					
Mass	—	2.74 (-6.37 – 10.05)	1.05 (-3.79 – 6.57)	4.47 (-6.40 – 11.21)	8.58 (-2.53 – 21.89)
Skull length	0.50 (-0.97 – 0.68)	—	$18.60*10^{-4}$ (-0.02 – 0.47)	$0.16*10^{-4}$ (-0.17 – 0.61)	0.01 (-0.20 – 1.05)
Skull width	0.43 (-0.67 – 0.74)	0.84 (-0.09 – 1.00)	—	0.16 (-0.05 – 0.57)	0.18 (-0.02 – 0.98)
Forearm length	0.44 (-0.56 – 0.72)	0.58 (-0.55 – 0.99)	0.80 (-0.05 – 0.99)	—	0.41 (-0.05 – 1.55)
Hind-foot length	0.48 (-0.11 – 0.78)	0.89 (-0.48 – 0.98)	**0.98 (0.25 – 1.00)**	**0.99 (0.34 – 1.00)**	—
Birth litter					
Mass	—	**37.05 (24.96 – 50.24)**	**31.33 (20.98 – 39.22)**	**47.37 (27.92 – 57.03)**	**40.57 (25.33 – 55.38)**
Skull length	**0.50 (0.37 – 0.63)**	—	**2.42 (2.03 – 3.27)**	**3.87 (3.33 – 5.32)**	**4.58 (3.85 – 6.64)**
Skull width	**0.57 (0.42 – 0.67)**	**0.84 (0.77 – 0.89)**	—	**2.85 (2.34 – 3.62)**	**2.92 (2.31 – 4.05)**
Forearm length	**0.59 (0.42 – 0.71)**	**0.98 (0.87 – 0.99)**	**0.89 (0.82 – 0.94)**	—	**4.82 (3.98 – 6.63)**
Hind-foot length	**0.44 (0.29 – 0.56)**	**0.93 (0.90 – 0.97)**	**0.79 (0.100 – 0.86)**	**0.92 (0.87 – 0.96)**	—
Permanent environment					
Mass	—	**11.07 (5.14 – 14.65)**	**3.92 (1.84 – 7.72)**	**11.00 (5.79 – 15.36)**	**13.85 (7.79 – 21.72)**
Skull length	0.99 **(0.88 – 1.00)**	—	**0.43 (0.17 – 0.55)**	**0.84 (0.51 – 1.20)**	**1.23 (0.70 – 1.54)**
Skull width	**1.00 (0.82 – 1.00)**	**0.99 (0.88 – 1.00)**	—	**0.42 (0.18 – 0.64)**	**0.63 (0.26 – 0.83)**
Forearm length	**1.00 (0.89 – 1.00)**	**1.00 (0.92 – 1.00)**	**1.00 (0.87 – 1.00)**	—	**1.38 (0.77 – 1.90)**
Hind-foot length	**1.00 (0.92 – 1.00)**	**0.99 (0.91 – 1.00)**	**1.00 (0.87 – 1.00)**	**1.00 (0.96 – 1.00)**	—

	Trait				
	Mass	Skull length	Skull width	Forearm length	Hind-foot length
Juvenile/sub-adult (*n*=10857)					
Additive genetic					
Mass	—	**36.91 (14.34 – 54.80)**	13.26 (-0.48 – 23.28)	**49.00 (18.89 – 63.94)**	**40.50 (7.41 – 63.66)**
Skull length	**0.79 (0.48 – 0.96)**	—	0.43 (-0.09 – 0.86)	**2.49 (0.95 – 3.23)**	**2.34 (0.77 – 3.40)**
Skull width	**0.97 (0.38 – 1.00)**	**0.92 (0.16 – 1.00)**	—	0.42 (-0.08 – 1.09)	0.57 (-0.09 – 1.27)
Forearm length	**0.83 (0.58 – 0.98)**	**0.99 (0.82 – 1.00)**	**0.98 (0.17 – 0.99)**	—	**2.65 (1.35 – 4.10)**
Hind-foot length	**0.56 (0.24 – 0.78)**	**0.81 (0.60 – 0.92)**	0.87 (-0.03 – 0.99)	**0.84 (0.71 – 0.92)**	—
Birth litter					
Mass	—	**38.47 (27.49 – 66.64)**	**31.83 (21.09 – 53.35)**	**26.61 (6.80 – 46.70)**	**30.93 (15.65 – 51.57)**
Skull length	**0.65 (0.42 – 0.81)**	—	**2.08 (1.40 – 2.98)**	**2.68 (1.42 – 3.56)**	**2.08 (1.29 – 3.43)**
Skull width	**0.46 (0.29 – 0.64)**	**0.72 (0.58 – 0.86)**	—	**2.21 (1.40 – 3.00)**	1.43 (0.60 – 2.22)
Forearm length	**0.36 (0.10 – 0.57)**	**0.91 (0.80 – 0.97)**	**0.73 (0.51 – 0.88)**	—	**2.35 (1.02 – 3.43)**
Hind-foot length	**0.43 (0.27 – 0.66)**	**0.83 (0.68 – 0.94)**	**0.50 (0.22 – 0.67)**	**0.83 (0.64 – 0.94)**	—
Permanent environment					
Mass	—	$-73.22*10^{-4}$ (-2.08 – 16.97)	8.27 (-0.03 – 14.68)	-0.03 (-0.93 – 18.97)	-0.09 (-4.55 – 21.23)
Skull length	0.99 (-0.42 – 1.00)	—	$24.56*10^{-4}$ (-0.06 – 0.45)	$1.53*10^{-4}$ (-0.05 – 0.65)	$7.07*10^{-4}$ (-0.06 – 0.81)
Skull width	**0.99 (0.51 – 1.00)**	0.97 (-0.49 – 1.00)	—	$40.62*10^{-4}$ (-0.04 – 0.49)	$23.36*10^{-4}$ (-0.09 – 0.57)
Forearm length	0.99 (-0.11 – 1.00)	0.98 (-0.47 – 1.00)	0.91 (-0.23 – 1.00)	—	$6.06*10^{-4}$ (-0.05 – 0.81)
Hind-foot length	0.99 (-0.78 – 1.00)	0.99 (-0.73 – 1.00)	0.98 (-0.63 – 1.00)	0.98 (-0.61 – 1.00)	—
Adult (*n*=6907)					
Additive genetic					
Mass	—	**22.71 (7.78 – 56.39)**	9.26 (-2.07 – 39.93)	**28.67 (15.07 – 74.65)**	**22.31 (10.92 – 70.36)**
Skull length	**0.69 (0.40 – 0.95)**	—	1.40 (-0.62 – 0.92)	**1.26 (0.60 – 2.95)**	**1.31 (0.63 – 2.85)**
Skull width	**0.70 (0.02 – 0.86)**	-0.09 (-0.75 – 0.47)	—	0.13 (-0.58 – 1.43)	-0.26 (-0.74 – 1.20)
Forearm length	**0.92 (0.32 – 0.98)**	**0.79 (0.46 – 0.91)**	0.10 (-0.47 – 0.63)	—	**2.37 (1.56 – 4.25)**
Hind-foot length	**0.77 (0.25 – 0.89)**	**0.67 (0.43 – 0.85)**	-0.16 (-0.57 – 0.50)	**0.92 (0.73 – 0.98)**	—
Birth litter					
Mass	—	**24.02 (10.02 – 43.01)**	3.42 (-14.02 – 21.08)	-3.56 (-13.04 – 18.33)	**28.72 (13.18 – 46.03)**
Skull length	**0.44 (0.23 – 0.64)**	—	**1.49 (0.080 – 2.00)**	**0.50 (0.12 – 1.07)**	**0.41 (0.03 – 0.96)**
Skull width	0.01 (-0.15 – 0.25)	**0.67 (0.50 – 0.83)**	—	**1.04 (0.38 – 1.55)**	0.12 (-0.27 – 0.74)
Forearm length	0.04 (-0.35 – 0.33)	**0.57 (0.15 – 0.82)**	**0.72 (0.40 – 0.97)**	—	0.06 (-0.15 – 0.59)
Hind-foot length	**0.99 (0.42 – 1.00)**	**0.58 (0.20 – 0.89)**	0.24 (-0.23 – 0.62)	0.28 (-0.21 – 0.66)	—
Permanent environment					
Mass	—	14.00 (-1.95 – 30.01)	21.16 (-1.61 – 32.07)	14.11 (-9.08 – 28.57)	21.96 (-2.17 – 36.69)
Skull length	0.59 (-0.35 – 0.97)	—	$8.78*10^{-4}$ (-0.03 – 0.95)	$58.18*10^{-4}$ (-0.02 – 1.28)	$-1.96*10^{-4}$ (-0.03 – 1.23)
Skull width	**0.65 (0.33 – 0.99)**	0.94 (-0.28 – 1.00)	—	$-34.34^{*}10^{-4}$ (-0.04 – 1.17)	$8.86*10^{-4}$ (-0.03 – 1.17)
Forearm length	0.41 (-0.76 – 0.72)	**0.95 (0.08 – 0.99)**	0.89 (-0.61 – 0.98)	—	$1.68*10^{-4}$ (-0.07 – 1.46)
Hind-foot length	**0.60 (0.16 – 0.95)**	0.99 (-0.28 – 1.00)	0.99 (-0.49 – 1.00)	0.92 (-0.63 – 0.99)	—

Table 4.11 Correlations between the random terms of additive genetic (V_A), birth litter (V_{BL}), maternal (V_M) and birth group (V_{BG}). Data are for complete datasets which combine all life stages (n=total number of records), and values in italics and bold respectively indicate the lowest and highest correlations for each trait.

	Variance component		
	V_{BL}	V_M	V_{BG}
Mass (n=18365)			
V_A	0.34	**0.53**	0.36
V_{BL}	—	0.41	*0.22*
V_M	—	—	0.41
V_{BG}	—	—	
Skull length (n=2439)			
V_A	0.34	0.56	0.53
V_{BL}	—	0.41	*0.30*
V_M	—	—	**0.66**
V_{BG}	—	—	—
Skull width (n=2473)			
V_A	0.33	0.54	0.51
V_{BL}	—	0.41	*0.29*
V_M	—	—	**0.66**
V_{BG}	—	—	—
Forearm length (n=1926)			
V_A	0.35	0.57	0.55
V_{BL}	—	0.42	*0.33*
V_M	—	—	**0.70**
V_{BG}		—	—
Hind-foot length (n=2452)			
V_A	0.33	0.56	0.53
V_{BL}	—	0.41	*0.30*
V_M	—	—	**0.66**
V_{BG}	—	—	—

4.5 Discussion

4.5.1 Additive genetic effects

My analyses revealed that additive genetic effects contribute to variation in all five meerkat morphological traits considered here in at least one life stage. These findings, to the best of my knowledge, are the first estimates of heritable variation for morphological traits in a population of a cooperative mammal. Heritability estimates for mass ranged from 0.18 to 0.24, and from zero to 0.52 for skeletal traits. Despite the fact that the sample sizes for both the number of individuals and observations were considerably larger for mass than for any of the skeletal datasets, the overall mean heritability estimates and range in 95% HPDIs (i.e. difference between lower and upper intervals) for mass and the skeletal traits were very similar (mass=0.25 ± 0.268 HPDI; skeletal traits=0.23 ± 0.269 HPDI). This is perhaps surprising as mass has a large component of immediate condition (which may be environmentally determined) in it and may have thus been expected to exhibit lower heritabilities than the skeletal traits. Taken as a whole, the mean heritability of meerkat morphological traits was 0.24, which is less than half that calculated by Mousseau and Roff (1987) in their meta-analysis of heritabilty estimates in wild populations (0.461 ± 0.004 S.E). Although heritability estimates for any population are dependent on the fixed effects considered and are thus difficult to compare between studies (Wilson *et al.* 2010), in the absence of any previous quantitative genetic analyses on cooperative mammals it is unclear whether my heritability estimates are typical of cooperative species. My results do, however, provide some evidence for morphological trait heritability in meerkats, indicating that these traits are evolvable.

4.5.2 Birth litter effects

My analyses also revealed that birth litter identity contributes substantially to variation in all morphological traits at all life stages. The proportion of variance in mass due to birth litter varied from 0.44 to 0.80, and from 0.11 to 0.61 for the skeletal traits, with the mean proportion of variance attributed to birth litter across all skeletal traits being 0.45. As differences between birth litters potentially reflect differences between birth group, birth year, birth time of year and group composition

at birth, it is perhaps not surprising that I found very little evidence for maternal or birth group effects over and above birth litter effects. It is consistent with this suggestion that all of the random terms I included were positively correlated and thus potentially confounded with one another. The variance components of birth group and maternal identity were particularly strongly correlated which may be explained by the fact that dominant females monopolise the vast majority of reproduction in their group, sometimes over several years (Griffin *et al.* 2003).

Further evidence for the composite nature of the birth litter term is provided by my analyses which exclude birth litter as a source of variation: consistently across the different traits and life stages, some of the variance (average=10.44%; range=0.02-21.92%) previously attributed to birth litter was instead attributed to maternal effects for most traits and life stages. Although not as strong as those between birth group and maternal identity, I also detected positive correlations between additive genetic and birth litter effects, suggesting that these terms are also confounded with one another. Consistent with this, when the birth litter term was excluded, the variance explained by additive genetic effects increased for most traits, especially in the earlier life stages. These findings support the notion that there are substantial challenges to accurately disentangling genetic from shared environment effects when individuals live in family groups of related individuals (Moore *et al.* 1997, Wolf *et al.* 1998, Bijma *et al.* 2007, Kruuk & Hadfield 2007, Bijma & Wade 2008). The large amount of variance explained by birth litter may, however, also reflect the important influence of the social environment on trait variation in cooperative species since this term also contains information on the helper composition of the group at birth.

4.5.3 The social environment

The social environment is particularly important for meerkat pups, which are baby-sat, provisioned with milk and prey items, and taught to forage prior to independence (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999, Clutton-Brock *et al.* 2001b, Scantlebury *et al.* 2002, Thornton & McAuliffe 2006). Consequently, and in line with my predictions of change, the proportion of variance explained by the social

environment (manifested in the birth litter term) decreased with meerkat age. This may reflect the decreasing importance of the social environment in determining morphological trait values and the harsher environment imposed by the discontinuation of helper care. Conversely, I found a general (but imperfect) tendency for heritability estimates to increase with age. Other previous studies on wild population of bighorn sheep (*Ovis canadensis*) also reported increases in heritability with age, which were attributed to declining levels of maternal and residual variation (Reále *et al.* 1999, Wilson *et al.* 2005) with age. As residual variances remained relatively constant over meerkat life stages, and because the social environment is thought, to some degree, to replace the maternal contributions typical of non-cooperative species (Jennions & Macdonald 1994, Russell *et al.* 2002), the general tendency for heritability to increase with age in meerkats is probably best explained by the concordant decreases in contributions of social environment effects. Making predictions for changes in heritability estimates across age are, however, notoriously difficult as there are valid explanations for changes in either direction (Wilson *et al.* 2005), and this was thus not something that I explicitly investigated in this chapter. The main conclusion is therefore that variance components changed with age and that different conclusions may have been reached if I had just averaged across all ages.

4.5.4 Inbreeding effects

Meerkats appear to suffer from the negative effects of inbreeding in later life. A previous study on the same population reported inbreeding depression on pup mass at emergence and hind-foot length as well as survival (Chapter 3). Although my results herein did not find inbreeding depression in either mass or hind-foot length for the emergence/pup life stages, it is important to note that both the data analysed and the fixed and random terms considered differed between studies. For example, whereas in Chapter 3 of this thesis I used single morphomometric measurements and restricted the data to only include measures that were obtained prior to a pup commencing foraging, this study used repeated mass measurements from pups aged 16 to 26 days and all hind-foot measures from birth until independence. Furthermore, in Chapter 3 I included a number of maternal and group-related fixed

effects, whereas I excluded these fixed effects in order to retain as many data points as possible and to more accurately apportion variance to group-related components. Putting these differences aside but considering the pup inbreeding depression previously reported (Chapter 3), coupled with my results herein, the magnitude of the inbreeding effects does appear to increase with age.

One of the few studies that has also investigated the effects of inbreeding across life stages found that inbreeding depression accumulated with age and ultimately reduced long-term fitness in takahes (*Porphyrio horchstetteri*) in New Zealand (Grueber *et al.* 2010). Grueber *et al.* (2010) also reported the positive effect of *F* on offspring recruitment and concluded that detection of inbreeding depression at single life-history stages is generally imprecise due to the high standard errors around the effect sizes. I also found positive effects of *F* on pup skull width, which may either represent an imprecise estimate, or alternatively, provide further support for my prediction that the effects of inbreeding increase with age and the broad transition from being a recipient to a provider of cooperative care (also see Chapter 3). As for Chapter 3, these results may provide some evidence that the stress imposed by a change in social environment (i.e. the discontinuation of helper provisioning) is analogous to the ecologically stressful environments reported in other animal populations. Furthermore, helpers may in fact direct more care to inbred pups in order to compensate or 'buffer' them from the effects of inbreeding prior to independence. Where significant, the effect size of inbreeding depression was small across all meerkat traits and life stages and in the absence of life-history analyses it is difficult to determine whether these effects translate into reduced fitness. As for the variance component analyses, continuing collection of data from the Kalahari Meerkat Project may eventually yield adequate samples sizes to make analyses of inbreeding depression in adult life-history traits possible.

4.5.5 Future work

In this study I have presented an initial quantitative genetic analysis of morphological traits in a wild population of cooperative mammals in a first attempt to decompose the variation of these traits into genetic and social environmental

components. One of the general themes to have emerged from this research is the difficulty in disentangling genetic from shared (social) environment effects within a traditional quantitative genetic framework. It is also possible that indirect genetic effects (IGE) may play a role in the phenotypic expression of meerkat traits (Moore *et al.* 1997, Wolf *et al.* 1998, Bijma *et al.* 2007, Bijma & Wade 2008). Contrary to traditional quantitative genetics, where trait expression is only determined by an individual's own gene, IGEs occur when an individual's phenotype is dependent on the genes expressed in its conspecifics (Moore *et al.* 1997, Wolf *et al.* 1998, Bijma *et al.* 2007, Bijma & Wade 2008). This has particular relevance to cooperative species where individuals live in groups consisting of closely related family members. Analyses that specifically consider IGEs are complex by nature and may require larger samples sizes than I currently have available. Recommended future work could focus on disentangling genetic from shared (social) environment effects and/or determining the role of IGEs in shaping the phenotypic expression of traits in meerkats, and other cooperatively breeding species.

Chapter 5

Genetic and non-genetic sources of variation in cooperative care in a wild population of meerkats (*Suricata suricatta*)



5.1 Abstract

Cooperation has always posed a 'special difficulty' to the theory of evolution via natural selection (Darwin 1859) and it continues to be the focus of much theoretical and empirical research. Some of this research has attempted to explain the consistent individual variation in contributions to cooperation that remain after all other possible state-dependent, ecological and/or social factors have been accounted for. Very few studies, however, have attempted to decompose the genetic and environmental sources of variation in cooperation in wild mammalian populations, or investigated whether being a cooperator has a genetic basis. Furthermore, we know very little about how inbreeding affects cooperative care. Here I use a Bayesian animal model approach, a multi-generation genetically-validated pedigree and behavioural records from a wild population of meerkats (*Suricata suricatta*) to show low heritable variation for two types of cooperative care: baby-sitting ($h^2 = 0.10$) and pup-feeding ($h^2 = 0.08$). Additionally, I provide evidence for positive genetic correlations between baby-sitting and pup-feeding ($r = 0.64$), and baby-sitting and adult mass ($r = 0.09$) and reveal the importance of other non-genetic sources of variation in these traits, such as recipient ($rl^2 = 0.06 – 0.23$) and birth litter effects ($bl^2 = 0.09 – 0.47$). Finally, I also find evidence for kin-directed provisioning in pup-feeding, show that inbred meerkats contribute more pup-feeding, but that contributions to baby-sitting are negatively associated with relatedness between helpers and recipient litters. My results provide some of the first evidence for heritable variation, and inbreeding and relatedness effects on individual variation in contributions to cooperative care in a wild mammalian species.

5.2 Introduction

Individual variation in behavioural phenotypes that is consistent over both time and across contexts has been documented for a wide range of taxa in laboratory and wild settings (Stirling *et al.* 2002, Réale *et al.* 2010, Taylor *et al.* 2012). Some consistency in behaviour can be explained by state-dependent models (Dingemanse & Wolf 2010, Wolf & Weissing 2010). 'Individual variation', however, tends to refer to the residual or unexplained differences among individuals after all other known or measured, morphological, physiological, neurobiological, ecological and/or social factors have been accounted for (Hayes & Jenkins 1997, Wilson 1998, Dingemanse & Wolf 2010). One current goal of evolutionary ecology is therefore to explain the existence of this residual variation and to determine the extent to which individual behaviour is governed by underlying genetic and/or non-genetic factors (Stirling *et al.* 2002, Réale *et al.* 2010, van Oers & Mueller 2010).

Cooperation generally describes a social behaviour where the action of the cooperator increases the fitness of the receiver, often at the expense of the cooperator's own fitness, and is one particularly interesting type of behaviour that is known to vary consistently between individuals (see examples in Bergmüller *et al.* 2010). Although historically, individual variation in cooperativeness was thought to be mainly explained by relatedness and/or the direct and indirect costs and benefits of kin selection, which includes condition-dependent responses (Hamilton 1964a, Koenig & Dickinson 2004, Dingemanse *et al.* 2010), evidence that variation in cooperative behaviour may also have a heritable basis is accumulating (Bergmüller *et al.* 2010). For example, it is likely, firstly, that cooperation is correlated with heritable personality traits (Bergmüller *et al.* 2010), and, secondly, that some cooperative behaviour is hormonally regulated (Kosfeld *et al.* 2005, Zak *et al.* 2005, Carlson *et al.* 2006a, Carlson *et al.* 2006b, Soares *et al.* 2010) and that genes linked to the expression of hormones ultimately influence cooperativeness (Knafo *et al.* 2008). Although some of these studies have investigated the hormonal mechanisms of care giving, very few have examined the genetic basis underlying this type of behaviour (but see examples in Freeman-Gallant & Rothstein 1999, MacColl &

Hatchwell 2003, Charmantier *et al.* 2007, Cesarini *et al.* 2008).

Two specific studies have revealed that individual variation in cooperation is, in part, explained, by genetic effects. Cesarini *et al.* (2008) used two independent datasets of human (*Homo sapiens*) mono- and di-zygotic twins to show evidence for the heritability of interpersonal trust (average $h^2 = 0.15 \pm 0.03$ - 0.38 C.I.) and trustworthiness (average $h^2 = 0.18 \pm 0.05$ - 0.32 C.I.). And in a quantitative genetic analysis of cooperation in a wild avian population, Charmantier *et al*. (2007) used an eight-generation social pedigree and an 'animal model' approach to show significant heritable variation in the propensity of facultatively cooperative western bluebirds (*Sialia mexicana*) to help ($h^2 = 0.76 \pm 0.25$ S.E.) and receive help ($h^2 = 0.32 \pm 0.13$ S.E.). In terms of parental care-giving behaviour, research has also revealed significant non-zero heritability estimates for the rate at which parents feed offspring in a long-tailed tits (*Aegithalos caudatus*) (MacColl & Hatchwell 2003) and Savannah sparrows (*Passerculus sandwichensis*) (Freeman-Gallant & Rothstein 1999). Although this collection of studies have greatly contributed to the field by demonstrating the environmental, social and genetic source of variation in cooperation and parental care-giving, to the best of my knowledge, research on the heritability of cooperation in wild mammals is currently lacking. In this study, I therefore present an analysis of the heritability of two types of cooperative behaviour in a wild population of meerkats in an explicit attempt to decompose the variation of these traits into environmental and genetic components.

Meerkats (*Suricata suricatta,* Schreber 1776) are small, diurnal, cooperatively breeding mongooses that inhabit the semi-arid regions of the Kalahari in southern Africa (Doolan & Macdonald 1999). They typically live in groups of 2 – 50 which consist of a dominant pair and a number of male and female subordinate helpers that are commonly either offspring from the dominant pair's previous breeding attempts, or immigrant males (Clutton-Brock *et al.* 1999b, Doolan & Macdonald 1999). Dominants monopolize 80 – 90% of the group's reproductive output (Griffin *et al.* 2003, Spong *et al.* 2008) and contribute comparatively little to the post-natal raising of their pups (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999). Most of the

care is instead provided by sub-adult and older subordinates (≥6 months) who baby-sit and suckle (allolactate) pups at the natal burrow from their birth until they begin to forage with the group (at approximately four weeks of age) (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999, Scantlebury *et al.* 2002), and who feed and teach pups to forage until they reach independence (at approximately three months of age) (Clutton-Brock *et al.* 2001b, Thornton & McAuliffe 2006). Helpers can also contribute to the group in general by participating in raised guarding during foraging periods, and social digging which acts to maintain the group's burrows and bolt-holes (Clutton-Brock *et al.* 2002).

Both baby-sitting and pup-feeding are accompanied by appreciable energetic costs to the helper: baby-sitters forgo feeding for up to 24 hours which leads to significant weight loss (Clutton-Brock *et al.* 1998b, Clutton-Brock *et al.* 2000) and pup-feeders sacrifice food items by provisioning pups (Brotherton *et al.* 2001). Previous research on the same population of meerkats that I consider herein has found substantial individual- and group-level factors that are likely to affect the costs of helping, such as sex, age, body condition, hormonal factors and group size (Clutton-Brock *et al.* 2000, Brotherton *et al.* 2001, Clutton-Brock *et al.* 2001a, Clutton-Brock *et al.* 2001b, Clutton-Brock *et al.* 2002, Carlson *et al.* 2006a). For instance, females are more likely to baby-sit, and feed pups more frequently than males, and contributions to these cooperative behaviours generally increases with age (Clutton-Brock *et al.* 2000, Brotherton *et al.* 2001, Clutton-Brock *et al.* 2001a, Clutton-Brock *et al.* 2002). Heavier individuals and more successful foragers also contribute comparatively more to baby-sitting, and helpers baby-sit and pup-feed less when more helpers are available (i.e. in larger groups) (Clutton-Brock *et al.* 2001b, Clutton-Brock *et al.* 2002). Variation in meerkat cooperative care has not, however, been found to be associated with the relatedness of helpers to the young they are raising (Clutton-Brock *et al.* 2000, Clutton-Brock *et al.* 2001a), although this finding was made at a time when there were insufficient generations available to construct a pedigree for the study population. In the years since, continued data collection from the same study population has enabled a genetic pedigree to be constructed and hence a

pedigree-derived relatedness coefficient-based investigation of whether meerkats exhibit kin-directed provisioning in baby-sitting and pup-feeding to be undertaken.

Even once all the known influencing factors are accounted for, a substantial amount of consistent between-individual variation in contributions to both baby-sitting (r^2 = 0.22) and pup-feeding (r^2 = 0.51) (English *et al.* 2010) remains. My aim here is to therefore quantify the contribution of genetic factors to this variation. I attempt to determine whether consistent variation in meerkat contributions to baby-sitting and pup-feeding is due to additive genetic variance and/or what components of the environment explain the high repeatability of these behaviours.

Although demonstrating a genetic basis for behaviour is the first step in understanding its evolution and for, theoretically, predicting a population's response to selection (Falconer & Mackay 1996, Lynch & Walsh 1998), even the absence of significant additive genetic variation for a trait can be interesting. In cooperative species such as meerkats, the social environment can be an important influencing factor on morphometric and behavioural traits (Russell *et al.* 2002, English 2009), and consequently one possibility is that additive genetic variance may play a much smaller role in shaping meerkat cooperation than components of the social environment, such as litter and/or group effects.

Early body mass is a predictor of an individual meerkat's chance of attaining dominance (Russell *et al.* 2007b) and also influences an individual's propensity to contribute to baby-sitting and pup-feeding (Clutton-Brock *et al.* 2000, Clutton-Brock *et al.* 2003). One advantage of using a quantitative genetic approach to investigating the genetic and non-genetic sources of variation in cooperative traits is the ability to determine the extent to which phenotypic correlations are reflected at the genetic level. In this way I can address whether individuals might be more or less predisposed to contribute cooperative care for reasons of early development, social environment or genetics and whether this in turn canalises them into different life-history trajectories for the remainder of their lives.

Genetic influences on individual behaviour can also be manifested as inbreeding depression, which may also be quantified if pedigree information is available (Pemberton 2008). The negative effects of inbreeding on multiple aspects of phenotype have now been demonstrated in a wide variety of laboratory, captive and wild populations (Crnokrak & Roff 1999, Keller & Waller 2002). However, to the best of my knowledge no study has specifically investigated the effect of inbreeding on cooperative behaviour. Furthermore, there is growing awareness that failure to include inbreeding coefficients (*F*) can have implications for quantitative genetic analyses by biasing estimates of variance components (de Boer & Arendonk 1992, Norberg & Sørensen 2007, Reid & Keller 2010). Relatively little research has been reported on the effects of inbreeding on animal behaviour and what currently exists has tended to report negative effects of *F* on traits such as mating performance (Ala-Honkola *et al.* 2009, Kuriwada *et al.* 2011) and aggression (Eklund 1996, Gallardo & Neira 2005).

Predictions for the effect of inbreeding on contributions to cooperation are complex. On one hand, the classic prediction is that because inbreeding has the effect of increasing the expression of recessive alleles (Charlesworth & Charlesworth 1999), the negative effects of inbreeding on individual performance or condition may act in such a way as to decrease contributions to cooperative care. On the other hand, in social species, such as meerkats, where greater levels of cooperative care are contributed by subordinates than dominants (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999) and where becoming dominant, or indeed roving as a subordinate, are highly costly (Clutton-Brock *et al.* 2006), (less fit) inbred individuals may be more likely to remain as lifelong subordinates. In doing so they may provide *more* cooperative care than non-inbred individuals. The meerkat study population has substantial rates of inbreeding and shows inbreeding depression on a variety of morphological and survival traits (Chapters 3 & 4). As part of this study, I investigate whether the effects of inbreeding extend to cooperative behaviour.

In this chapter I investigate the genetic and non-genetic sources of variation in two kinds of meerkat helping behaviour, babysitting and pup-feeding. I consider

associations with the inbreeding coefficient of both the helper and the recipients, and with the relatedness between them, and estimate the additive genetic variance in an animal model framework, which also includes other potential explanatory variables and sources of variance. Since previous studies have found a phenotypic correlation between helping behaviour and body mass (Clutton-Brock *et al.* 2001b, Clutton-Brock *et al.* 2002), I also conduct a parallel analysis of adult body mass and test whether these phenotypic correlations have a genetic basis. This is in an attempt to determine whether individuals are canalised into different life-history trajectories based on their mass.

5.3 Material and methods

5.3.1 Study population

All data in this study come from the Kalahari Meerkat Project, which is located at the Kuruman River Reserve, South Africa (26º5'S, 21º49'E), and spans the period of time from the project's inception in 1993 to March 2009. Professor T. H. Clutton-Brock (University of Cambridge) initially established the study site and it continues to be maintained by T. H. Clutton-Brock and Professor M. B. Manser (University of Zurich). Resident meerkats are habituated to close observation (<1 m), given individually recognisable fur dye marks (Thornton 2008), and each group is visited up to two times a day (on average three times a week) in order for individual key life-history events, changes in group composition, behavioural observations and mass measurements to be recorded (Sharp & Clutton-Brock 2010). The majority of meerkats in the study population have been trained to stand on an electronic top-pan balance (±1 g) and are weighed up to three times daily (Clutton-Brock *et al.* 2000). A 2-5 mm tissue sample from the tail tip of each pup is taken at emergence for genetic analysis (Griffin *et al.* 2001, Spong *et al.* 2008). Other individuals are either sampled post-mortem or under anaesthetic (i.e. for recently habituated or immigrant adults) so that approximately 86% of the total recorded population has now been sampled for tissue and genotyped at up to 18 microsatellite markers (Chapter 2). Further details of the study area and system are provided elsewhere (Clutton-Brock *et al.* 1999a, Russell *et al.* 2002).

5.3.2 Pedigree, inbreeding coefficients and relatedness

The pedigree for this study population was reconstructed using a combination of microsatellite data and phenotypic descriptors and two parentage inference programs: COLONY2 (Wang 2004, Wang & Santure 2009) and MASTERBAYES (Hadfield *et al.* 2006). Full details of the molecular and pedigree construction methods used can be found in Chapter 2. In brief, the full pedigree contains 2,147 individuals, spans up to eight generations, and contains 1,531 individuals with both parents known and a further 411 individuals with only maternity known (Table 5.1). There are a total of 175 different mothers and 180 different fathers in the pedigree,

with an average of 11 ± 16.26 S.D. and 9 ± 10.41 S.D. offspring respectively, although the strong reproductive skew in favour of dominant meerkats means the maximum total sib-ship sizes in the population are large (maternal = 83; paternal = 54). Full pedigree statistics, as retrieved by PEDANTICS (Morrissey & Wilson 2010), including the number of individuals that are informative with respect to the specified phenotypic traits can be found in Table 5.1. Any parentage assignment errors in the pedigree are predicted to be minimal as all paternities were genetically-validated and there was a high level of confidence (average individual parentage assignment confidence = 95.57%) and correspondence of parents inferred between the two programs (Chapter 2). Any parentage mis-assignments are furthermore expected to act in a conservative manner as they reduce estimates of the covariance between relatives and hence of heritability (Charmantier & Réale 2005).

Table 5.1 Summary statistics for the entire Kalahari Meerkat Pedigree ('full' pedigree) utilised herein and as produced by the PEDSTATSUMMARY function in the R-package PEDANTICS. The 'pruned' pedigrees for baby-sitting, pup-feeding, and adult mass consist of only those individuals that are informative with respect to the specified phenotypic traits (i.e. the individual has been measured for the trait or links individuals which have been measured for the trait).

Statistic	No. of links in full pedigree	No. of links in pruned pedigrees		
		Baby-sitting	Pup-feeding	Adult mass
Records	2147	1830	1809	1060
Maternities*	1942	1757	1737	1008
Paternities*	1531	1420	1403	797
Full sibs	11165	10932	10866	4547
Maternal sibs	32820	32208	32100	1089
Maternal half sibs	21655	21276	21234	6542
Paternal sibs	15441	15088	14744	6084
Paternal half sibs	4276	4156	3878	1537
Maternal grandmothers	1701	1619	1620	974
Maternal grandfathers	1624	1474	1479	923
Paternal grandmothers	1009	969	962	563
Paternal grandfathers	861	821	823	430
Maximum pedigree depth (generations)	8	7	7	7
Founders	205	73	72	52
Mean maternal sibship size	10.10	12.92	13.16	8.26
Mean paternal sibship size	8.51	9.04	8.94	6.59

*Maternities and paternities include 'dummy' parents as assigned by COLONY2 (see Chapter 2 for further details).

The resultant pedigree was visualised and Wright's inbreeding coefficients (*F*) calculated in PEDIGREEVIEWER (http://www-personal.une.edu.au/~bkinghor/pedigree.htm) (Kinghorn 1994). *F*-values for founders and immigrants with unknown parents could not be accurately determined and were thus excluded from my analyses involving *F*. Furthermore, *F*-values were only calculated for individuals when at least two grandparents had been identified (Chapter 3). These restrictions meant that 19% of the total population were not assigned a *F*-value. Since the pedigree is incomplete and limited in depth, there will be error in the *F*-values and they are likely to be downward-biased.

Pair-wise relatedness coefficients (*R*) between all individuals in the pedigree were obtained using the R software package PEDANTICS (http://wildevolution.biology.ed.ac.uk/awilson/pedantix.html) (Morrissey & Wilson 2010). Due to inbreeding in the population (Chapter 3), relatedness coefficients can exceed 0.5.

5.3.3 Study traits

5.3.3.1 Response variables

Baby-sitting

From birth until when pups commenced foraging (at approximately four weeks of age), groups were visited every morning and evening in order for the identity of the baby-sitter(s) to be recorded. Twice-daily records were necessary as baby-sitters sometimes changed in the afternoon, especially if the group returned to the burrow after a morning of foraging (English 2009). Baby-sitting contributions were calculated on a per-litter basis, where 'litter' refers to maternal litters of the same age and natal group. Occasionally, multiple females in a group gave birth at a similar time and pups born to these combined 'mixed maternity' litters receive baby-sitting care simultaneously and were thus deemed a single litter for the purposes of this study (English 2009). I estimated the amount of time individual helpers spent baby-sitting as a binomial trait composed of the number of half-days a specific helper remained at the breeding burrow (*x*; average = 4.90 ± 5.07 S.D.) and the number it did not (*t*-*x*; average = 38.83 ± 11.15 S.D.), where *t* was the total possible half days

the helper could have baby-sat a particular litter (i.e. when any baby-sitter was present from birth to foraging of each particular litter; average = 43.73 ± 11.15 S.D.) (English 2009). A helper could baby-sit more than one litter over the course of its life and thus the dataset contains repeated measures of baby-sitting, one for each litter that each helper could potentially have baby-sat (i.e. those litters that were born into the group in which the helper was currently resident in). In total, the full baby-sitting dataset contained 4,242 unique baby-sitting records involving 938 different baby-sitters (female = 443; male = 495) and 566 different recipient litters (Table 5.2).

Table 5.2 Means and samples sizes for the baby-sitting, pup-feeding, and adult mass datasets. Full data is described here although reduced datasets were subsequently analysed due to missing fixed effect data for some records.

Model	No. records	No. dominants[*] / no. individuals	No. of recipient litters	Mean ± S.D. (and range) no. of records per individual[†]	Mean ± S.D. (and range) of measures[‡]
Baby-sitting	4242	152/938	566	4.52 ± 3.69 (1 – 26)	0.11 ± 0.11 (0 – 0.78)
Female	1964	60/443	283	4.43 ± 3.40 (1 – 26)	0.12 ± 0.12 (0 – 0.78)
Male	2278	92/495	283	4.60 ± 3.93 (1 – 24)	0.11 ± 0.11 (0 – 0.63)
Pup-feeding	3187	142/887	420	3.59 ± 2.77 (1 – 20)	6.54 ± 9.11 (0 – 105.22)
Female	1474	57/425	210	3.47 ± 2.66 (1 – 20)	7.36 ± 9.88 (0 – 105.22)
Male	1713	85/462	210	3.71 ± 2.87 (1 – 18)	5.84 ± 8.34 (0 – 85.75)
Adult mass (g)	2803	89/619	—	4.53 ± 2.11 (1 – 11)	648.07 ± 77.78 (384 – 961)
Female	1308	36/297	—	4.40 ± 2.09 (1 – 11)	641.29 ± 75.11 (428 – 907)
Male	1495	53/322	—	4.64 ± 2.11 (1 – 11)	654.13 ± 79.63 (384 – 961)

[*]No. of dominants: the number of individuals that eventually became dominant at any point in its life.
[†]Mean no. of records per individual: baby-sitting and pup-feeding = mean number of litters baby-sat or pup-fed by an individual; adult mass = mean number of morning mass records.
[‡]Mean of measures: baby-sitting = number of half days a baby-sitter remained at the breeding burrow divided by the total possible half days it could have baby-sat; pup-feeding = number of pup-feeds directed to any member of the same litter by a specific helper divided by the logarithm of the total number of minutes that the helper could have been observed pup-feeding; adult mass = mean mass in the 543 - 553 day window.

Pup-feeding

Once pups started foraging with their group (at approximately 4 weeks of age), data for pup-feeding rates were recorded *ad libitum* whenever field-based observers accompanied a meerkat group on foraging trips (English 2009). During each field observation period, the total number of pup-feeding events and the identities of the helper and recipient pup involved in each pup-feed event were recorded (English 2009). Additionally, the total field observation period (mins) during which a pup-feeding event could have been detected was noted (English 2009). Relative pup-feeding rates were recalculated to a per litter value and, because a helper could pup-feed more than one litter over the course of its life, the dataset contains repeated measures (English 2009). The total number of pup-feeds a helper directed to any particular litter (x; average = 11.25 ± 16.41 S.D.) was scaled by the logarithm of the total observation period (t) in which the helper could have been observed to pup-feed (i.e. when the helper was foraging with the group containing the relevant litter; average t = 43.08 ± 21.66 S.D.) in order to take into account the positive relationship between total feeds and total observation period. In total, the full pup-feeding dataset contained 3,187 unique pup-feeding records involving 887 different pup-feeders (female = 425; male = 462) and 420 different recipient litters (Table 5.2).

Adult mass

Final or asymptotic body mass is complicated to measure in meerkats, as some individuals have a clear final body mass whereas others continue to grow, albeit slowly (S. English, *pers. comm.*). Consequently I used mass at 18 months (548 days) as a proxy for final adult mass in meerkats. In order to limit the effect of differences in daily foraging success I only used morning mass measures, but in order to make full use of the extensive mass data I considered all measures of morning mass from age 543 to 553 days. The adult mass dataset therefore contains repeated measures (average number of mass records used to calculate adult mass = 4.53 ± 2.11 S.D.) (Table 5.2). As not all individuals survived to reach, or were weighed at, 548 days of age (Sharp & Clutton-Brock 2010), my adult mass dataset was smaller than that for either baby-sitting and pup-feeding (number of records = 2,803; number of unique individuals = 619) (Table 5.2). The adult mass dataset is analogous to that of

Chapter 4 but is also included in this set of analyses in order to test for genetic and non-genetic correlations between cooperative care and mass.

5.3.3.2 Explanatory variables

Fixed effect terms

Previous studies on the same population of meerkats (e.g. Clutton-Brock *et al.* 2000, Brotherton *et al.* 2001, Clutton-Brock *et al.* 2001a, Clutton-Brock *et al.* 2001b, Clutton-Brock *et al.* 2002, English 2009) have revealed a number of variables that influence an individual's baby-sitting and pup-feeding contributions (see section 5.2), and mass at emergence and independence (Chapter 3 of this thesis, Russell *et al.* 2002, Hodge *et al.* 2008). I therefore included the following as fixed effects in my initial preliminary models (Tables 5.2 & 5.3):

- *Sex* (male/female). All individuals in the three datasets could be sexed based on morphology as they were all juveniles or older.
- *Age* of the helper (days) at the time of the recording of the cooperative event in the behavioural models and of the adult at weighing in the adult mass model. Age in the behavioural models was taken as the average age of the helper over the litter-specific period it could have baby-sat or pup-fed and was modelled as a linear effect in all three models.
- *Current (helper/adult) status* (dominant/subordinate) at the time of the measurement record. Dominants are generally larger than subordinates (Clutton-Brock *et al.* 2006, Hodge *et al.* 2008) and although subordinates contribute more care to pups they do not do so exclusively. Dominants have also been shown to baby-sit and/or pup-feed, albeit at reduced rates compared to subordinates (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999). In my datasets, approximately 15% of both the baby-sitting periods and pup-feeds were conducted by a dominant.
- *Ultimate (helper/adult) status* (dominant/subordinate). Indicating whether the individual eventually became dominant at any point in its life.
- *Current group size.* Calculated as the average number of helper-age individuals (≥6 months) in the group during the window of time in which the

cooperative behaviour was recorded or during the 11-day window in which mass measures were taken.

- *Litter size*. Although the size of the recipient litter has previously been shown to have an effect on pup-feeding but not on baby-sitting (Clutton-Brock *et al.* 2000, Clutton-Brock *et al.* 2001a), I included it as a fixed effect in both behavioural trait models. Because this variable was specific to recipients of helping behaviour, it was not included in the adult mass analyses.
- *Relatedness (R)*. Although previous studies on the same population found that individual contributions to cooperative care are not correlated with degree of kinship (Clutton-Brock *et al.* 2001a), I further tested this finding with this enlarged dataset and improved pedigree by including the pedigree relatedness of the helper to the recipient litter. In cases of litters with mixed maternity or paternity, pedigree relatedness was taken as the average across all pups in the litter. Because this variable was specific to recipients of helping behaviour, it was not included in the adult mass analyses.
- *Individual inbreeding coefficient (F)*. In order to test for any associations between inbreeding and cooperative behaviour, the pedigree-derived *F*-value of each helper was included. In the adult mass models this variable represented the *F* of the weighed adult.
- *Recipient litter inbreeding coefficient (F).* There is some evidence to suggest that the social (helper) environment of meerkats may ameliorate the negative effects of inbreeding depression in early life (Chapters 3 & 4). I therefore tested whether cooperative behaviour varied in relation to the inbreeding coefficient of pups by including the average *F* of all pups in a litter in models of cooperative care. Because this variable was specific to recipients of helping behaviour, it was not included in the adult mass analyses.
- *Season.* A two-level factor (cold-dry, May-September; hot-wet, October-April) indicating the season in which the measurement record was taken.
- *Rainfall.* Total accumulated rainfall (mm) in the nine months prior to measurement record was extracted for the study site (26°58'S, 21°49'E) from the National Aeronautics and Space Administration online Rainfall Analysis

Tool (http://disc2.nascom.nasa.gov/Giovanni/tovas/rain.GPCP.shtml). (Bateman *et al.* 2011, English *et al.* 2012).

- *Observation time.* As pup-feeding counts followed a Poisson distribution, I accounted for the fact that longer observation periods were likely to result in more observed pup-feeds by including the log of the total observation period (mins) as an additional fixed effect.

Note that the aim here is to investigate the associations between baby-sitting, pup-feeding and adult mass by estimating the covariances between them in a multivariate model, rather than by including mass as a fixed effect in the models of helping behaviour as has been done in previous analyses (Clutton-Brock *et al.* 2000, Clutton-Brock *et al.* 2003).

Table 5.3 Summary statistics for the fixed effect variables included in the baby-sitting, pup-feeding, and adult mass analyses. Full data (*n*) is described here although reduced datasets were subsequently analysed due to missing fixed effect data for some records.

	Baby-sitting (*n* = 4242)	Pup-feeding (*n* = 3187)	Adult mass (*n* = 2803)
Fixed effect	Mean ± S.D. (and range)	Mean ± S.D. (and range)	Mean ± S.D. (and range)
Age (days)	660.62 ± 552.47 (99 – 4405)	671.79 ± 583.69 (94.33 – 4171)	548.02 ± 3.14 (543 - 553)
Current group size	19.45 ± 7.97 (4 – 38)	18.63 ± 7.66 (4 – 37)	18.89 ± 7.94 (3 - 58)
Litter size	3.96 ± 1.48 (1 – 9)	3.78 ± 1.69 (1 - 9)	— —
Relatedness (*R*)	0.32 ± 0.16 (0 – 0.69)	0.34 ± 0.16 (0 – 0.61)	— —
Individual *F*	0.02 ± 0.04 (0 – 0.19)	0.03 ± 0.04 (0 – 0.19)	0.03 ± 0.05 (0 – 0.22)
Recipient litter *F*	0.03 ± 0.04 (0 – 0.20)	0.03 ± 0.04 (0 – 0.20)	— —
Rainfall (mm)	219.52 ± 96.45 (66.20 – 472.99)	214.62 ± 89.27 (66.20 – 470.45)	240.71 ± 104.00 (56.90 – 472.99)
Observation time (log(mins))	— —	1.58 ± 0.22 (1.06 – 2.02)	— —

Random effect terms

I fitted a number of random effects in the models in order to investigate the genetic and non-genetic sources of variation in the study traits, account for repeated measures, control for the non-independence of data, and to minimise erroneous inflation of additive genetic variance. As both baby-sitting and pup-feeding

measures were recorded on a per-litter basis, *recipient litter* (*RL*) identified the litter of investment that the cooperative behaviour was directed toward, and was fitted in the behavioural models. The following random effect terms were included in models of all three traits. The litter into which an individual was born has been shown to explain a large proportion of variance in morphological traits in meerkats (Chapter 4) and thus the *birth litter* (*BL*) of the helper (for baby-sitting and pup-feeding) or adult (for adult mass) was also included to test for the relative contributions of this term to behavioural traits. Both the *RL* and *BL* terms implicitly encapsulate any temporal variation between the years in which the measures were recorded (*RL*), and when helpers/adults were born (*BL*) and, as such, year of measurement and helper/adult birth year were not modelled explicitly. I did, however, include *maternal identity* (*M*) and *birth group* (*BG*) of the helper/adult to explicitly test for long-term maternal and natal group effects respectively. A *permanent environment* (*PE*) effect can arise as a result of differences between individuals other than those accounted for by the other random terms and can be estimated from repeated measures of a trait on the same individual (Kruuk & Hadfield 2007). I thus included *PE* in all models as all datasets included repeated measures.

I also considered the possibility that the *current group* (*CG*) of which an individual was member when it was measured might account for trait variation. However, few individuals in the datasets actually moved into different groups. Furthermore, preliminary analyses on data that included only the individuals that did move into different groups showed very little difference between models excluding ($V_{PE} + V_A$ ($+ V_{RL}$) $+ V_{BL} + V_M + V_{BG}$) versus including current measurement group ($V_{PE} + V_A$ ($+ V_{RL}$) $+ V_{BL} + V_M + V_{BG} + V_{CG}$) (mean ΔDIC between the models = 0.30). Consequently I did not include a *CG* random term in any of my models as it would be largely confounded with *BG*.

5.3.4 Statistical analyses

The most powerful and accurate way to estimate heritabilities in the wild is to use an 'animal model' (Kruuk 2004, Wilson *et al.* 2010). Traditionally, maximum likelihood (or frequentist) animal models have been used, but this approach is limited

by the fact that it typically does not model non-Gaussian response variables properly (Hadfield 2009), and hence is inappropriate for the many behavioural traits which typically show non-Gaussian distributions. A more appropriate Bayesian animal model approach has now become the analysis of choice when dealing with non-normally distributed traits in wild populations (McFarlane *et al.* 2010, Charmantier *et al.* 2011, Lane *et al.* 2011, Taylor *et al.* 2012), and thus all my analyses were conducted using the Markov Chain Monte Carlo for Generalized Linear Mixed Models (MCMCGLMM) (Hadfield 2009) package in R (R Development Core Team).

Fixed effects

Initially, I evaluated the influence of all the fixed effects in models where only *A* and *PE* random terms were fitted (Tables 5.2 & 5.3) (Wilson *et al.* 2010). Fixed effect terms were deemed significant when the posterior distribution of the 95% Highest Probability Density Intervals (HPDI), the Bayesian equivalent of confidence intervals, did not include zero (Wilson *et al.* 2010). Only the fixed terms that were significant were retained in subsequent analyses.

Random effects

I attempted to investigate the variance components of each trait using two different types of models: 1.) *full models* (FM), which included all random effect terms, and 2.) *reduced models* (FM_{-BL}), which included all random terms except for *BL*. This comparative approach was chosen in order to 1.) investigate the relative effect of each random term, and 2.) investigate the degree to which the *BL* term was confounded with the *A* term (or other variance components).

Model selection

As variances are bounded above zero (and hence all 95% HPDIs will exclude zero), I established the importance of each random term by comparing changes in Deviance Information Criterion (DIC) values between models where different random effect terms were excluded (Wilson *et al.* 2010). Random terms were only retained if they improved the model fit. Within the Bayesian framework, DIC is analogous to the Akaike Information Criterion (AIC), where smaller values indicate a more

parsimonious model fit (Taylor *et al.* 2012), although randomization tests have shown that a ΔDIC of five is the most suitable threshold value to use in the model selection process (S. Drobniak, pers. comm.). DIC values are, however, not especially well suited to model selection of non-Gaussian traits (i.e. baby-sitting and pup-feeding), and consequently I simultaneously visually checked that the posterior modes of the random terms were not in the lowest 25% quartile of the range. A posterior mode in the lowest 25% quartile of the range indicates low levels of variance explained by the random term and thus, visual inspection of the posterior modes is the most suitable substitute where DIC analysis is inappropriate.

Variance component analysis

I estimated the variance components (conditional on the significant fixed effects) of contributions to baby-sitting and pup-feeding, and adult mass from univariate models where the FMs took the form:

Baby-sitting and pup-feeding: $\boldsymbol{y}^{1} = \boldsymbol{Xb} + \boldsymbol{Z_1}\mathbf{pe} + \boldsymbol{Z_2}\mathbf{a} + \boldsymbol{Z_3}\mathbf{rl} + \boldsymbol{Z_4}\mathbf{bl} + \boldsymbol{Z_5}\mathbf{m} + \boldsymbol{Z_6}\mathbf{bg} + \boldsymbol{e}$

Adult mass: $\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Z_1}\mathbf{pe} + \boldsymbol{Z_2}\mathbf{a} + \boldsymbol{Z_3}\mathbf{bl} + \boldsymbol{Z_4}\mathbf{m} + \boldsymbol{Z_5}\mathbf{bg} + \boldsymbol{e}$

where:

- $\boldsymbol{y}$ = vector of observed phenotypic values
- $\boldsymbol{b}$ = vector of fixed effects
- $\boldsymbol{a}$ = vector of additive genetic effects
- $\boldsymbol{rl}$ = vector of recipient litter efects
- $\boldsymbol{bl}$ = vector of helper/adult birth litter effects
- $\boldsymbol{m}$ = vector of helper/adult maternal effects
- $\boldsymbol{bg}$ = vector of helper/adult birth group effects
- $\boldsymbol{pe}$ = vector of permanent environment effects
- $\boldsymbol{e}$ = vector of residual effects
- $\mathbf{X}$ and $\mathbf{Z_{1\text{-}6}}$ = corresponding design matrices

Variance components (conditional on the fixed effects) were estimated as the mode of the posterior distribution, with accompanying HPDI intervals (necessarily bounded above zero), and the total phenotypic variance (V_P) for each trait was subsequently partitioned as the following in the FMs:

Baby-sitting and pup-feeding: $V_P = V_{PE} + V_A + V_{RL} + V_{BL} + V_M + V_{BG} + V_R$

Adult mass: $V_P = V_{PE} + V_A + V_{BL} + V_M + V_{BG} + V_R$

where:

- V_P = total phenotypic variance
- V_A = additive genetic effect variance
- V_{RL} = recipient litter effect variance
- V_{BL} = helper/adult birth litter effect variance
- V_M = helper/adult maternal effect variance
- V_{BG} = helper/adult birth group effect variance
- V_{PE} = permanent environment effect variance
- V_R = residual effect variance

Narrow sense heritabilities and the proportion of variance attributed to the other effects were then calculated for each MCMC sample by dividing the relevant variance component by the total phenotypic variance (e.g. heritability $h^2=V_A/V_P$). Because the levels of the other components of variance differ between traits, I also calculated the coefficient of additive genetic variance (CV_A) where the additive genetic variance was scaled by the trait mean (x) rather than the total phenotypic variance: $CV_A = 100*\sqrt{(V_A)}/x$.

The repeatability (i.e. the proportion of the variance in the trait that was due to variation among rather than within individuals) was estimated for all FMs as:

$$r^2 = ([V_{PE} + V_A + V_{BL} + V_M + V_{BG})/]/V_P$$

V_{RL} was not included in estimates of repeatability for the behavioural traits as it generates within, rather than between, individual variation.

Correlations between traits

Whereas estimates for the proportion of variance attributed to the random effect terms were from univariate models, genetic and non-genetic covariance and correlation estimates were calculated from a multivariate animal model with only the consensus random terms (i.e. applicable to all traits) of *A*, *BL*, *M*, *BG*, and *PE* included. All significant fixed effect terms were included but coded so they were specific only to the trait in which they were found to be relevant (see Table 5.4).

Covariances were rescaled to correlations following: $r = \text{COV}_{a,b}/\sqrt{(V_a * V_b)}$ and both covariances and correlations were taken to be statistically different from zero when 95% HDPIs around the posterior distributions excluded zero. Because the random terms differ slightly from those included in the univariate models, the multivariate model is not directly analogous to the univariate models.

Program settings

Parameter expansion speeds up the mixing and rate of convergence of Markov chains (Hadfield 2009) and thus, I used parameter expanded priors in MCMCGLMM in order to avoid poor mixing:

univariate models: V = 1, nu = 1, alpha.mu = 0, alpha.V = 1000

multivariate model: V = diag(3), n = 2, alpha.mu = rep(0,3), alpha.V = diag(3)*1000)

All models were preceded by a burn-in of 15,000 (univariate) or 30,000 (trivariate) iterations, and then were run for 65,000 (univariate) or 130,000 (trivariate) iterations and estimates of every 50th (univariate) or 100th (trivariate) iteration were stored. The convergence of the MCMC sampling was assessed by visual inspection of the variance component chains. Note that as residual multivariate variance structures cannot have parameter expansion in MCMCGLMM, these are not presented.

5.4 Results

I analysed the sources of variance in contributions to baby-sitting and pup-feeding, and adult mass and found that the FMs for all traits had the lowest DIC values, indicating that models with all random terms retained were the most parsimonious (Table 5.5). However, because DIC values are not especially well suited to model selection of non-Gaussian traits (i.e. baby-sitting and pup-feeding), I visually checked the posterior modes of the random terms to see whether they were in the lowest 25% quartile of the range (additive genetic posterior probability density functions are shown in Fig. 5.1). This revealed that some terms explained extremely low levels of variance and were thus likely to be non-significant and I summarise these for each trait below. I also refer to the FM_{-BL} results as they provide insight into the degree to which the birth litter (*BL*) term was confounded with the other variance components. In this Results section, I present analyses of each trait in turn; I then consider results for the different fixed and random effects across traits in the Discussion.

Table 5.4 Parameter estimates of the fixed terms in the baby-sitting, pup-feeding, and adult mass univariate, repeated measure animal models. Models included both additive genetic (V_A) and permanent environment (V_{PE}) random effect terms. Statistics are reported for restricted datasets, where any records with missing fixed term values were excluded (n = total number of records), and were generated by the R-package MCMCGLMM. Fixed effect terms were deemed significant (indicated in bold) when the posterior distribution of the 95% Highest Probability Density Intervals (HPDI; in parentheses) did not overlap zero. Significant fixed terms were retained in subsequent models for which variance components are shown in Tables 5.6 – 5.9.

	Baby-sitting (n = 2813)		Pup-feeding (n = 2108)		Adult mass (n = 2122)	
Fixed effect	Coefficient	95% HPDI	Coefficient	95% HPDI	Coefficient	95% HPDI
Sex (male)	**-0.27**	**-0.40 – -0.20**	**-0.26**	**-0.33 – -0.09**	8.92	-4.48 – 17.02
Age	**$7.61*10^{-4}$**	**$6.25*10^{-4}$ – $8.67*10^{-4}$**	**$5.17*10^{-4}$**	**$4.00*10^{-4}$ – $6.48*10^{-4}$**	**0.28**	**0.07 – 0.51**
Current status (subordinate)	**3.11**	**2.90 – 3.39**	**0.58**	**0.42 – 0.81**	**-56.41**	**-104.40 – -2.58**
Ultimate status (subordinate)	$-51.98*10^{-4}$	-0.16 – 0.12	0.11	-0.07 – 0.26	-7.06	-21.29 – 8.37
Current group size	**-0.06**	**-0.06 – -0.05**	**-0.07**	**-0.08 – -0.06**	**-0.97**	**-1.57 – -0.02**
Litter size	**0.06**	**0.04 – 0.10**	**0.04**	**0.01 – 0.08**	—	—
Relatedness	**-0.45**	**-0.78 – -0.12**	**0.25**	**0.01 – 0.64**	—	—
Individual F	-0.26	-2.00 – 1.49	**8.24**	**6.24 – 10.57**	**-509.15**	**-702.90 – -350.69**
Recipient litter F	0.27	-0.89 – 1.70	0.21	-1.33 – 1.62	—	—
Season (hot-wet)	**-0.09**	**-0.18 – -0.01**	0.08	-0.01 – 0.24	**37.76**	**26.55 – 50.29**
Rainfall	$-2.29*10^{-4}$	$-5.87*10^{-4}$ – $2.47*10^{-4}$	**$9.60*10^{-4}$**	**$3.14*10^{-4}$ – $16.51*10^{-4}$**	**0.23**	**0.18 – 0.30**
Observation time*	—	—	**3.44**	**3.22 – 3.67**	—	—

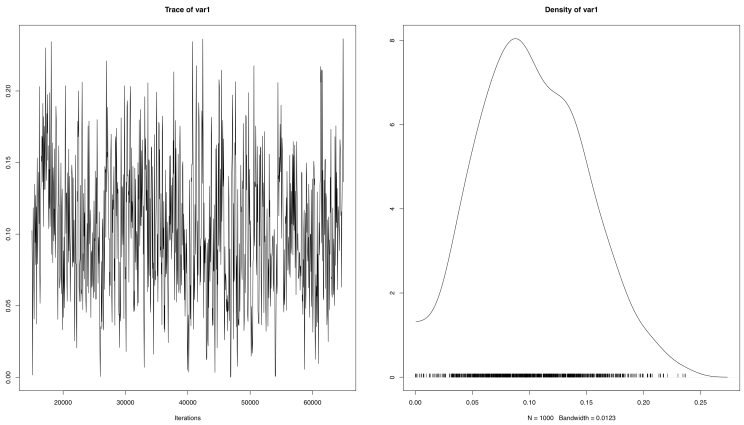
*Log(mins).

Table 5.5 Significance of the random effect terms derived from the baby-sitting, pup-feeding and adult mass univariate, repeated measure MCMCGLMM animal models. Models were analysed including all relevant fixed effects (see Table 5.4) and significance of the random terms was based on the Deviance Information Criterion (DIC) where smaller DIC values are preferred. Number of records (*n*) is given and the random effects are permanent environment (V_{PE}), additive genetic (V_A), recipient litter (V_{RL}), birth litter (V_{BL}), maternal (V_M), and birth group (V_{BG}). V_{RL} is only applicable to the behavioural measures, and V_{BL}, V_M, and V_{BG} are all at the level of the helper (behavioural measures)/adult (adult mass). The full (FM; where all possible random effects were included) and reduced (FM_{-BL}; excluding birth litter) models are indicated. Because DIC values are not especially well suited to model selection of non-Gaussian traits (i.e. behavioural measures), visual checks of the posterior distributions of the random terms were also simultaneously performed (see Fig. 5.1).

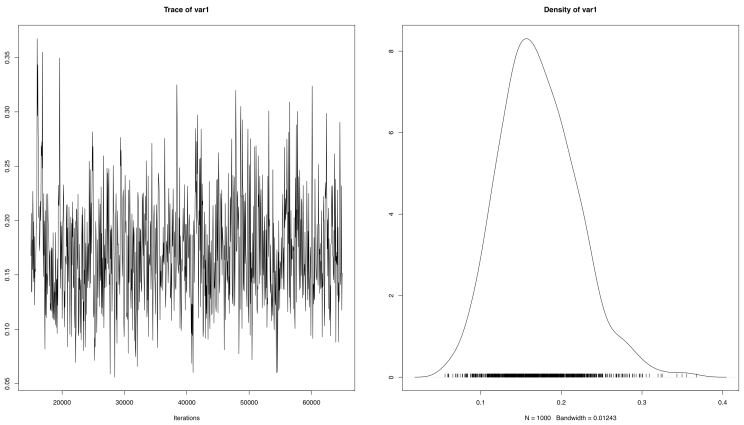
Model		DIC
Baby-sitting (n = 3804)*		
FM	$\sim V_{PE} + V_A + V_{RL} + V_{BL} + V_M + V_{BG}$	**99221.74**
	$\sim V_{PE} + V_A + V_{BG}$	99283.01
	$\sim V_{PE} + V_A + V_M$	99287.12
	$\sim V_{PE} + V_A + V_{BL}$	99264.97
	$\sim V_{PE} + V_A + V_{RL}$	99255.54
	$\sim V_{PE} + V_{BL}$	99268.60
	$\sim V_{PE} + V_A$	99288.53
FM_{-BL}	$\sim V_{PE} + V_A + V_{RL} + V_M + V_{BG}$	99244.00
Pup-feeding (n = 2108)		
FM	$\sim V_{PE} + V_A + V_{RL} + V_{BL} + V_M + V_{BG}$	**10487.07**
	$\sim V_{PE} + V_A + V_{BG}$	10545.79
	$\sim V_{PE} + V_A + V_M$	10537.72
	$\sim V_{PE} + V_A + V_{BL}$	10534.18
	$\sim V_{PE} + V_A + V_{RL}$	10507.18
	$\sim V_{PE} + V_{BL}$	10531.73
	$\sim V_{PE} + V_A$	10552.53
FM_{-BL}	$\sim V_{PE} + V_A + V_{RL} + V_M + V_{BG}$	10495.94
Adult mass (n = 2122)		
FM	$\sim V_{PE} + V_A + V_{BL} + V_M + V_{BG}$	**17727.89**
	$\sim V_{PE} + V_A + V_{BG}$	17731.69
	$\sim V_{PE} + V_A + V_M$	17731.97
	$\sim V_{PE} + V_A + V_{BL}$	17728.83
	$\sim V_{PE} + V_{BL}$	17728.61
	$\sim V_{PE} + V_A$	17732.14
FM_{-BL}	$\sim V_{PE} + V_A + V_M + V_{BG}$	17730.77

*Sample size is larger than that in Table 5.4 as records that were previously omitted due to non-significant fixed effect terms are now included.

a.) i.)


Trace of var1
Iterations
Density of var1
N = 1000 Bandwidth = 0.0123

ii.)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Iterations
Density of var1
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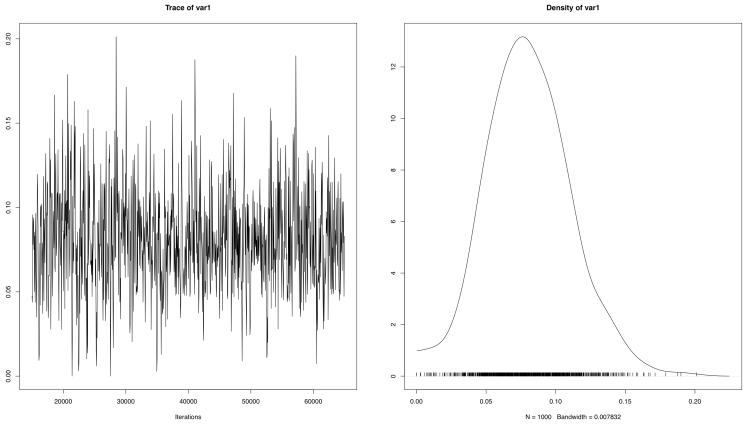
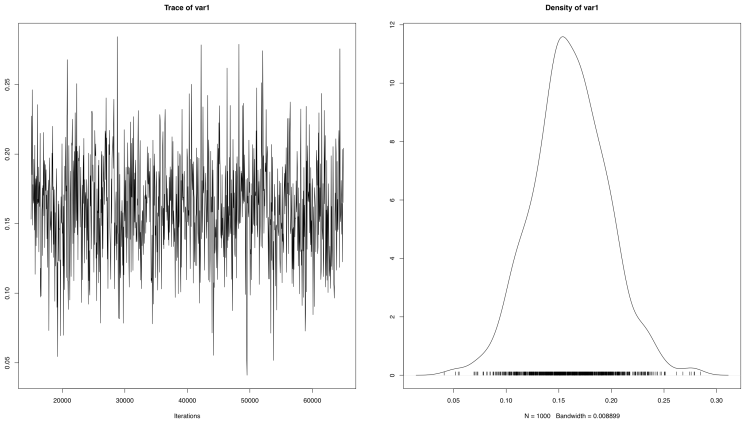
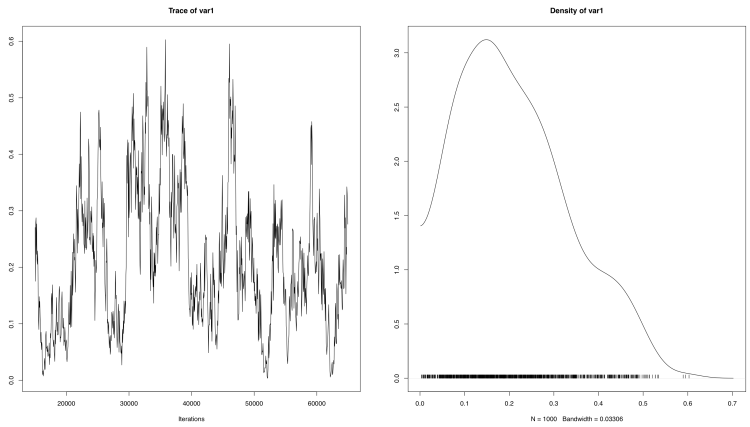
b.) i.)


Trace of var1
Iterations
Density of var1
N = 1000 Bandwidth = 0.007832

ii.)


Trace of var1
Iterations
Density of var1
N = 1000 Bandwidth = 0.008899

c.) i.)



ii.)

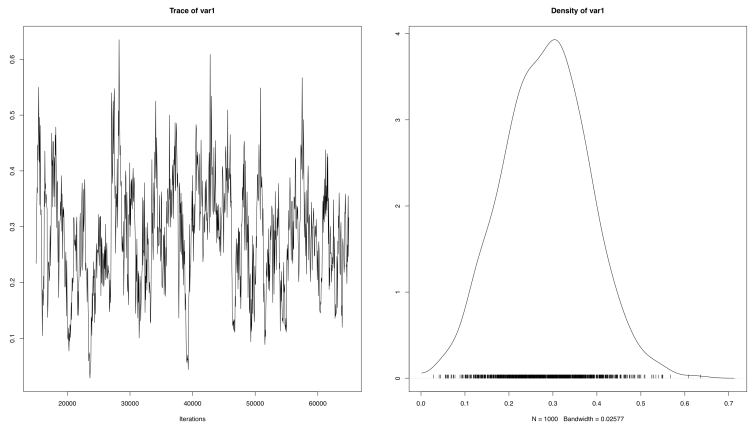


Figure 5.1 The posterior probability density functions for additive genetic variance (V_A) in *a.*) baby-sitting, *b.*) pup-feeding, and *c.*) adult mass as estimated by the R-package MCMCGLMM (Hadfield 2009). Data are from the i.) full (FM; where all possible random effects were included) and ii.) reduced (FM_{-BL}; excluding birth litter) models.

5.4.1 Baby-sitting

Fixed effects

Analysis of the fixed effects in the preliminary model, which included only *A* and *PE* random effect terms, revealed that females, older individuals and subordinate individuals contributed more to baby-sitting than did males, younger individuals and dominants respectively (Table 5.4). I also found significant effects of both the size of the current group and of the recipient litter: individuals were more likely to baby-sit when there were fewer individuals of helper age in the group and to larger litters (Table 5.4). I also found that the more related a helper was to a litter, the less likely it was to baby-sit, and that baby-sitting contributions were higher in cold-dry periods of the year (Table 5.4). There was no significant effect of ultimate helper status, rainfall or inbreeding coefficient (of either the helper or the litter) (Table 5.4).

Variance components

Full model (FM)

Although the MCMC sampling for the additive genetic chains converged in the FM (Fig. 5.1*a*), the narrow-sense heritability estimate for baby-sitting was low (h^2 = 0.10) (Table 5.6 & Fig. 5.2). The estimates for the recipient litter (rl^2 = 0.06) and permanent environment effects (pe^2 = 0.05) were also low, whereas helper birth litter (bl^2) explained as much as 12% of the variance in baby-sitting contributions (Table 5.6 & Fig. 5.2). The posterior modes of the maternal and birth group effects were in the lowest 25% quartile of the range (results not shown) and did not explain appreciable levels of variance (Table 5.6 & Fig. 5.2). The majority of the variance in baby-sitting remained unexplained as is apparent from the large residual variation (e^2 = 0.61). The repeatability of effort (r^2) was 0.28 (Table 5.6).

Reduced model (FM_{-BL})

MCMC sampling for the additive genetic chains converged in the FM_{-BL} (Fig. 5.1*a*) and the narrow-sense heritability estimate for baby-sitting explained a larger proportion of variance than in the FM (h^2 = 0.17) (Table 5.6 & Fig. 5.2). The permanent environment effect (pe^2 = 0.08) also explained slightly more variance than in the FM, whereas the proportion of variance explained by both the recipient litter

(rl^2 = 0.05) and residual effects (e^2 = 0.62) were slightly reduced in comparison to the FM (Table 5.6 & Fig. 5.2). The posterior modes of the maternal and birth group effects remained in the lowest 25% quartile of the range (results not shown) and did not explain appreciable levels of variance (Table 5.6 & Fig. 5.2). The repeatability of effort (r^2 = 0.29) was similar to the FM (Table 5.6).

Table 5.6 Quantitative genetic parameters for baby-sitting. Estimates from the full (FM; where all possible random effects were included) and reduced (FM_{-BL}; excluding birth litter) models are provided. Models were analysed including all significant fixed effects (see Table 5.4). V_A = additive genetic, V_{RL} = recipient litter, V_{BL} = birth litter, V_M = maternal, V_{BG} = birth group, V_{PE} = permanent environment, V_R = residual, and V_P = total phenotypic variance; CV_A = coefficients of additive genetic variance ($CV_A = 100*\sqrt{(V_A)}/x$), and r^2 = repeatability. 'Variance' gives the mode of the posterior distributions for each parameter whereas 'source/V_P' gives the ratio of the effect to the total phenotypic variance, i.e. narrow-sense heritability (h^2) = V_A/V_P and proportion of variance explained by residual effects (e^2) = V_R/V_P. Values are accompanied by their 95% HPDI credible intervals (in parentheses), which are bounded above zero and values in bold indicate where posterior modes of the random terms were in the lowest 25% quartile of the range.

	FM		FM_{-BL}	
Source	Variance (95% HPDI)	Source/V_P (95% HPDI)	Variance (95% HPDI)	Source/V_P (95% HPDI)
V_A	0.08 (0.01 – 0.20)	0.10 (0.02 – 0.19)	0.18 (0.08 – 0.28)	0.17 (0.09 – 0.28)
V_{RL}	0.06 (0.03 – 0.09)	0.06 (0.03 – 0.09)	0.06 (0.03 – 0.08)	0.05 (0.03 – 0.08)
V_{BL}*	0.12 (0.07 – 0.17)	0.12 (0.07 – 0.18)	— —	— —
V_M*	**$1.04*10^{-4}$ ($0.13*10^{-6}$ – 0.03)**	**$0.97*10^{-4}$ ($0.14*10^{-6}$ – 0.03)**	**$1.15*10^{-4}$ ($0.02*10^{-6}$ – 0.05)**	**$2.24*10^{-4}$ ($0.02*10^{-6}$ – 0.05)**
V_{BG}*	**$7.32*10^{-4}$ ($0.13*10^{-8}$ – 0.08)**	**$4.04*10^{-4}$ ($0.15*10^{-8}$ – 0.08)**	**0.03 ($0.58*10^{-4}$ – 0.15)**	**0.03 ($0.62*10^{-4}$ – 0.14)**
V_{PE}	0.06 ($0.02*10^{-4}$ – 0.11)	0.05 ($0.03*10^{-4}$ – 0.12)	0.08 (0.01 – 0.16)	0.08 ($79.67*10^{-4}$ – 0.16)
V_R	0.60 (0.55 – 0.66)	0.61 (0.55 – 0.69)	0.60 (0.55 – 0.67)	0.62 (0.52 –0.68)
V_P	0.92	—	0.95	—
CV_A	257.13	—	385.69	—
r^2	—	0.28	—	0.29

*Of the helper.

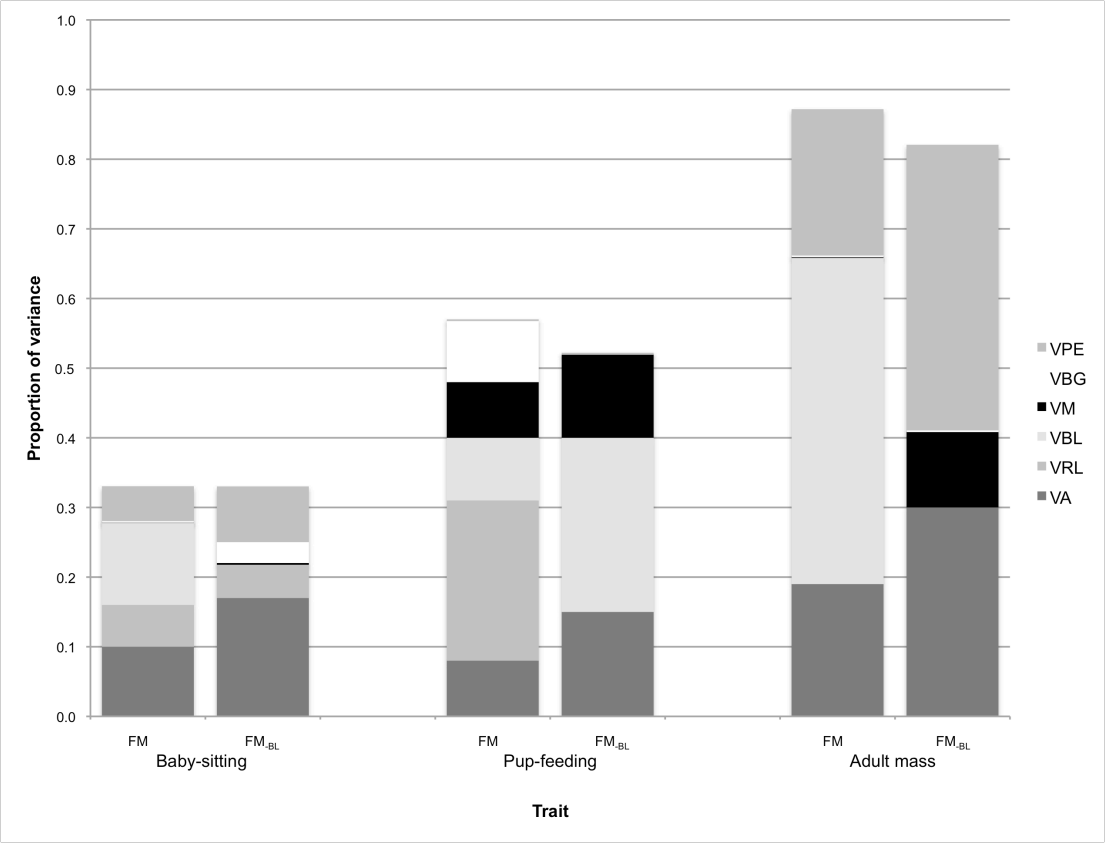


Figure 5.2 Proportion of variance explained by each of the components for baby-sitting, pup-feeding, and adult mass. V_A = additive genetic, V_{RL} = recipient litter, V_{BL} = birth litter, V_M = maternal, V_{BG} = birth group, and V_{PE} = permanent environment. Data are from the full (FM; where all possible random effects were included) and reduced (FM_{-BL}; excluding birth litter) models.

5.4.2 Pup-feeding

Fixed effects

The significance of the fixed effects in contributions to pup-feeding were similar to those found for baby-sitting such that females, older individuals, subordinates and members of smaller groups contributed more to pup-feeding than males, younger individuals, dominants and members of larger groups (Table 5.4). Similarly, individuals were more likely to feed pups in larger litters. In contrast to baby-sitting, however, individuals were significantly more likely to feed pups that were more related to them. There was also a positive association with the amount of pup-feeding and the accumulated rainfall in the previous 9 months, and observation time (Table 5.4). In addition, I found positive effects of helper *F* on pup-feeding (Table 5.4).

Variance components

Full model (FM)

The MCMC sampling for the additive genetic chains converged in the FM (Fig. 5.1*b*) and the narrow-sense heritability estimate was similar to baby-sitting (h^2 = 0.08) (Table 5.7 & Fig. 5.2). In contrast to baby-sitting, however, the estimate for the recipient litter effect was higher (rl^2 = 0.23), whereas the residual variance was lower (e^2 = 0.33) (Table 5.7 & Fig. 5.2). Helper birth litter and maternal effects explained similar magnitudes of variance (bl^2 = 0.09; m^2 = 0.08), whereas the posterior modes of the birth group and permanent environment effects were in the lowest 25% quartile of the range (results not shown) and did not explain appreciable levels of variance (Table 5.7 & Fig. 5.2). The repeatability of pup-feeding effort was higher than for baby-sitting (r^2 = 0.36) (Table 5.7).

Reduced model (FM_{-BL})

As for baby-sitting, the MCMC sampling for the additive genetic chains converged in the FM_{-BL} (Fig. 5.1*b*) and the narrow-sense heritability estimate for pup-feeding explained a larger proportion of variance than in the FM (h^2 = 0.15) (Table 5.7 & Fig. 5.2). The recipient litter (rl^2 = 0.25) and maternal effects (m^2 = 0.12) also explained slightly more variance than in the FM, whereas the proportion of variance

explained by the residual effect (e^2 = 0.33) remained unchanged in comparison to the FM (Table 5.7 & Fig. 5.2). The posterior modes of the birth group and permanent environment effects remained in the lowest 25% quartile of the range (results not shown) and did not explain appreciable levels of variance (Table 5.7 & Fig. 5.2). The repeatability of effort (r^2 = 0.32) was lower than in the FM (Table 5.7).

Table 5.7 Quantitative genetic parameters for pup-feeding. Estimates from the full (FM; where all possible random effects were included) and reduced (FM_{-BL}; excluding birth litter) models are provided. Models were analysed including all significant fixed effects (see Table 5.4). V_A = additive genetic, V_{RL} = recipient litter, V_{BL} = birth litter, V_M = maternal, V_{BG} = birth group, V_{PE} = permanent environment, V_R = residual, and V_P = total phenotypic variance; CV_A = coefficients of additive genetic variance ($CV_A = 100*\sqrt{(V_A)}/x$), and r^2 = repeatability. 'Variance' gives the mode of the posterior distributions for each parameter whereas 'source/V_P' gives the ratio of the effect to the total phenotypic variance, i.e. narrow-sense heritability (h^2) = V_A/V_P and proportion of variance explained by residual effects (e^2) = V_R/V_P. Values are accompanied by their 95% HPDI credible intervals (in parentheses), which are bounded above zero and values in bold indicate where posterior modes of the random terms were in the lowest 25% quartile of the range.

	FM		FM_{-BL}	
Source	Variance (95% HPDI)	Source/V_P (95% HPDI)	Variance (95% HPDI)	Source/V_P (95% HPDI)
V_A	0.09 (0.03 – 0.16)	0.08 (0.02 – 0.14)	0.19 (0.11 – 0.26)	0.15 (0.09 – 0.24)
V_{RL}	0.28 (0.21 – 0.39)	0.23 (0.17 – 0.35)	0.29 (0.21 – 0.41)	0.25 (0.18 – 0.36)
V_{BL}*	0.09 (0.05 – 0.16)	0.09 (0.04 – 0.15)	— —	— —
V_M*	0.09 (0.01 – 0.27)	0.08 (0.02 – 0.23)	0.12 (0.05 – 0.31)	0.12 (0.06 – 0.26)
V_{BG}*	**0.09 (0.01*10^{-4} – 0.46)**	**0.09 (0.01*10^{-4} – 0.32)**	**18.47*10^{-4} (0.49*10^{-6} – 0.35)**	**18.54*10^{-4} 0.42*10^{-6} – 0.25)**
V_{PE}	**1.59*10^{-4} (0.10*10^{-8} – 0.02)**	**1.06*10^{-4} (0.08*10^{-8} – 0.02)**	**2.24*10^{-4} (0.09*10^{-6} – 0.04)**	**2.03*10^{-4} (0.09*10^{-6} – 0.03)**
V_R	0.36 (0.32 – 0.41)	0.33 (0.24 – 0.40)	0.37 (0.32 – 0.42)	0.33 (0.24 – 0.39)
V_P	1.00	—	0.97	—
CV_A	4.59	—	10.70	—
r^2	—	0.36	—	0.32

*Of the helper.

5.4.3 Adult mass

Fixed effects

Adult mass was positively influenced by age, subordinate status, hot-wet seasons and rainfall (Table 5.4). There was also evidence for negative effects of current group size, and for apparent inbreeding depression in the form of a negative association with the inbreeding coefficient (Table 5.4). No other fixed effects were found to be significant (Table 5.4).

Variance components

Full model (FM)

The narrow-sense heritability estimate ($h^2 = 0.19$) for adult mass was higher than for either baby-sitting or pup-feeding, as were the estimates for the birth litter ($bl^2 = 0.47$), and permanent environment ($pe^2 = 0.21$) effects (Table 5.8 & Fig. 5.2). In consequence the residual variation ($e^2 = 0.04$) was substantially lower than for either the baby-sitting or pup-feeding models (Table 5.8 & Fig. 5.2). The posterior modes of the maternal and birth group effects were in the lowest 25% quartile of the range (results not shown) and did not explain appreciable levels of variance (Table 5.8 & Fig. 5.2). The repeatability of adult mass was very high ($r^2 = 0.95$) (Table 5.8).

Reduced model (FM_{-BL})

The MCMC sampling for the additive genetic chains converged in the FM_{-BL} (Fig. 5.1*c*) and the narrow-sense heritability estimate for adult mass explained a larger proportion of variance than in the FM ($h^2 = 0.30$) (Table 5.8 & Fig. 5.2). The permanent environment effect ($pe^2 = 0.41$) also explained more variance than in the FM, whereas the proportion of variance explained by the residual effect ($e^2 = 0.04$) remained unchanged in comparison to the FM (Table 5.8 & Fig. 5.2). The posterior mode of the birth group remained in the lowest 25% quartile of the range (results not shown) and did not explain appreciable levels of variance (Table 5.8 & Fig. 5.2). Conversely, whereas the posterior mode of maternal effect was in the lowest 25% quartile of the range in the FM, it was not in the FM_{-BL} and instead explained 11% of the total variance in adult mass (Table 5.8 & Fig. 5.2). The repeatability of adult mass remained very high ($r^2 = 0.96$) (Table 5.8).

5.4.4 Correlations between traits

There were significant strong positive genetic correlations between baby-sitting and pup-feeding ($r = 0.64$) and baby-sitting and adult mass ($r = 0.94$) (Table 5.9). There was also a significant strong positive correlation between baby-sitting and adult mass for the birth litter source of variation ($r^2 = 0.78$; Table 5.9). This means that within the variation due to helper birth litter, heavy individuals did more baby-sitting.

Table 5.8 Quantitative genetic parameters for adult mass. Estimates from the full (FM; where all possible random effects were included) and reduced (FM_{-BL}; excluding birth litter) models are provided. Models were analysed including all significant fixed effects (see Table 5.4). V_A = additive genetic, V_{BL} = birth litter, V_M = maternal, V_{BG} = birth group, V_{PE} = permanent environment, V_R = residual, and V_P = total phenotypic variance; CV_A = coefficients of additive genetic variance ($CV_A = 100*\sqrt{(V_A)}/x$), and r^2 = repeatability. 'Variance' gives the mode of the posterior distributions for each parameter whereas 'source/V_P' gives the ratio of the effect to the total phenotypic variance, i.e. narrow-sense heritability (h^2) = V_A/V_P and proportion of variance explained by residual effects (e^2) = V_R/V_P. Values are accompanied by their 95% HPDI credible intervals (in parentheses), which are bounded above zero and values in bold indicate where posterior modes of the random terms were in the lowest 25% quartile of the range.

	FM		FM_{-BL}	
Source	Variance (95% HPDI)	Source/V_P (95% HPDI)	Variance (95% HPDI)	Source/V_P (95% HPDI)
V_A	658.03 (27.00 – 2436.88)	0.19 (0.01 – 0.46)	1550.08 (499.49 – 2477.00)	0.30 (0.10 – 0.47)
V_{BL}	2138.13 (1441.78 – 2812.29)	0.47 (0.32 – 0.57)	— —	— —
V_M	**5.49 (0.13*10^{-6} – 739.69)**	**7.51*10^{-4} (0.32*10^{-10} – 0.14)**	724.76 (284.95 – 1654.05)	0.11 (0.06 – 0.30)
V_{BG}	**7.19 (1.81*10^{-4} – 639.93)**	**10.83*10^{-4} (0.04*10^{-6} – 0.13)**	**7.32 (0.16*10^{-4} – 912.93)**	**7.35*10^{-4} (0.38*10^{-8} – 0.16)**
V_{PE}	1115.30 (237.53 – 1610.00)	0.21 (0.03 – 0.34)	2046.65 (1555.31 – 2903.45)	0.41 (0.28 – 0.62)
V_R	198.20 (187 – 213.96)	0.04 (0.03 –0.05)	199.32 (186.60 – 213.37)	0.04 (0.03 – 0.05)
V_P	4122.34	—	4528.13	—
CV_A	3.96	—	6.08	—
r^2	—	0.95	—	0.96

Table 5.9 Additive genetic and non-genetic covariances (above diagonal) and correlations ($r = Cov_{a,b}/\sqrt{(V_a * V_b)}$; below diagonal) of baby-sitting, pup-feeding, and adult mass. The multivariate, repeated measure model was analysed including the consensus random effect terms of additive genetic (V_A), birth litter (V_{BL}), maternal (V_M), birth group (V_{BG}), and permanent environment (V_{PE}), and all significant trait-specific fixed terms (see Table 5.4). All values are accompanied by their 95% HPDI credible intervals (in parentheses) and are indicated in bold where significant. Note that because the random terms differ slightly from those included in the univariate models, the multivariate model is not directly analogous to the univariate models and, as residual multivariate variance structures cannot have parameter expansion in MCMCGLMM, these are not presented.

		Baby-sitting	Pup-feeding	Adult mass
Additive genetic	Baby-sitting	—	0.03 ($-1.72*10^{-4}$ – 0.05)	**11.53 (2.05 – 17.55)**
	Pup-feeding	**0.64 (0.16 – 0.98)**	—	3.04 (-0.22 – 5.68)
	Adult mass	**0.94 (0.56 – 0.99)**	**0.95 (0.25 – 1.00)**	—
Birth litter*	Baby-sitting	—	0.02 (-0.01 – 0.04)	**25.36 (16.72 – 37.97)**
	Pup-feeding	0.27 (-0.12 – 0.45)	—	3.72 (-1.31 – 9.39)
	Adult mass	**0.78 (0.61 – 0.87)**	0.25 (-0.06 – 0.42)	—
Maternal*	Baby-sitting	—	$63.15*10^{-4}$ (-0.01 – 0.06)	$-90.25*10^{-4}$ (-2.61 – 20.73)
	Pup-feeding	0.58 (-0.22 – 0.99)	—	1.19 (-5.79 – 10.46)
	Adult mass	0.77 (-0.48 – 0.99)	0.0.29 (-0.50 – 0.64)	—
Birth group*	Baby-sitting	—	$54.80*10^{-4}$ (-0.01 – 0.09)	-0.02 (-1.72 – 15.97)
	Pup-feeding	0.97 (-0.15 – 1.00)	—	-0.01 (-3.21 – 11.72)
	Adult mass	0.94 (-0.45 – 1.00)	0.80 (-0.61 – 1.00)	—
Permanent environment	Baby-sitting	—	$-0.03*10^{-4}$ ($-24.52*10^{-4}$ – $34.32*10^{-4}$)	$-40.93*10^{-4}$ (-0.59 – 1.40)
	Pup-feeding	0.97 (-0.83 – 0.99)	—	$28.94*10^{-4}$ (-0.29 – 0.37)
	Adult mass	0.44 (-0.81 – 0.96)	0.55 (-0.84 – 0.95)	—

*Of the helper (behavioural measures)/adult (adult mass).

5.5 Discussion

The aim of this study was to characterize the genetic and non-genetic sources of variance influencing two kinds of cooperative behaviour, baby-sitting and pup-feeding, in a wild population of meerkats. Since body mass is also implicated in helping behaviour at the phenotypic level (Clutton-Brock *et al.* 2000, Clutton-Brock *et al.* 2003), I conducted a parallel analysis of adult body mass and investigated the covariances between adult body mass and these two cooperative behaviours in order to ascertain whether there was a genetic basis to the phenotypic correlations. Simultaneously, I investigated the roles of inbreeding and relatedness on cooperative behaviour, and of inbreeding on adult mass. My results provide some of the first estimates of the heritability of cooperation in a wild mammalian population and of the effect of inbreeding on behavioural traits, while concurrently highlighting the importance of considering the effect of non-genetic sources of variation in contributions to cooperative care.

5.5.1 Fixed effects

The initial fixed term model selection process identified a number of variables that influenced contributions to baby-sitting and pup-feeding, and adult mass, which were broadly consistent with previous findings on the same population. For example, my results confirmed that subordinates, females, older individuals and members of small groups (i.e. with fewer helpers) were more likely to baby-sit or pup-feed than dominants, males or members of large groups (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999, Clutton-Brock *et al.* 2000, Brotherton *et al.* 2001). Adult mass was also positively influenced by age and negatively affected by status (i.e. subordinates weighed less) and the number of helper-age individuals in the group (Chapter 4 of this thesis, Hodge *et al.* 2008, Spong *et al.* 2008). Furthermore I confirmed that helpers provisioned pups more often when the recipients were in larger litters (Clutton-Brock *et al.* 2001a), and provided new evidence for the positive effects of litter size on baby-sitting contributions (a previous study on the same population of meerkats found no effect of litter size on contributions to baby-sitting, Clutton-Brock et al., 2000).

In addition to social factors, I found that contributions to cooperative care and adult mass were influenced by abiotic factors such as season and lagged, accumulated rainfall. Seasonal variation and the lagged effects of rainfall are known to influence invertebrate dynamics which form the majority of the meerkat's diet and meerkat growth is highest in mid-summer when prey are most abundant (English *et al.* 2012). In line with this, adult mass was higher in the summer months and was positively affected by lagged, accumulated rainfall. By extension, pups appeared to be provisioned more after periods of higher rainfall, and thus during periods of increased prey abundance.

Inbreeding effects

I found positive effects of inbreeding on contributions to pup-feeding but evidence for negative effects, i.e. inbreeding depression, on adult mass, which can be viewed as an extension of previous findings on mass in the same population (Chapters 3 & 4). To the best of my knowledge, the results herein represent the first empirical estimates of the effect of inbreeding on cooperative behaviour. Given the large degree of competition for dominant positions (Hodge *et al.* 2008, Spong *et al.* 2008), my results suggest that physically smaller, inbred individuals may be more likely to remain as subordinates. Inbred subordinates may also be less likely than their more out-bred, subordinate counterparts to increase their chances of securing mating opportunities by participating in the costly behaviour of roving into neighbouring groups (Young *et al.* 2007, Mares 2012). In summary, inbred individuals may be more likely to remain as subordinate helpers, ultimately creating a positive relationship between inbreeding coefficient and pup-feeding contributions. The positive effect of inbreeding could be further emphasised if subordinates, which are physically small (due to inbreeding or other factors), need to contribute more in order to avoid aggression and/or eviction by the dominant (Kutsukake & Clutton-Brock 2006), and if increased pup-feeding rates are a way in which subordinates were able to maximise their inclusive fitness when routes to direct fitness are unlikely.

The positive effects of inbreeding on contributions to pup-feeding provides some

tentative support for the theory that increased kinship within social groups that results from inbreeding may in fact promote or maintain cooperative behaviours in line with kin selection theory (Hamilton 1963, West *et al.* 2002, Griffin & West 2003, Rossiter *et al.* 2005). Rather than leading to inbreeding depression, inbreeding among meerkats may in fact be at an adaptive group-level optimum, such that it might be advantageous to have a low level of inbreeding if it creates more 'unambitious', helpful offspring (Kokko & Ots 2006). Although the same arguments could apply to baby-sitting contributions, I did not find that contributions to baby-sitting were influenced by inbreeding. I also did not find any evidence to suggest that inbred pups successfully elicited more care from helpers (i.e. significant effects of recipient litter *F*). Further work on the relationship between inbreeding, dominance acquisition, male roving frequency and subordinate female breeding would help to further elucidate the relationship between inbreeding and cooperative care.

Relatedness effects

Contrary to previous findings (Clutton-Brock *et al.* 2000, Clutton-Brock *et al.* 2001a), where relatedness was not a factor in determining contributions to cooperative care, I found opposing effects of relatedness on baby-sitting (negative) and pup-feeding (positive) with this much increased dataset. The positive association between helper-recipient relatedness and pup-feeding provides some evidence for kin selection (Hamilton 1964a) as it appears that meerkats preferentially provision to more related individuals. This furthermore implies that meerkats may be able to discriminate individuals based on their level of kinship (Griffin & West 2003, Leclaire *et al.* in press).

The negative association between helper-recipient relatedness and baby-sitting is more difficult to explain, however. While, research has shown that stronger discrimination occurs where the benefits of helping are greater (Griffin & West 2003), possibly suggesting that pup-feeding is accompanied by greater benefits than baby-sitting, this does not explain the negative association. One hypothesis is that the negative association between baby-sitting and kinship is driven by (less related)

immigrants contributing more to baby-sitting than (more related) natal group members. In an attempt to explore this possibility, I re-analysed the data in two ways where: 1.) the data was restricted to include only those individuals where $R > 0$, and 2.) pair-wise relatedness coefficients were substituted for immigrant/natal status. The negative association between relatedness and baby-sitting remained in the first re-analysis and immigrant/natal status was not a significant factor explaining variation in baby-sitting in the second re-analysis. While further investigation is undoubtedly required, the negative association between relatedness and baby-sitting may be taken as possible evidence for either 'pay-to-stay' or 'social prestige'. The pay-to-stay hypothesis describes how group members help in order to be allowed to stay in the group and potentially gain breeding positions later in life (Kokko *et al.* 2002), whereas the social prestige hypothesis states that cooperative behaviours showcase social status and provide direct benefits to the helper by increasing the respect it receives from other group members (Zahavi 2003). Both of these hypotheses have the potential to create negative associations between helper-recipient relatedness and baby-sitting, although extensive further investigation is required before either, or both, hypotheses are supported.

5.5.2 Additive genetic effects

I found low but significant heritabilities for both baby-sitting ($h^2 = 0.10 \pm 0.02 – 0.19$ 95% HPDI) and pup-feeding ($h^2 = 0.08 \pm 0.02 – 0.14$ 95% HPDI). As neither of the 95% Highest Probability Density Intervals (HPDI) for these estimates include 0.30, they are both significantly less than the average ($h^2 = 0.30$) reported for behavioural traits (see reviews of Mousseau & Roff 1987, Stirling *et al.* 2002). My estimates of heritability are, however, somewhat similar to the heritability estimates of trust (average $h^2 = 0.15 \pm 0.03$ - 0.38 C.I.) and trustworthiness (average $h^2 = 0.18 \pm 0.05$ - 0.32 C.I.) in humans (*Homo sapiens*), which also accounted for the effects of the common environment (Cesarini *et al.* 2008).

In an analogous study, Charmantier et al. (2007) reported very high heritabilities for both the propensity to help ($h^2 = 0.76 \pm 0.25$ S.E.) and to receive help ($h^2 = 0.32 \pm 0.13$ S.E.) in cooperative western bluebirds (*Sialia mexicana*). Contrary to the

western bluebird study, however, my analyses also included estimates of the proportion of variance in cooperation that were explained by litter effects (since some birds can have many clutches per year in same physical nest, birth litter is analogous to clutch effects in avian populations). I accounted for both recipient litter (*rl*) and helper birth litter (*bl*) effects, with the latter suggesting the importance of considering the effects of early life conditions on later life cooperative contributions (English 2009). In meerkats, subordinate helpers are thought to replace the maternal effects that are commonly found to govern the fitness of non-cooperative vertebrates and are known to have important long-term fitness effects on the offspring they help to raise (Jennions & Macdonald 1994, Russell *et al.* 2002). Furthermore, litter effects have been shown to explain a large amount of variance in meerkat morphological traits (Chapter 4), and here I also find some evidence for the influence of birth litter on later life traits, especially on baby-sitting and adult mass. Although litter effects will encompass aspects of the abiotic environment (e.g. season and rainfall), given the highly social nature of meerkat societies, litter effects may be best viewed as representing an individual's early-life social environment, composed of both their litter mates and adult helpers. This finding therefore emphasises the importance of considering the effect of conspecifics, beyond parents, in social animal systems.

There are a number of reasons why my estimates of heritability might be smaller than that typically reported for behavioural traits (Mousseau & Roff 1987, Stirling *et al.* 2002). First, theory predicts that directional selection on fitness will tend to drive beneficial alleles to fixation and thus traits under strong selection should have lower levels of additive genetic variance than those under weak selection (Fisher 1958). There is, however, little evidence to suggest that baby-sitting and pup-feeding are directly positively related to individual fitness – in fact, both behaviours are known to be accompanied by appreciable energetic costs to the helper (Clutton-Brock *et al.* 1998b, Clutton-Brock *et al.* 2002, Clutton-Brock *et al.* 2004). However, since I here report a significant positive effect of relatedness on pup-feeding which contrasts with previous research (Clutton-Brock *et al.* 2000, Clutton-Brock *et al.* 2001a) it is possible that paths towards accruing indirect fitness benefits may be more complex

than thought hitherto. Alternatively, small heritabilities may be due to large environmental variances (Wilson *et al.* 2010). The residual variance component estimates I report herein are indeed relatively high and do not exclude the possibility that unmeasured factors may be explaining much of the variance in my traits. The most likely explanation, however, is that the large contributions from shared environmental factors, such as recipient and birth litter, have necessarily reduced estimates of heritability as a proportion.

5.5.3 Correlations between traits

I found strong positive genetic correlations between baby-sitting and pup-feeding and baby-sitting and adult mass indicating that contributions to cooperative care are not necessarily wholly condition-dependant plastic responses. This suggests that heavier individuals may be more genetically predisposed to contribute cooperative care. However, heavier individuals are also more likely to obtain dominance status (Hodge *et al.* 2008, Spong *et al.* 2008), and thus heavier individuals do not appear to be canalized into a life-time subordinate trajectory. The fact that ultimate status was not a significant factor in contributions to cooperative care further supports this theory and suggests that subordinates that contribute relatively high levels of cooperative care in early life can still obtain dominance status in later life. Further work determining to what extent dominance acquisition is genetically mediated would be of interest and it is possible that continued data collection and increased samples size of dominants will enable this to be addressed in the future.

5.5.4 Future work

The genetic architecture of cooperative behaviour is likely to be complex, especially in social animals where the effect of another individual's genotype on the phenotype of the focal individual, or indirect genetic effects (IGEs), may need to be properly considered (Wolf *et al.* 1999, Wilson *et al.* 2011). IGEs can manifest at a variety of levels, for example they may be important to consider at the social interaction level (i.e. helper-recipient) but also at the litter, maternal and group levels. In line with most previous research, I have assumed that cooperative care is a trait determined primarily by helper-level rather than recipient-level effects but I appreciate the

possibility that certain recipients may be predisposed to, or may elicit, a certain degree of care. These points highlight the potential benefits of investigating the role of IGE in meerkat cooperative care. Models that consider IGE are, however, substantially more complex than those I present herein and thus, this study provides a first step towards a more complete understanding of the factors influencing individual variation in cooperative care. It furthermore highlights the need to consider both inbreeding and the relatedness between helpers and recipients in any future attempts to investigate contributions to cooperative care.

Conclusion



Each chapter of this thesis has its own individual discussion of results. Here I summarise these findings and suggest specific future research directions in turn.

6.1 Meerkat pedigree construction

A seven-generation deep pedigree for the Kalahari Meerkat Project was successfully constructed. In Chapter 2, I presented a pedigree for the Kalahari Meerkat Project, which I constructed using a combination of behavioural observations, genotyping data and two parentage inference programs. To my knowledge, this is the first available genetically-validated pedigree for a wild population of cooperatively breeding mammals. The main findings from its construction were that: a) the microsatellite kit used proved to be an efficient and reliable tool for the high-throughput genotyping of meerkats; b) the combinatorial approach of re-constructing the pedigree was generally successful; but that c) in future, the use of more informative phenotypic descriptors may better inform the MASTERBAYES parentage inference process and potentially ameliorate the reported 'sib paradox' effects.

The meerkat microsatellite kit was effective at inferring parentage. The microsatellite kit was initially specifically developed and optimized for use on the Kalahari Meerkat Project population by W. Goodall-Copestake, and using it, I genotyped 82% of the population. All 18 microsatellite loci showed adequate levels of variability for their application in inferring parentage and although deviations from the Hardy-Weinberg equilibrium were reported for 13 loci, these deviations were generally small (<0.020) and due to heterozygote excess. Heterozygote excess is thought to be promulgated by the exchange of breeders among genetically divergent lines and may thus provide evidence for some degree of inbreeding avoidance. Heterozygote excess has also been reported in other species characterized by large kin groups and some degree of inbreeding avoidance (Chesser 1991, Dobson *et al.* 1998, Spong *et al.* 2008). Meerkats are also characterized by large kin groups and female philopatry, which is thought to partly act as an inbreeding avoidance mechanism (Clutton-Brock *et al.* 2002, Clutton-Brock & Lukas 2011, Lukas & Clutton-Brock 2011). Although in Chapter 3 I show that

meerkats may not avoid inbreeding as much as previously thought, I did report some evidence for the avoidance of close inbreeding (i.e. no evidence of parent-offspring or full-sibling matings), which may be facilitated, to some extent, by male dispersal and female philopatry.

A combinatorial approach to pedigree construction was successfully used. For pedigree construction, I followed a combinatorial approach similar to Walling et al. (2010) and recovered meerkat parentages using two different software packages: one Bayesian, MASTERBAYES (Hadfield *et al.* 2006), and one likelihood, COLONY2 (Wang 2004), based. I concluded by suggesting that, due to that fact that COLONY2 recovered more parentages than MASTERBAYES and because of its ability to assign 'dummy' parents, this program may be better suited to infer parentage in social animals such as meerkats, especially in wild settings where complete sampling of all candidate parents is unrealistic. The continued combinatorial approach to parentage inference in meerkats is, however, recommended as mis-matches between MASTERBAYES and behavioural maternal identifications may be useful in identifying sampling, labeling or genotyping errors, and in resolving instances where COLONY2 either fully recovers or splits large true sibships while simultaneously failing to confidently assign the correct genotyped parent. Note that, following conclusions in Walling et al. (2010), I did not attempt to infer parentages using CERVUS so cannot comment on how appropriate this program may be for cooperatively breeding mammals.

I suggest that improved phenotypic descriptors are developed if MASTERBAYES continues to be used for meerkat parentage inference. MASTERBAYES was instrumental in revealing the likely existence of 'sib paradox' effects, in which siblings were erroneously assigned as parents if, as can occur by chance, they shared more alleles with the focal offspring than were shared between the true parent and the focal offspring. Approximately 5% of all the assignments were erroneously assigned to siblings rather than the true parents. My findings highlight the fact that the sib paradox is likely to be an important consideration when using genetics to infer parentage in any population of organisms that is characterised by reproductive

skews, large non-dispersing sibship groups and overlapping generations. In Chapter 2 I suggested a number of specific ways in which the meerkat phenotypic descriptors provided to MASTERBAYES could be improved in order to increase both the number of parentages this program assigns and to mitigate the effects of the sib paradox in the future.

Microsatellites may soon be superseded by single nucleotide polymorphisms (SNPs). One other way in which the erroneous effects of the sib paradox could be mitigated in the future is by including more microsatellite loci in the marker set. These loci could either be developed using cloning techniques (Queller *et al.* 1993), or by using primers that have since been developed or applied in closely related species such as banded (*Mungos mungo*) and small Indian (*Herpestes palustris*) mongooses (H. Nichols & B. Graw *pers. comm.*). However, the rapid advances and the concomitant decreases in the financial costs of molecular methods may soon herald in the routine use of SNPs as the marker of choice in pedigree reconstruction (Pemberton 2008). Although SNPs are less individually informative than microsatellites, they are the most abundant type of genetic polymorphism in most genomes and the large number of loci typically considered in SNP-based analyses more than compensates for any lack of individual allelic diversity (Pemberton 2008, Slate *et al.* 2009). SNP-based analyses are also more likely to sample loci where full siblings do not share alleles and are thus a powerful means to discriminating both individuals and parents (Pemberton 2008, Slate *et al.* 2009). They are also less likely to be scored erroneously (Pemberton 2008, Slate *et al.* 2009). SNP panels have been successfully used to infer parentage in humans and agricultural species (e.g. Phillips *et al.* 2007, Rohrer *et al.* 2007) and are already being applied to some wild populations (J. Pemberton *pers. comm.*). While, unlike wild sheep, meerkats do not benefit from molecular methods developed for use in closely related agricultural species, advances in DNA sequencing and typing technologies may nevertheless result in SNPs soon providing the best way to infer parentage and to mitigate the effects of the sib paradox in non-model cooperatively breeding mammals such as meerkats.

The genetically-validated parentages and pedigree are valuable resources. In additional to being used to facilitate investigations into inbreeding and inbreeding depression, and the sources of variance in a number of different traits (this thesis), the parentages and pedigree-derived metrics can be applied to a great variety of other evolutionary questions. To date, the genetically-inferred maternities have been incorporated into models of social group dynamics (Bateman *et al.* in press), and pair-wise relatedness values have been used to provide some evidence that dominant females may be able to discriminate the scent of kin from non-kin (Leclaire *et al.* in press). Coefficients of relatedness have also been used to show that close relatives are more likely to engage in dominance interactions with each other (Madden *et al.* 2012), but that patterns of cooperation between individuals are not simply explained by individuals interacting with close kin (Madden *et al.* in review).

6.2 Inbreeding and inbreeding depression in meerkats

Chapters 3, 4 and 5 all dealt, to some degree, with inbreeding and inbreeding depression effects in meerkats: Chapter 3 described the extent of inbreeding in the study population, and then associations between inbreeding and variation in a range of traits, and for a number of different life stages, were investigated in Chapters 3, 4 and 5. The main conclusions of this work are that: a) meerkats inbreed to some extent; and that this inbreeding has b) negative consequences on a range of morphometric and survival measures; but c) is positively associated with pup-feeding behaviour; and d) that there was some evidence to suggest that the magnitude of inbreeding depression increases as a pup moves from being provisioned to provisioning itself. Below, I discuss each of these findings in turn.

I found that 44% of the study population was inbred to some extent. The fact that more than half of the breeding pairs in the study population were related and that subordinates were statistically more likely than dominants to produce inbred offspring indicates that inbreeding was widespread rather than simply being the result of a few highly productive, but inbreeding, dominant pairs. At first glance these findings suggest that meerkats do not avoid inbreeding, but further inspection revealed that the majority of consanguineous matings were between more distantly related kin, rather than close relatives, which resulted in low to moderate *F*-values (average population F=0.078). Furthermore, I found no evidence of very close inbreeding between either parents and their offspring or between full siblings, suggesting that the male-biased dispersal and lack of intra-group matings between natal members may act as an effective way of avoiding mating between closely related meerkat kin (Griffin *et al.* 2003). These means of avoiding inbreeding do not, however, preclude the existence of other mechanisms of inbreeding avoidance and research has already begun to investigate whether meerkats discriminate the scent of their kin (Leclaire *et al.* in press) and might thus be capable of actively avoiding breeding with them. Further research could also specifically investigate whether meerkats actively avoid mating with kin by comparing simulated datasets of mating patterns to see if the observed level of inbreeding differs from that expected

under random mating (Szulkin *et al.* 2009, Rioux-Paquette *et al.* 2010a, Stopher 2011). However, defining the appropriate null models for testing inbreeding avoidance is complex for non-cooperative species (Pärt 1996, Szulkin *et al.* 2009), and defining them in cooperatively breeding species may be a considerably more complicated process.

From evolutionary theory, inbreeding will only be avoided if it leads to net negative fitness consequences. In Chapter 3 I provided evidence that inbred meerkat pups weigh less, have shorter hind-foot length and grow slower than outbred pups and that this translates into reduced juvenile survival. Although the related analyses in Chapter 4 did not further support these effects on pup mass or hind-foot length, I did find evidence for the effects of inbreeding depression on mass, skull length and width, and forearm and hind-foot length at later life stages. Further research on whether these later life inbreeding effects translate into reduced fitness would be the most obvious next area of research interest. The on-going collection of data from the Kalahari Meerkat Project may eventually yield adequate samples sizes to make these analyses possible in the future.

I found a positive relationship between individual inbreeding coefficient and contributions to pup-feeding. As this is the first study to investigate the effects of inbreeding on a cooperative behaviour it is difficult to make comparisons or even to determine the likely expectation of the effect of inbreeding. I nevertheless concluded by suggesting that the positive association between inbreeding and pup-feeding most likely reflects that (smaller) inbred offspring are less competitive than outbred individuals and are thus more likely to remain as cooperative subordinate helpers rather than attempt to obtain dominant position or rove between groups, both of which are costly behaviours (Young *et al.* 2007, Hodge *et al.* 2008, Spong *et al.* 2008, Mares 2012). Investigating whether inbred individuals are indeed less likley to obtain dominance and/or whether they rove less frequently or over shorter distances would be interesting lines of future research. Investigating the effects of inbreeding on other meerkat behaviours, such as contributions to guarding or social digging behaviours, and on the cooperative behaviours of other social species would also be

of interest. The continued collection of data from the Kalahari Meerkat Project and from other cooperatively breeding species may make these analyses possible in the future.

Although not an explicit aim, in some ways Chapters 3 and 4 represent preliminary research on whether the magnitude of inbreeding depression changes depending on the social environment, as both chapters provided some tentative evidence to suggest that the early social environment when pups are provisioned ameliorates inbred individuals from the effects of inbreeding depression. In Chapter 3 I showed that allolactor number (a component of the social environment) had a positive effect on pup emergence mass and buffered (but did not completely compensate for) inbred pups from the negative effects of inbreeding. And in Chapter 4 I found evidence for inbreeding depression in all five adult morphometric traits (mass, skull length and width, and forearm and hind-foot length) but no evidence for it in any of the early life stages. This further suggests that the care young meerkats receive in early life may be buffering them from inbreeding depression, the effects of which are only revealed once they become independent and are not direct recipients of cooperative care. Upon reaching independence, the magnitude of inbreeding depression in mass did not appear to increase, suggesting that the main differences in contributions from the social environment were between pre- and post-independence. However, it has not escaped me that the results of Chapters 3 and 4 are somewhat contradictory. Having found evidence for inbreeding depression in pup mass and hind-foot length in Chapter 3, I had expected to find evidence for it in the same traits in my Chapter 4 analyses. As the data analysed and the fixed and random terms considered varied between the chapters, it would be beneficial to further investigate what factors are contributing to the differences in these results.

Inbred matings may offer positive effects on the inclusive fitness of parents (Kokko & Ots 2006). While the negative effects of inbreeding are generally thought to drive the evolution of inbreeding avoidance mechanisms, one alternative hypothesis is that the increased kinship within social groups that results from inbreeding may in fact promote or maintain cooperative behaviours in line with kin selection theory

(Hamilton 1963, West *et al.* 2002, Griffin & West 2003, Rossiter *et al.* 2005). While this hypothesis is currently not generally well accepted, my finding that inbred meerkats contribute more pup-feeding provides tentative evidence that inbreeding in meerkats may be at an adaptive optimum, such that it might be advantageous to have a low level of inbreeding if it indeed creates more 'unambitious', helpful offspring. Further research on whether similar relationships between inbreeding coefficients and cooperative care are found in other social animals would clearly be of great interest.

6.3 Relatedness

Kin selection dictates that individuals should preferentially direct cooperative care towards kin (Hamilton 1964a). Determining whether individuals interact based on their degree of relatedness is another additional strand of interrogation afforded by the pedigree (e.g. Madden *et al.* 2012, Madden *et al.* in review). In this thesis I found a positive relationship between the relatedness of helpers and recipient litters with regards to pup-feeding, which is in line with kin selection. While previous studies on the same population of meerkats did not provide concrete support for kin directed contributions to pup-feeding (Clutton-Brock *et al.* 2000, Clutton-Brock *et al.* 2001a), this research was conducted using a much reduced dataset than the one I present herein. The surprising, significant negative relationship between helper-recipient relatedness and baby-sitting cannot, however, be explained by kin selection. While this finding would undoubtedly benefit from further investigation, one hypothesis is that the negative association between baby-sitting and kinship is driven by 'pay-to-stay' or 'social prestige' mechanisms. Determining the factors influencing why immigrants may be more likely to baby-sit would be he next step in this line of research.

6.4 The genetic and social influences on meerkats

In Chapters 4 and 5 I used animal models to analyse a variety of morphometric traits and two helping behaviours in order to apportion the variance in these traits to genetic and non-genetic sources. The main conclusions from these analyses were that: a) morphometric and helping behaviour do, to some extent, have a genetic component to them and are thus heritable; b) the variance components of meerkat morphometric traits change with life stages which may be related changes in the social environment; and c) that the highly social nature of meerkats make separating additive genetic from social environment effects difficult within a traditional quantitative genetic framework.

I found that additive genetic effects contribute to variation in meerkat mass, skull length and width, forearm and hind-foot length and contributions to both baby-sitting and pup-feeding. Although my estimates were generally less than the averages previously found for both morphometric and behavioural traits (see reviews of Mousseau & Roff 1987, Stirling *et al.* 2002), non-zero heritability nevertheless suggests that these traits are evolvable. Despite a large amount of research on the Kalahari Meerkat Project population, few analyses have so far specifically focused on selection. Analysing the the direction and strength of selection on these traits would redress the lack of research in this area.

The low heritability estimates I report herein might be a consequence of the fact that the social environment (birth litter) explained a relatively large proportion of the variance in both morphometric and behavioural traits. A wide array of potential factors is embodied in the birth litter identifiers, including mother, territory, year and season identifiers. Birth litter, however, also contains information on the social components of the group pups are born into, including the number and identity of helpers. Due to the large number of other factors embodied in it, birth litter is best viewed as a reflection, rather than a direct measure, of the social environment. The social environment is known to be an important component of meerkat groups, where offspring are baby-sat, provisioned with milk and prey items, and taught to

forage prior to independence by older group members (Clutton-Brock *et al.* 1998b, Doolan & Macdonald 1999, Clutton-Brock *et al.* 2001b, Scantlebury *et al.* 2002, Thornton & McAuliffe 2006). Accordingly, I found that the proportion of morphometric trait variance that was explained by birth litter decreased with meerkat age, which I took to be a reflection of the decreasing importance of the social environment in determining morphometric trait values. The associated increases in heritability estimates with meerkat age are probably a consequence of the decreases in variance explained by birth litter effects. Determining whether the patterns in additive genetic and 'social' effects that I observed are also true of other cooperatively breeding species would be most interesting.

The small heritability estimates I reported may also reflect the challenges involved in accurately disentangling genetic from shared environment effects when individuals live in family groups of related individuals. Due to the highly social nature of meerkats, trait expression is probably not only determined by an individual's own genes but also by the genes expressed in its conspecifics (Moore et al. 1997, Wolf et al. 1998, Bijma et al. 2007, Bijma & Wade 2008). While I recognized that indirect genetic effects (IGE) may play an important role in determining the phenotypic expression of meerkat traits, I did not specifically model them in my analyses due to the complexities involved in such analyses and the large sample sizes required. My research herein is thus best viewed as initial quantitative genetic analyses and a preliminary attempt to decompose the variation of meerkat traits into genetic and social environmental components. Future work incorporating IGEs is recommended in order for genetic and social environmental components to be disentangled appropriately.

6.5 General conclusions

Darwin himself recognized the 'special difficulty' that cooperative societies posed to his theory of evolution by natural selection (Darwin 1859). A large and growing body of both theoretical and empirical research on cooperation over the last 150 years is evidence of the additional challenges involved in studying evolutionary processes in cooperative species. In some small way, the research that has so far been facilitated by the construction of a genetically-validated pedigree for the Kalahari Meerkat Project has contributed to a slightly greater understanding of the evolutionary genetics of one specific species of cooperative mammal. Moreover, the variety of research areas that can be further investigated by pedigree-derived data in meerkats is now extensive. Having been largely ignored to date, on the grounds that there would be inbreeding avoidance in cooperative breeders, I have shown that inbreeding is likely to be an important and fruitful area of research in cooperative breeders. Furthermore, I conclude that apportioning trait variance, especially to additive genetic effects, has especial problems in cooperative breeders, highlighting a new 'special difficulty' involved in the study of cooperative societies.

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Appendices



A1 The annotated R code script used to run the offspring MASTERBAYES model. Annotations are in bold and follow the hash symbols.

```
library(MasterBayes)  # load MasterBayes
G<-read.csv('/ # file path # /GdataMasterBayes.csv') # load genotype data (Gdata)
P<-read.csv('/ # file path # /PdataMasterBayes.csv') # load phenotype data (Pdata)
P$groupref<-as.factor(P$groupref) # convert group identifier (groupref) to a factor
G<-G[,c("id", names(G)[grep("A1$|A2$", names(G))])] # extract individual identifiers and genotypes from the
Gdata file
G<-G[which(G$id%in%P$id),] # only use genetic data for individuals that have phenotypic data
P$censusdate<-as.Date(as.character(P$censusdate), format="%d/%m/%Y") # convert monthly dates (censusdate) to a
character
P$timevar<-as.numeric(as.factor(P$censusdate)) # create column called timevar giving the censusdate as a
numeric factor
P<-P[order(P$censusdate),] # order Pdata by censusdate
bdate<-P$timevar[which(P$offspring==1)] # calculate the birth date (bdate) for each offspring record
P$bdate<-bdate[match(P$id, P$id[which(P$offspring==1)])] # create column called bdate listing the bdate of each
offspring record
P$juv<-(P$timevar-P$bdate)<6 # calculate which offspring when the difference between timevar and bdate is <6
months
P$juv[which(is.na(P$juv))]<-FALSE # give TRUE when the difference between timevar and bdate is <6 months
rownames<-c("Ssu11_12", "Ssu12_1", "Ssu13_8", "Ssu13_9", "Ssu14_14", "Ssu10_4", "Ssu7_1", "Ssu8_5", "MmAAAC",
"Mm18_1", "Mm2_10", "Mm18_2", "Mm7_5", "FCA77", "Ssu14_18", "AHT130", "FCA045", "HG810") # loci as row names
(rownames)
colnames<-c("E1","E2") # error class I and II as column names (colnames)
labels<-list(rownames,colnames) # use the rownames and colnames
error<-as.data.frame(matrix(c(rep(0.015,18), rep(0.015,18)),18, dimnames=labels)) # create a matrix called
error; both columns have 18 rows and the matrix labels are given by rownames and colnames
error[1,1]<-0.019 # replace values in 1st row and 1st column with 0.040 (Ssu11_12 error I)
error[1,2]<-0.019 # replace values in 1st row and 2nd column with 0.040 (Ssu11_12 error II)
error[3,1]<-0.040 # set the per locus error rates for the remaining loci as above…
error[3,2]<-0.040
error[4,1]<-0.040
error[4,2]<-0.040
error[5,1]<-0.025
error[5,2]<-0.025
```

```
error[6,1]<-0.017
error[6,2]<-0.017
error[7,1]<-0.014
error[7,2]<-0.014
error[8,1]<-0.009
error[8,2]<-0.009
error[9,1]<-0.014
error[9,2]<-0.014
error[10,1]<-0.011
error[10,2]<-0.011
error[11,1]<-0.011
error[11,2]<-0.011
error[12,1]<-0.007
error[12,2]<-0.007
error[13,1]<-0.030
error[13,2]<-0.030
error[14,1]<-0.009
error[14,2]<-0.009
error[15,1]<-0.016
error[15,2]<-0.016
error[16,1]<-0.009
error[16,2]<-0.009
error[17,1]<-0.013
error[17,2]<-0.013
error[18,1]<-0.010
error[18,2]<-0.010
res1<-expression(varPed(x="groupref",gender="Female",relational="OFFSPRING",restrict="==", lag=c(-3,0))) #
maternal restriction: candidate mothers must have been recorded as member of the same natal group as the
offspring in the 3 months prior to offspring birth
res2<-expression(varPed(x="id", relational="OFFSPRING",restrict="!=")) # offspring restriction: an offspring
cannot be parent to itself
res3<-expression(varPed(x="juv", restrict=FALSE)) # individuals <6 months old cannot be parent to an offspring
var1<-expression(varPed(x="dominant",gender="Male", lag=c(-3,0))) # paternal variable: dominance status of
candidate males in the 3 months prior to offspring birth as a phenotypic predictor
var2<-expression(varPed(x="dominant",gender="Female", lag=c(-3,0))) # maternal variable: dominance status of
candidate females in the 3 months prior to offspring birth as a phenotypic predictor
```

```
var3<-expression(varPed(x="gavebirth",gender="Female", lag=c(-3,0))) # maternal variable: gestational status of
candidate females in the 3 months prior to offspring birth
var4<-expression(varPed(x="roved",gender="Male", lag=c(-3,0))) # paternal variable: roving behaviour of
candidate males in the 3 months prior to offspring birth
var5<-expression(varPed(x="groupref", gender="Male", relational="OFFSPRING", lag=c(-3,0))) # paternal variable:
group membership of candidate males in the 3 months prior to offspring birth
PdP<-PdataPed(list(res1, res2, res3, var1, var2, var3, var4, var5),data=P,USsire=TRUE,USdam=TRUE) # store
phenotypic predictors in PdP and form equations using the following restrictions and variables and the Pdata

GdP<-GdataPed(G, perlocus=TRUE) # store genotypes in GdP
sP<-startPed(estG=FALSE, E1=error$E1, E2=error$E2) # use the specified per locus error rates
pP<-priorPed(beta=list(mu=c(0,0,0,0,0), sigma=diag(c(rep(1+pi^2/3,5))))) # specify this is a 6 parameter model
tP<-tunePed(beta=rep(0.5,5), USdam=0.5, USsire=0.5) # tuning in order to improve mixing of the model
m1<-MCMCped(PdP,GdP, sP=sP, pP=pP, tP=tP, write_postP="JOINT", jointP=FALSE) # build 1st model and save pedigree
after each iteration
m2<-MCMCped(PdP,GdP, sP=sP, pP=pP, write_postP="JOINT", DSapprox=TRUE, jointP=FALSE, nitt=13000*10, thin=10*5,
burnin=3000*10) # build 2nd model with the following iterations, burn-in iterations and thinning intervals
Pedm2<-modeP(m2$P, threshold=0.8) # store the parentage inferences with >=80% confidences in Pedm2
write.csv(cbind(Pedm2$P, Pedm2$prob, Pedm2$prob.male), "~/ # file path # /Parentages.csv", row.names=FALSE) #
list the parentages (>with =80% confidences) and associated probabilities in a Comma Separated Values file
```

A2 The annotated .dat file outlining the parameters used to run the offspring COLONY2 model. Annotations are in bold and follow the exclamation mark symbols.

```
1494 ! Number of offspring in the sample
18 ! Number of loci
1234 ! Seed for random number generator
0 ! 0/1=Not updating/updating allele frequency
2 ! 2/1=Dioecious/Monoecious species
0 ! 0/1=Diploid species/HaploDiploid species
0  0 ! 0/1=Polygamy/Monogamy for males & females
0 ! 0/1=with/without sibship size prior
1 ! 0/1=Unknown/Known population allele frequency
9 11 13 10 9 11 10 15 3 7 10 8 12 5 12 11 11 13 ! Number of alleles per locus
      109      111      113      115      119      121      123      125      127 ! allele sizes of locus 1
  0.13150  0.11520  0.40050  0.09490  0.11320  0.06150  0.00920  0.06940  0.00460 ! frequency of allele at
locus 1  sizes
      161      163      165      167      169      171      175      177      179      181      183 ! allele
sizes of the remaining loci as above…
  0.11010  0.00170  0.00070  0.08020  0.05890  0.00170  0.16670  0.30070  0.16870  0.10580  0.00500 ! frequency
of alleles at the remaining loci as above…
      213      217      219      221      223      225      227      229      231      233      235      237
239
  0.00100  0.00070  0.00070  0.01290  0.00070  0.00590  0.00530  0.12950  0.42110  0.04590  0.04000  0.27080
0.06570
      255      259      263      265      267      269      271      277      279      281
  0.00070  0.00100  0.00070  0.01860  0.24060  0.42410  0.30950  0.00070  0.00300  0.00130
       72       74       76       78       80       82       84       86       88
  0.00070  0.17400  0.14490  0.06590  0.03070  0.25780  0.04700  0.27420  0.00490
      120      122      124      130      132      134      135      137      140      143      147
  0.25480  0.09340  0.01060  0.34720  0.12210  0.01680  0.00330  0.00100  0.13170  0.00330  0.01580
      156      158      163      165      167      169      171      173      175      177
  0.00070  0.07100  0.00650  0.25900  0.20500  0.07720  0.02810  0.23380  0.11640  0.00230
      205      209      211      215      217      219      223      225      228      230      232      234
235      238      240
  0.04080  0.05450  0.00800  0.12270  0.01070  0.04450  0.08530  0.18230  0.12610  0.01240  0.13910  0.10300
0.02470  0.02240  0.02340
```

```
      113       117       121
  0.42560   0.47640   0.09800
      140       142       150       152       154       160       164
  0.84770   0.01150   0.00070   0.00100   0.00030   0.13660   0.00230
      170       175       177       182       184       186       188       190       192       194
  0.00200   0.00030   0.00030   0.09410   0.04540   0.47930   0.25920   0.11840   0.00070   0.00030
      251       261       265       267       269       271       273       275
  0.00030   0.00370   0.28120   0.02840   0.36120   0.10540   0.21510   0.00470
      180       182       186       192       198       203       207       209       212       215       220       223
  0.00830   0.03920   0.00070   0.00860   0.24150   0.54380   0.02650   0.01230   0.00130   0.06040   0.04780   0.00960
      110       121       123       125       127
  0.00190   0.00030   0.68860   0.30170   0.00750
      122       126       128       131       133       135       137       139       141       143       145       148
  0.00070   0.00550   0.02200   0.24570   0.44680   0.13180   0.02200   0.00030   0.00310   0.01000   0.11080   0.00140
      136       138       139       143       145       147       149       151       153       155       158
  0.00700   0.41910   0.00100   0.00280   0.22370   0.08930   0.07190   0.08720   0.05370   0.00210   0.04220
      159       161       163       165       167       169       171       173       175       179       181
  0.00070   0.04100   0.03000   0.15170   0.33100   0.06100   0.22930   0.12900   0.00070   0.01970   0.00590
      193       196       201       203       213       216       219       221       223       225       228       229
233
  0.07100   0.00270   0.41400   0.01940   0.00070   0.00440   0.00070   0.17070   0.14360   0.03180   0.08130   0.05820
0.00170

1          ! Number of runs
2          ! Length of run
1          ! 0/1=Monitor method by Iterate#/Time in second
1          ! Monitor interval in Iterate#/Time in seconds
1          ! Windows version
1          ! Full Likelihood method
2          ! Precision 1/2/3=low/medium/High

    Ssu11_12      Ssu12_1      Ssu13_8      Ssu13_9     Ssu14_14      Ssu10_4      Ssu7_1      Ssu8_5
MmAAAC      Mm18_1      Mm2_10       Mm18_2      Mm7_5       FCA77     Ssu14_18      AHT130      FCA045
HG810 ! locus names
           0           0           0           0           0           0           0           0
0           0           0           0          0           0           0           0           0          0
```

```
0.01877608  0.015447155  0.040137615  0.040302265  0.025333335  0.017054265  0.01397327  0.00930626
0.013972055
0.01075269  0.01096491  0.006640105  0.0300813  0.00909091  0.015580735  0.00866051  0.01272455  0.0102459 !
class I error at locus 1
  0.01877608  0.015447155  0.040137615  0.040302265  0.025333335  0.017054265  0.01397327  0.00930626
0.013972055  0.01075269  0.01096491  0.006640105  0.0300813  0.00909091  0.015580735  0.00866051  0.01272455
0.0102459 ! class II error at locus 1

! All offspring genotypes !

.75  .75 ! probability of true father and mother being in the candidate lists
636  557 ! number of male and female genotypes

! All candidate father and mother genotypes !
```