

BEHAVIORAL ECOLOGY OF THE STRIPED HYENA (*Hyaena hyaena*)

by

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To my family, then and now.

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TABLE OF CONTENTS

1. GENERAL INTRODUCTION.....	1
Preface & Organization.....	1
Introduction.....	3
Prior Research.....	3
Distribution.....	3
Habitat.....	4
Abundance.....	5
Adaptations.....	5
Foraging and Food.....	6
Social Behavior.....	6
Reproduction.....	7
Predators and Mortality.....	7
Evolutionary Ecology in the Hyaenidae and New <i>Hyaena</i> Study	8
Chapter References.....	16
2. ESTIMATING RELATEDNESS AND RELATIONSHIPS USING MICROSATELLITE LOCI WITH NULL ALLELES	20
Abstract.....	20
Introduction.....	21
Relationships.....	24
Relatedness (r)	29
Null alleles.....	30
Comparison of analytical methods.....	36
Conclusion.....	49
Chapter References.....	50
Appendix.....	52
3. SPATIAL GROUPING IN A BEHAVIORALLY SOLITARY CARNIVORE: SPATIAL, SOCIAL, AND GENETIC STRUCTURE OF A STRIPED HYENA POPULATION.....	55
Introduction.....	55
Permissive Conditions.....	56
Females as a Resource.....	59
Interpretation & Expectations.....	60
Promoting Conditions.....	62
Study Aims, Constraints, and Definitions.....	64

TABLE OF CONTENTS – CONTINUED

Methods.....	65
Study Area.....	65
Trapping.....	66
Animal Handling & Sample Collection.....	68
Radio Tracking & Spatial Data.....	69
Home-ranges & Space-use.....	71
Spatial Patterns of Association.....	73
Temporal Patterns of Association.....	74
Overlap in Space-Use.....	75
Genetic Analyses.....	76
Patterns of Relatedness Across Geographic Distances.....	77
Patterns of Relatedness Across Social Distances.....	79
Maternity and Paternity.....	80
Diet.....	81
Results.....	83
Trapping.....	83
Home-ranges & Space-Use.....	86
Spatial & Temporal Patterns of Association.....	91
Overlap in Space-use.....	95
Patterns of Relatedness Across Geographic Distances.....	97
Patterns of Relatedness Across Social Distances.....	101
Maternity and Paternity.....	108
Diet.....	111
Discussion.....	118
Spatial Grouping & Behavioral Isolation.....	118
Foraging & Feeding.....	118
Paternity.....	121
Coalitions.....	122
Females as a Resource.....	124
Relatedness Across Social Distances.....	127
Female Tolerance of Multiple Males.....	131
Resources and Group Formation.....	134
Conclusion.....	135
Chapter References.....	139

TABLE OF CONTENTS – CONTINUED

4. TRANSIENT GENITAL ABNORMALITIES IN STRIPED HYENAS.....	148
Abstract.....	148
Introduction.....	148
Methods.....	150
Results	151
Discussion.....	156
Chapter References.....	159
5. CONCLUSIONS.....	162
Striped Hyena Ecology and Species Account.....	162
Taxonomy	162
Description.....	162
Geographic Variation.....	164
Similar Species.....	164
Distribution.....	165
Habitat.....	166
Abundance.....	166
Adaptations.....	167
Foraging and Food.....	169
Social and Reproductive Behaviour.....	172
Reproduction and Population Structure.....	174
Predators, Parasites and Diseases.....	176
Conservation.....	176
Chapter References.....	179

LIST OF FIGURES

Figure		Page
1.1.	Global Distribution of <i>Hyaena hyaena</i>	4
1.2.	Postulated Evolutionary Development of <i>Crocuta</i> and <i>Hyaena</i>	14
2.1.	Probability of False Exclusion of Parent-Offspring Relationships by Competing Methods.....	45
2.2.	Probability of Large Errors in Relatedness Estimation by Competing Methods.....	46
3.1.	Trapping Effort and Capture Success.....	84
3.2.	Locations of Capture by Age and Sex.....	85
3.3.	Variation in Home-range Size Estimates from Simulations.....	87
3.4.	Plot of Areas of Use for Radio-collared Hyenas.....	89
3.5.	Plot of Hyena Home-ranges.....	90
3.6.	Point Locations and Use of Common Areas by Hyenas within Spatial Groups.....	92
3.7.	Degree of Relatedness Over Geographic Distance.....	98
3.8.	Degree of Relatedness Over Social Distance.....	103
3.9.	Predicted vs Actual Number of Territorial Overlaps.....	104
3.10.	Plotted Space-use Polygons and Harmonic Centers for Individuals Within and Across Spatial Groups.....	105
3.11.	Proportions of Individuals in Same, Adjacent, or Non-adjacent Groups that are Related.....	106
4.1.	Genital Characteristics of Juvenile <i>Hyaena</i>	152
4.2.	Testosterone Concentrations by Age*Sex Class.....	154

LIST OF TABLES

Table	Page
2.1. k -Coefficients for Relationships.....	28
2.2. Probabilities of Observable Genotypes.....	29
2.3. Probabilities of all Possible Genotypes when Null Alleles Occur.....	35
2.4. Observed Microsatellite Genotypes in a Putative <i>Hyaena</i> Family.....	37
2.5. Estimates of Relationship and Relatedness in a Population Subset by Competing Methods.....	38
2.6. Measured Error in Relatedness Estimation Due to Null Alleles for Competing Methods.....	43
2.7. Accuracy in Relationship Identification Due to Null Alleles for Competing Methods.....	44
2.8. Accuracy of Relationship and Relatedness Estimation in the Absence of Null Alleles.....	48
2.A. Calculation of Genotype Probabilities Given Multiple (non-IBD) Null Alleles.....	54
3.1. <i>Hyaena</i> Captures, Recaptures, and Losses by Year.....	86
3.2. Total Number of Individual <i>Hyaena</i> Captured by Age at First Capture.....	86
3.3. Fixed-Kernel Home-range Size Estimates.....	91
3.4. Levels of Association within Spatial Groups.....	93
3.5. Comparison of Levels of Association by Dyad Type from simulations.....	95
3.6. Overlap in 50% fixed-Kernel Home-ranges.....	96
3.7. Overlap in 95% fixed-Kernel Home-ranges.....	96
3.8. Test of Geographic Distance Effect on Relatedness.....	100

LIST OF TABLES – CONTINUED

Table	Page
3.9. Test of Social Distance Effect on Relatedness.....	107
3.10. Maternity of Offspring.....	109
3.11. Paternity of Offspring.....	110
3.12. Diet as Determined by Hair and Bone Identification from Feces.....	114
3.13. Average Minimum Number of Individuals and Species Represented in Fecal Samples.....	115
3.14. Composition of Den-bone Collections Compared to Fecal Hairs Identified in Samples from the Same Site.....	115
3.15. Reference Hairs Used in Diet Analysis.....	116
4.1. Circulating Testosterone Concentrations in Adult Male and Female Mammals.....	155

LIST OF TEXT BOXES

Text Box	Page
1.1. Definition of terms.....	15
3.1. Definition of terms.....	136
3.2. Hypotheses of group formation: promoting and permitting factors.....	138

GENERAL INTRODUCTION

Preface & Organization

The ecology of the striped hyena (*Hyaena hyaena*) is little understood and has only marginally been investigated. This study was originally designed, in part, to fill in ‘gaps’ in our understanding of the social ecology (defined in Box 1.1) of the species and provide a better understanding of the evolution of social organization (Box 1.1) in the Hyaena family as a whole. Unexpected discoveries (transient masculinization) and methodological issues that arose in the course of the project modified this basic plan. To accommodate the original plan and the issues that were incorporated as my research proceeded, my dissertation is broken into five main sections: prior research on this species, analytical methods for analyzing microsatellite data containing null alleles, species’ diet, social, and spatial structure (Box 1.1), genital morphology, and a comprehensive species account.

The remainder of this first chapter contains two parts: a broad review of pre-existing ecological and behavioral data on this species and a discussion of the connections between the later chapters. Chapter 2 is an expansive discussion of genetic methods developed specifically to address analytical problems raised by the data collected in this study, namely the presence of null alleles in microsatellite data. This problem is common in other studies, but was not adequately addressed by the analytical methods currently in use. Chapter 3 then describes the social, genetic, and spatial structure of the study population (Box 1.1), and relates them to one another to evaluate

several broad hypotheses about the evolution of social organization, particularly in carnivores. The analytical methods developed in Chapter 2 were applied in Chapter 3, but were too detailed to include solely within the methods section of the latter. Chapter 4 evaluates theories regarding the evolution of genital masculinization in the spotted hyena, *Crocuta crocuta*, in light of new information on the genital morphology and ecology of the striped hyena. I found that genital appearance in juvenile striped hyena is transiently masculinized, in females, and feminized, in males, and the existence of these traits does not seem to support evolutionary models of masculinization in *Crocuta* which rely on masculinization originating within that species. Chapter 5 is a synthetic review of the biology of the striped hyena. This final chapter integrates all of the information collected in every preceding section. Chapter 5 stands, as the title of this dissertation reflects, as the most comprehensive description available of the characteristics and ecology of this species.

Chapters 2 through 5 each represent published or submitted (or soon to be submitted) manuscripts that have been adapted for this format. Each chapter draws from the data presented and conclusions drawn in previous chapters. For example, genital morphology, discussed in Chapter 4, is considered in the context of striped hyena social ecology, which is established in Chapter 3, and the genetic analyses in Chapter 3 utilize the analytical methods established in Chapter 2.

Given the limited degree to which striped hyenas are known and understood and the wide range of ecological traits relevant to questions addressed in later chapters, the

first part of the following Introduction is a brief summation of the previously available information on the species.

Introduction

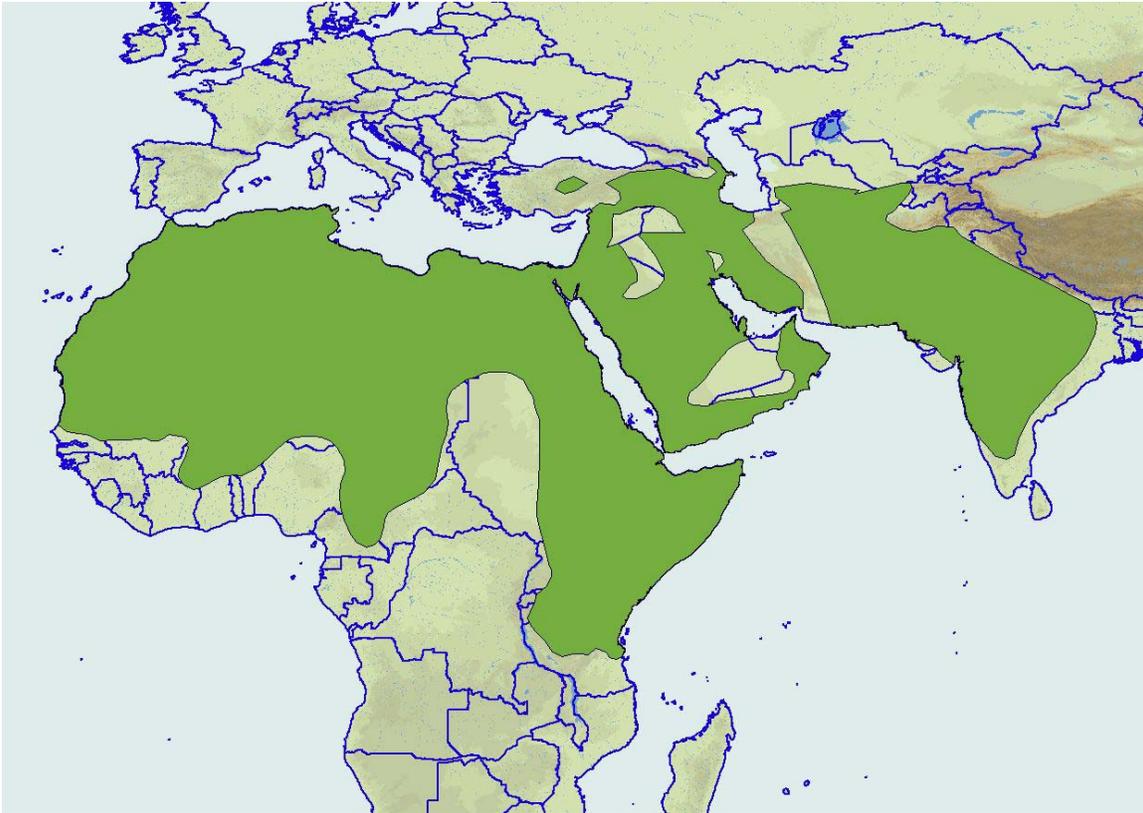
Prior Research

Although reviewed in multiple publications, there have been only two previous studies of striped hyenas in Africa: the first was a study by Kruuk (1976) in the Serengeti and the second by Leakey *et al.* (1999) in northern Kenya. Outside of the continent, studies have been conducted in Israel (Macdonald 1978, Bouskila 1984, Ilani 1975, Kerbis-Peterhans and Horwitz 1992, Skinner and Ilani 1979), India (Davidar 1990), and in captivity (Rieger 1978, 1979a & 1979b). Because all of these studies were brief and relatively informal, there is very little information available on the species ecology, behaviour and social organization, and much of what is available is anecdotal.

Distribution

The current distribution of the striped hyena was reviewed extensively by Hofer & Mills (1998). Broadly, the striped hyena has a very large range extending from East and North-east Africa, through the Middle East, Caucasus region, Central Asia, and into the Indian subcontinent (Fig. 1.1).

Figure 1.1. Global distribution of the striped hyena adapted from survey results in Hofer & Mills (1998).



Habitat

Striped hyenas generally favor arid to semi-arid environments (Prater 1948, Kruuk 1976, Rieger 1978, Leakey 1999) where water is available within 10 km (Rieger 1979a), appear to avoid open desert and dense thickets and forests (Rieger 1979a, Heptner and Sludskii, 1980), and have only been found below 3,300 m (Roberts 1977, Rieger 1979a). Striped hyenas generally favour and will consistently revisit larger caves for resting (Kruuk 1976, Rieger 1979a, Leakey 1999).

Abundance

Throughout the species' range, striped hyenas occur at low densities. The only quantitative estimate of striped hyena density in Africa comes from the Serengeti National Park, Tanzania where, based on observations of a limited number of individuals, density was estimated as greater than 0.02 per km² (Kruuk 1976). For comparison, spotted hyenas in the same ecosystem have been estimated to exceed 1 individual per km², and 0.02/km² is substantially lower than the densities of spotted hyenas, lions in most ecosystems, and even lower than the density of endangered African wild dogs in some ecosystems (Creel & Creel 1996).

The total African population estimate of 2,450 to 7,850 individuals (Hofer & Mills 1998) represents roughly half of the total worldwide estimated population. Only Egypt and Kenya have estimated populations over 1,000 (accounting for 51% of the maximum African population estimate and 82% of the minimum estimate).

Adaptations

The high sagittal crest of the skull increases the area of origin for the temporal muscles and the well developed masticatory muscles facilitate seizing and crushing of prey (Buckland-Wright 1969).

Ducts from the anal glands open into an anal pouch dorsal to the anus. The pouch is inverted during scent marking (pasting) and greeting behaviour (Brehm 1927, Holzapfel 1939, Fox 1971, Kruuk 1976, Rieger 1977, 1978, 1981). It is not known if pasting is used to mark territories.

Where studied, there is no sexual dimorphism in body measurements and weight (Mendelssohn and Yom-Tov 1988, Kruuk 1976).

Foraging and Food

Striped hyenas have been reported to consume a wide variety of vertebrates, invertebrates, vegetables, fruit, and human originated organic wastes (Flower 1932, Novikov 1962, Harrison 1968, Ilani 1975, Kruuk 1976, Macdonald 1978, Leakey 1999) and this limited data has led to the interpretation that striped hyenas are essentially omnivorous scavenger.

In Israel, groups of hyenas converge at feeding sites (Kruuk 1976, Macdonald 1978, Bouskila 1984), but relatedness of observed groups has not been investigated. Foraging activity in Tanzania was restricted entirely to night-time (Kruuk 1976). Striped hyenas have also been described as raiding human grave sites and carrying away bones (Horwitz 1988, Leakey 1999), and fruit and vegetable crop raiding is considered a serious problem in Israel (Kruuk 1976).

Social Behavior

Striped hyenas have been considered solitary, though my observations (Chapter 3) do not support this conventional wisdom. The only home range size estimates in Africa of 44 and 72 km² come from the Serengeti for one male and one female, respectively (Kruuk 1976). Very little has been recorded regarding direct social interactions outside of captive situations. Kruuk (1976) noted that the meeting ceremony between greeting pairs involved mutual sniffing of the face, neck, and anal regions. During these

encounters, the anal pouch was protruded during sniffing and either both hyenas were standing or one would lie down while exposing the anal region.

Reproduction

Gestation period is 90-91 days and there is no apparent seasonal pattern (Pocock 1941, Ronnefeld 1969, Heptner & Sludskii 1980). Litter sizes in the captivity range from 1-5 cubs (Rieger 1981). Weaning in captivity takes place after eight weeks. Sexual maturity is reached at 2-3 years.

Striped hyena cubs are reared in dens and intense digging behaviour in the females announces parturition (Rieger 1979a). Dens may be holes dug by the mother, holes formed and abandoned by other species (Prater 1948, Roberts 1977) or deep, natural, and sometimes complex, caves (Heptner & Sludskii 1980, Kerbis-Peterhans *et al.* 1992, Leakey *et al.* 1999). Mothers carry food back to the den for their cubs (Kruuk 1976, Davidar 1985, Davidar 1990) and prepare meat for cubs by biting off pieces (Rieger 1979a).

Predators and Mortality

Humans are consistently indicated as the major source of mortality throughout the evaluated range (Hofer 1998). The striped hyena is considered subordinate to lions, *Panthera leo*, and spotted hyenas, *Crocuta crocuta*, although Kruuk (1976) described a mutual 'attraction' between the two Hyaenids.

Evolutionary Ecology in the Hyaenidae and New *Hyaena* Study

The aardwolf, *Proteles cristatus*, is a highly specialized forager on termites that lives in socially monogamous, territorial pairs with only their most recent dependent offspring (Richardson 1987, Richardson & Coetzee 1988). Social pairs cooperate in raising young, but females commonly mate outside the pair-bond with neighboring males. Foraging in aardwolves is concentrated in time, as they eat a large number of termites in a very short interval (Gittleman & Harvey 1982). The aardwolf's diet is thought to constrain the evolution of social groups (Mills 1989).

Brown hyenas live in small, female-bonded social groups that share and defend a common territory and den (Owens & Owens 1979a & 1979b, Mills 1978 & 1989). Brown hyenas are well adapted to utilizing varied and sparse resources. They feed on carcasses and small prey which tend to be rare, widely dispersed, and provide food for only one individual (Owens & Owens 1978, Mills 1989 & 1990, Frank 1996). Because of their diet, foraging is primarily solitary, they do not cooperate in killing large prey, and there is no apparent benefit to foraging in groups. In brown hyenas, 33% of adult males become permanently nomadic and these males father the majority of cubs (Mills 1982). Resident, non-breeders of both sexes care for young at communal den sites, adults provision cubs other than their own offspring, and mothers occasionally suckle the cubs of other females (Owens & Owens 1979a & 1979b). The solitary foraging behavior in brown hyenas may have constrained the development of larger groups and the rank-related bias in reproductive success typical of many social carnivores (Mills 1983 & 1989).

The spotted hyena is a communal hunter and scavenger of large mammals that lives in matrilineal, territorial social groups of up to one hundred individuals (Kruuk 1972), and is the only hyaena in which females are dominant over males. Spotted hyena females stay in their natal 'clan' for life and form the stable core of the social group (Frank 1996). Immigrant males father the majority of offspring in spotted hyena clans, there is a dominance hierarchy among males, and reproductive success of males is positively correlated with social rank and clan tenure (Mills 1989 & 1990, Frank *et al.* 1995, Engh *et al.* 2002). Reproductive success is also linked to rank in females, but in a manner unusual for carnivores, because younger females are dominant to older members of the same lineage, as in some primates (Frank *et al.* 1995). Across matrilineal groups, age does not predict dominance (all of the descendants of the alpha female are dominant to all of the females in other lineages). Spotted hyenas do not suckle cubs of other females and do not provision the cubs of others at dens (Mills 1989). *Crocuta* lactate for more than a year, in comparison to lactation periods of a few weeks to a few months in most carnivores. Prolonged maternal suckling of offspring has been interpreted as either a constraint on, or effect of, intense competition for feeding access at carcasses (van Jaarsveld 1993). Spotted hyenas specialize in feeding on relatively large prey items that provide enough food for more than one individual and the benefits of cooperative foraging (being greater than the costs of feeding competition) are considered to be the initial selective pressures favoring group formation in the species (Frank 1996, Van Horn *et al.* 2004).

Consequently, within this small family, there is one species with complete behavioral sex-role reversal that lives in the largest social groups of any carnivore, one species with both group-living and nomadic males of which only nomadic males reproduce, and one species with a very specialized diet that lives in social pairs (Box 1.1), but this pairing does not produce a monogamous mating system as might be expected. In contrast to what is known about these species, the most complete study of striped hyena ecology was compiled by Kruuk (1976) from admittedly “scanty” observations recorded over a one-year period, so little is known about the ecology and behavior of this last species of hyena.

Despite the lack of information on the ecology of the striped hyena, the potential significance of collecting such information has not gone unnoted and researchers (e.g. Mills 1989, Mills & Hofer 1998) have long recognized the potential value of a more thorough description of the species’ ecology. Seminal studies by Kruuk (1976), Mills (1990), and MacDonald (1978) related carnivore social organization to the distribution of resources, drawing on inter-specific comparisons between the well-studied brown and spotted hyenas. More broadly, these authors argue that, because social organization spans a broad range within this small family, the different social structures and foraging behaviors of hyenids probably result from the different characteristics of their food resources, so the Hyaenidae provide a good test case for hypotheses about relationships between social evolution and resource use (Kruuk 1976, Mills 1990). Striped hyenas were believed to be strictly solitary foragers, brown hyenas live in groups but hunt alone, spotted hyenas live and hunt in clans, and aardwolves live in pairs but forage alone.

These differences in social foraging among hyenas should reflect adaptations of social structure to the variation of resources, as well as any benefits or costs of group hunting and living.

We developed a three-year study of striped hyenas, collecting data on their social population structure, diet and foraging behavior, and genetic population structure. Describing the striped hyena social and foraging systems adequately required very broad investigations, much of which were carried out through analysis of radio-tracking data and identification of prey remains from collected fecal samples. We also sought to describe the population in terms of the degree of genetic relatedness between individuals that were spatially associated and individuals separated by distances. These investigations were developed to elucidate if and how individuals distribute themselves in relation to kin. However, to carry out the analytical portion of this investigation required developing some new analytical techniques.

Briefly, the first step to describe the degree of genetic relatedness between individuals (or the specific relationship between them, e.g. father-son, sibling, etc) requires accurate genotypes for the individuals being considered. Microsatellite loci are commonly used for this purpose. To determine microsatellite genotypes one must develop homologous primers (those designed specifically for the species they are applied to) or apply heterologous primers (those designed for other, typically closely related, species). The use of heterologous primers is common in studies that relate genetic data to behavior and ecology. No primers have been developed for striped hyenas, so we evaluated each of 24 available primers developed for *Crocuta* for use in *Hyaena*. Of

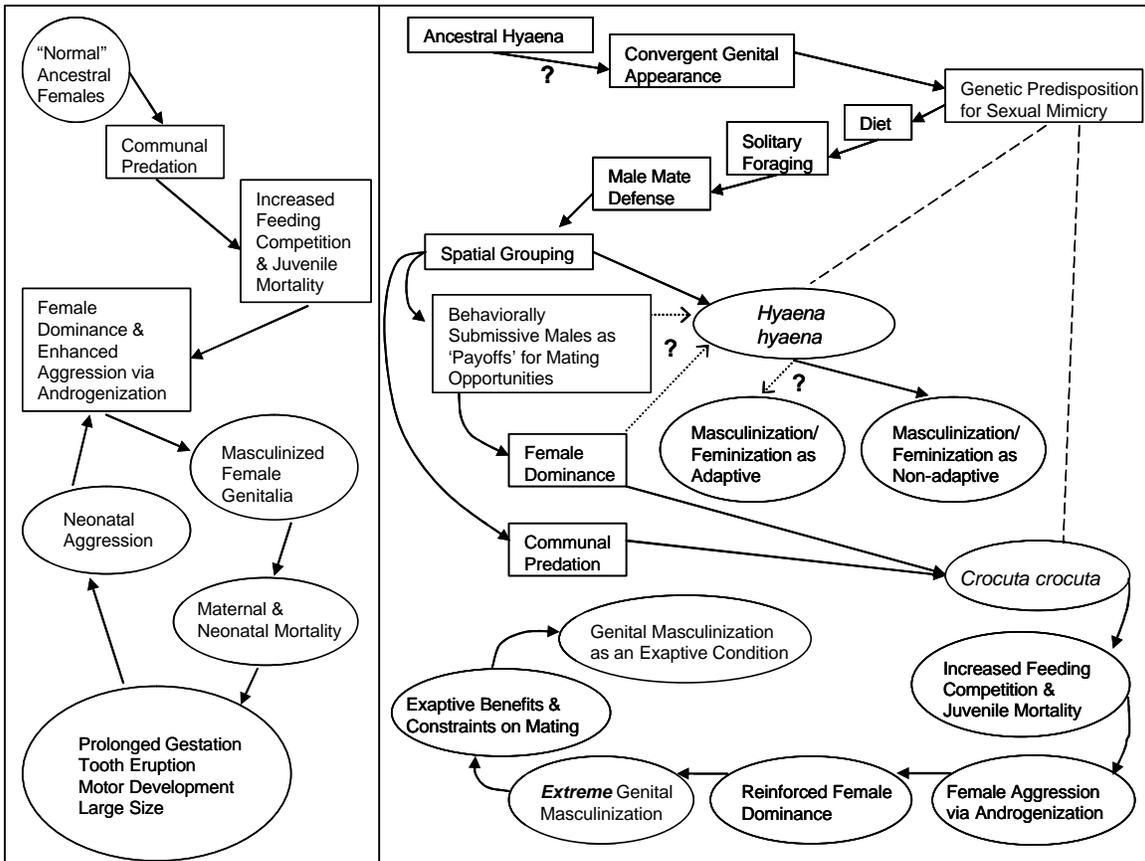
those, we were successful in applying eight in *Hyaena*. However, three of these resulted in null alleles, alleles that fail to amplify during PCR, at the targeted loci. When null alleles occur, any genotype observed as a homozygote may, in fact, contain one observable allele and one null allele and the genotypes observed may not be accurate representations of the true genotypes. When null alleles occur, researchers may either choose to ignore the problem, drop the affected loci from consideration, or redesign and optimize the primers to eliminate null alleles. All of these options are commonly adopted (Dakin & Avise 2004). The latter solution requires significant additional time and expense and is often, as it was in this study, an impractical solution. The first option may create errors (for instance, falsely excluding parent-offspring relationships) while the second option reduces the power to discriminate between competing relationships or to accurately estimate relatedness. To circumvent these short-comings, we developed a statistical approach that accounts for the possibility of a null allele occurring within any observed genotype and, effectively, allows for evaluation of relationship and relatedness probabilities even if null alleles occur. After demonstrating that this approach works well, we applied the method in our evaluation of the genetic structure of striped hyena populations.

A description of the striped hyena social system is also of interest because it is relevant to investigation of the evolutionary origins of masculinization in female spotted hyenas. We discovered, much to our surprise, that there is a marked convergence in the appearance of genitalia in juveniles of both sexes of *Hyaena*. Explanations for the evolution of masculinization in *Crocuta* have consistently relied on the unusual aspects of

the social ecology of that species to explain the evolution of these morphological traits. However, given the descriptions of the social ecology of *Hyaena* that we present here, none of the explanations developed for *Crocuta* can be applied to *Hyaena*, without significant modification. Consequently, the discovery of transient genital anomalies in *Hyaena* should lead researchers to reevaluate hypotheses for the evolution of masculinization in *Crocuta* and these evaluations should consider the ecological characteristics of both species (Fig. 1.2).

This study allowed for developing a much better understanding of striped hyena ecology and the application of the study findings linked analytical methods for relationship and relatedness estimation to descriptions of the *Hyaena* diet and social and genetic population structure to evolutionary ideas about sociality in carnivores to the evolution of genital masculinization and female dominance in *Crocuta* (Fig. 1.2). Although this diversity may superficially appear somewhat disjoint, it simply demonstrates the inherent inter-dependence of these sub-disciplines of ecology.

Figure 1.2. Postulated evolutionary development of female masculinization and dominance as proposed by, and adapted from, Frank (1996) (left panel) and after modifications suggested by findings from striped hyenas (right panel) (discussed in Chapters 3 & 4). The ultimate cause of masculinization / feminization in the ancestral hyena, the final links from the ancestral hyena to *Hyaena*, and the function, if any, of convergent juvenile genital appearance in striped hyenas are unclear. These uncertain links are indicated by question marks in the figure. However, evidence from striped hyenas does suggest a path by which female dominance and female aggression in *Crocota* could have developed independently, and that genital masculinization need not have originated in the species, but likely became more “extreme” in *Crocota*. Evidence of behaviorally submissive males and/or female dominance in striped hyenas would clarify the ancestral links (indicated by dotted lines). Evidence of infanticide would clarify the function of convergent genital appearance in striped hyenas as adaptive (indicated by dotted line). In the absence of that evidence, the most supported links are as shown (indicated by solid lines). In addition, the expression of these unusual genital characteristics in *Hyaena* and *Crocota* may represent expression of preadaptations for unusual genital development (indicated by dashed lines).



Text Boxes

Box 1.1. Definition of terms, as used.*

genetic structure	the way in which genetic relatedness varies within a population and across space
mating system	1) the way in which individuals obtain mates (including number of mates, promiscuousness), 2) the characteristics of the mating pair (incl. relatedness, where they typically range outside of breeding periods), and 3) patterns of parental care (incl. degree and means of parental involvement)
population structure	the composition of a population (incl. size, age structure, sex composition)
social ecology	the relationships between individuals, social groups, and their environments (incl. social systems, social organization, mating systems, population structures)
social organization	the way in which the components of a population are organized in space and time in relation to one another
social pair	individuals that consistently remain spatially (and temporally) associated; differentiated from mating pair by lack of copulation
social structure	the composition of a population (e.g. population structure) and the way in which that population distributes and arranges itself across space, time, and scales (e.g. groups, neighbors, non-neighbors)
social system	categorized descriptors of social structures (e.g. solitary, cooperative); may encompass mating systems

*Other definitions provided as needed within each chapter and in Chapter 3, Box 3.1.

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ESTIMATING RELATEDNESS AND RELATIONSHIPS USING MICROSATELLITE LOCI WITH NULL ALLELES

Abstract

Relatedness is often estimated from microsatellite genotypes that include null alleles (Dakin & Avise 2004). When null alleles are present, observed genotypes represent one of several possible true genotypes. If null alleles are detected, but analyses do not adjust for their presence (i.e., observed genotypes are treated as true genotypes), then estimates of relatedness and relationship can be incorrect. The number of loci available in many wildlife studies is limited, and loci with null alleles are commonly a large proportion of data that cannot be discarded without substantial loss of power. To resolve this problem, we present a new approach for estimating relatedness and relationships from data sets that include null alleles. Once it is recognized that the probability of the observed genotypes is dependent on the probabilities of a limited number of possible true genotypes, the required adjustments are straightforward. The concept can be applied to any existing estimators of relatedness and relationships. We review established maximum likelihood estimators and apply the correction in that setting. In an application of the corrected method to data from striped hyenas, we demonstrate that correcting for the presence of null alleles affect results substantially. Finally, we use simulated data to confirm that this method works better than two common approaches, namely ignoring the presence of null alleles or discarding affected loci.

Introduction

Microsatellite genotypes are useful for estimating the relationship and relatedness between individuals of unknown ancestry. Current relationship/relatedness estimators either assume that genotypes are error-free (Thompson 1991) or that genotyping error is rare (Boehnke & Cox 1997; Marshall *et al.* 1998). Genotype data, however, often do contain errors, resulting in discrepancies between the *observed* individual genotypes and the *true* underlying genotypes (Dakin & Avise 2004).

A significant source of such genotyping errors that is not accounted for in current methods for estimating relationship/relatedness is the occurrence of null alleles—alleles that fail to amplify during PCR, often due to mutation within a primer site. Null alleles cause two types of genotyping problems. First, if an individual is homozygous for a null allele (nn , where n is a null allele), genotyping will fail. Second, if an individual is a heterozygote with one null allele (in , where i is an ordinary non-null allele), the observed genotype will be indistinguishable from a true homozygote (ii).

Null alleles complicate the interpretation of all data on coancestry, but the problem is most apparent in parentage analysis (Blouin 2003). Null alleles may eliminate potential parents as possible candidates, even when they occur at very low frequencies. Parents and offspring must share one identical allele at every locus. If the observed genotypes at one locus show no identical alleles between a potential parent and offspring, the probability of the parent-offspring relationship is zero (Blouin 2003), regardless of the number of loci considered. For example, a candidate parent with the observed genotype ii is excluded as the parent of an offspring with the observed genotype jj . If

there is a null allele at this locus, however, the parent and offspring may share a null allele: true genotypes could be *in* for the parent, and *jn* for the offspring (Paetkau & Strobeck 1995). These genotypes are consistent with a parent-offspring relationship, so measuring the frequency of null alleles and taking them into account is clearly necessary to avoid false exclusion of a parent in cases such as this. This problem also affects estimates of relatedness where including genotypes with null alleles may cause underestimation of the coefficient of relatedness between individuals.

The occurrence of null alleles is widely acknowledged and many papers report the results of diagnostic tests for the presence of null alleles (Dakin & Avise 2004), but options for dealing with null alleles are limited. When null alleles are detected, researchers may eliminate them by redesigning the primer for the affected locus or circumvent the problem by developing new primers for alternate loci that do not contain null alleles. However, these solutions involve additional time and expense, and are not readily available to many investigators who seek to apply microsatellite data to questions in behavioral ecology and conservation biology. Dakin & Avise (2004) summarized 233 studies that detected null alleles in microsatellite data, often at frequencies up to 0.25 (and occasionally as high as 0.70 – 0.75). Through simulations, they demonstrated that dropping loci with null alleles is better than including them in analyses and recommended that strategy. However, they did not consider the overall number of loci available. A large number of loci may be required to differentiate between relationship categories or to accurately estimate relatedness (Queller *et al.* 1993; Blouin *et al.* 1996; Sancristobal & Chevalet 1997; Milligan 2003), but wildlife biologists are often restricted in the number

of loci by the availability of pre-existing primers (Blouin 2003). Dropping data from problem loci may then prove an impractical option as any omission of loci would substantially reduce inferential and discriminatory power (Marshall 1998).

Consequently, many studies have simply included loci with null alleles in their analyses (Dakin & Avise 2004) without explicitly considering the consequences.

A better option for correcting for errors caused by null alleles would be to accommodate them in data analysis (Sobel *et al.* 2002). In this paper, we account for null alleles by modifying well-established maximum likelihood approaches for estimating relationship and relatedness (r) (Thompson 199; Marshall *et al.* 1998; Blouin 2003). We account for null alleles by distinguishing between an observed genotype and the set of true genotypes that may have produced that observation. We determine the probability of observing the genotype pair ii / ii , for example, as the sum of the probabilities that the true genotypes are ii / ii , in / ii , ii / in , or in / in —the four true genotypes that would be observed as ii / ii . In addition to describing these calculations in detail, we use microsatellite genotypes from striped hyenas (*Hyena hyaena*) to show that ignoring null alleles can have a substantial impact on estimation of relatedness and inferences concerning population biology. Finally, we use a set of simulations to demonstrate that this technique provides more accurate results than the methods most commonly used in recent papers, while utilizing all available data.

Relationships

Before showing how maximum likelihood estimators of relationship and relatedness are derived for loci with null alleles, we review the maximum likelihood formulae for estimating genealogical relationships and relatedness from genotypic data not affected by null alleles. We begin with estimating relationship.

In practice, estimating relationship usually means identifying the most likely of a small set of potential relationships that might exist between a pair of individuals, e.g. parent-offspring, full-siblings, half-siblings, or unrelated. If R represents a potential relationship between individuals and G_1 / G_2 represents the pair of genotypes observed at a homologous locus in two individuals, by definition, the likelihood of R , $L(R)$, is the probability of observing G_1 / G_2 in two individuals having the relationship R . These probabilities have been described previously (Thompson 1991), but they are subtly complex and are essential to understand our estimators—so we present their derivation in detail.

The probability of observing G_1 / G_2 in two individuals having relationship R is calculated by conditioning on the number of alleles in the pair that are identical by descent (IBD) (Cotterman 1941; Thompson 1975, 1991). Every pair of individuals will have 0, 1, or 2 alleles IBD at each locus. The probability of observing genotypes G_1 / G_2 in a pair of individuals is equal to the probability of observing G_1 / G_2 if there are zero alleles identical by descent, plus the probability of observing G_1 / G_2 if one allele is IBD, plus the probability of observing G_1 / G_2 if two alleles are identical by descent. This

approach works because the probability that a pair of individuals has either 0, 1, or 2 alleles IBD is determined by the genealogical relationship between the individuals. Let m represent the number of alleles IBD between individuals and let k_m represent the probability that the individuals with genealogical relationship R have m alleles IBD (Table 2.1 lists k_m values for relationships commonly of interest; Cotterman 1941; Thompson 1975).

If, for example, two individuals are unrelated, all loci within the pair of individuals will have no alleles IBD ($k_0 = 1, k_1 = 0, k_2 = 0$). If two individuals are parent-offspring, all loci will share one allele IBD ($k_0 = 0, k_1 = 1, k_2 = 0$). And if two individuals are full-siblings, loci may share 0, 1, or 2 alleles IBD ($k_0 = 0.25, k_1 = 0.5, k_2 = 0.25$). Where $k_0, k_1,$ and k_2 are the k -coefficients for the relationship R , the probability of observing G_1 / G_2 , given R , is calculated by:

$$P(G_1 / G_2 | k_0, k_1, k_2) = P(G_1 / G_2 | m = 0)k_0 + P(G_1 / G_2 | m = 1)k_1 + P(G_1 / G_2 | m = 2)k_2 \quad (1)$$

All the terms on the right hand side of Equation 1 are straightforward to calculate (e.g. Thompson 1991). Three of these depend on the genealogical relationship between the individuals— $k_0, k_1,$ and k_2 . The remaining probabilities in Equation 1 [$P(G_1 / G_2 | m = 0), P(G_1 / G_2 | m = 1), P(G_1 / G_2 | m = 2)$] depend on the genotypes in the individuals and are calculated from the allele frequencies in the population. Expressions for $P(G_1 / G_2 | m)$ are provided in Table 2.2 for all possible genotype pairs, assuming no

inbreeding and no null alleles (Thompson 1975). These probabilities have been presented in two basic forms: one in which the individuals are ordered and one in which they are not ordered (i.e., G_1/G_2 is not distinct from G_2/G_1). Either approach is valid, but the approach used affects the probabilities and it is necessary to be consistent. Here we use the ordered approach for individuals, although the positions of alleles within individuals remain unordered.

The derivations of the probabilities in Table 2.2 differ according to the number of alleles IBD. If $m=0$, the two genotypes being considered are independent, so that the probability of obtaining the pair of genotypes is simply the product of obtaining each of the two individual genotypes:

$$P(G_1 / G_2 | m = 0) = P(G_1)P(G_2). \quad (2a)$$

If $m=2$, the two genotypes are identical and therefore completely dependent, so that the probability of obtaining the genotypes is the probability of obtaining either genotype once:

$$P(G_1 / G_2 | m = 2) = P(G_1) = P(G_2) \quad (2b)$$

Determining the probability of obtaining the observed genotypes under $m=1$ is more difficult and is best explained by example. The most complex situation occurs when $m=1$ and both individuals are homozygous for the same allele (G_1 and $G_2 = ii$).

Let p_i indicate the frequency of allele i in the population. For $m=1$, the probability of the individuals having the pair of genotypes ii / ii is given by:

$$\begin{aligned} P(G_1 = ii / G_2 = ii | m=1) &= P(G_1 = ii)[P(G_2 = ii | G_1 = ii / m=1)] \\ &= p_i^2 [p_i \cdot \frac{1}{2}(1) + \frac{1}{2}(1)p_i] = p_i^3 \end{aligned} \quad (2c)$$

In equation 2c, the probability of the first ii genotype is calculated directly from allele frequencies, but the probability of obtaining a second ii genotype must then take into account that one allele is IBD to an allele in the first individual. Thus, the probability for the second individual's genotype is the product of the probability of the second individual having one i allele (p_i) and, for the second allele in the second individual, the probability (=1) that the IBD allele is an i , and the probability (=1/2) that IBD allele is in the second position. This is the first term within square brackets. The second term within brackets accounts for the alternative possibility that the IBD allele is in the first position. Probabilities are calculated for the IBD allele being in each of the two possible positions in the second individual and then summed, giving $p_i^2 p_i = p_i^3$ (Table 2.2).

Using the same approach, the probability of two individuals having the pair of genotypes ij / ik when $m = 1$ would then consider the probability of getting an i and j in the first individual in either configuration ($p_i p_j + p_j p_i = 2p_i p_j$). Probabilities for the second individual are dependant on the probability of having a k allele ($=p_k$) and there being one allele IBD with the first individual: the probability that the IBD allele is an i is $1/2$, given

that an i or j could be IBD, while the probability of that IBD allele being in either the first or second position is $1/2$:

$$\begin{aligned} P(G_1 = ij / G_2 = ik \mid m = 1) &= P(G_1 = ij)[P(G_2 = ik \mid G_1 = ij / m = 1)] \\ &= 2p_i p_j [\frac{1}{2} \frac{1}{2} * p_k] + 2p_i p_j [p_k * \frac{1}{2} \frac{1}{2}] = p_i p_j p_k \end{aligned} \quad (2d)$$

Similar logic can be used to determine the remaining seven probabilities for $m = 1$ in Table 2.2, of which four have zero probability because a pair of genotypes with no alleles in common cannot have one allele IBD, given that we are not (yet) allowing for null alleles in ‘observed’ genotypes.

Once the probabilities of Table 2.2 are defined, relationships are evaluated using Equation 1, so that the likelihood of the genotypic data is calculated for each candidate relationship. The values for $P(G_1 / G_2 \mid k_0, k_1, k_2)$ are multiplied across loci to yield the likelihood of the relationship, $L(R)$. By definition, the maximum likelihood relationship between two individuals is the relationship for which the observed data is most probable.

Table 2.1. A list of k -coefficients for common relationship categories. k_m represents the probability that two individuals share m alleles IBD under a given relationship.

	k_0	k_1	k_2
Parent-Offspring	0	1	0
Full-siblings	0.25	0.50	0.25
Half-siblings			
Grandchild-Grandparent	0.50	0.50	0
Niece/Nephew-Uncle/Aunt			
First Cousin	0.75	0.25	0
Unrelated	1	0	0

Table 2.2. A list of all possible pairs of observed genotypes and the probability of each pair given the number of alleles identical by descent (m). The individual genotypes are ordered, so that ii / ij is distinct from ij / ii , because ordering affects the probabilities for genotype pairs. p_x represents the observed frequency of the allele x in the population. This table assumes null alleles are not present.

Genotypes	Probability given m genes IBD		
	$m = 0$	$m = 1$	$m = 2$
ii / ii	p_i^4	p_i^3	p_i^2
ii / ij	$2p_i^3 p_j$	$p_i^2 p_j$	0
ij / ii	$2p_i^3 p_j$	$p_i^2 p_j$	0
ii / jj	$p_i^2 p_j^2$	0	0
ii / jk	$2p_i^2 p_j p_k$	0	0
jk / ii	$2p_i^2 p_j p_k$	0	0
ij / ij	$4p_i^2 p_j^2$	$p_i p_j (p_i + p_j)$	$2p_i p_j$
ij / ik	$4p_i^2 p_j p_k$	$p_i p_j p_k$	0
ij / kl	$4p_i p_j p_k p_l$	0	0

Relatedness (r)

Relatedness (r) may be interpreted as the proportion of genes IBD between two individuals or groups of individuals (Cotterman 1941). For outbred individuals, r is given by (Thompson 1975):

$$r = \frac{k_1}{2} + k_2 \quad (3)$$

The maximum likelihood estimate of r , $ML(r)$, is equal to the maximum likelihood estimate of $\frac{k_1}{2}$ plus the maximum likelihood estimate of k_2 . Maximum likelihood estimates of k_1 and k_2 can be obtained from genotypic data by varying k_0 , k_1 , and k_2 through all possible values (subject to the constraint that they sum to one) to find the set of k -coefficients that maximize the product of $P(G_1 / G_2 | k_0, k_1, k_2)$ (Equation 1) across all loci. Note the difference between estimates of relationship and estimates of relatedness. When estimating relationship, values for k_0 , k_1 , and k_2 are determined by the genealogy of the relationship (Table 2.1) and then used in Equation 1. When estimating r , Equation 1 is used to find the optimum values of k_1 and k_2 that are then used in Equation 3. If r is being calculated for an evaluation of the relatedness of one individual to a group, the individual of interest is first paired with each group member and an average of the pairwise r -values is used.

Null Alleles

The formulae above show how to estimate relationship and relatedness assuming genotypes have no null alleles. In other words, the above formulae show how to calculate the probability if the *true* genotypes in two individuals are G_1 and G_2 . If null alleles are present at a locus, however, the probability of *observing* G_1 and G_2 , $P(\text{Observe } G_1 / G_2 | k_0, k_1, k_2)$, needs to be determined. Only observed homozygotes may have null alleles. If G_1 or G_2 is an observed heterozygote (e.g. ij), we assume that the observed genotype is correct. However, if G_1 or G_2 is an observed homozygote, it can

be a true homozygote (e.g. ii) or a heterozygote with one null and one non-null allele (e.g. in). If there are no homozygotes observed in the pair, G_1 / G_2 , the only possible true genotype pair is identical to the observed pair. However, if one homozygote is observed, there are two possible genotype pairs (e.g. the observed ii / ij may actually be ii / ij or in / ij). Further, if two homozygotes are observed, there are four possible true genotype pairs (e.g. the observed ii / ii may actually be ii / ii , in / ii , ii / in , or in / in). Genotype pairs, therefore, may have either 0, 1, or 2 null alleles, depending on how many homozygotes are observed. Because up to four true genotype pairs can have the same observed genotype, the likelihood of an observed genotype pair is calculated by summing the probabilities of all the genotype pairs that have the same observed genotype. For example, the probability of observing ii / ii is calculated by summing the probabilities of two individuals actually having genotypes ii / ii (no null allele), in / ii (null allele in first individual), ii / in (null allele in second individual), and in / in (null allele in both individuals).

Table 2.3 lists the true genotypes that may produce each of the nine possible observed genotype pairs and the corresponding probabilities under each value of m . Once these new probabilities are determined, the probability of the observed genotypes is still calculated following Equation 1 by listing all true genotype pairs that would be observed as G_1 / G_2 and then summing $P(\text{Observe } G_1 / G_2 \mid k_0, k_1, k_2)$ values for each possible true genotype. In essence, all this entails is using the multiple probabilities for the true genotype pairs in Table 2.3, rather than the probabilities in Table 2.2. For example, if the observed genotypes are ii / ii , then the true underlying genotypes are

taken from Table 2.3 and the probability of the observed genotypes, accounting for the possible presence of null alleles at a single locus, is thus:

$$\begin{aligned} P(\text{Observe } G_1 = ii / G_2 = ii | k_0, k_1, k_2) &= P(\text{Observe } ii / ii | k_0, k_1, k_2) \\ &= P(ii / ii | k_0, k_1, k_2) + P(in / ii | k_0, k_1, k_2) + P(ii / in | k_0, k_1, k_2) + P(in / in | k_0, k_1, k_2) \end{aligned} \quad (4a)$$

The four probabilities listed in the right-hand side of Equation 4a are calculated using Equation 1. For example,

$$P(ii / ii | k_0, k_1, k_2) = P(ii / ii | m = 0)k_0 + P(ii / ii | m = 1)k_1 + P(ii / ii | m = 2)k_2 \quad (4b)$$

and

$$P(in / ii | k_0, k_1, k_2) = P(in / ii | m = 0)k_0 + P(in / ii | m = 1)k_1 + P(in / ii | m = 2)k_2 \quad (4c)$$

For those true underlying genotypes having null alleles ($n = 1$ or 2), the probabilities are determined following the same logic used in Table 2.2 (where $n = 0$). For example, $P(in / ii | m = 0)$, $P(in / ii | m = 1)$, and $P(in / ii | m = 2)$ are calculated in the same way as was $P(ij / ii | m)$ for $m = 0, 1$, or 2 . To be more specific, $P(in / ii | m = 0)$, $P(in / ii | m = 1)$, and $P(in / ii | m = 2)$ are equal to $2p_i^3 p_n$, $p_i^2 p_n$, and 0 (respectively, Table 2.3). Note that although p_n is a total null allele frequency, we make no assumptions about the number of different null alleles at a locus or about whether any null alleles are IBD. We need only to account for the possibilities that there are 0 or 1 or 2 null alleles in the pair (summing along columns in Table 2.3) and that there are 0 or 1 or 2 null or non-null

alleles IBD (summing along rows in Table 2.3). As is true for all alleles, the probability under $m = 0$ and the partial probability under $m = 1$ account for the possibility that the null allele is not IBD, while the alternative that there is an IBD null allele is accounted for by the partial probability under $m = 1$ and the probability under $m = 2$ (see Appendix for a demonstration that having multiple non-IBD null alleles does not affect the probability of observing any particular genotype).

Calculating $P(\text{Observe } G_1 / G_2 | k_0, k_1, k_2)$ requires knowing the frequency of the null allele, p_n . In practice, p_n will not be known, but it can be estimated with several approaches (Chakraborty *et al.* 1992; Brookfield 1996; Summers & Amos 1997; Kalinowski & Taper *in press*) that have been implemented in programs such as Genepop (Raymond & Rousset 1995), Cervus (Marshall *et al.* 1998), Micro-Checker (Van Oosterhout *et al.* 2004) and ML-Relate (Kalinowski *et al.* *in press*) or can be programmed into an Excel spreadsheet (Kalinowski & Taper *in press*). Frequencies for observed non-null alleles may be corrected accordingly. As a source of typing error, inaccurate estimates of p_n would affect probabilities of false exclusion in parentage analysis (Marshall 1998; Sancristobal & Chevalet 1997). The probabilities above and those in Table 2.3 assume genotypes are observed in each case and that there are no homozygotes for a null allele producing ‘blank’ genotypes. The maximum likelihood approach developed by Kalinowski & Taper (*in press*) and implemented in ML-Relate (Kalinowski *et al.* *in press*) uses an EM algorithm and performs better under this assumption than the approaches of Summers & Amos (1997) and Chakraborty *et al.* (1992).

One aspect of Table 2.3 is particularly note worthy. When parents and offspring are being considered, definitive exclusion (as opposed to the relative considerations made below) of the true PO -relationship occurs when the likelihood of that relationship is zero ($L(PO) = 0$). By this measure, false exclusion of the true relationship would occur when, at any locus, parent and offspring are true heterozygotes with one common null allele and distinct non-null alleles (in / jn) but are observed as homozygotes for different alleles (ii / jj). The probability of false exclusion is then equal to the probability of having in / jn when one allele is IBD ($m = 1$). From Table 2.3, this is $p_i p_j p_n$, which is just the probability of having any two different alleles ($p_i p_j$) multiplied by the frequency of the IBD null allele. By definition, this is equivalent to the observed heterozygosity (He_{obs}) multiplied by p_n , so the probability of false exclusion of a parent-offspring relationship at a single locus if null alleles are ignored is $He_{obs} p_n$.

Table 2.3. A list of all possible observed genotypes for a pair of individuals, the underlying true genotypes that can produce the observed genotypes given the possible number of null alleles (n), and the probability of each underlying genotype pair given the number of alleles identical by descent (m). p_x represents the frequency of allele x in population corrected for the presence of null alleles (that is, p_n is considered when summing allele frequencies to 1). The genotypes within a pair are ordered, e.g. ii / ij is distinct from ij / ii . The probability of each observed genotype pair at each locus is obtained by summing the probabilities of the possible underlying true genotypes.

Observed genotypes	True genotypes	n	Probability of true genotypes given m genes IBD		
			$m = 0$	$m = 1$	$m = 2$
ii / ii	ii / ii	0	p_i^4	p_i^3	p_i^2
	ii / in	1	$2p_i^3 p_n$	$p_i^2 p_n$	0
	in / ii	1	$2p_i^3 p_n$	$p_i^2 p_n$	0
	in / in	2	$4p_i^2 p_n^2$	$p_i p_n (p_i + p_n)$	$2p_i p_n$
ii / ij	ii / ij	0	$2p_i^3 p_j$	$p_i^2 p_j$	0
	in / ij	1	$4p_i^2 p_j p_n$	$p_i p_j p_n$	0
ij / ii	ij / ii	0	$2p_i^3 p_j$	$p_i^2 p_j$	0
	ij / in	1	$4p_i^2 p_j p_n$	$p_i p_j p_n$	0
ii / jj	ii / jj	0	$p_i^2 p_j^2$	0	0
	ii / jn	1	$2p_i^2 p_j p_n$	0	0
	in / jj	1	$2p_i p_j^2 p_n$	0	0
	in / jn	2	$4p_i p_j p_n^2$	$p_i p_j p_n$	0
ii / jk	ii / jk	0	$2p_i^2 p_j p_k$	0	0
	in / jk	1	$4p_i p_j p_k p_n$	0	0
jk / ii	jk / ii	0	$2p_i^2 p_j p_k$	0	0
	jk / in	1	$4p_i p_j p_k p_n$	0	0
ij / ij	ij / ij	0	$4p_i^2 p_j^2$	$p_i p_j (p_i + p_j)$	$2p_i p_j$
ij / ik	ij / ik	0	$4p_i^2 p_j p_k$	$p_i p_j p_k$	0
ij / kl	ij / kl	0	$4p_i p_j p_k p_l$	0	0

Comparison of Analytical Methods

As discussed at the outset, there have been two alternative approaches to the analysis of microsatellite data that include null alleles when redesigning existing or developing new primers is not an option. One approach is to drop the data from affected loci. Another approach is to use the data from affected loci and proceed with estimation of relatedness or relationship using Table 2.2, ignoring the existence of null alleles. Above, we developed a new approach that explicitly accounts for null alleles by using Table 2.3. We now use empirical data to show how the results of these approaches differ.

Table 2.4 shows microsatellite genotypes at eight loci from a putative family of striped hyaenas (*Hyaena hyaena*) (unpublished data), within which the adult female (F09) was thought to be the mother of the three cubs (cubs 30, 31, & 32). Ignoring null alleles and using Table 2.2 & Equation 1, we tested the hypothesized parent-offspring relationship for F09 to each of the three cubs. F09 is immediately ruled out as the potential mother for two of the three cubs (cub 30 & cub 31), because the female and cubs share no alleles identical in state (and therefore none IBD) at locus CCR5. At this locus, $P(G_1 / G_2 | k_0, k_1, k_2) = 0$ and, since probabilities are multiplied across loci to determine the probability of the relationship, the entire probability of maternity is 0. This is a good illustration of the general problem that null alleles can easily create observed genotypes at one locus that are impossible under the hypothesized relationship even if genotypes at other loci strongly support that relationship.

Table 2.4. Observed microsatellite genotypes at eight loci for a group of striped hyenas. Numbers in the table indicate specific observed alleles, expressed as number of base-pairs in the allele. Loci with null alleles are indicated by an asterisk (*).

Individual ID	Locus							
	CCR4	CCR6	CCROC01	CCRA5*	CCROC05	CCRA3*	CCROC06	CCR5*
Female09	114/130	114/116	199/203	143/149	159/167	143/143	161/169	148/148
Cub30	114/130	114/114	203/203	143/143	159/167	143/143	161/169	152/152
Cub31	114/114	114/116	203/203	149/149	157/159	143/143	169/169	150/150
Cub32	114/130	114/116	203/203	143/143	157/159	143/143	169/169	148/150

In this case, null alleles were detected at three of the evaluated loci (CCRA5, CCRA3, and the critical CCR5) and, for loci where null alleles were detected, adjusted and null allele frequencies were calculated following Kalinowski and Taper (*in press*). Although the null allele frequency at CCR5 was relatively low (0.074), it appears to have created problems for assigning maternity. The characteristics of this data set illustrate a common problem contributing to the prevalence of studies that include, but do not correct for, loci with null alleles (Dakin & Avise 2003): in many existing data sets from wildlife studies researchers are restricted to using existing primers, only a limited number of loci are available, null alleles are present, but retention of inferential and discriminatory power requires salvaging those problem loci.

Table 2.5 summarizes conclusions about the maternity of the cubs using the three approaches. For the female and each cub, the probability of the genotypes for the three adult-cub pairs was calculated for parent-offspring vs. unrelated relationships, although any hypothesized relationships could be used for comparison. Likelihood ratios were

used to evaluate the relative degree of support for the competing relationships. A ratio >1 indicates that the relationship in the numerator is more likely, whereas a ratio <1 favors the denominator. Clearly, support for the parent-offspring hypothesis is highly dependent on the approach that is employed. Accounting for the occurrence of null alleles can give very different results than ignoring them (cubs 30 & 31) or discarding them (cubs 31 & 32). Only our approach, applying a correction for the presence of null alleles, retains enough information and correctly interprets the observed genotypes to indicate that F09 is likely to be the mother of all three cubs.

Table 2.5. Relationship likelihood ($L(R)$) ratios and maximum likelihood calculations of relatedness ($ML(r)$) for the population subset. $L(R)$ ratios are the probability of the observed genotypes given the hypothesized parent-offspring (PO) relationship vs the alternative unrelated (UR) relationship. $ML(r)$ was determined between F09 and each cub and all cubs as a group. Calculations were made without considering null alleles (Table 2.2), without using loci for which null alleles were detected, and with all loci corrected for the probabilities of null alleles at each locus (Table 2.3).

		$L(R)$ Ratio		
			$\frac{PO}{UR}$	$ML(r)$
Individuals	Approach Used			
F09	cub30	Nulls not considered	0	0.44
		Loci with nulls not used	14.91	0.70
		Correction for nulls applied	15.09	0.61
F09	cub31	Nulls not considered	0	0.33
		Loci with nulls not used	4.27	0.50
		Correction for nulls applied	20.50	0.50
F09	cub32	Nulls not considered	15.21	0.50
		Loci with nulls not used	3.25	0.39
		Correction for nulls applied	10.17	0.50
F09	Cubs as a Group	Nulls not considered		0.43
		Loci with nulls not used	NA	0.53
		Correction for nulls applied		0.54

We also used the three competing approaches to calculate the coefficient of relatedness between female F09 and each of the three cubs, and to the cubs as a group (Table 2.5). In general, this example shows that correcting for null alleles sometimes gives the same result as dropping loci with null alleles (cub 31), sometimes gives the same result as ignoring null alleles (cub 32), and sometimes differs from both of these methods (cub 30). When the presence of null alleles is not considered and all loci are used, the observed genotype probabilities for the F09-cub 31 pair under-estimates the relatedness of the female to her own cub by more than 20%. When cubs are viewed as a group, support for this female as the mother of this litter would be greatly reduced based on this result. Using the corrected approach, however, the female would still be considered a likely candidate.

In addition to the empirical tests above, we used computer simulation to further test the effectiveness of our method for accommodating null alleles in relationship and relatedness estimation. We did this by repeatedly simulating genotype data for pairs of related individuals and evaluating how accurately our method could estimate the relationship between individuals, in comparison to the commonly employed methods of ignoring null alleles or discarding problem loci. Each iteration of the simulation began by simulating parametric allele frequencies in a randomly mating population, using broken stick random numbers. This method produces allele frequencies that are uniformly distributed in multidimensional space (e.g. [0.2, 0.2, 0.2, 0.2, 0.2] is as likely as [0.96, 0.01, 0.01, 0.01, 0.01]) (Devroye 1986). All simulations had five alleles per locus, which resulted in an average observed heterozygosity of 0.66 (which was reduced

to 0.59 when null alleles were introduced). With these allele frequencies, genotypes of the adults in the population were simulated by multinomial sampling. We simulated relatedness within the population by forming monogamous mating pairs from the adults and then simulating genotypes for two offspring per mating pair. For example, most of our simulated data had 96 individuals in 24 families, each with a dam, sire, and two offspring. We simulated null alleles by choosing one allele at a locus to be a null allele. Loci that were homozygous for null alleles were treated as missing data. Note that the frequency of null alleles in our simulated data is a random variable. Null alleles that have a high frequency in a population are more likely to interfere with relationship estimation than null alleles having a low frequency. Therefore, we binned simulated data according to the frequency of null alleles, with a bin width of 0.10 (e.g. data that had null alleles with a frequency greater than or equal to 0.15 and less than 0.25 were placed in the “0.2” bin). In some cases, we simulated data for multiple loci having null alleles. Here, data were binned according to the average frequency of null alleles at all loci with null alleles.

We used two statistics to measure how accurately relatedness and relationship could be estimated. The accuracy of estimates of relatedness was measured by the root mean squared error (RMSE) of the estimates. The accuracy of estimates of relationship was measured by the proportion of simulated data that successfully identified the correct relationship from among four possibilities: unrelated, half-siblings, full-siblings, and parent-offspring. Under each set of conditions, at least one thousand simulated data sets were used to estimate these statistics.

We examined the effect of the following variables upon the accuracy of estimates of relatedness and relationship: sample size ($N_{Samples}$), total number of loci (N_{Loci}), number of loci having null alleles (N_{Nulls}), and the frequency of null alleles (p_{null}) (Tables 2.6 & 2.7). In addition, we evaluated six methods for estimating relatedness and relationship—methods that spanned the range of options available to geneticists encountering null alleles. For example, the first method, IGNORE, simply ignored null alleles. The next three methods are variations of the maximum likelihood approach we present above. ML-APRIORI assumes that the user knows *a priori* which loci have null alleles. ML-DETECTED assumes the user does not know which loci have null alleles, and therefore must test for them. We used a Monte-Carlo randomization test (Guo & Thompson 1992) for excess homozygosity and the U-statistic (Rousset & Raymond 1995) to detect null alleles. Loci that had a one-tailed p -value of less than 0.05 divided by the number of loci in the data were classified as having null alleles. ML-ALL assumed null alleles were present at all loci. This strategy may appear unreasonable, but if a locus did not have a null allele, the estimated frequency of a null allele at the locus was usually small. Last, we tested two variants of removing loci with null alleles. REMOVE-APRIORI assumed that loci having null alleles were identified *a priori*. REMOVE-DETECTED used the randomization test described above to detect loci having null alleles. In each case, such loci were removed from the data.

Our method of correcting for the presence of null alleles (ML-DETECTED, ML-APRIORI, or ML-ALL) improves the accuracy of relatedness identification for full-siblings (Table 2.6), but the differences can appear subtle in this context. However, the ML-

DETECTED method improves RMSE by up to 6.2% over IGNORE (average improvement = 2.2%) and represents up to a 14.0% improvement in relatedness estimation over REMOVE-DETECTED (average = 7.6%). Across simulated conditions, increasing the number of loci considered has the greatest impact on reducing error in relatedness estimation and it is only when more than 6 loci are considered that REMOVE-DETECTED approaches the accuracy of the IGNORE or ML approaches. Our method also performs the best in relationship estimation, improving the ability to correctly identify parent-offspring relationships (Table 2.7). The biggest improvements over IGNORE occur when null allele frequencies are high or when many loci are available. The largest improvements in accuracy of our method relative to REMOVE methods occur when null alleles are present at multiple loci.

We also considered the probabilities of drawing false conclusions using two simple ways of evaluating population genotype data. For the same three methods applied in Table 2.5 (IGNORE, ML-DETECTED, and REMOVE-DETECTED), we determined the percentage of simulated parent-offspring pairs for which the likelihood of the true parent-offspring relationship was less than the likelihood of being unrelated (likelihood ratio less than one), as a measure of the probability of reaching a false conclusion in relationship estimation (Fig. 2.1). In every case, the probability of drawing false conclusions is highest when null alleles are ignored: IGNORE leads to false conclusions 7.4 to 19.1% of the time. Applying our correction for null alleles also reduces the probability of drawing a false conclusion relative to REMOVE methods, except when a large number of loci are considered: in this case the accuracy of the two approaches is equivalent. We also used

the percentage of calculated r -values for all parent-offspring pairs that deviated by more than $\pm 20\%$ (± 0.1) from the true value of 0.5 as a further test of these three competing methods (Fig. 2.2). For relatedness, up to 7% (IGNORE) or 4% (REMOVE-DETECTED) more of the calculations over or under-estimated r by $>20\%$ than when the correction for null alleles is applied.

Table 2.6. The root mean square error of estimates of relatedness between full-siblings under simulated conditions varying the sample size ($N_{Samples}$), total number of loci (N_{Loci}), number of loci having null alleles (N_{Nulls}), and the frequency of null alleles (p_{null}), as indicated in the first four columns. Lower values indicate greater accuracy in relatedness estimation.

N_{Loci}	N_{Nulls}	$N_{Samples}$	p_{null}	Statistical method					
				Ignore	ML	ML	ML	Remove	Remove
<i>No Null alleles</i>									
6	none	96	-	0.210	0.210	0.210	0.210	0.210	-
<i>Vary number of loci having null alleles</i>									
6	1	96	0.2	0.216	0.214	0.214	0.214	0.226	0.224
“	2	“	“	0.221	0.216	0.216	0.216	0.236	0.243
“	3	“	“	0.226	0.220	0.220	0.220	0.253	0.268
<i>Vary frequency of null allele</i>									
6	2	96	0.1	0.217	0.215	0.214	0.214	0.228	0.243
“	“	“	0.2	0.221	0.216	0.216	0.216	0.236	0.243
“	“	“	0.3	0.225	0.218	0.218	0.218	0.239	0.243
“	“	“	0.4	0.226	0.219	0.219	0.219	0.242	0.243
<i>Vary sample size</i>									
6	2	48	0.2	0.225	0.221	0.220	0.220	0.238	0.243
“	“	96	“	0.221	0.216	0.216	0.216	0.236	0.243
“	“	192	“	0.218	0.212	0.212	0.212	0.235	0.243
<i>Vary total number of loci</i>									
6	1	96	0.2	0.216	0.214	0.214	0.214	0.226	0.224
12	“	“	“	0.160	0.159	0.158	0.159	0.164	0.164
24	“	“	“	0.116	0.116	0.116	0.116	0.118	0.118

Table 2.7. Proportion of simulated data sets successfully able to identify the relationship between a parent-offspring pair when differing characteristics of the data set are varied: sample size ($N_{Samples}$), total number of loci (N_{Loci}), number of loci having null alleles (N_{Nulls}), and the frequency of null alleles (p_{null}). Higher values indicate greater accuracy in relationship estimation.

N_{Loci}	N_{Nulls}	$N_{Samples}$	p_{null}	Statistical method					
				Ignore	ML Detected	ML Apriori	ML All	Remove Detected	Remove Apriori
<i>No null alleles</i>									
6	none	96	-	0.781	0.780	0.780	0.779	0.780	-
<i>Vary number of loci having null alleles</i>									
6	1	96	0.2	0.718	0.757	0.758	0.759	0.740	0.751
“	2	“	“	0.663	0.728	0.730	0.731	0.706	0.705
“	3	“	“	0.624	0.711	0.713	0.716	0.676	0.653
<i>Vary frequency of null allele</i>									
6	2	96	0.1	0.698	0.731	0.739	0.739	0.715	0.705
“	“	“	0.2	0.663	0.728	0.730	0.731	0.706	0.705
“	“	“	0.3	0.644	0.724	0.725	0.725	0.714	0.705
“	“	“	0.4	0.636	0.718	0.719	0.719	0.707	0.705
<i>Vary sample size</i>									
6	2	48	0.2	0.660	0.714	0.721	0.723	0.703	0.705
“	“	96	“	0.663	0.728	0.730	0.731	0.706	0.705
“	“	192	“	0.663	0.734	0.735	0.736	0.711	0.705
<i>Vary total number of loci</i>									
6	1	96	0.2	0.718	0.757	0.758	0.759	0.740	0.751
12	“	“	“	0.827	0.883	0.889	0.888	0.877	0.884
24	“	“	“	0.898	0.965	0.974	0.974	0.963	0.965

Figure 2.1. Probability of falsely concluding that the likelihood of unrelated is greater than the likelihood of the true parent-offspring relationship from the simulated data using the three competing approaches: ignoring problem loci and including loci with null alleles without applying a correction (IGNORE), removing loci where null alleles were detected from data analysis (REMOVE-DETECTED), and applying our correction for null alleles at loci where they were detected (ML-DETECTED). Vertical dotted lines separate sub-sets of the simulated data within which one characteristic of the data set was varied (as described in Table 2.7). y-axis indicates the percentage of parent-offspring pairs for which $L(PO)/L(UR)$ was incorrectly determined to be <1.0 .

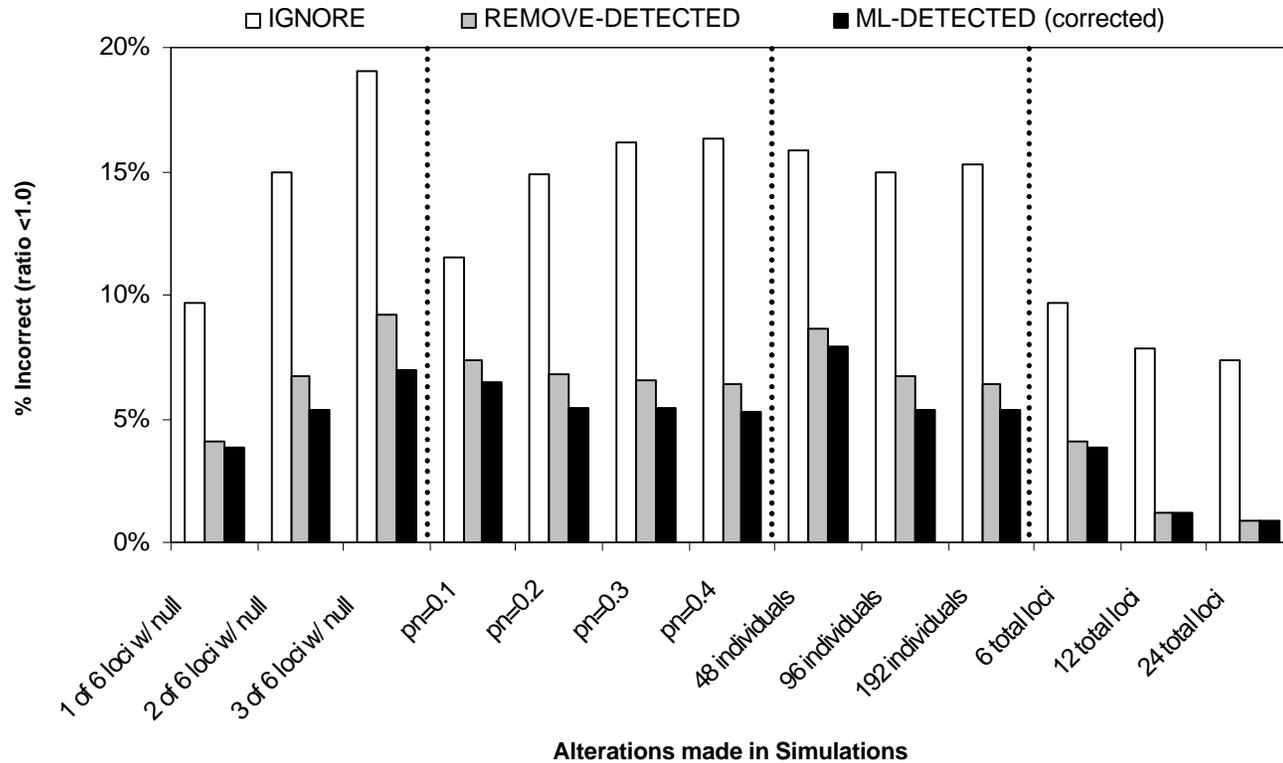
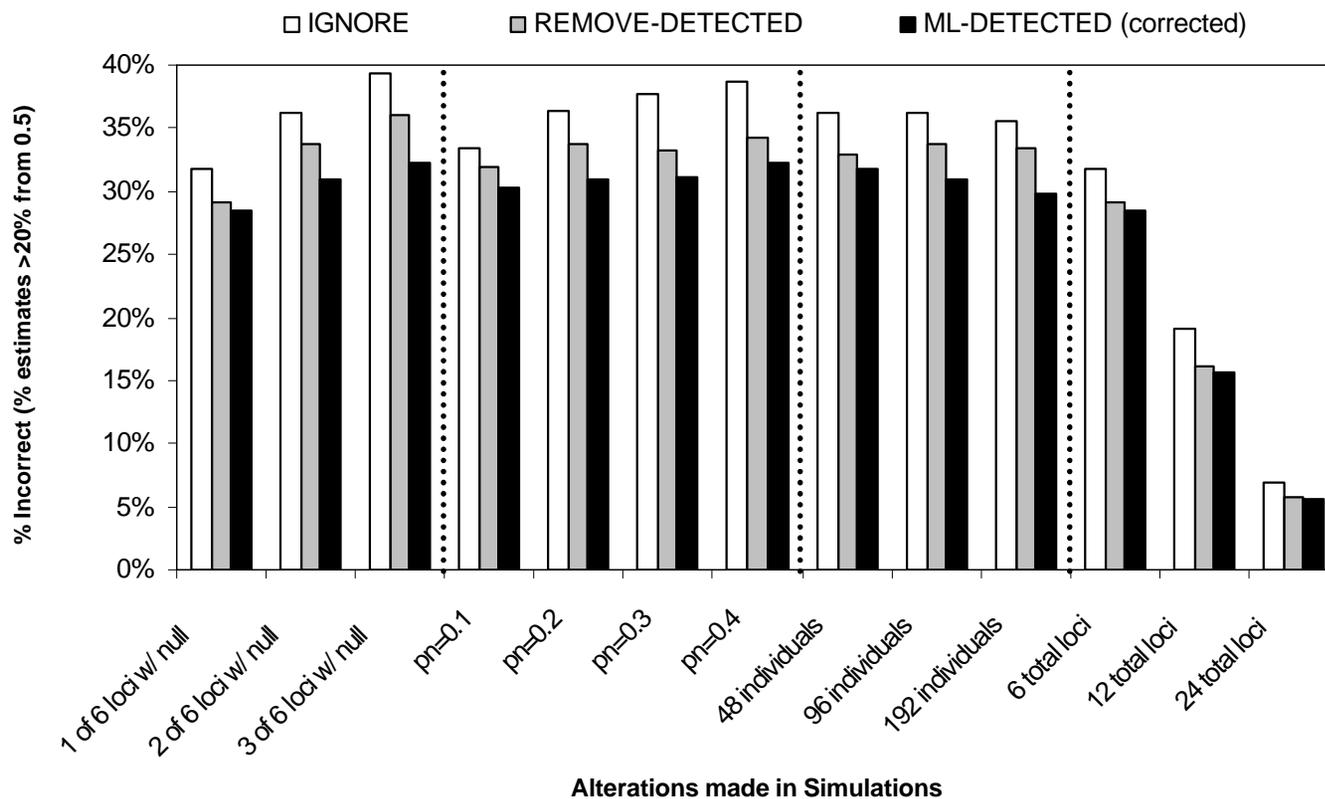


Figure 2.2. Probability of over or under-estimating relatedness for parents and offspring by more than 20% from the simulated data using three competing approaches: IGNORE, REMOVE-DETECTED, and ML-DETECTED. Vertical dotted lines separate sub-sets of the simulated data within which one characteristic of the data set was varied (Table 2.7). y-axis indicates the percentage of parent-offspring pairs for which r estimates deviated by more than $\pm 20\%$ from the true value.



Finally, we evaluated the effect of the number of loci (N_{Loci}) on the accuracy of relationship and relatedness estimates from our simulated genetic data having no null alleles (Table 2.8). For a given number of loci, Table 2.8 indicates the levels of accuracy that could be expected if the data are 'error-free and shows that accuracy improves when the number of loci increases. Comparing the results in Table 2.8 to Tables 2.6 & 2.7 also allows for evaluation of the performance of our method to two methods that would eliminate null alleles from the data. First, researchers might consider replacing loci containing null alleles with loci having no null alleles. However, if additional primers are developed, loci with null alleles should be retained and our method applied: accuracy would be improved by retaining all loci and applying our correction rather than replacing loci because the latter would reduce the total number of loci considered (compare ML results for N_{Loci} given N_{Nulls} , in Tables 2.6 or 2.7, to results for $N_{Loci} - N_{Nulls}$ in Table 2.8). Second, if researchers consider redesigning existing primers, instead of replacing loci, comparing the results in Table 2.8 to the ML results in Tables 2.6 & 2.7 shows that, for the same N_{Loci} , the differences between the accuracy achieved using error-free data or applying our corrected method are small and diminish as the number of loci increase. When few loci are available, the costs of redesigning primers should then be evaluated relative to the costs (slightly reduced accuracy) of applying our corrected method to the imperfect data at hand. When many loci are available, researchers would receive little benefit by redesigning primers rather than applying our correction.

Table 2.8. The accuracy of simulated genetic data in estimating relationship or relatedness when no null alleles are present. The first column shows the number of loci (N_{Loci}) in the simulated data, the second the proportion of simulated data sets that successfully identified parent offspring pairs (R_{PO}), the third the root mean square error (RMSE) of estimates of relatedness between full sibs (r_{FS}).

N_{Loci}	Proportion R_{PO} Correct	RMSE r_{FS}
1	0.44	0.42
2	0.59	0.31
3	0.65	0.27
4	0.71	0.24
5	0.75	0.22
6	0.78	0.21
7	0.81	0.20
8	0.83	0.19
9	0.85	0.18
10	0.87	0.17
11	0.88	0.16
12	0.90	0.16
13	0.91	0.15
14	0.92	0.15
15	0.92	0.14
16	0.93	0.14
17	0.94	0.14
18	0.94	0.13
19	0.95	0.13
20	0.95	0.13
21	0.96	0.12
22	0.96	0.12
23	0.96	0.12
24	0.97	0.12

Conclusion

Failure to correct for the presence of null alleles in microsatellite data can produce badly biased estimates of relatedness and incorrect assessments of relationships. Even at low frequencies, null alleles can have a large impact. Dropping data from problem loci altogether can significantly alter the likelihoods of competing relationships and this solution needlessly discards valuable information. Even under relatively simple scenarios, dropping loci does not perform as well as, and never performs better than, correcting for null alleles, so it cannot be considered the ‘conservative’ approach. Inclusion of loci at which null alleles are present, without correcting for them, is the approach most likely to lead to false conclusions in relatedness and relationship estimation.

Our method for including null alleles in calculations of relationship probabilities and relatedness values is easy to apply to co-dominant genotype data. Once null alleles are detected and their frequency estimated, all of the information required for these adjusted calculations is present in the original genotyping data, so application of our method bears no additional costs. Little modification is required to the methods already in place for evaluating relationships and relatedness: all that is required is to use Table 2.3 rather than Table 2.2 when applying Equation 1. This new approach provides a means by which a previously recognized and wide-spread problem, predominantly discussed as a theoretical or conceptual issue, can be corrected in practice.

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Appendix

We have shown how having a null allele at a locus affects the probabilities of observing each possible pair of genotypes. However, it is possible to have multiple, distinct (non-IBD) null alleles at a single locus. If multiple null alleles are present at a locus, this expands the number of true genotypes that may have produced an observed genotype and it reasonable to question how this might affect the probabilities of observing each genotype. If there is no effect, the sum of the probabilities of all possible true genotypes when two distinct null alleles are considered should equal the sum of the probabilities in Table 2.3 (for the same observed genotypes).

For example, if there are two null alleles at a locus (n_1 and n_2) and ij / ik are the genotypes observed, true genotypes may be ij / ik or in_1 / ik or in_2 / ik . The sum of the probabilities under $m = 0$ for ij / ik with two distinct null alleles is:

$$\begin{aligned}
 P(G_1 = ij / G_2 = ik | m = 0) &= P(\text{Observe } ij / ik | m = 0) \\
 &= P(ij / ik | m = 0) + P(in_1 / ik | m = 0) + P(in_2 / ik | m = 0) \\
 &= 2p_i^2 p_j p_k + 4p_i p_j p_k p_{n_1} + 4p_i p_j p_k p_{n_2} = 2p_i^2 p_j p_k + 4p_i p_j p_k (p_{n_1} + p_{n_2})
 \end{aligned} \tag{A-1}$$

Recall that we defined p_n as the total frequency of all null alleles at a locus, so $p_n = p_{n_1} + p_{n_2}$, in this example. The sum of the final quantities in the above equation is then identical to the sum of the probabilities of the true genotypes under $m = 0$ for ij / ik given in Table 2.3.

A more complex situation occurs when two homozygotes for the same allele are observed (ii/ii). For this set of observed genotypes, there are now nine possible true genotypes that can produce that observation (see Table 2.A) and we break up the possible true underlying genotypes by the expanded set that corresponds with the true genotypes in Table 2.3. For example, ii/in expands to ii/in_1 and ii/in_2 and, for $m = 0$, the probabilities of those true genotypes are $2p_i^3 p_{n_1}$ and $2p_i^3 p_{n_2}$, respectively. Summing these quantities gives:

$$P(ii/in_1 | m=0) + P(ii/in_2 | m=0) = 2p_i^3 p_{n_1} + 2p_i^3 p_{n_2} = 2p_i^3 (p_{n_1} + p_{n_2}) = 2p_i^3 p_n \quad (\text{A-2a})$$

Similarly, in/in now expands to in_1/in_2 , in_2/in_1 , in_1/in_1 , and in_2/in_2 . For $m = 0$, these probabilities are $4p_i^2 p_{n_1} p_{n_2}$, $4p_i^2 p_{n_1} p_{n_2}$, $4p_i^2 p_{n_1}^2$, and $4p_i^2 p_{n_2}^2$, respectively.

Summing these quantities gives:

$$\begin{aligned} & P(in_1/in_2 | m=0) + P(in_2/in_1 | m=0) + P(in_1/in_1 | m=0) + P(in_2/in_2 | m=0) \\ &= 4p_i^2 p_{n_1} p_{n_2} + 4p_i^2 p_{n_1} p_{n_2} + 4p_i^2 p_{n_1}^2 + 4p_i^2 p_{n_2}^2 = 4p_i^2 (2p_{n_1} p_{n_2} + p_{n_1}^2 + p_{n_2}^2) \\ &= 4p_i^2 p_n^2 \end{aligned} \quad (\text{A-2b})$$

For in/in , when $m = 1$:

$$\begin{aligned} & P(in_1/in_2 | m=1) + P(in_2/in_1 | m=1) + P(in_1/in_1 | m=1) + P(in_2/in_2 | m=1) \\ &= p_i p_{n_1} p_{n_2} + p_i p_{n_1} p_{n_2} + p_i p_{n_1} (p_i + p_{n_1}) + p_i p_{n_2} (p_i + p_{n_2}) \\ &= 2p_i p_{n_1} p_{n_2} + (p_i^2 p_{n_1} + p_i p_{n_1}^2) + (p_i^2 p_{n_2} + p_i p_{n_2}^2) \\ &= p_i (2p_{n_1} p_{n_2} + p_i p_{n_1} + p_{n_1}^2 + p_i p_{n_2} + p_{n_2}^2) = p_i (p_n^2 + p_i (p_{n_1} + p_{n_2})) \\ &= p_i (p_n^2 + p_i p_n) = p_i p_n (p_i + p_n) \end{aligned} \quad (\text{A-2c})$$

The probabilities given in each cell of Table 2.3 can similarly be shown not to be affected by the number of distinct null alleles actually present at a locus.

Table 2.A. A list of all possible true genotypes for a pair of individuals, where ii / ii was the observed genotype, given the possible number of null alleles (n) when null alleles are considered IBD (column 1) or distinct (column 2), and the probability of each underlying genotype pair given the number of alleles identical by descent (m) when two distinct null alleles are present. p_x represents the frequency of allele x in population corrected for the presence of null alleles (that is, p_n or $p_{n_1} + p_{n_2}$ is considered when summing allele frequencies to 1). The probability of the observed genotype pair is obtained by summing the probabilities of the possible underlying true genotypes. Note that the sum of the quantities in this table is the same as the sum of the true genotype probabilities for ii / ii in Table 2.3.

True genotypes from Table 2.3	True genotypes with two null alleles	n	Probability of true genotypes, given m genes IBD, when two distinct null alleles are at a locus		
			$m = 0$	$m = 1$	$m = 2$
ii / ii	ii / ii	0	p_i^4	p_i^3	p_i^2
ii / in	ii / in_1	1	$2p_i^3 p_{n_1}$	$p_i^2 p_{n_1}$	0
	ii / in_2	1	$2p_i^3 p_{n_2}$	$p_i^2 p_{n_2}$	0
in / ii	in_1 / ii	1	$2p_i^3 p_{n_1}$	$p_i^2 p_{n_1}$	0
	in_2 / ii	1	$2p_i^3 p_{n_2}$	$p_i^2 p_{n_2}$	0
in / in	in_1 / in_2	2	$4p_i^2 p_{n_1} p_{n_2}$	$p_i p_{n_1} p_{n_2}$	0
	in_2 / in_1	2	$4p_i^2 p_{n_1} p_{n_2}$	$p_i p_{n_1} p_{n_2}$	0
	in_1 / in_1	2	$4p_i^2 p_{n_1}^2$	$p_i p_{n_1} (p_i + p_{n_1})$	$2p_i p_{n_1}$
	in_2 / in_2	2	$4p_i^2 p_{n_2}^2$	$p_i p_{n_2} (p_i + p_{n_2})$	$2p_i p_{n_2}$

SPATIAL GROUPING IN A BEHAVIORALLY SOLITARY CARNIVORE:
SPATIAL, SOCIAL, AND GENETIC STRUCTURE
OF A STRIPED HYENA POPULATION

Introduction

Sociality and group-living (defined below and in Box 3.1) can be related to the shared use of space, feeding in groups, foraging in groups, or breeding in groups (Gittleman 1989). Patterns of behavior, like grouping, are most appropriately understood in terms of the selective benefits (Rubenstein & Wrangham 1986) of maximizing individual fitness (Wrangham & Rubenstein 1986) directly, or indirectly through kin selection (Hamilton 1964). To understand group formation, it is necessary to identify the factors that allow group formation by lowering costs (*permitting* conditions), and the factors that actively favor grouping and sociality by providing benefits (*promoting* conditions) (see Box 3.2). Generalizations about these permissive and promoting conditions are difficult (i.e. important influences may differ between different groups and at different times), but they are necessary to derive a general understanding of the evolution of grouping strategies.

There is no single theory unifying all of the permissive and promoting conditions underlying social evolution, but many studies have identified conditions that may initially facilitate group formation or subsequently favor sociality itself (Wrangham & Rubenstein 1986). In general, food type and size is correlated with spacing patterns in carnivores and the influence of food resources is expected to be greatest on females (Wrangham & Rubenstein 1986, Sandell 1989). Accordingly, Wrangham & Rubenstein (1986) propose a series of questions that should be addressed to understand the evolution of a species'

social system. Briefly, does the nature of (food) resources permit or promote group foraging, group travel, or group living (among females)? And (how) does the resulting distribution of females limit male distribution options?

Most terms applied in such evaluations refer to mating systems, but are incomplete (Wrangham & Rubenstein 1986) and require clarification. In applying these questions to striped hyenas *Hyaena hyaena*, we will consider ‘grouping’ to have occurred when two or more individuals of either sex share the same defended space. Whether *groups* will form is related to the costs and benefits of *tolerance*. ‘Association’ or ‘associating’ simply implies close physical proximity. ‘Sociality’ is a special, and more advanced, case of grouping that is generally more permanent, implies frequent direct interaction and cooperation, and may be much more complex. Generally, sociality is demonstrated by groups that remain associated over long periods of time. Whether *sociality* evolves is related to the costs and benefits of *active cooperation beyond the shared defense of space*. This paper is primarily concerned with the mechanisms of group formation (permissive conditions), and not, necessarily, sociality (although the former is expected to be a precondition for the evolution of the latter). So, we begin with a discussion of hypotheses about factors that permit group formation in carnivores.

Permissive Conditions

Diet and the dispersion of food resources are widely recognized as determinants of group formation (Crook 1965, Alexander 1974, Wilson 1975, Mills 1989)—the way food is distributed plays a strong role in determining whether grouping carries costs or provides benefits. Consequently, diet and food are commonly considered the factors of

greatest importance in understanding group formation (Gittleman 1989). Overlapping home-ranges, but not necessarily social cooperation, is predicted based on the quality or richness of resources (MacDonald 1983, Waser & Waser 1985). Home-ranges may overlap, and groups subsequently form, when resources can be shared with little impact on foraging success (a permissive condition). Macdonald's (1983) Resource Dispersion Hypothesis (RDH) encapsulates the underlying logic by predicting that the resource-related costs of group size are determined by the abundance of resources (Johnson *et al.* 2002). Temporal or spatial variation in resource abundance within defended territories can act as the primary factor permitting group formation because the minimum viable range for a pair may also support a group. For instance, if resources occur in patches and production within those patches is asynchronous, an animal might require access to several patches (so that patch dispersion determines territory size), but any one patch that is productive at any one time may be able to support multiple users (so that mean patch quality determines group size). In this way, groups can form even in the absence of conditions promoting group formation (via group foraging or any other mechanism) (MacDonald 1983, MacDonald & Carr 1989, Johnson *et al.* 2002).

Some carnivore species live in 'spatial groups' with highly overlapping, congruent home ranges, but forage alone within their shared range (Macdonald 1983). Species with solitary foragers living in spatial groups clearly show that resources can support multiple users in the absence of cooperative foraging and its benefits (e.g. red fox *Vulpes vulpes*, arctic fox *Alopex lagopus*, black-backed jackal *Canis mesomelas*, domestic cat *Felis catus*, European badger *Meles meles*: Johnson *et al.* 2002, and brown

hyena *Parahyaena brunnea*: Mill 1982, Mills 1989 & 1990). For these species, diet likely plays a permissive role in allowing group formation, but the effects of active cooperation on foraging success clearly do not explain group living (Johnson *et al.* 2002). For example, grouping in red foxes is accompanied by clear and amicable relationships between individuals sharing a single, well-defined group territory (MacDonald 1983). There is no cooperation in foraging, but group members probably benefit through increased anti-predator defense and boundary surveillance.

Whereas food abundance confines group size, the distribution of food resources determines territory size (Kruuk 1978, MacDonald 1983, MacDonald & Carr 1989, Moehlman 1989). Territoriality will develop when resources are defendable (Wrangham & Rubenstein 1986), and territory size is determined by both the spatio-temporal distribution of food resources and the ability to defend those resources. With temporal variation in resource abundance, territory size is correlated with the minimum experienced 'bottleneck' resource availability—range size should reflect an ability to retain sufficient resources when resources are low (MacDonald 1983, Sandell 1989, Johnson *et al.* 2002). The RDH predicts that increases in group size do not necessitate changes in territory size and similar resource levels should be contained within territories of different sizes. Further, the RDH allows for competition to increase due to depletion of resources in the face of grouping, provided that the benefits of grouping still outweigh the costs.

The RDH is inclusive of many hypotheses, but cannot account for all circumstances of group formation based on prey characteristics. For instance, the RDH

predicts groups will not form when resources are low in density and evenly dispersed, regardless of the types of resource being considered (food or mates). However, Waser's (1981, see Box 3.2) Resource Renewal Hypothesis does allow for group formation when resources are evenly dispersed provided that the rate of resource renewal is sufficient to reduce the effects of local food competition. This hypothesis was developed for and has been applied specifically to address permissive conditions in insectivorous carnivores (e.g. mongooses), because harvested invertebrates can renew at very high rates relative to even small vertebrate prey. As in other studies, we remain primarily concerned with more widely applicable hypotheses 'of what may be broadly called the RDH' (Carr & MacDonald 1986). Overall, the RDH predicts that 1) resource distribution and abundance determine whether grouping carries foraging costs through local food competition and 2) that concentrations of resources in space or time that exceed the metabolic needs of the primary inhabitants in a territory (i.e. a solitary resident or mating pair) allow group formation, but do not necessitate or promote such development. That is, abundant and patchy resources might reduce the costs of sharing space with conspecifics, but do not necessarily provide a benefit to sharing space.

Females as a Resource

In evaluating hypotheses regarding group formation, explicit identification of the population sub-set of interest (e.g. males vs. females, subordinates vs. dominants, adults vs. juveniles) is necessary because different sub-sets may not behave similarly or react to the same resources or selection pressures (Mills 1978a, von Schantz 1984, Van Orsdol *et al.* 1985, Gittleman 1989, Revilla 2003). Most importantly, female behavior will reflect

an attempt to meet the demands of the environment, while male behavior is adapted to maximize mating opportunities (Jarman 1974). Because males compete for access to mates, while females generally do not, the distribution of females (and food) may largely determine the spacing patterns of males (MacDonald 1983, Johnson *et al.* 2002). If this argument is correct, males are expected to maintain adequate food ranges while maximizing mating ranges. Male home-ranges are typically larger than females' ranges, because female home-ranges should correspond only to metabolic needs, while competition for access to females may result in male territories that encompass food resources exceeding their energy requirements (Sandell 1989).

Interpretation & Expectations

The RDH does not necessarily predict that members of a group will utilize the same resource simultaneously (Carr & MacDonald 1986, Johnson *et al.* 2002) even when there is only one large, persistent resource patch (e.g. Horn 1968, Bradbury & Vehrencamp 1976, Chapman *et al.* 1994). Individuals may defend a shared range but forage independently with little probability of encountering one another, or even with active avoidance of occupied feeding sites. Consequently, solitary foraging does not require exclusive, non-overlapping home-ranges and spatial grouping is known to occur in species that do not forage together or even meet frequently (Waser & Waser 1985, Carr & MacDonald 1986, Wrangham & Rubenstein 1986). For instance, a brown hyena may feed alone on a carcass and a different individual may feed on the same carcass sequentially (Mills 1982). European badgers may also forage from the same patch of worms at different times (Kruuk 1978). Neither of these behaviors is in opposition to the

predictions of the RDH, which makes no explicit predictions about social vs. solitary foraging within a shared range.

Application of the RDH to explain group living is not restricted to carnivores. The importance of resource heterogeneity in space and time has been used to explain group size and degree of social relationships in primates. Black and white colobus *Colobus uellensis* and purple-faced langurs *Presbytis senex* live in small groups with only one or two males, while red colobus *C. tephrosceles* and grey langurs *P. entellus* live in larger groups containing multiple males (Carr & MacDonald 1986). For the former two, food is dispersed evenly, but in the latter species, there is spatial and temporal variability in food resources such that those resources can support more users.

Grouping may carry costs including increased probability of detection by predators, increased occurrence or transmission of diseases, and increased local competition and aggression (Gittleman 1989). Even when grouping strategies appear to be favored, they may represent only one option available to solve a particular problem. For instance, leopards *Panthera pardus* and cheetahs *Acinonyx jubatus* illustrate alternative strategies to grouping in response to inter-specific competition: rapid consumption of prey (cheetahs), a shift in period of activity (cheetahs are crepuscular, while their competitors are more strictly nocturnal), or caching of prey items in trees (leopards) (Schaller 1972). However, the RDH is facilitative, not causal: it identifies conditions under which the costs of sharing resources may be low enough to permit group formation *if* benefits to grouping exist. The RDH does not insist that groups will *always*

form when permissive resource conditions exist, but that groups are more likely to form when those conditions exist.

Promoting Conditions

In general, prior studies suggest that the distribution, abundance, and renewal of food (or other resources) set the limits under which the factors promoting group living operate. Once resources *permit* groups to form at little cost, the benefits of other behaviors, such as group hunting, defense against predators, and defense against conspecifics, are more likely to exceed the costs of sharing resources (MacDonald 1983, Creel & Creel 1995). Selection pressures can then *promote* the evolution of sociality and group formation, group living, and cooperation through increased offspring production (Gittleman 1989, Sandell 1989), predator defense (Rasa 1986, Rood 1986), exploitation of food and other resources (Kruuk 1972, Schaller 1972, Caraco & Wolf 1975, Lamprecht 1981, MacDonald 1983, Creel & Creel 1995), defense of resources (Owens & Owens 1984, Packer 1986), or mating success (Gittleman 1989, Sandell 1989) (Box 3.2).

The mechanisms just listed all provide a benefit to grouping through some form of collective action. A final mechanism that also promotes group living but does not directly involve cooperation is the high cost associated with dispersal (Waser 1996, Wrangham & Rubenstein 1986). For most carnivores (and other taxa), dispersers are more likely to die than individuals not involved in dispersal (Lucas *et al.* 1994, Waser 1996, Creel & Creel 2002). Consequently, the evolution of group living is often discussed in the context of group formation as a result of natal philopatry or non-dispersal of offspring (MacDonald 1983, Lindstrom 1986, Blackwell & Bacon 1993, Johnson *et al.*

2002). For instance, consistent with the RDH, Lindstrom's (1986) Territory Inheritance Hypothesis proposes that low costs associated with natal philopatry (permitting) relative to high costs of dispersal (promoting) may be a common mechanism of group formation in carnivores. Group living in lions, for example, may be favored because of the high costs associated with solitary living while dispersing (e.g. a low ability to defend kills) (Owens & Owens 1984, Packer 1986). Additional factors, such as social dominance, which can increase the costs of 'staying' or decrease the relative costs of dispersal, may also regulate group size (Wrangham & Rubenstein 1986), but it remains the nature of resources that permit group development.

Importantly, the current benefits of grouping may be an evolutionary consequence of grouping, rather than the force that favored the initial evolution of grouping (Waser 1981, MacDonald 1983). For example, a current benefit of grouping in some species is increased foraging success due to active cooperation in hunting (Creel 2001). By itself, this current benefit does not establish that sociality arose due to cooperative hunting. Evidence for this comes from the prevalence of carnivores that feed routinely on large prey without forming foraging groups (e.g. leopard, mountain lion *Puma concolor*, tiger *Panthera tigris*) and the frequency in which spatial group size exceeds foraging group size (MacDonald 1983, Gittleman 1989). The evolutionary origins of carnivore sociality cannot be resolved purely from its current functions (Packer *et al.* 1990). Consequently, it is very difficult to evaluate the RDH in social species within which the benefits of sociality have already been realized and are at work. Rather, the most ideal conditions

for evaluating hypotheses about social evolution are to study ‘solitary’ species with intra-specific variation in group sizes (Johnson 2002).

Study Aims, Constraints, and Definitions

Inter-specific and intra-specific variability (e.g. in spotted hyenas *Crocuta crocuta*), in diet and social organization among the Hyaenidae makes them useful for studies of the links between diet and social organization (Mills 1989)—seminal studies relating carnivore social organization to the distribution of resources have successfully drawn on inter-specific comparisons of spotted hyenas, brown hyenas, and aardwolves *Proteles cristatus* (Kruuk 1976, Mills 1978a, 1989 & 1990, MacDonald 1978). However, our understanding of social organization within the hyaenids remains incomplete because, although three of the four extant species have been well studied, descriptions of striped hyena ecology have been based on very limited observations.

We developed a multi-year study of striped hyenas, collecting data on their population structure, patterns of relatedness, and diet and foraging behavior. Here we present data from that study and interpret the results under the expected influences of resources and resource utilization on social organization, described primarily by the RDH.

A priori, I could not design this study to explicitly test hypotheses regarding the regulation of specific aspects of striped hyena social ecology (because they were not known). However, we can expect striped hyenas to follow the principles regulating group formation as demonstrated in, and deduced from, other carnivores: (1) Diet (through effects of resource abundance and dispersion) should influence group size and

individual distribution. (2) If ranges are shared, group structures and social interactions should reflect a balance between fitness costs and payoffs to behaviors such as the formation of cooperative coalitions of males to defend access to mates (Caro 1994), or cooperative hunting groups (Creel & Creel 1995).

In this chapter, I first describe the basic spatial organization, patterns of behavioral interaction, and genetic structure of a striped hyena population in Laikipia District, Kenya. I then compare each of these three aspects of the species' biology with similar traits in other carnivores. Through these comparisons, I evaluate hypotheses about social evolution in carnivores (summarized in Box 3.2). Finally, I discuss how the individual aspects of the species' ecology may interact as adaptations to the constraints imposed by their diet.

Methods

Study Area

The study was conducted from August 2000-October 2003 on private and communally owned ranch land in Laikipia District, Kenya. The primary study area of 480 km² was area centered on the 284 km² Loisaba Wilderness, a privately held livestock ranch and wilderness reserve (36° 50 East, 0° 63 North, altitude range 1400 to 1800m). The primary study area included portions of 9 properties, but other properties in the district were used opportunistically for limited capture and radio-tracking activities. The area is semi-arid with mean annual rainfall on Loisaba of 464 (± 37) mm from 2001-2003. The northwestern portion of this primary study area was relatively flat, open grassland

with scattered shrubs and light acacia bush. The Owaso Narok and Owaso Nyro rivers pass through the southern and eastern edges of the area and large, rocky escarpments and dry drainages dominate much of the western, eastern, and southern portions of the study area with the largest escarpment following roughly the 1700m contour. Outside of the northwestern portion of the study area, the landscape was a mixture of small grassland patches and heavy acacia shrubland with frequent intermixing of rocky outcrops and boulder fields. Livestock densities on Loisaba were typical for the region, ranging from 4.3-5.9 cattle per km², but grazing was heavily concentrated on and regulated to the larger open tracts of grasslands in the northern and western extremes of the ranch. Cattle were contained within bomas (pens) at night.

Trapping

Trapping and handling procedures followed Kenya Wildlife Service and IACUC approved guidelines. We caught striped hyenas using soft-catch foot-hold traps having 3/8" thick jaws offset by 3/8". We filed the jaws of the traps smooth and covered them in rubber to avoid hurting the foot. Additionally, we fitted a spring damper into the anchoring chain to further reduce leg and foot strain. We used three trap types: #4 Sterling 4-coil, #4 Northwoods double-coil, and #4 McBride long-springs. To control hyenas' approach to each trap, we either built a backing from available scrub or cut a U-shaped pattern into the base of a bush. We then set traps at the top-center of the U. We spread two to four small pieces of bait in an arc along the bottom of the U at a distance from the trap approximating the distance from the tip of a hyena's nose to its front paw. We used bait that typically came from livestock that had died on the ranch (which

otherwise would have been disposed of in an open pit accessible to hyenas), but also used road killed hares. Occasionally neighboring ranches caught hyenas in their own cage traps, which we then processed. The Laikipia Predator Project (LPP) also caught and processed a number of hyenas on other properties in cage traps or foot snares set for lions. Consequently, hyenas were captured and processed throughout the district.

We set foot-hold traps just before dusk, checked them twice during the night and once at dawn, and closed them in the daytime. We reset/rebaited any sprung traps or traps with bait taken were at each check. Each foot-hold trap site consisted of three to four traps spread over an area of ~50m in diameter. We set up three to five sites (12-21 traps in total) on a given night, with 100m to 1km between sites. So that we could check traps often, we limited all trapping activities to areas accessible by roads and trails. Cage traps and foot snares were typically restricted to one site and set at dusk and checked at dawn.

For most of the study period, we set foot-hold traps opportunistically across the study area at locations where striped hyenas had recently been seen. Beginning January 2003, we trapped using a spatially systematic approach. Over 10 months, we set traps in a pattern radiating outward from the center of the study area along roads and tracks (which striped hyenas often follow). We set traps at each site for a minimum of three nights. If any previously unmarked hyenas were caught, we kept traps active until no 'new' hyenas were caught for two consecutive nights. For each year of the study, we measured trapping effort by 'trap-nights' – the number of individual traps set multiplied by the number of nights each trap was active. Our high trapping and collaring effort

within the primary study was intended to catch and sample all adult hyenas and most of the older juveniles.

Animal Handling & Sample Collection

We anesthetized trapped animals using a blowpipe or dart gun and a 3cc Telinject or Daninject dart, with either Telazol at a dose of approximately 2.5 mg/kg, or with a combination of Ketamine (0.036cc/kg) and Metatomadine (0.06 cc/kg) delivered IM. Immobilization occurred within 10 minutes of injection. We removed immobilized animals from the trap and immediately treated their eyes with ophthalmic ointment, and then covered them with a cloth. Overheating was not an apparent problem because trapping was conducted at night and dawn only. We tied the feet of anesthetized hyenas to protect personnel in case of early arousal. We individually ear-notched each animal, using a scalpel, in a simple numerical identification code. We made no more than two one-half inch notches in each ear and we stopped any bleeding with a hemostat, which we could normally remove, without bleeding, within five minutes. We fitted all adult and young adult hyenas captured within the primary study with VHF radio collars (Telonics, Inc or SirTrack).

At each capture, we drew blood into evacuated tubes from the jugular vein. We collected tissue samples from ear notching only at first capture, but not at recaptures. We recorded body and tooth measurements at every capture along with weight as indexes of age. Body measurements recorded included weight, head-body and hind-foot lengths, neck circumference, chest girth, and shoulder height. Tooth measurements taken were designed to give an indication of wear, using upper and lower third premolar height,

length, and breadth. We also recorded reproductive condition (parous/nulliparous and lactation status for females, testes size for males). After data accumulated, we subsequently assigned each animal to age class based on known dates of birth or estimates from body measurements, weight, and tooth wear (cub: < 6 months, juvenile: 6mos to 1year, young adult: 1 to 3 yrs, adult: 3+ yrs). For each individual, we also took photos of genitalia, the left body profile, and head.

After marking and sampling were completed, for Metatomidine+Ketamine treated animals, we waited until the effects of the Ketamine appeared to wear off (typically ~45 min post-injection, as marked by more rapid breathing and/or muscle or ear twitching) and administered Atipamezole (0.25mg/kg) IM to reverse the Metatomidine. Recovery occurred in 5-10 minutes with the animal rising to its feet and walking away. No drug antagonist was used with Telazol treated animals. In all cases, we followed the animal in a vehicle until fully recovered from anesthesia, to protect it from predators.

We froze collected tissue samples in individual cryovials. We prepared blood samples in the field, according to protocols supplied by the National Cancer Institute's Laboratory for Genomic Diversity, to separate blood components. We always processed blood samples the morning after collection and froze the isolated components, along with tissue samples, within 12 hours of collection. We later transported samples on dry ice and, thereafter, kept samples in a 40°C freezer until removed for laboratory processing.

Radio Tracking & Spatial Data

Striped hyenas move and forage throughout the night and usually rest with limited movement during the day. We could directly follow and observe hyenas only on foot and

only in the daytime (0630-1829 hours). Consequently, radio-tracking was our primary tool for inferring nighttime locations and activity patterns. Within the primary study area, we based nighttime (1830-0629 hours) locations on triangulation of radio-collar signals taken from a vehicle, typically restricted to roads and tracks. We took bearings with a handheld three element yagi-antenna directed toward the peak of the signal, using a hand compass to identify the bearing of the signal. We recorded the position from which each bearing was taken by GPS (Magellan GPS 4000XL). We took two signal bearings and calculated the final position using the triangulation option in the GPS unit or by using LOAS 3.0.4 (Ecological Software Solutions, Urnäsch, Switzerland). We always selected the positions from which the bearings to the radio-collar signal were taken to allow the relative bearing between the two fixes used for each location to be as close to 90° as possible ($\pm 15^\circ$, in practice). The maximum time allowed between fixes for a valid location was 10 minutes. If the time between fixes exceeded 10 minutes, we recorded a new set of fixes. In addition to recording the location of the hyena being tracked, we scanned for the frequencies of all other hyenas to determine if any were in the area. We determined whether a hyena was active or inactive based on activity sensors in the radio-collars and changes in the direction and clarity of signals. For every location, we recorded the time of each bearing, whether the hyena was active or inactive, and the presence or proximity of any other hyenas. We collected locations for all hyenas within the primary study area at least once per month. In 2003, we collected locations for hyenas in the primary study area at least once per week. For hyenas outside of the primary study area, supplementary aerial locations (daytime only) were provided by the

LPP. We used ArcGIS 8.3 and ArcView 3.2a (ESRI, Redlands, California, USA) to plot and analyze spatial data. A base GIS layer of property boundaries within the district was provided by the Laikipia Research Centre.

Home-ranges & Space-use

Based on highly overlapping home-ranges and field observations of direct interactions, we identified distinct groups with stable membership (see Results). We selected six focal hyenas from three social groups in the primary study area for intensive radio-tracking from February to November 2003 (one male and one female from each of the Northern, Eastern, and Western groups). For each focal hyena, we recorded locations for every hour of the day according to a randomized (by time and individual) schedule with no more than one daytime and one nighttime location recorded for each individual within a 24-hour period. For every location, we checked the frequencies of all other hyenas, and if detected, triangulated the positions of those hyenas. We also used daytime walk-ins to record sightings of known/unknown hyenas in the immediate vicinity. We repeated this cycle seven times, from a new randomized schedule each time, yielding locations and activity patterns with seven observations for each individual in each of the 24 hours ($7 \times 6 \times 24 = 1008$ total observations taken on this schedule).

We determined the minimum number of locations needed to reliably estimate home-range size by identifying the asymptote in the relationship between calculated home-range size and the number of locations considered. For each of three males and three females with ≈ 150 locations, we identified the asymptote using a bootstrap routine (Seaman et al. 1999) to select locations, in steps of 10 locations, up to the maximum

number of locations available divisible by 10 (e.g. if 139 locations were available, only 13 sets of iterations were conducted). We randomly selected each set of points for each sample size (N=10, 20, 30...) from the full data set(s), using the Bootstrap file creator in the ArcView Animal Movement 2.0 extension (Hooge and Eichenlaub 1997), in 30 iterations with replacement between iterations. We determined a home-range for each iteration by the 95% fixed-Kernel method using the Home Range Extension for ArcView (Rodgers 1998). We then calculated the mean and variance of the home-range size (km²) for each individual at each sample size. We used the point at which the home-range size estimate and variance changed little with any increase in sample size (80 locations, see Results) as the minimum number of locations required to calculate home-range size for less intensively sampled individuals. For those with enough locations available, we calculated fixed-Kernel home-range size at 50, 75, and 95%.

In addition to plotting fixed-Kernel home-ranges, we plotted space-use polygons for every hyena with >20 locations available using the 95% fixed-Kernel method. Although these space-use polygons are not useful to estimate home-range sizes, they are still useful to examine patterns in the shared use of space. Using the fixed-Kernel method, home-range or space-use polygon size estimates diminish with an increase in the number of locations used. Consequently, space-use polygons are larger than, and fully encompass, actual home-ranges and are conservative estimators as applied in this study. All but one of the hyenas for which fixed-Kernel home-ranges were not determined were short-lived or were not a part of the targeted Northern, Eastern, and Western groups in

the primary study area in 2003. For individuals with <20 locations available, we plotted only point locations.

Spatial Patterns of Association

We calculated levels of association for all pairs of individuals that overlapped spatially and temporally. Here, we define ‘association’ as the proportion of observation periods in which a pair of hyenas was together. We calculated association levels as the number of occasions (nights or days) that the pair of hyenas was known to be together (resting at the same site or traveling together), divided by the total number of occasions at which the presence/absence of both hyenas was known. We did not need to know the exact location of both hyenas at each observation to classify them as located but not together: all that was required in this case was to confirm that the second member of the pair was not in the same location as the first (confirming that an animal is not in a specific location is easier than fixing its true location). We calculated association independently for observations made in the nighttime and made in the daytime. For daytime observations, we considered hyenas ‘together’ if we saw both hyenas simultaneously or found them within 50 meters of each other. At night, direct observations were not possible and hyenas we considered ‘together’ if their triangulated locations were within 200 meters of each other. We did not restrict nighttime locations to those observations taken after hyenas had clearly begun moving or foraging. Thus, there are some nighttime observations in which hyenas were considered together that represent a delay in separation for the night rather than actively joining together while foraging.

We further evaluated association by the characteristics of the pair: male-male or male-female. Because adult females did not share ranges, association for female-female pairs was, by definition, zero.

Temporal Patterns of Association

To evaluate differences in levels of association during the nighttime and daytime and between male-male pairs and male-female pairs, we used bootstrap simulations implemented with PopTools 2.6.2 (Hood 2003). We used bootstrap simulations to avoid pseudo-replication: the observations of association are repeated measures, but they are nested within dyads, rather than individuals, so that the common method of including individual identity as a random effect cannot be employed. Because some individuals appear in more of the pairs than others, different pairs with one individual in common cannot be considered independent, and the distribution of the observed data was non-normal. Similar considerations lead to developing additional bootstrap simulations in analyses of relatedness and space-use (see Patterns of Relatedness Across Social and Genetic Distances, below). To test for differences between day and nighttime association for male-female pairs, we randomly assigned each observed level of association as either a day or nighttime observation and calculated the mean level of association for those assigned to day and nighttime. This random assignment and mean calculation was iterated 1000 times. We then compared the observed difference between nighttime and daytime mean levels of association to the distribution of the simulated differences. We used the proportion of the frequency distribution for the simulated data more extreme than the observed difference to evaluate the significance of the observed difference. If

>5% ($\alpha=0.05$) of the simulated values were more extreme than the observed difference, we did not consider that difference to be significant. We repeated this bootstrap simulation for all pairs (male-male and male-female pairs considered together). For male-male pairs alone, however, there were only seven pairs of individuals to consider. With seven observations that can be assigned to two different categories (night and day), there are only 2^7 or 128 possible combinations. Rather than using a random system of assigning the observations to day and night (and repeating many combinations many times), we simply considered each of the possible 128 arrangements once because any randomization procedure would converge on this distribution.

To evaluate the difference between levels of association in male-male pairs and male-female pairs, we conducted a similar bootstrap simulation in which we randomly classified each observed level of association as either a male-male or male-female. In 1000 simulations, we calculated the male-male and male-female mean levels of association. We compared the observed difference between male-male and male-female levels of association to the frequency distribution of the simulated differences in the means. Again, we used the percentage of simulated values more extreme than the observed to evaluate the significance of the observed difference.

Overlap in Space-Use

For those individuals with enough locations to calculate accurate home-ranges in the primary study area, we calculated the proportions and area of 50% and 95% fixed-Kernel home-ranges that overlapped between individuals, for individuals living in the same social group and between those living in adjacent social groups. Because home-

ranges drift through time, we only considered spatial overlap for pairs of individuals with fixes that overlapped temporally. The percentage of spatial overlap for any two individuals is a relative measure and changes when calculated as a proportion of the home-range size of each of the two individuals being considered. Consequently, we used a full matrix to represent percent overlap relative to each individual. This was not necessary for area of overlap (km^2), an absolute measure.

Genetic Analyses

For all hyenas sampled, we used polymerase-chain-reaction (PCR) to amplify DNA extracted from tissue or blood samples. We evaluated primers for 23 microsatellite loci developed for spotted hyenas for use (Ccr11-17, Libants *et al.* 2000; Ccroc01-10, Wilhelm *et al.* 2003; ccr01-07, ccrA3, ccrA5, Funk & Engh unpublished). Eight of those primers (ccr04-06, Ccroc01&05-06, ccrA3, ccrA5) performed well and were utilized for genotyping. We detected null alleles at three loci (ccr05, ccrA3&A5) using ML-Relate (Kalinowski *et al. In press*). In ML-Relate, we applied a correction for the presence of null-alleles (Chapter 2, Wagner *et al. In review*) at those three loci for further calculations of relatedness (r) and the probability of relationships. This program uses a maximum likelihood approach and the degree of relatedness between individuals is on an absolute scale (0 to 1), not a relative scale as with other programs (e.g. Kinship). The program allows for pairwise calculations of Wright's (1922) coefficient of relatedness (r), as well as the likelihood of specific patterns of relationship. Here, we evaluated unrelated, half-sib, full-sib, and parent-offspring relationships, using likelihood ratio tests. The correction for null alleles improves the accuracy of relatedness and relationship

estimation, in general, and eliminates the problem of falsely excluding parents when a null allele causes an apparent mismatch between truly matching genotypes in maternity or paternity analysis.

Patterns of Relatedness Across Geographic Distances

We determined the geographic distance between pairs of individuals in two ways. First, we calculated the central balancing point (harmonic mean, HM) of each space-use polygon of each individual, in ArcView. We calculated the distance (km) between every combination of two harmonic means (HM distance) as a continuous measure of the distance expected to separate a pair of individuals. Second, we categorized every possible pair of individuals as to whether they lived in the same social group and used the same areas, lived in adjacent territories, or lived greater than one territory away from each other (non-adjacent). In both cases, we considered distances for all possible pairwise comparisons, and separated by sex class (all pairs, male-male, male-female, and female-female pairs— for the case of comparisons among groups, female-female pairs are possible). We then compared the degree of relatedness (r) between each pair of individuals to the geographic distance between them measured categorically (same, adjacent, non-adjacent) or continuously (HM distance).

We tested the significance of relationships between HM distance and relatedness by first fitting a least-squares linear regression to the data evaluated for each pair-type. Within each pair-type, we then developed a randomization procedure by which each observed r -value was selected at random, without replacement, and assigned to an observed HM distance. We then fit a least-squares regression to the randomized data.

We repeated this procedure in 1000 iterations (using PopTools) and the frequency distribution of the slopes determined. We evaluated the significance of the observed slope based on the percentage of simulated slopes more extreme than the observed slope.

Although the actual distance between the centers of activity of any two individuals may seem a reasonable predictor of relatedness (relatives should live closer together than non-relatives), the relationship between HM distance and degree of social isolation (number of home-ranges or territories between individuals) can be complex, particularly at close distances. For example, the configuration of individual territories can cause a segment of a given length (distance) drawn from one harmonic mean to cross less than one territory, if projected in one direction, but more than one territory if projected in another direction. Consequently, the categorical measure of 'group distance' (same, adjacent, non-adjacent) could be more sensitive even though categorization of continuous data typically reduces power.

We evaluated the usefulness of HM distance to represent social isolation between two individuals by first determining the frequency distribution of the number of extra-territorial overlaps observed for 28 individuals. For each individual, we then drew a circle of 69 km^2 (equal to the mean home-range size), centered on the harmonic mean of the individual's range, and determined the number of extra-territorial overlaps predicted. We then evaluated the ability of a continuous measure to reflect effective distance between any two individuals by comparing the number of actual territorial overlaps to the predicted number. If distance, in km, is a good measure of effective distance, the

frequency distributions of predicted and actual and the counts of territorial overlaps for each individual should be similar.

Patterns of Relatedness Across Social Distances

To test whether spatial and genetic population structure were associated, we tested whether related individuals were more likely to live in the same, adjacent, or non-adjacent groups. In our data, ‘unrelated’ was the most likely relationship (relative to half-sib, full-sib, and parent-offspring relationships) for all pairs of hyenas for which r was less than 0.15. Conversely, no individuals with $r=0.15$ were classified as unrelated. Consequently, we then used an r -value of 0.15 to separate related from unrelated pairs of individuals and considered the proportions of related pairs of individuals that lived together, or in adjacent or non-adjacent territories.

We tested for differences in the proportions of related individuals living in each spatial-category by bootstrap simulation. We calculated the differences in the observed proportion (ppn) of adult pairs living in each group class that were related (e.g. ppn related living in same group minus ppn related living in adjacent groups) as our test statistic. We isolated those observations in the data that were categorized by the two spatial-categories being compared. We then selected at random, without replacement, each observed r -value and assigned it to one of the two spatial-categories. Finally, we calculated the difference between the simulated proportions of individuals that were related in each spatial-category. We repeated this simulation in 1000 iterations and, from the frequency distribution of the simulated differences, determined the percentage of

simulated values more extreme than the observed. We conducted bootstrapping for every possible spatial-category comparison within each of the three possible pair-types.

Maternity and Paternity

We genotyped fourteen young hyenas (cubs and juveniles) from within the primary study area. Our criteria for assigning maternity and paternity were conservative in the sense of not excluding potential parents when the evidence was equivocal. We assigned maternity of each offspring based on the likelihood of the parent-offspring (L(PO)) relationship as determined in ML-Relate. We evaluated every adult female within three territories from the location at which the offspring was captured. Of those, we considered only females for which L(PO) was greater than zero to be viable maternal candidates. We assigned maternity only if each of those criteria were met. We further compared maternal assignments with our best guess of maternity based on field observations. We also considered the ability to assign maternity (and paternity, below) for each offspring as a test of the success of our trapping efforts for capturing all adults within the primary study area.

We assigned paternity in much the same way as maternity. However, since there were more males in any area than females, there were more paternal candidates, so we evaluated the relative degree of support for assigning paternity to each male. For each offspring, we considered the L(PO) for every paternal candidate relative to the highest L(PO) among all candidates. We considered individual candidate fathers to be viable only if paternity of the top candidate was less than 15-times more likely. Although this method does not allow for definitive assignment of paternity when multiple males meet

all of those criteria, likelihood ratios still show the degree of support for the top candidate relative to the others.

Diet

We used a combination of bone fragments and hairs taken from fecal samples to evaluate striped hyaena diet. We collected fecal samples at den sites ($n = 7$), trap sites ($n = 11$), and opportunistically in the field ($n = 10$). We could not separate samples collected at den sites into individual scats, so we grouped these samples according to the location collected. We processed samples following methods described in (Marker *et al.* 2003): we placed scats in individual nylon stockings and washed them thoroughly, we dried each stocking in a conventional oven at 65° C, we then spread each sample evenly over a 5X5 grid of 10cm squares, and selected one hair from the same area of each grid cell. In some instances, fecal samples contained fewer than 25 hairs and we selected all of the hairs in the sample (minimum of 20). In other instances, hairs were too badly damaged to retain diagnostic characteristics (the medulla or cuticle scales were missing) and we selected and used replacement hairs from the same sample. We also collected all visible bone fragments from within each sample.

We created cuticle scale imprints of sample hairs by placing hairs on plastic cover slips sandwiched between two glass microscope slides with four binder clips used as clamps. We then placed the slides in a conventional oven at 107° C for 5-10 minutes. Afterward, we allowed the slides to air cool and then peeled the hair sample off of the plastic cover slips with forceps. We retained the plastic cover slips for the cuticle imprints and the sample hairs for whole mounting. We sent bone fragments to the

Osteology Department at the National Museums of Kenya for identification (identification was made by department staff and by B. Pobiner, Anthropology Department, Rutgers University).

We collected reference hairs from 62 species of mammals (see Table 3.15) in the field or from skins stored at the National Museums of Kenya. Different cuticle scale and medullary patterns appear on hairs taken from different areas of an animal's body and along different longitudinal sections of the same hair. We imprinted representative sample of several reference hairs for each species and permanently whole mounted reference hairs on microscope slides using synthetic balsam in xylene or temporarily using lab-grade mineral oil. We created a photographic catalog of all of the different scale and medulla patterns apparent within each hair type available from each species. We then compared the sample hairs with the library of reference hairs and identified each based on general and medullary coloration, cuticular color banding, medullary pattern (e.g. simple, fragmental, lattice, globular, ladder), hair shape and shielding (e.g. spatulate, round), classification as hairs or 'spines', presence/absence and extent of protruding cuticular scale spines, form of scale margins (e.g. smooth, crenate), distance between scale margins (e.g. distant, near), number of scales across hair width, and scale patterns (e.g. simple, mosaic, waved) (adapted from descriptors and methods in: Hausman 1920; Mayer 1952; Brunner & Coman 1974; Moore *et al.* 1974; Perrin & Campbell 1980; Palenik 1983; Keogh 1983; Buys & Keogh 1984; Oli 1993; Riordan 1997). We assigned identified prey species remains to size classes based on typical adult body weight (class I:

<22.7 kg, class II: 22.7 to 113.4, class III: 113.4 up to 340.2, class IV: 340.2 to 907.2, class V: 907.2 up to 2721.6 kg).

Results

Trapping

Within the primary study area, from 2000 through 2003, there were 48 captures in 2729 trap-nights (Fig. 3.1-3.2, Table 3.1), and 54 captures in the remainder of the district (Fig. 3.2). We captured five cubs at den sites without the use of traps. In total, we caught 65 individual hyenas throughout Laikipia district (Table 3.2). Within the primary study area, we caught no new adults in 2003 (Table 3.1) despite a substantial increase in trapping effort, indicating that we had captured, identified, and sampled most adults in the population. With only six captures, adult re-captures in 2003 were below expectations. This can be attributed to the shift to systematic trapping, rather than trapping at locations where hyenas were seen, as in previous years. Hyenas that had been captured in previous years may also have learned to avoid traps by this time, but habituation is not likely to have affected hyenas that had never been trapped. Paternity and maternity analysis confirmed that mothers and fathers could be identified for the sampled population of young hyenas, which also indicated that the majority of adult hyenas were sampled within the main study area (see Maternity & Paternity, below). Within Loisaba, seven adults either died during the course of the study or were lost and not recaptured. Hyenas were lost due to either collar failure or, as evidenced by the fact that they were not recaptured or resighted, emigration.

Figure 3.1. Capture success and trapping effort within the primary study area for each study year. New captures are separated from recaptures and both are separated further by age class (adults and young). ‘Young’ hyenas include cubs, juveniles, and young adults. The values depicted for each class indicate the number of capture events, not the number of unique individuals caught. Trapping effort was measured by trap-nights: the number of individual traps set multiplied by the number of nights each trap was active. In the final year of the study, no new adult individuals were caught within the primary study area.

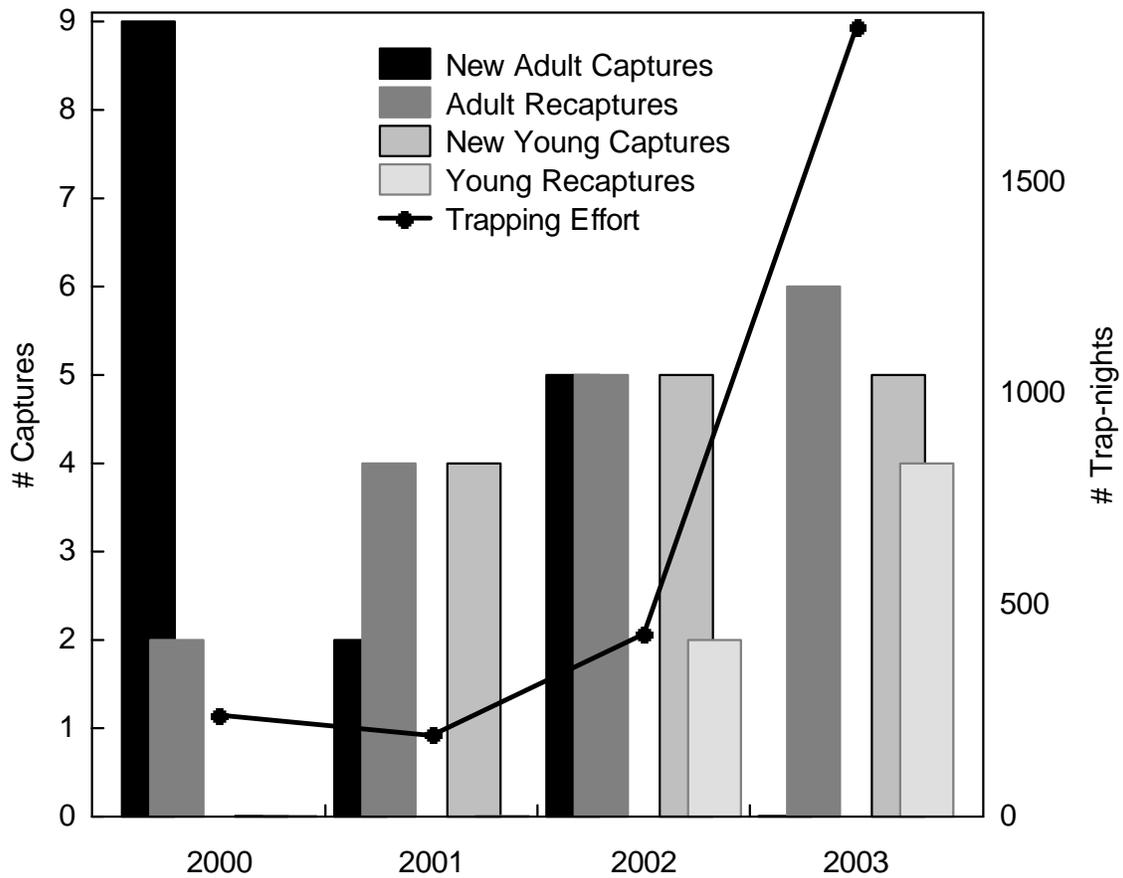


Figure 3.2. Locations of captures throughout Laikipia District. Inset and area shaded and outlined in the main map indicate the primary (Loisaba) study area. Text on the main map indicates individual property or ranch names. Point styles and colors indicate the sex and age class, respectively, of each hyena captured. Numbers in the figure key (N) represent the number of captures within each age*sex class, not individuals.

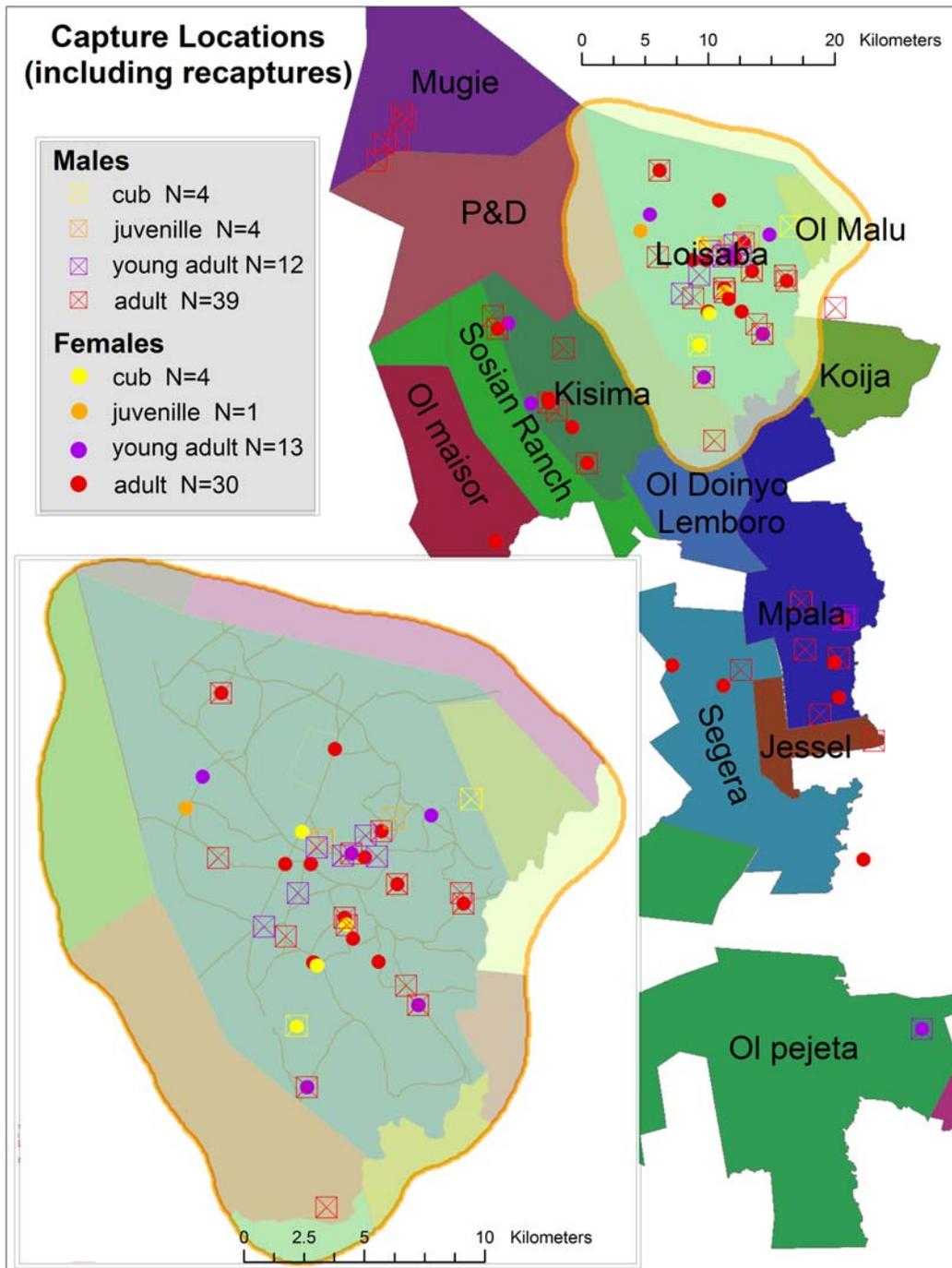


Table 3.1. Number of individual hyenas caught per year of the study as new captures or recaptures with the number of adults that were lost (collar failure) or died within each year and the trapping effort applied. Trapping effort was the number of traps set in a year multiplied by the number of nights each trap was kept open. All values in the table are from the primary study site only.

Year	Adult		Young		Adult deaths & loses	Trapping effort
	New	Recapture	New	Recapture		
2000	9	2	0	0	1	240
2001	2	4	4	0	2	192
2002	5	5	5	2	4	432
2003	0	6	5	4	0	1865

Table 3.2. Total number of individual male and female hyenas caught over the duration of the study, separated according to the age class assigned when each individual was first caught. Ages represented by each age class are indicated in parenthesis.

Age at First Capture	Males	Females
Adult (3+ yrs)	23	17
Young adult (1-3 yrs)	8	9
Juvenile (6 mos-1yr)	1	1
Cub (<6 mos)	4	2

Home-ranges & Space-Use

Hyenas' 95% fixed-Kernel home-range sizes stabilized when 70 or more locations were used for (Fig. 3.3 A&B). The variance of bootstrapped home-range size estimates also stabilized at 70 or more locations. In general, the fixed-Kernel method was robust to changes in the number of locations used. However, we chose 80 as a conservative minimum number of locations needed from each individual to estimate home-range.

Figure 3.3. 95% fixed Kernel (A) home-range (HR) size and (B) variance in home-range size estimates from bootstrap simulations of HR size for three males and three females with $n=150$ locations recorded. The maximum number of locations used for each individual was limited to the number of locations available. HR size was calculated in 30 simulations at each step of 10 points and the (A) mean and (B) variance calculated for each individual. Note that in Kernel methods for home-range estimation, home-range size tends to decrease with increasing number of fixes, in contrast to the patterns seen in other HR estimators like minimum convex polygon (MCP). Lines within the plot indicate the best fit log-function for the bootstrapped data for each individual. Legend indicates the symbols and lines corresponding to each of the six individuals evaluated.

Figure 3.3A.

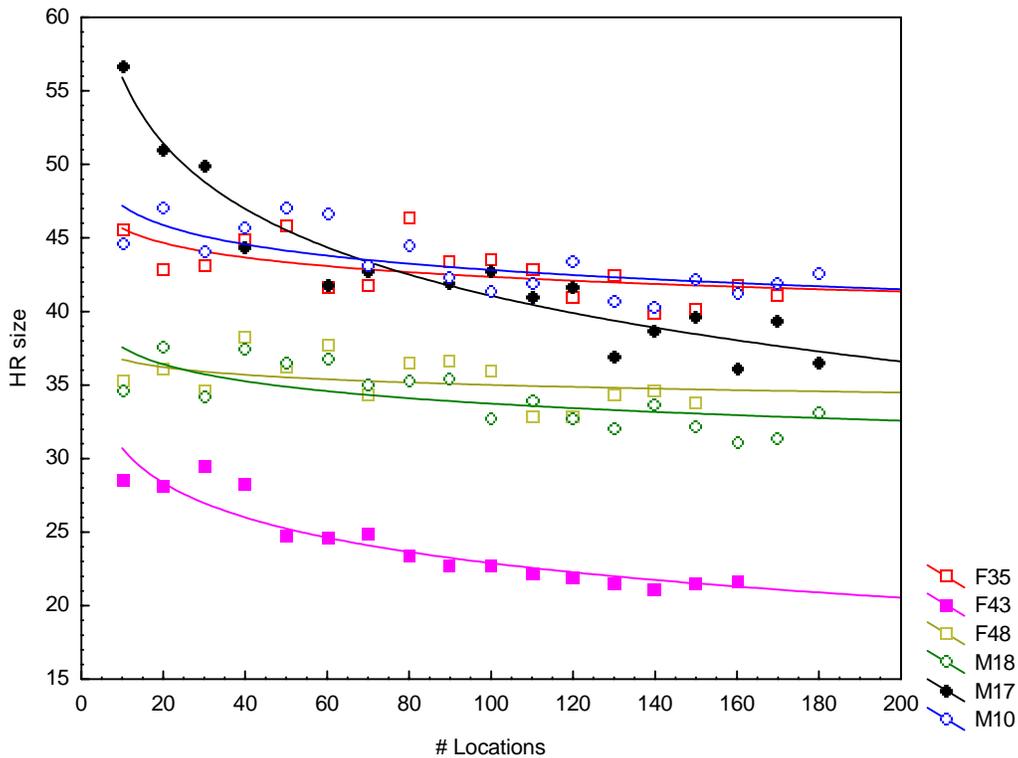
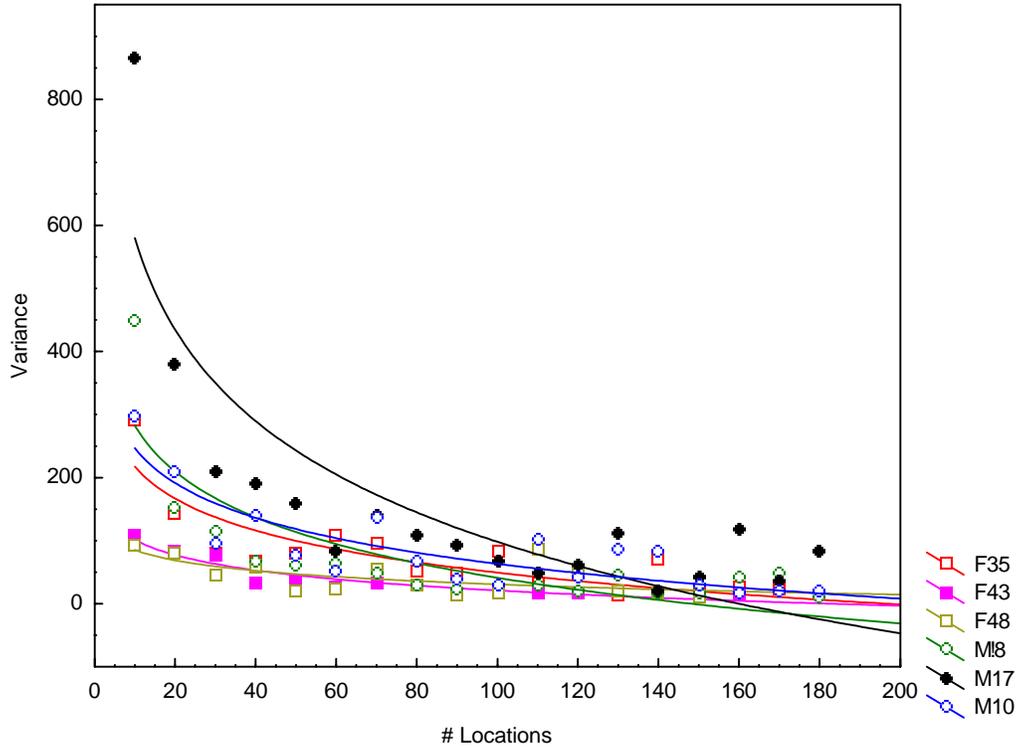
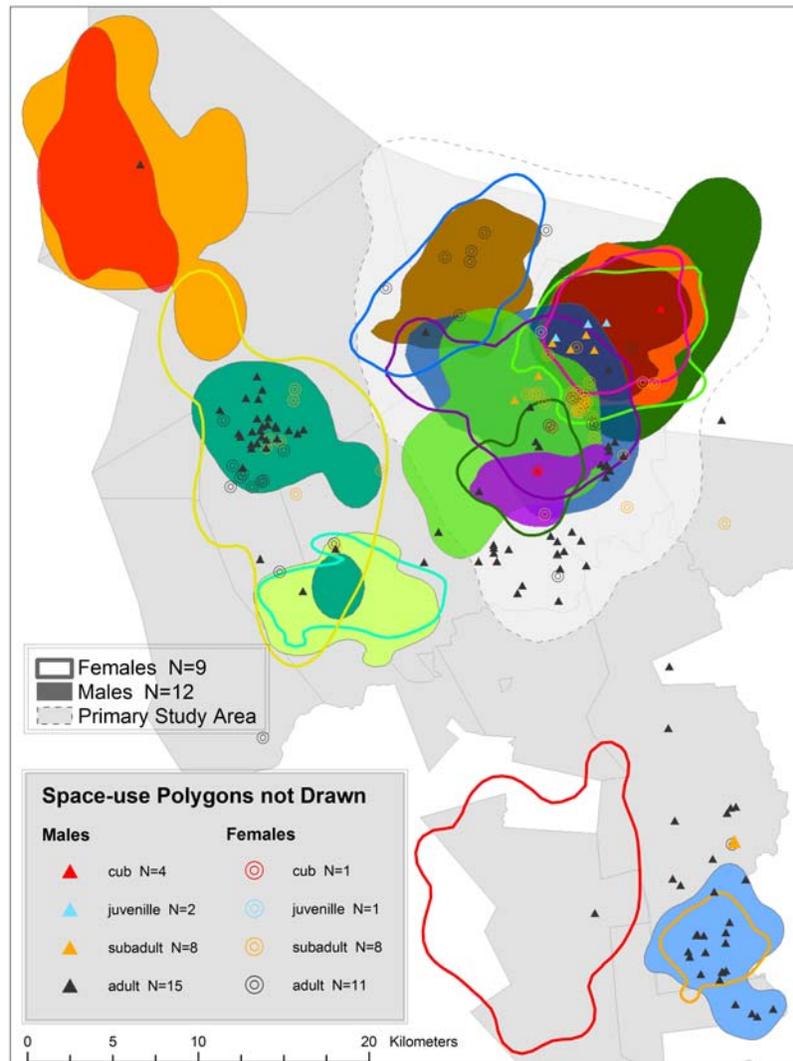


Figure 3.3B.



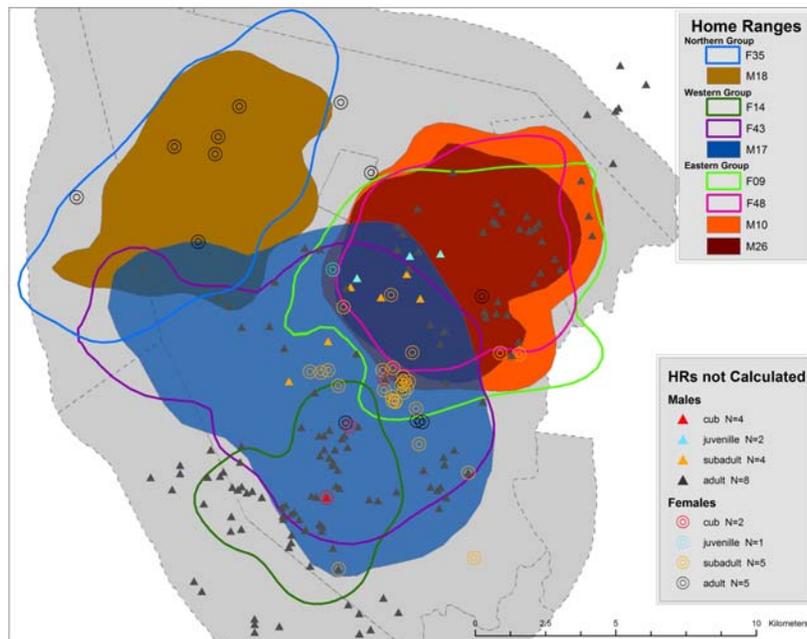
Group membership and the structure of social groups can be identified, in part, by the space-use polygons plotted for all individuals in the district with >20 locations (Fig. 3.4). Note that we distinguish ‘home-ranges’ (limited to those individuals with =80 locations) from ‘areas of use’, ‘space-used’, or ‘space-use polygons’. The latter terms are used for all individuals when we do not intend to imply that actual home-ranges were determined. Plots of home-ranges for individuals living in the primary study area (Fig. 3.5) show one adult female and one to three adult males living in three distinct social groups: Eastern, Western, and Northern.

Figure 3.4. Space-use polygons for every collared hyena for the duration of the study (2000-2003) and point locations for those individuals with ≤ 20 locations, including trap locations. Shaded polygons represent individual collared adult males and open polygons represent collared adult females. The number (N) of individual males and females plotted is indicated in the upper figure key. Point locations are not separated by individual, but are separated by sex and age class. The lower figure key indicates the point style and color for each sex and age class, respectively, and the number of individuals (N) within each age*sex class. Dark grey areas indicate different properties within the district. Light grey area, outlined by a dashed line, indicates the primary study area. Although calculated by the 95% fixed Kernel method, space-use polygons do not necessarily represent actual home-ranges as a requisite number of locations (≥ 80) were not available for some individuals. This map does not account for time, so some individuals with spatial overlap may not have overlapped temporally (e.g. no two females had significant areas of overlap in both space and time).



Our data extended over multiple years, but Fig. 3.4 does not account for temporal overlap or variation in space-use over time within Loisaba. Fig. 3.5 paints a clearer picture of *both* temporal and spatial overlap in the primary study area. However, note that F48 & F09 did not overlap temporally, nor did F14 & F43 (see Table 3.4 for accounting of temporal overlap among spatially overlapping individuals).

Figure 3.5. 95% fixed Kernel home-ranges for individuals with ≥ 80 locations within the primary (Loisaba) study area. Locations for individuals with fewer than 80 observations are plotted as points, differentiated by sex and age class point styles and colors. Upper figure key indicates the individual IDs and the shaded (males) or outline (females) patterns used for each. Lower figure key indicates the number of individuals (N) within each age*sex class represented as points. A third male (M11) utilized the Eastern portion of the study area and a second male (M42) utilized the Western portion of the region, but adequate numbers of locations were not available (M11: $n=45$, M42: $n=43$) to plot accurate HRs for these two individuals (space-use for M11 and M42 is represented by the dark green and light green polygons, respectively, in Fig. 3.4). Note that F48 and F09 did not overlap temporally, nor did F14 and F43 (see Table 3.4). Point locations are indicated for five adult females, but these females did not remain in the area or did not overlap in time with resident adult females.



50, 75, and 95% fixed-Kernel home-ranges were calculated for the ten individual adults that had 80 or more locations (Table 3.3). The mean \pm se 95% fixed-Kernel home-range size was $68.91 \pm 7.80 \text{ km}^2$ with no detectable difference between sexes (two-sample t-test: $p=0.49$; females: $n=6$, $\bar{x}=64.18 \pm 10.35$; males: $n=4$, $\bar{x}=76.02 \pm 12.67$), though male home-ranges were estimated to be 19% larger than those of females.

Table 3.3. Home-range (HR) sizes for those individuals having ≥ 80 location observations recorded. HR sizes were calculated by the fixed-Kernel method at 50, 75, and 95%.

ID	% Fixed Kernel (km^2)		
	95	75	50
F09	77.40	35.29	17.21
M26	51.77	26.48	13.93
M10	72.18	31.76	15.32
F48	54.55	26.76	14.52
M18	64.71	24.54	12.03
F35	72.33	33.88	16.63
M17	115.41	45.27	18.40
F43	100.63	40.15	15.19
F14	36.00	18.43	8.57
F21	44.16	19.96	9.70

Spatial & Temporal Patterns of Association

Plotting each location recorded in 2003 for two individuals in each of the three Loisaba groups shows a high degree of space-use overlap within groups, but low overlap between groups (Fig. 3.6). This shows that group mates occupied shared home-ranges, but does not address the question of whether they associated with one another within the shared range. Estimated levels of association within social groups (groups of individuals that overlapped spatially in a given year) were very low (Table 3.4). Males rested with other males on only 4% of days and males rested with females on 8% of days. At night

(when foraging and other activity occurs), males were never found together and males were found with females 8% of the time (Table 3.4). Thus a picture emerges of largely independent movements and solitary foraging within a shared home-range. The distinction between spatial and social groups is clear for striped hyenas, as in some other ‘proto-social’ carnivores such as white-tailed mongooses *Ichneumia albicauda* and slender mongooses *Herpestes sanguineus* (Waser & Waser 1985; Waser *et al.* 1994; see Discussion). This distinction is important when considering the selection pressures that might favor the initial evolution of group living, relative to the selection pressures that operate once grouping is established.

Figure 3.6. Point locations, from 2003, representing space-use for three males and three females that resided in the primary study area at the same time. IDs, point colors used, and the number of observations for each individual (N) are indicated in the figure key. Despite the low frequency at which individual members of the same group are found together (Table 3.4), groups and group members are readily differentiated by the high degree of overlap in space-used (M10 and F48; M18 and F35; M17 and F43) and the low degree of overlap with adjacent group members in space-used.

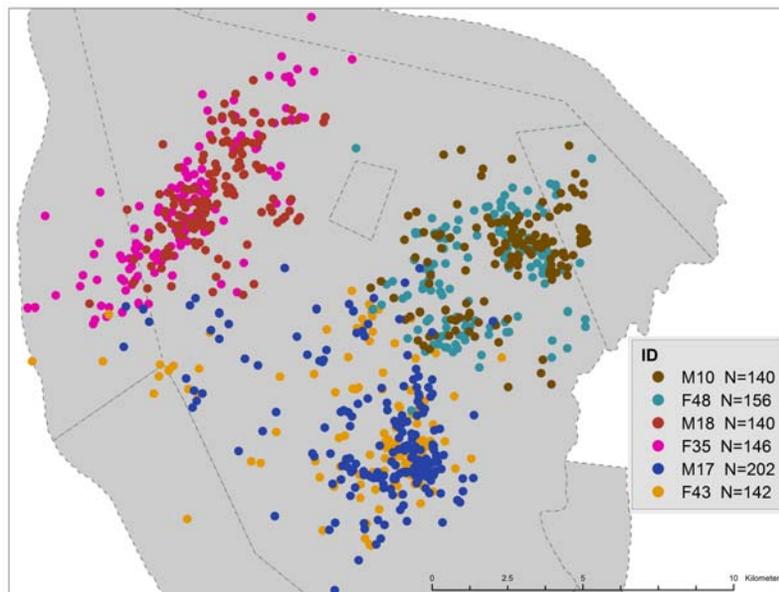


Table 3.4. Pairs of individual adults that overlapped spatially and temporally in the primary study area with the dates for which their space use overlapped, the number of days within that period, the total number of occasions (Total obs) at which the individuals were known to be together or apart, the number of those occasions for which those individuals were together, and the resulting levels of association (Assoc. = Together / Total obs) for each pair for those observations recorded in the daytime and nighttime, and the overall level of association (Overall = Together day + Together night / Total day + night obs). There were no simultaneous nighttime locations available for the M11-M26 and F14-M23 pairs.

ID1	ID2	Overlapping Dates	Overlapping Days	Day			Night			Overall
				Total obs	Together	Assoc.	Total obs	Together	Assoc.	
F09	M10	16-Aug-00 to 01-Aug-02	715	77	3	0.04	8	2	0.25	0.06
F09	M11	16-Aug-00 to 14-Feb-02	547	55	2	0.04	7	0	0.00	0.03
F09	M26	13-Jun-01 to 01-Aug-02	414	61	1	0.02	1	0	0.00	0.02
M10	M11	16-Aug-00 to 14-Feb-02	547	49	3	0.06	7	0	0.00	0.05
M10	M26	13-Jun-01 to 09-May-03	695	99	5	0.05	8	0	0.00	0.05
M10	F48	12-Feb-03 to 27-Nov-03	288	64	10	0.16	26	3	0.12	0.14
M11	M26	13-Jun-01 to 14-Feb-02	246	31	0	0.00	0	-	-	0.00
M26	F48	12-Feb-03 to 09-May-03	86	17	3	0.18	8	0	0.00	0.12
F14	M23	08-Jun-01 to 22-Jul-02	409	49	4	0.08	0	-	-	0.08
M18	F35	10-Dec-02 to 27-Nov-03	352	53	8	0.15	26	2	0.08	0.13
M17	F43	15-Jul-02 to 27-Nov-03	500	76	10	0.13	31	3	0.10	0.12
M17	M42	14-Jul-02 to 27-Nov-03	501	61	1	0.02	17	0	0.00	0.01
F43	M42	14-Jul-02 to 27-Nov-03	501	68	2	0.03	21	0	0.00	0.02

From the bootstrap simulations, there were no differences detected in the levels of association in the daytime vs. the nighttime for males with males, females with males, and all individuals pooled (Table 3.5). These results should be viewed with caution, however, as individuals were categorized as ‘together’ during the day only when they were seen or found together from a close observation distance. At night, individuals were classified as ‘together’ whenever the triangulated positions from each radio-collar were within 200 meters. If a finer spatial scale could have been applied to the nighttime observations, nighttime levels of association might have been lower, which would further reinforce the conclusion that striped hyenas typically forage alone within their shared ranges. Further bootstrap simulations showed that males were more likely to associate with females than with other males (bootstrap $p = 0.029$). We were not able to isolate periods of male-female association that may have fallen during mating periods, but the higher levels of association for inter-sexual pairs are probably attributable to reproduction. Low levels of male-male association within a group does not support a view of groups as highly organized, interactive social units as in some other social carnivores (e.g. African wild dogs, dwarf mongooses, meerkats).

Table 3.5. Mean levels of association for adult male-male, male-female, and all pairs of individuals that overlapped spatially and temporally within the primary study area. Differences for each pair type between nighttime and daytime levels of association were tested with bootstrap simulations. The observed difference between mean levels of night and daytime associations (= Night Mean assoc. – Day Mean assoc.) was compared with the frequency distribution of the differences in the mean night and daytime associations from the simulations. The percentile of the frequency distribution where the observed differences fell indicate levels of association were not significantly different, at $\alpha=0.05$, between night and day for male-male, male-female, and all pairs. In each of the three cases, $>5\%$ of the simulated values were less than the observed difference.

	Day		Night		Obs. Night – Day Assoc.	Percentile of the Randomized Distribution
	Mean assoc.	n	Mean assoc.	n		
All pairs	0.068	13	0.062	11	-0.006	0.227
Male-male	0.038	4	0	3	-0.038	0.070
Male-female	0.083	9	0.078	8	-0.004	0.277

Overlap in Space-use

The 50% fixed-Kernel home-ranges (home-range ‘cores’) of adjacent groups did not overlap at all (Table 3.6), but overlap in the core ranges of group-mates averaged $73 \pm 2\%$ ($n=14$) or $11.07 \pm 0.59 \text{ km}^2$ ($n=7$). The 95% fixed-Kernel home-ranges of adjacent groups overlapped by an average of $22 \pm 2\%$ ($n=24$) or $17.17 \pm 2.11 \text{ km}^2$ ($n=12$) (Table 3.7), for those adjacent groups where overlap was non-zero. The mean overlap of 95% home-ranges for group-mates was $85.26 \pm 3\%$ or $59.52 \pm 6.08 \text{ km}^2$.

Table 3.6. Proportion of overlaps in 50% fixed-Kernel home-ranges within and between social groups in the primary study area for those individuals with =80 location observations (Fig. 3.5), presented by individual (across rows and columns). Both F48 and F09 were in a group with the same three males (M10-M26-M11; M11 not shown due to low number of locations), but the two females were not present at the same time. Bold outlines indicate within group overlaps by group. The full matrix is needed as overlap must be evaluated relative to each individual considered. The average within group overlap was 0.73 (se=0.02). No home-ranges in adjacent groups overlapped.

	F09	M26	M10	F48	M18	F35	M17	F43
F09	-	0.70	0.81	**	0.00	0.00	0.00	0.00
M26	0.57	-	0.68	0.72	0.00	0.00	0.00	0.00
M10	0.72	0.75	-	0.73	0.00	0.00	0.00	0.00
F48	**	0.75	0.69	-	0.00	0.00	0.00	0.00
M18	0.00	0.00	0.00	0.00	-	0.59	0.00	0.00
F35	0.00	0.00	0.00	0.00	0.82	-	0.00	0.00
M17	0.00	0.00	0.00	0.00	0.00	0.00	-	0.92
F43	0.00	0.00	0.00	0.00	0.00	0.00	0.76	-

Table 3.7. Overlaps in 95% fixed-Kernel home-ranges within and between social groups in the primary study area for those individuals with =80 location observations (Fig. 3.5), presented by individual. F48 and F09 were not present in the Eastern group at the same time. Bolded box contents indicate same group members. The average within group overlap was 0.85 (se=0.03). Average between group overlap was 0.13 (se=0.02). Northern (M18-F35) and Eastern (F09/F48-M10-M26-M11) groups were adjacent, but did not overlap. Average home-range overlap for those living in adjacent groups where overlap was >0 (Northern-Western and Eastern-Western), was 0.22 (se=0.02).

	F09	M26	M10	F48	M18	F35	M17	F43
F09	-	0.96	0.88	**	0.00	0.00	0.40	0.27
M26	0.64	-	0.72	0.87	0.00	0.00	0.25	0.16
M10	0.82	1.00	-	0.98	0.00	0.00	0.29	0.19
F48	**	0.91	0.74	-	0.00	0.00	0.22	0.14
M18	0.00	0.00	0.00	0.00	-	0.79	0.15	0.07
F35	0.00	0.00	0.00	0.00	0.88	-	0.14	0.09
M17	0.40	0.37	0.31	0.31	0.18	0.15	-	0.93
F43	0.36	0.16	0.27	0.26	0.11	0.13	0.82	-

Patterns of Relatedness Across Geographic Distances

Using HM distances (see Methods), the spatial distance between all pairs of hyenas, between pairs of males, or between males and females cannot be predicted by the degree of genetic relatedness between those individuals (Fig. 3.7A-C). However, the degree of relatedness between females appears inversely related to the distance between them (Fig. 3.7D). The bootstrap simulations used to test for a significant relationship between HM distance and relatedness only detected a significant effect ($p=0.003$) for females (Table 3.8). Consequently, females living farther apart appear, on average, to be more closely related than females living adjacent to one another.

Figure 3.7. Geographic distance (kilometers) between the central harmonic mean of individual space-use polygons (HM distance) compared to the degree of genetic relatedness (r) between individuals. Horizontal bars beneath each plot indicate the range of HM distances observed within each categorical descriptor of distance (space-use distance; Fig. 3.11): living in the same group, living in adjacent groups, and living in non-adjacent groups. Fitted least-squares regression lines are included within each plot.

- A) All pair-wise comparisons for the entire adult population.
- B) Males compared to other males only.
- C) Males compared to females.
- D) Females compared to other females.

Relatedness among females living close together (low HM distance) appears lower than for those living farther apart (high HM distance). No patterns are apparent for male-male pairs, male-female pairs, or the population as a whole.

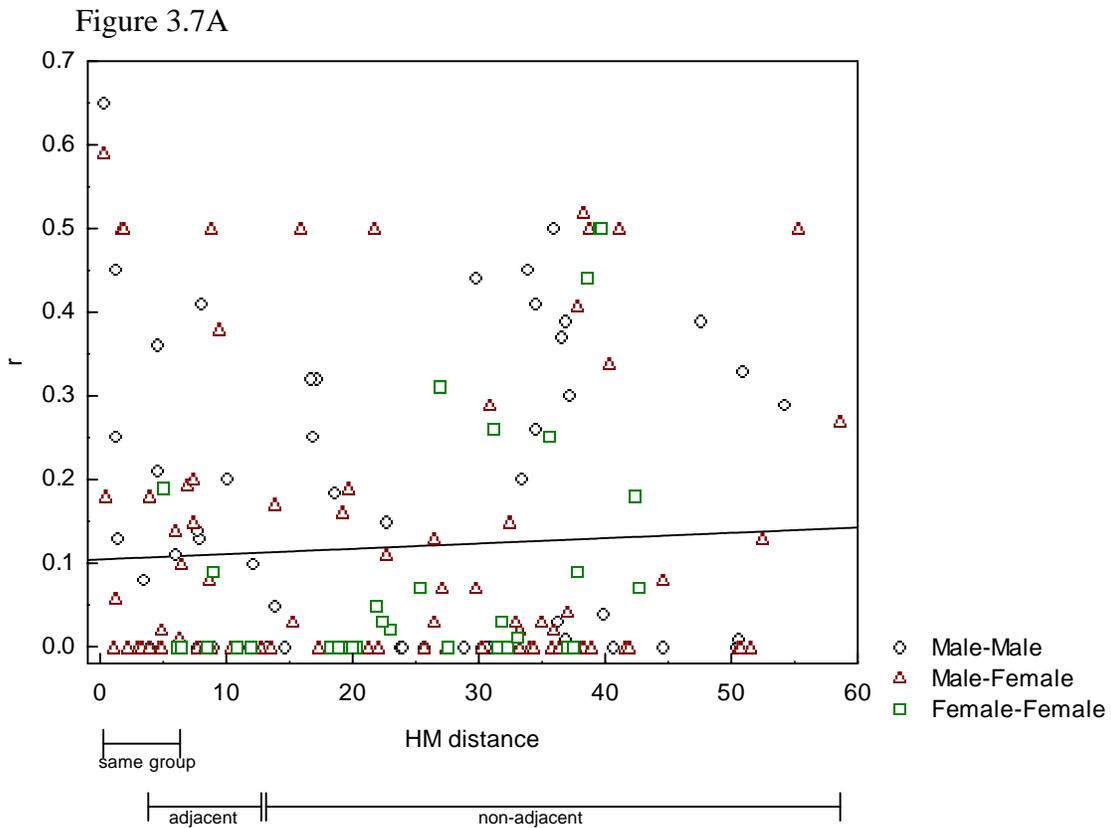


Figure 3.7B.

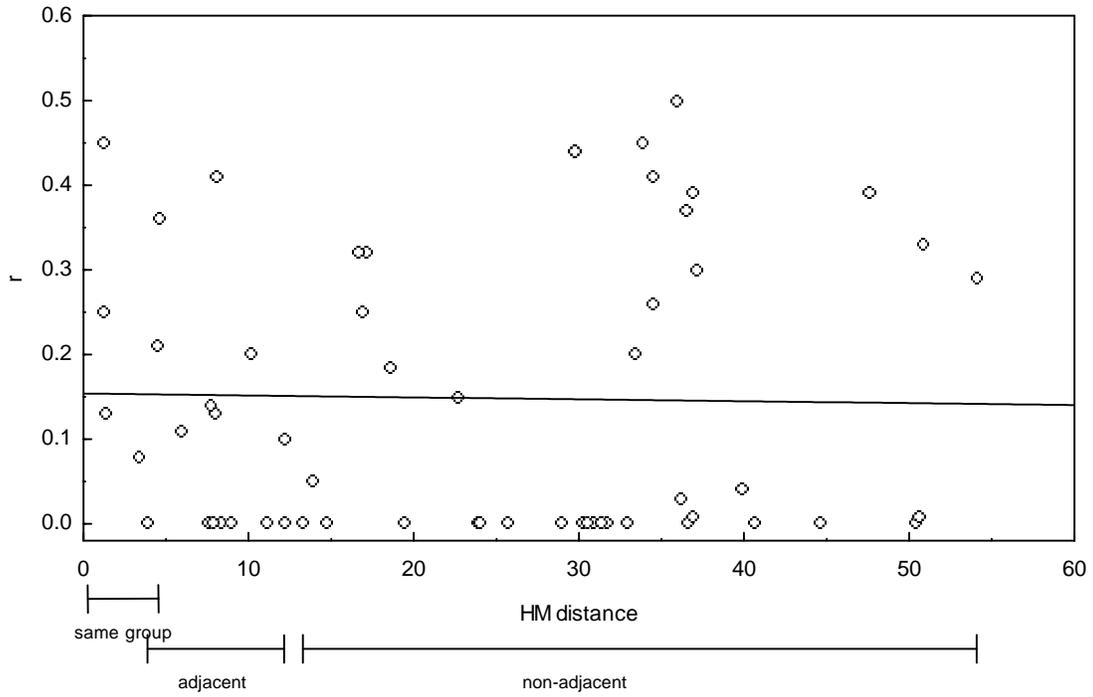


Figure 3.7C.

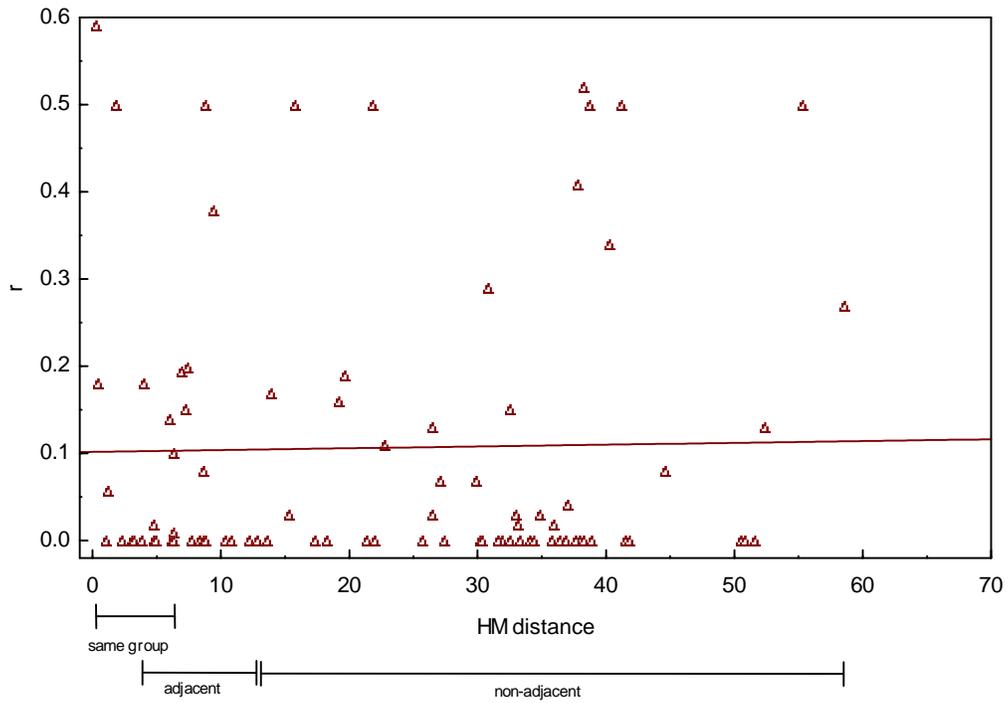


Figure 3.7D.

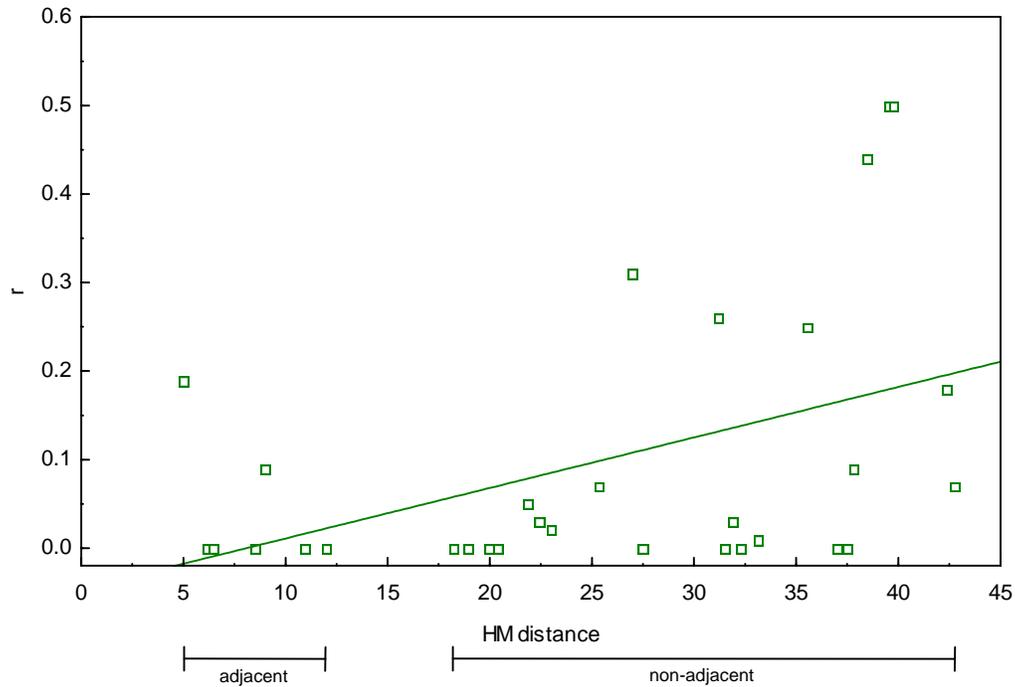


Table 3.8. The slope of the least-squares regression line from plots of the distance between harmonic-means of space-use polygons and the degree of relatedness (Fig. 3.7B-D) compared to the slopes of the best fit lines from simulations. The simulated slopes were determined by separating the male-male, female-female, or male-female pairs, randomly selecting, without replacement, and assigning each observed r -value, within each sex-pair class, to one of the HM distances observed for the same sub-set of the data, and calculating the slope of the best fit line. Slopes were calculated in 1000 simulations for each sex and group class and the percentage of slopes more extreme than the observed slope determined.

	Observed Slope	Percentile of the Randomized Distribution
Males	-0.0002	0.479
Females	0.0057	0.003
Males-Females	0.0002	0.404

Patterns of Relatedness Across Social Distances

When distance between areas of space-use is translated into categories (living in the same, adjacent, non-adjacent groups), the degree of relatedness between individuals is highest for those living in the same group and lowest for those living in adjacent groups for male-male and male-female comparisons (Fig. 3.8; mean r -values for male-male dyads: same=0.30, adjacent=0.08, non-adjacent=0.14; for male-female dyads: same=0.14, adjacent=0.04, non-adjacent=0.12). For males sharing the same group, mean relatedness was high, falling between that of non-inbred half-sibs (0.25) and full-sibs or parent-offspring (0.5). This level of relatedness is similar to that seen in groups of highly social, cooperatively breeding carnivores (e.g. dwarf mongooses *Helogale parvula*: Creel & Waser 1994, lions *Panthera leo*: Packer *et al.* 1991) as well as other hyaenids (brown and spotted hyenas: Mills 1989). Adult females were never found living together in one group, but relatedness also tended to be lower in adjacent groups when compared to non-adjacent groups, as it was for males and inter-sexual pairs (mean r -values for female-female dyads were 0.04 and 0.12 in adjacent and non-adjacent categories, respectively).

If two hyenas live in adjacent groups, the HM distance between the centers of their areas of space-use may not reflect this ‘social-proximity’, because of irregular home-range shapes and configurations. Differences in the patterns observed in Fig. 3.7 and Fig. 3.8 are partially attributable to the lack of a clear relationship between the ‘social’ isolation between two individuals and the HM distance between them. For example, when an artificial territory (represented by a circle of a set radius centered on the harmonic mean of space-used) is created for each of 28 individuals from this study

population and the number of territory overlaps is compared with the actual observed number of overlapping space-use polygons, there is little apparent relationship (Fig. 3.9). The uncertain relationship between HM distance and social isolation between two individuals can be seen clearly when the HM centers are plotted for as subset of space-use polygons from the primary study site (Fig. 3.10). The actual distance between same group members (e.g. F09 & M11) may be the same as the distance for individuals living in adjacent groups (e.g. F09 & M42).

Maximum likelihood tests showed that pairs of individuals with an estimated coefficient of relatedness less than 0.15 were more likely to be unrelated than any other relationship considered (half-sibs, full-sibs, parent-offspring). Plotting the proportion of individuals that were related ($r = 0.15$) for dyads of hyenas in the same, adjacent, and non-adjacent groups, we found a strong association between spatial and genetic population structure for males, but weaker association for other types of dyads (Fig. 3.11). Related males are most likely to live in the same group. If related males do not live in the same group, it is (surprisingly) more likely that they will live in non-adjacent groups than adjacent groups. For males, the proportions of related individuals living in same, adjacent, or non-adjacent groups differed at $\alpha=0.05$ (Table 3.9). Although the patterns are weaker and not significant at $\alpha=0.05$, related male-female pairs were also more likely to live in the same group and females were most likely to live in territories distant from those of related females. From this pattern it appears that males prefer to share ranges with relatives and, if unable to do that, to orient themselves more than one home-range away from relatives. Because approximately twice as many related females

live in non-adjacent ranges than in adjacent ranges, related females also appear to prefer living in ranges non-adjacent to relatives. However, related males and females are no more likely to live far from each other than to live in adjacent ranges.

Figure 3.8. Pair-wise genetic relatedness (r) of individuals relative to the categorical distance between areas of space-use (same group, adjacent groups, non-adjacent groups) for male-male, female-female, and male-female pairs. Horizontal lines indicate the mean degree of relatedness within each distance and pair-type category. Size of each point in the plot reflects the number of observations (minimum count=1, maximum=7). No two females live in the same groups, but for male-male and male-female comparisons, the highest level of relatedness is found between individual living in the same group. For all pair-types, those living in non-adjacent groups appear more closely related, on average, than those living in adjacent groups.

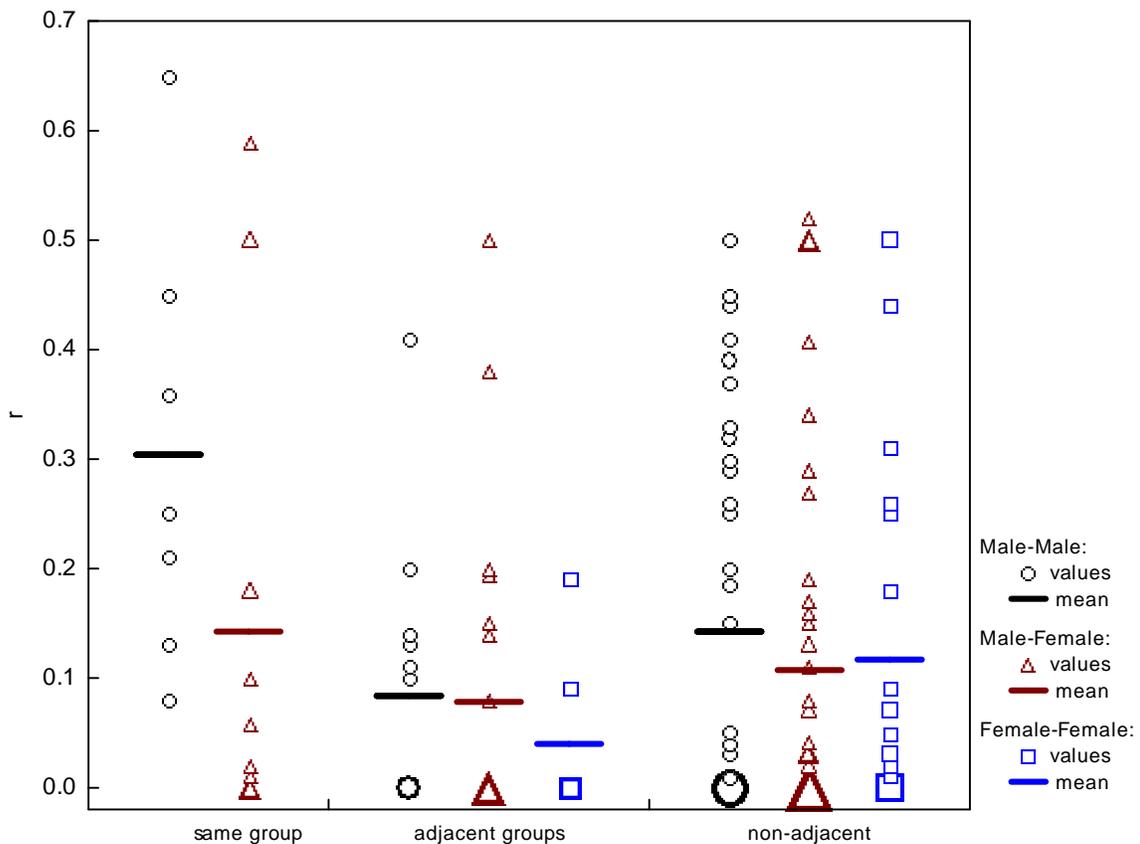


Figure 3.9. Predicted vs. actual number of extra-territorial overlaps for 28 individuals illustrated as frequency distributions of the number of extra-territorial overlaps predicted by using a circle of area equal to the mean *Hyaena* home-range size (69 km^2) centered on the harmonic mean of space-use polygons and the true number of overlaps based on actual polygons. The discrepancies between the two sets of bars illustrate that geographic distance does not accurately measure effective distance between any two individuals (number of territories between two individuals).

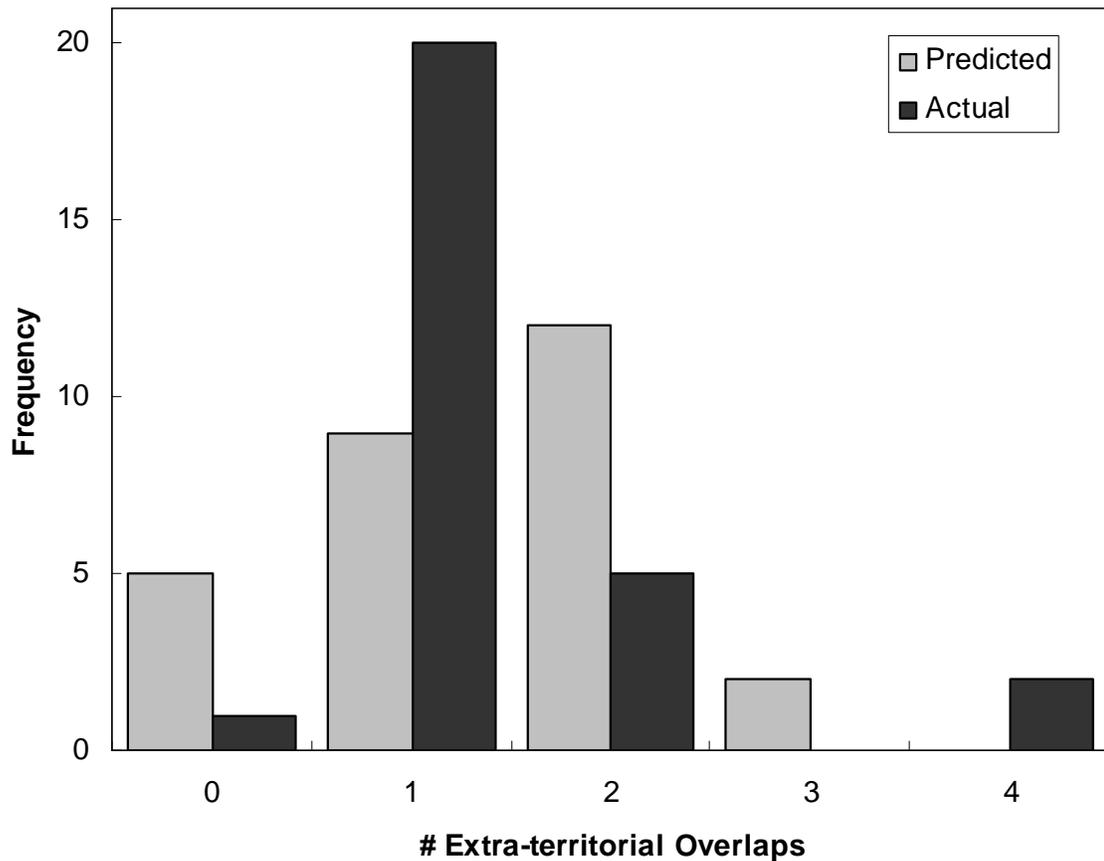


Figure 3.10. Space-use polygons for the Eastern and Western groups for the duration of the study. Points and labels indicate the harmonic mean of space-use for each individual. Note the lack of a clear relationship between HM distance and effective distance: the HM distance between F09 or M26 and M42 in the adjacent group is almost the same as the distance to members in the same group (M10, M11). M42 is also as close to non-group members (F09, M26) as to group members (F43, M17).

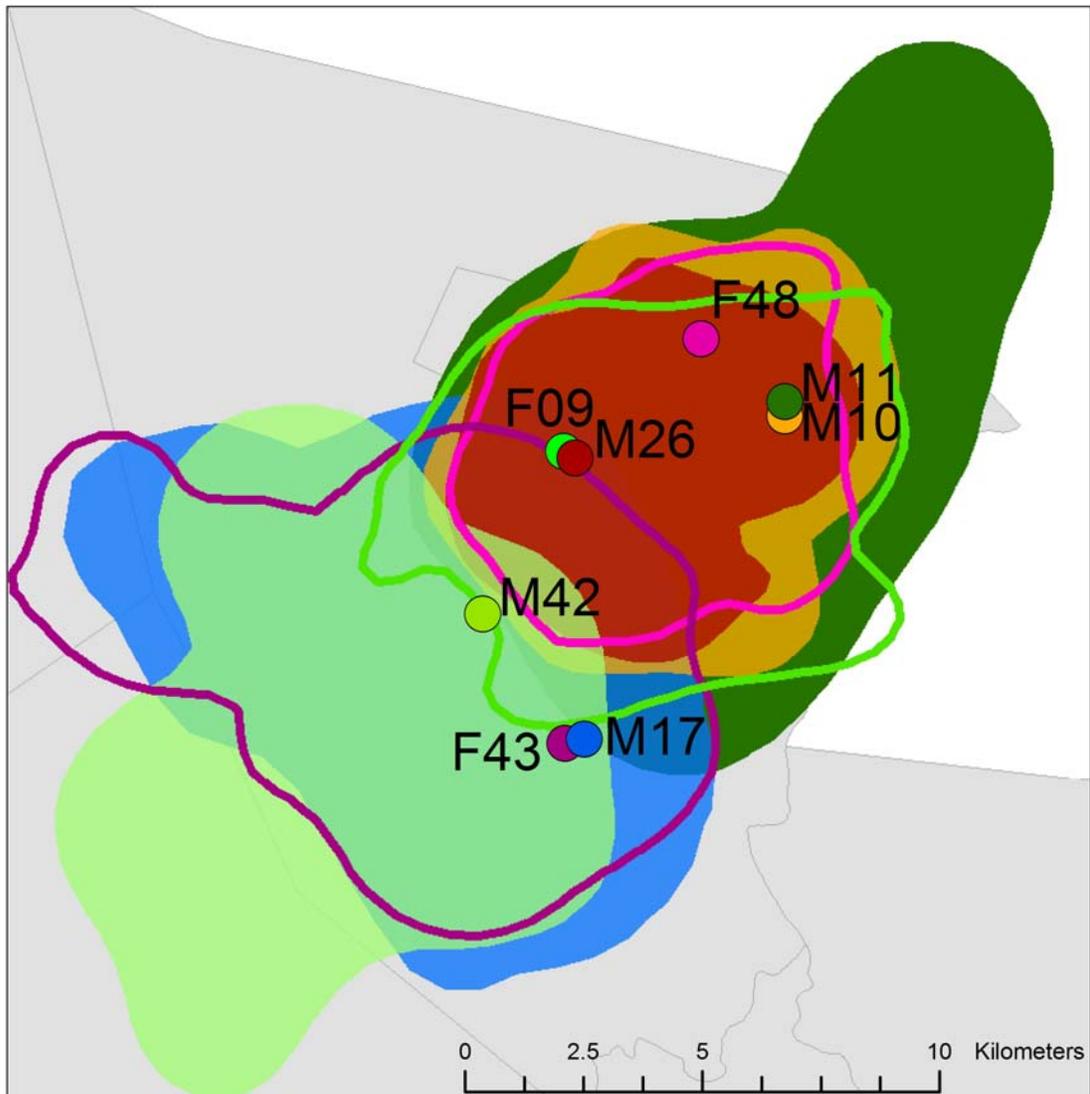


Figure 3.11. Proportions of (A) male-male, (B) male-female, (C) female-female pairs of individuals living in the same, adjacent, or non-adjacent groups that are related ($r=0.15$) or unrelated ($r<0.15$). Within a group, males are likely to be related to each other, but not to females. Males also tend to either live with relatives or disperse >1 territory away from relatives, but not to territories adjacent to relatives. This likely reflects a preference for forming coalitions with relatives or, alternatively, distributions across adjacent and non-adjacent areas in accordance with availability (see Discussion). Females, which do not live with other females, also tend to live >1 territory away from relatives, reflecting a preference for remaining in adjacent areas (somewhat counter-intuitively; see Discussion).

Figure 3.11A.

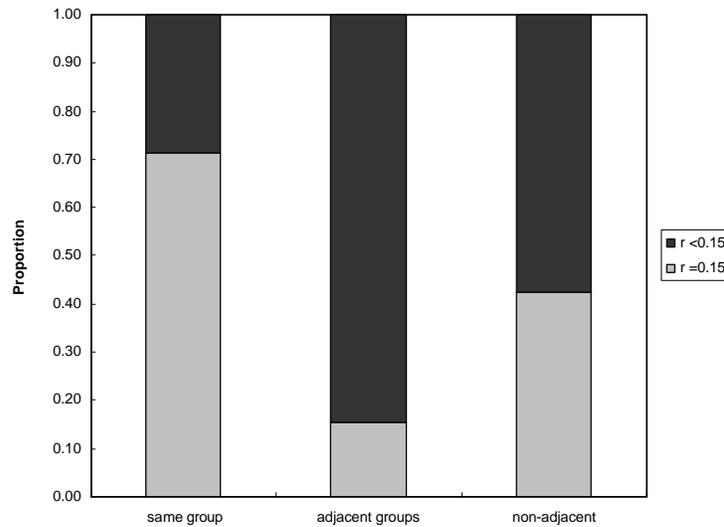


Figure 3.11B.

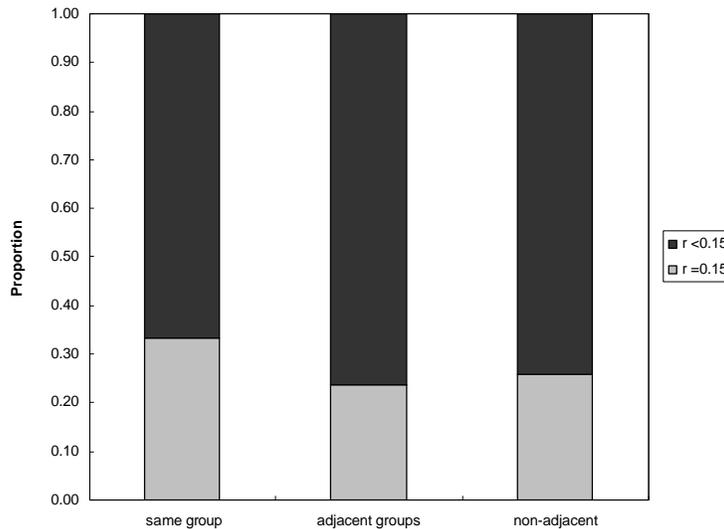


Figure 3.11C.

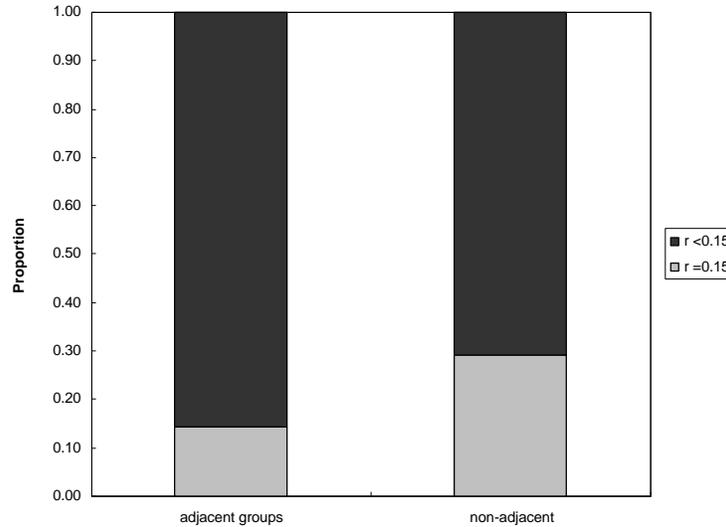


Table 3.9. The observed differences in the proportion of adult pairs within each group class (same, adjacent, non-adjacent), separated also by sex, that are related ($r=0.15$; comparing proportions between bars but within each of Figures 3.11A-C) and the percentage of simulated differences having values more extreme than the observed. The simulated differences were determined by separating the male-male, female-female, or male-female pairs, randomly selecting, without replacement, each observed r -value within each group (and sex) class being considered, assigning each r -value randomly to one of the two group classes, and calculating the difference between the proportions of r -values= 0.15 in each group class. Differences were calculated in 1000 simulations for each sex and group class and the percentage of observations more extreme than the observed difference determined. Adult females only live in adjacent or non-adjacent groups.

			Observed Difference	Percentile of the Randomized Distribution
Males	same group	– adjacent groups	0.56	0.002
	same group	– non-adjacent groups	0.289	0.059
	adjacent groups	– non-adjacent groups	-0.271	0.041
Females	adjacent groups	– non-adjacent groups	-0.149	0.199
Males-Females	same group	– adjacent groups	0.095	0.22
	same group	– non-adjacent groups	0.074	0.275
	adjacent groups	– non-adjacent groups	-0.021	0.443

Maternity and Paternity

Maternity could be assigned to all but one of the young hyenas evaluated (Table 3.10). In 13 of 14 cases, the maternal candidate thought to be the mother based on field observations was also the most likely maternal candidate based on microsatellite genotypes. Viewed another way, the offspring was also found in the area used by the most likely mother in 13 of 14 cases. In three cases (F41, F49, M30), parent-offspring (PO) was not the most likely relationship for the assigned parent, but the most likely relationship was less than one-and-a-half times the likelihood of PO. In one of those three cases (M30), two litter-mates (M31 & M32) had the same mother identified, both with PO most likely. The assignment of maternity to the female living in the same area for all offspring tends to confirm that no undetected females utilized the primary study area.

Paternity was successfully assigned to a sampled adult male for all of the offspring considered (Table 3.11). For maternity, only one female satisfied our selection criteria in each case, but for paternity, up to three males were considered viable candidates. In some cases, there was much stronger support for one male than for other viable candidates (e.g. most likely father of F35 was more than seven-times more likely than the next best candidate: Table 3.11), but in many cases the relative degrees of support for each father are roughly equivalent. Despite the difficulty of separating paternal candidates, in two of the four cases where offspring from the same litter were considered, multiple paternity of litters is apparent. Additionally, the most likely paternal candidate did not live in the same group as the female (or cub) in five of fourteen cases

and no males from the same group were potential fathers in three of those cases. One male (M17) also appears to have bred more successfully with females in his own group (F12: mother of F15, F16; F43: mother of M56, M57, M58, F41) relative to the other male in the group (M42: paternal candidate in only two cases). Biases in male reproductive success are also common for more social carnivores (see Discussion).

Table 3.10. Offspring, the possible mothers for each offspring, the degree of relatedness (r) for the parent-offspring pair, the relationship most consistent with the genotypes (ML(R)), the ratio of the most likely relationship to the likelihood of a parent-offspring relationship (ML(R)/L(PO)), the best guess of maternity based on field observations (Best Guess), and the geographic distance (measured in number of territories) between the offspring and maternal candidate. Bold outlines indicate probable same-litter siblings. Only those mothers where $L(R=PO) > 0$ and that lived < 3 territories from the area used by the offspring were considered candidates. F15 was missing at the time that M34 was found and is unlikely to be the true parent. With one exception, the most likely maternal candidate based on field observations is genetically consistent with being the mother of each offspring sampled.

Offspring	Maternal Candidate	r	ML(R)	ML(R)/L(PO)	Best Guess	same?	# Territories apart
F15	F12	0.5	PO	1.00	F12	Yes	0
F16	F12	0.5	PO	1.00	F12	Yes	0
F24	F14	0.59	PO	1.00	F14	Yes	0
F35	F21	0.6	PO	1.00	F21	Yes	0
F41	F43	0.7	FS	1.21	F43	Yes	0
F49	F09	0.28	HS	1.23	F09/F43	Yes	0
M30	F09	0.61	FS	1.26	F09	Yes	0
M31	F09	0.5	PO	1.00	F09	Yes	0
M32	F09	0.5	PO	1.00	F09	Yes	0
F33a	F14	0.52	PO	1.00	F14	Yes	0
M34	F15*	0.45	PO	1.00	F14	No	
M56	F43	0.59	PO	1.00	F43	Yes	0
M57	F43	0.59	PO	1.00	F43	Yes	0
M58	F43	0.59	PO	1.00	F43	Yes	0

Table 3.11. Offspring, the possible fathers for each offspring, the degree of relatedness (r) for the parent-offspring pair, the relationship most consistent with the genotypes, the ratio of the most likely relationship to the likelihood of a parent-offspring relationship, the best guess of paternity based on field observations, and the geographic distance between the offspring and paternal candidate. Bold outlines indicate probable same-litter siblings. Only those fathers where $L(R=PO) > 0$, $L(PO)$ for the paternal candidate relative to the paternal candidate with the highest $L(PO)$ was < 15 (e.g. $L(PO)_{best}/L(PO)_x < 15$), and that lived < 3 territories from the area used by the offspring were considered candidates. The sampled population contained a male genetically consistent with paternity for all offspring.

Offspring	Paternal Candidate	r	ML(R)	ML(R)/L(PO)	L(PO) _{best} / L(PO) _x	Best Guess	same?	# Territories apart
F15	M17	0.5	PO	1.00	1.00	M17	Yes	0
	M23	0.5	PO	1.00	1.68			
	M26	0.26	HS	1.27	4.71			
F16	M17	0.5	PO	1.00	1.00	M17	Yes	0
	M23	0.5	PO	1.00	3.19			
F24	M44	0.5	PO	1.00	1.00	M23	No	1
	M36	0.5	PO	1.00	2.27			
F35	M42	0.5	PO	1.00	1.00	M22	No	1
	M39	0.26	HS	1.21	7.32			
	M46	0.27	HS	1.25	9.49			
F41	M17	0.5	PO	1.00	1.00	M17/M42	Yes	0
F49	M46	0.27	HS	1.19	1.00	M10/M11/ M26/M42	No	1
M30	M26	0.51	PO	1.00	1.00	M10/M11/ M26	Yes	0
	M46	0.42	PO	1.00	1.80			
M31	M17	0.41	PO	1.00	1.00	M10/M26/ M11	No	1
	M10	0.46	PO	1.00	1.60			
M32	M46	0.5	PO	1.00	1.00	M10/M26/ M11	No	1
	M26	0.5	PO	1.00	3.25			
F33a	M42	0.5	PO	1.00	1.00	M17	No	0
M34	M17	0.5	PO	1.00	1.00	M17	Yes	0
M56	M17	0.5	PO	1.00	1.00	M17/M42	Yes	0
M57	M17	0.5	PO	1.00	1.00	M17/M42	Yes	0
M58	M17	0.5	PO	1.00	1.00	M17/M42	Yes	0

Diet

The patterns apparent in many hairs are remarkably similar across species, particularly when the origin (in terms of location on the body) cannot be known. For example, impala, Grants gazelle, and Thomson's gazelle cannot be distinguished with any great degree of confidence. This is particularly problematic in circumstances, such as this, where very little is known about the species diet and there is little basis to limit the prey species under consideration. Consequently, some of the hairs examined were identified to small groups of species (impala / Grants gazelle / Thomson's gazelle; suni / duiker; goat / sheep), not individual species. Species within these groups tended to be of similar size and the characteristics of those prey species that are relevant to predatory selection and behaviour tended to be similar. Bone fragments from larger species are probably more likely to be consumed as small fragments while bone from smaller species may retain more of their diagnostic characteristics. Consequently, bone fragments are more readily identifiable to species when the species represented are small. Larger species were typically distinguishable by size class alone, but may be under-represented in the bone fragment analysis.

All of the 28 fecal samples collected contained identifiable hairs and 13 contained bone fragments identifiable to species (Table 3.12). Even with the difficulties inherent in these methods, the diet analysis indicates that smaller species, those more likely to be killed than scavenged, represent a significant proportion of the striped hyena diet. Smaller species are more likely to represent killed items than to represent scavenged carcasses because remains of smaller prey species, particularly the skins (hair), would not

persist for long periods in the environment. On the other hand, logic also dictates that a zebra, impala, or gazelle is more likely to represent scavenging in the striped hyena diet than hunting. Finally, patterns indicative of hunts of large prey apparent in other radio-tracking studies of carnivores (sudden, rapid, and erratic changes in signal directions: S. Creel *pers. comm.*) were also not detected in this study. Accordingly, a conservative separation of prey species indicates killed and scavenged food items are represented in roughly comparable proportions: at least 54% of striped hyena fecal samples contained likely prey and at most 61% contained remains of prey likely to be scavenged.

Importantly, most represented prey items were very small, not favoring foraging or feeding group formation (this may not be true of large carcasses, but they are extremely rare—in 743kms of walking transects bones and small potential prey were frequently encountered, but no carcasses were encountered: *unpublished*). Additionally, a number of very different types of prey are represented and, when all represented items (prey, bones, and carcasses) are considered as a single group, the expected ‘summed-food-resource’ distribution would be more uniform than patchy.

The minimum number of species (MNS) identified by analysis of bone fragments averaged (\pm se) 2.27 ± 0.28 per fecal sample and 1.52 ± 0.13 for hair analysis (Table 3.13). The minimum number of individuals (MNI) identifiable by bone fragments averaged 2.27 ± 0.28 . The two methods of analysis identified at least one of the same prey species in 77% of the samples from which both hair and bone fragments were identified (37% of all samples, including those for which no bone fragments were collected). The two methods also identified prey from the same size classes in 93% of

samples where both hair and bone fragments were available (52% when all samples are considered). Finally, hair analysis identified, on average, 60% ($se = 0.10$) of the species identified by bone fragments in the same sample, while bone fragments, when found, identified $63\% \pm 0.10$ of the same species identified by hair analysis ($34\% \pm 0.08$ overall, including samples without bone fragments). Consequently, the two methods compliment each other well, but application of both methods offers a broader description of dietary components.

We also collected bones from two den sites (A and B) from which fecal samples containing both identifiable hairs and bone fragments had been collected (Table 3.14). These collections, however, represented a much broader range of prey than did scat analysis and significant portions of bone assemblages were very old bones unlikely to represent scavenging from fresh kills. In addition, larger mammals were represented far less often in the analysis of hairs than would have been expected based on den bone collections alone. In general, den site bone collections represent bones collected from large mammalian species and interpretation of that data alone may cause overestimation of the importance of scavenging. Consequently, it seems unwise to rely on den bone collections for diet determination. Even with this potential bias, however, size class I species account for 55% of species represented. At site A, the minimum number of species (MNS) represented was nine of which 56, 33, and 22% were in size classes I, II, and III, respectively. At site B, MNS was 11 with 55, 18, 9, and 27% in size classes I, II, III, and IV. Note that site A was outside of the primary study area (specifically on OI Pejeta, see Fig. 3.2) and samples from site A were not considered in any other analyses.

Table 3.12. Components of striped hyena diet as determined by identification of prey remains in fecal samples. Size class typically refers to mammalian body sizes, but the same classifications are applied to the birds (B) and reptiles (R) represented here. Bone fragments that could be identified to size class, but not species, were only included if the size class identified did not represent the same class as other bone fragments identified to species in the same sample. Multiple species were frequently represented in the same fecal sample, so the percentage of samples containing prey items do not sum to one. The overall percentage of prey remains that likely represent active hunting (Killed), scavenging (Scavenge), or could represent either behavior (Either) is given for the species listed below each classification. These categorizations are meant to be conservative and are generally based on the size, rarity, and frequency of occurrence of the prey species.

Bone Fragments (n = 13)				Hairs (n = 28)			
Common name	Scientific name	size class	% samples	Common name	Scientific name	size class	% samples
Killed			85				54
suní	<i>Neotragus moschatus</i>	I	38	suní / duiker	<i>Neotragus moschatus</i> / <i>Sylvicapra grimmia</i>	I	29
duiker	<i>Sylvicapra grimmia</i>	I	23	Kirk's dik-dik	<i>Madoqua kirkii</i>	I	18
francolin		B / I	23	scrub hare	<i>Lepus saxatilis</i>	I	7
bat		I	15	ground squirrel	<i>Xerus rutilus</i>	I	7
Kirk's dik-dik	<i>Madoqua kirkii</i>	I	15	hyrax	<i>Procavia johnstoni</i>	I	4
hare	<i>Lepus saxatilis</i>	I	15	rodent	<i>Muridae</i>	I	4
guinea fowl		B / I	15				
rodent	<i>Muridae</i>	I	8				
rat	<i>Muridae</i>	I	8				
small reptile		R / I	8				
steinbok	<i>Raphicerus campestris</i>	I	8				
hyrax	<i>Procavia johnstoni</i>	I	8				
Either			46				18
warthog	<i>Phacochoerus aethiopicus</i>	II	31	warthog	<i>Phacochoerus aethiopicus</i>	II	11
unknown mammal	size class I	I	23	baboon	<i>Papio anubis</i>	II	7
Thomson's gazelle	<i>Gazella thomsoni</i>	I	8				
Scavenged			23				61
unknown mammal		II	15	impala / Grant's gazelle / Thomson's gazelle	<i>Aepyceros melampus</i> / <i>Gazella granti</i> / <i>Gazella thomsoni</i>	I / II	39
unknown mammal		II or III	8	plains zebra	<i>Equus burchelli</i>	III	11
				goat / sheep	<i>Capra hircus</i> / <i>Ovis aries</i>	I	11
				giraffe	<i>Giraffa camelopardalis</i>	IV	4
				striped hyena	<i>Hyaena hyaena</i>		32

Table 3.13. The average, per sample, minimum number of individuals (MNI) identified by fecal bone fragments, the minimum number of species (MNS) identified by fecal bone fragments and hairs for each prey size class (I through V), and the overall average MNI and MNS identified for all prey size classes.

	Bone Fragments		Hairs
	MNI	MNS	MNS
class I	2.00	1.87	0.81
class I or II	0	0	0.22
class II	0.33	0.33	0.33
class II or III	0.07	0.07	0
class III	0	0	0.11
class IV	0	0	0
class V	0	0	0.04
Overall average (\pm s.e.)	2.40 \pm 0.38	2.27 \pm 0.28	1.52 \pm 0.13

Table 3.14. Fecal bone fragments, fecal hairs, and bones collected at two den sites. At each site, all fecal samples and bones apparent were collected and all bones and fecal bone fragments examined. 25 fecal hairs were identified from 200 and 17 fecal pellets collected at sites A and B, respectively.

Site	Den Bones		Fecal Bone Frags		Fecal Hairs	
	Common name	size class	Common name	size class	Common name	size class
A	maribou stork	B / I	duiker	I	sun / duiker	I
	grey crowned crane	B / I	sun	I	warthog	II
	Thomson's gazelle	I	warthog	II	zebra	III
	Kirk's dik-dik	I				
	sheep / goat	I				
	impala	II				
	Grant's gazelle	II				
	warthog	II				
	plains zebra	III				
	zebra / donkey	III				
B	kori bustard	B / I	warthog	II	warthog	II
	Gabon viper	R / I			zebra	III
	klipspringer	I			striped hyena	
	steinbok	I				
	duiker	I				
	Kirk's dik-dik	I				
	impala	II				
	warthog	II				
	plains zebra	III				
	Cape buffalo	IV				
	domestic cow	IV				
	mammal	IV				

Table 3.15. Reference hairs used in fecal sample identification.

Order	Family	Species	Common Name
Artiodactyla	Bovidae	<i>Aepyceros melampus</i>	Impala
Artiodactyla	Bovidae	<i>Alcelaphus buselaphus</i>	Hartebeest
Artiodactyla	Bovidae	<i>Gazella granti</i>	Grant's gazelle
Artiodactyla	Bovidae	<i>Gazella thomsoni</i>	Thomsons Gazelle
Artiodactyla	Bovidae	<i>Litocranius walleri</i>	Gerenuk
Artiodactyla	Bovidae	<i>Madoqua kirkii</i>	Kirk's Dik-dik
Artiodactyla	Bovidae	<i>Oreotragus oreotragus</i>	Klipspringer
Artiodactyla	Bovidae	<i>Syncerus caffer</i>	Buffalo
Artiodactyla	Bovidae	<i>Taurotragus oryx</i>	Eland
Artiodactyla	Bovidae	<i>Tragelaphus scriptus</i>	Bushbuck
Artiodactyla	Bovidae	<i>Bos taurus</i>	Cow
Artiodactyla	Bovidae	<i>Capra hircus</i>	Goat
Artiodactyla	Bovidae	<i>Ovis aries</i>	Sheep
Artiodactyla	Bovidae	<i>Oryx beisa</i>	Oryx
Artiodactyla	Bovidae	<i>Kobus ellipsiprymnus</i>	Waterbuck
Artiodactyla	Camelidae	<i>Camelus dromedarius</i>	Dromedary Camel
Artiodactyla	Giraffidae	<i>Giraffa camelopardalis</i>	Giraffe
Artiodactyla	Suidae	<i>Phacochoerus aethiopicus</i>	Warthog
Carnivora	Canidae	<i>Canis megalotus</i>	Bat-eared fox
Carnivora	Canidae	<i>Canis mesomelas</i>	Black-backed jackal
Carnivora	Canidae	<i>Canis lupus familiaris</i>	Domestic dog
Carnivora	Canidae	<i>Canis adustus</i>	Side-striped jackal
Carnivora	Canidae	<i>Lycaon pictus</i>	Wild dog
Carnivora	Felidae	<i>Acinonyx jubatus</i>	Cheetah
Carnivora	Felidae	<i>Felis caracal</i>	Caracal
Carnivora	Felidae	<i>Felis domesticus</i>	Domestic cat
Carnivora	Felidae	<i>Felis serval</i>	Serval
Carnivora	Felidae	<i>Felis sylvestris</i>	Wildcat
Carnivora	Felidae	<i>Panthera leo</i>	Lion
Carnivora	Felidae	<i>Panthera pardus</i>	Leopard
Carnivora	Herpestidae	<i>Helogale parvula</i>	Dwarf mongoose
Carnivora	Herpestidae	<i>Herpestes ichneumon</i>	Egyptian mongoose
Carnivora	Herpestidae	<i>Herpestes pulverulenta</i>	Slender mongoose
Carnivora	Herpestidae	<i>Ichneumia albicauda</i>	White-tailed mongoose
Carnivora	Herpestidae	<i>Mungos mungo</i>	Banded mongoose
Carnivora	Hyaenidae	<i>Crocuta crocuta</i>	Spotted hyaena
Carnivora	Hyaenidae	<i>Hyaena hyaena</i>	Striped hyaena
Carnivora	Hyaenidae	<i>Proteles cristatus</i>	Aardwolf
Carnivora	Mustelidae	<i>Ictonyx striatus</i>	Zorilla
Carnivora	Mustelidae	<i>Mellivora capensis</i>	Honey badger
Carnivora	Viverridae	<i>Genetta genetta</i>	Common Genet
Hyracoidae	Procaviidae	<i>Procapra johnstoni</i>	Rock hyrax

Table 3.15 (continued).

Order	Family	Species	Common Name
Lagomorpha	Leporidae	<i>Lepus saxatilis</i>	Scrub hare
Macroscelididae	Elephantulus	<i>Elephantulus rufescens</i>	Lesser Elephant shrew
Perrissodactyla	Equidae	<i>Equus asinus</i>	Donkey
Perrissodactyla	Equidae	<i>Equus caballus</i>	Horse
Perrissodactyla	Equidae	<i>Equus burchelli</i>	Plains zebra
Primates	Cercopithecidae	<i>Papio anubis</i>	Olive baboon
Primates	Cercopithecinae	<i>Cercopithecus pygerythrus</i>	Vervet (blue) monkey
Primates	Galagonidae	<i>Galago crassiceudatus</i>	Bush baby
Rodentia	Hystriidae	<i>Hystrix cristata</i>	Porcupine
Rodentia	Muridae	<i>Acomys wilsoni</i>	Spiny mouse
Rodentia	Muridae	<i>Acomys percivali</i>	Spiny mouse
Rodentia	Muridae	<i>Arvicanthis abyssinicus</i>	Unstriped grass rats
Rodentia	Muridae	<i>Arvicanthis niloticus</i>	Unstriped grass rats
Rodentia	Muridae	<i>Mus musculus</i>	Common mouse
Rodentia	Muridae	<i>Tatera robusta</i>	Large Naked-Soled Gerbils
Rodentia	Muridae	<i>Aethomys lundae</i>	
Rodentia	Muridae	<i>Dasymys incomptus</i>	Shaggy swamp rat
Rodentia	Muridae	<i>Grammomys ibeanus</i>	Narrow-footed woodland mice
Rodentia	Muridae	<i>Grammomys macmillani</i>	Narrow-footed woodland mice
Rodentia	Muridae	<i>Mastomys natalensis</i>	Multimammate rats
Rodentia	Muridae	<i>Saccostomus mearns</i>	Pouched rat
Rodentia	Muridae	<i>Thamnomys gigas</i>	
Rodentia	Myocastoridae	<i>Myocastor coypus</i>	Coypu
Rodentia	Myoxidae	<i>Graphiurus murinus</i>	African dormouse
Rodentia	Paraxerus	<i>Paraxerus palliatus</i>	Red-bellied coast squirrel
Rodentia	Sciuridae	<i>Xerus rutilus</i>	Unstriped Ground Squirrel
Tubulidentata	Orycteropodidae	<i>Orycteropus afer</i>	Aardvark

Discussion

Spatial Grouping & Behavioral Isolation

Striped hyenas form stable, spatially associated population groups composed of one adult female and up to three adult males. Although it is difficult to demonstrate territoriality within a population (Gittleman & Harvey 1982), the low degree of home-range overlap between these groups does indicate that group members operate within common, exclusive ranges (compare to similar degrees of inter and intra-group overlap in the pack-living Ethiopian wolf *Canis simensis*: Sillero-Zubiri & Gottelli 1995a).

Despite spatial grouping in striped hyenas, group members are behaviorally solitary. Levels of inter and intra-sexual association are very low and group members remain physically isolated more than 90% of the time. This is similar to the levels of association found in the red fox, another behaviorally solitary carnivore that forms spatial groups. Members of red fox groups, composed of a single male and multiple females, come into direct contact with each other for only a few minutes per day (Macdonald 1983).

Foraging & Feeding

Within groups, striped hyenas remain separated at night and appear to be strictly solitary foragers and feeders. A difference in the size of different functional group types is not unusual. Spatial group size is not necessarily expected to correlate with foraging group size because different factors affect the two: spatial group size is limited by prey availability (Waser 1981, Macdonald & Carr 1989), while foraging group size is linked to

the ability to successfully locate, pursue, and kill prey (Gittleman 1989). For species in which cooperative hunting is a benefit of grouping, the relationship between foraging group size and spatial group size is strong (e.g. wild dogs *Lycaon pictus*: Creel & Creel 1995). Ethiopian wolves and banded mongooses *Mungos mungo* and are among those species that live in groups, but typically forage alone (on insects or rodents, respectively) (Rood 1975 & 1986, Creel 1996, Sillero-Zubiri & Gottelli 1995b). A size difference between spatial and foraging groups is also demonstrated in spotted hyenas, lions, and coatis *Nasua narica* where foraging group size is commonly smaller than spatial group size (Kruuk 1972, Schaller 1972, Mills 1989 & 1990, Gompper 1997).

Solitary foraging in striped hyenas should be a reflection of diet, because prey characteristics largely determine whether a solitary foraging strategy is favored. Solitary foraging is favored when conspecifics would interfere with hunting through, for example, prey disturbance (not permitted) (Sandell 1989) and when there are simply no benefits to group foraging to offset local feeding competition (not promoted). As would be predicted for a species with small population group sizes and solitary foraging habits, striped hyenas consume a wide variety of small food items, some of which are extremely rare. Diet in brown hyenas is similarly varied, consisting largely of small mammals and bones. Like striped hyenas, they also live in groups, but forage alone and food items in their diet can generally provide enough food for only one individual (Owens & Owens 1978, Mills 1978a, 1989 & 1990). It is the size, scarcity, and widely dispersed nature of these food sources that are credited with limiting feeding and foraging group sizes in brown hyenas, and the same is probably true for striped hyenas.

In addition to foraging alone, striped hyenas also feed alone. In carnivores, feeding group size is linked to foraging group size and varies with prey size (Gittleman 1989). When prey items are small, solitary feeding is favored due to the depletion of food that would result from large feeding groups, but large prey items may allow larger feeding groups, even exceeding the size of foraging groups. In the brown hyena, feeding groups are larger than foraging groups when individuals converge on large carcasses (Owens & Owens 1978). Similarly, spatial groups in “fission-fusion” species such as lions and spotted hyenas may split to forage, but form larger feeding groups when feeding on large prey items (Kruuk 1972, Schaller 1972, Mills 1989 & 1990). Kinkajous *Potos flavus* will also spend long periods of time alone, but may converge in groups at large feeding trees (Kays & Gittleman 2001). In contrast to these species, striped hyenas do not form large feeding groups even when large prey items are available. Similar to European badgers at large feeding sites (rich earthworm patches in plowed fields: Kruuk 1978), several striped hyenas may visit the same carcass over a long period, but temporal spacing maintains solitary feeding in striped hyenas (*pers. obs.*). For example, during this study, a giraffe killed by lions in the zones of overlap for the Eastern and Western groups was scavenged over a period of six weeks by at least three adult and three juvenile striped hyenas. The three (related) juveniles visited the carcass at the same time on several occasions, but no two adults were observed visiting the carcass simultaneously.

It is worth noting that this observation stands in contrast to feeding group formation recorded for this species at man-made feeding stations in Israel (Macdonald 1978). Based on those observations, striped hyenas were specifically cited as a case

where a large and clumped food resource may have allowed for large feeding groups and those feeding groups may then lead to formation of larger spatial groups (Macdonald 1978, Mills 1989, Gittleman 1989). This determination was based, in part, on Kruuk's (1976) observation that striped hyenas in East Africa were omnivorous scavengers that were strictly solitary spatially and in foraging and feeding. Our observations of spatial grouping in a different East African population do not disprove the thesis, but do demonstrate that other factors can interact to influence grouping outside of feeding situations. Size and quality of food items combined with the persistent nature of feeding stations probably do account for the feeding group size observed in Israel, but not necessarily spatial group size. Moreover, many species of carnivores will adopt unusual spatial and social organizations in response to artificially clumped food, as was the case in the Israeli population of striped hyenas. While the Israel study does reveal that foraging in striped hyenas is not solitary under all circumstances, it does not address the normal organization of striped hyenas without anthropogenic disturbance.

Paternity

In this population of striped hyenas, males living in a group fathered most of the resident female's offspring. However, extra-group paternity was possible in one-fifth to one-third of litters. Patterns of paternity by non-resident males are also known for the closely related aardwolf (Richardson 1987, Richardson & Coetzee 1988), the Ethiopian wolf (Sillero-Zubiri 1994, Sillero-Zubiri *et al.* 1996), and the red fox (Baker *et al.* 2004). In the former two species, females overtly copulate even more with neighboring males than with resident males.

Multiple paternity was possible in three of four striped hyena litters, and probable in one. Although the frequency with which it occurs varies, multiple paternity of litters is common across carnivore species (Gompper & Wayne 1996), including lions (Packer *et al.* 1991), dwarf mongooses (Creel & Waser 1994, Keane *et al.* 1994), Ethiopian wolves (Sillero-Zubiri 1994, Sillero-Zubiri *et al.* 1996), Eurasian badgers (da Silva *et al.* 1994), African wild dogs (Girman *et al.* 1997), grey wolves *Canis lupus* (Lehman *et al.* 1992) and red foxes (Baker *et al.* 2004). Among group-living striped hyena males, paternity was not distributed evenly. However, lifetime reproductive success, which we could not measure in this study, may be more evenly distributed among group-living males than short-term reproductive success (as demonstrated in dwarf mongooses: Creel 1998).

Coalitions

It is difficult to find precise parallels to the social organization found in striped hyenas. In the social carnivores, males may share a home-range, but dominance hierarchies among males determine which males will mate with the females within those territories (e.g. palm-civets : Waser *et al.* 1994, spotted hyenas: Frank *et al.* 1995, banded and dwarf mongooses: Creel & Creel 1991, Creel 1996). However, *Hyaena* are behaviorally solitary, there is no indication of a male dominance hierarchy, and little opportunity for such a system to operate given the low degree of direct contact between males.

The multi-male, single-female use of common, exclusive ranges in striped hyenas also distinguishes them from other behaviorally solitary species. Most commonly among carnivores that, like *Hyaena*, are behaviorally solitary, either a single male defends one or

more females, or males have overlapping home-ranges larger than females. In the latter case, male relationships are highly competitive with aggressive competition for mating opportunities within areas of overlap (e.g. bears, mustelids, most cats, white-tailed mongooses) (Waser & Waser 1985, Waser *et al.* 1994). The same general strategy also accounts for nomadic brown hyena males (Mills 1978b).

Male space-use patterns within striped hyena groups seem to more closely resemble a third sub-set of carnivores that form cooperative multi-male coalitions to secure mate access. In these species, male coalitions are believed to improve access to females by out-competing solitary males or smaller coalitions. The specific mechanism by which male coalitions compete can vary within and among species, but include coalition group size, territorial takeovers, differences in number of female ranges defended, differences in female group sizes defended, and defense of resource hot-spots to which females are attracted (Caro & Kelly 2001). In the behaviorally solitary slender mongoose, coalitions of up to four males will share a territory that encompasses or overlaps those of up to six females (Waser *et al.* 1994). In lions, males form coalitions to maintain exclusive access to prides of females (Packer *et al.* 1991). In cheetahs, male home-ranges are small, female ranges are comparatively large, and male coalitions establish their territories in areas utilized by several females (Caro & Collins 1987). Like the coalitions of lions and slender mongooses, cheetah coalitions are usually composed of relatives, but (also like these other species) include non-relatives in 30% of cases (Caro 1994). However, the equal sizes of, and almost complete overlap in, male and female home-ranges in striped hyenas distinguishes them from the inter-sexual differences in

territory size in cheetahs and slender mongooses. The presence of only one female in striped hyena spatial groups also contrasts with the multi-male, multi-female grouping in lions and the access of males to multiple females (in different ranges) in slender mongooses and cheetahs.

Females as a Resource

To our knowledge, the essentially polyandrous spatial organization of striped hyenas, combined with little direct social interaction, is unique among the Carnivora. Male-group formation in carnivores has been explained by the benefits of male cooperation in defending or providing access to *several* females. Following long-established logic for the evolution of mating systems (Jarman 1974), females should establish the minimum defendable territory with enough resources to provide food for herself and her offspring. In response, males, alone or in groups, generally either establish larger fixed ranges and attempt to try to monopolize a number of females, or roam and compete with other males for mating with several females in heat (Sandell 1989). Accordingly, exclusive male territories are predicted (outside of monogamous systems) only if multiple females can be defended simultaneously (Macdonald 1983, Sandell 1989, Johnson *et al.* 2002). Somewhat prophetically, after male coalitions in slender mongooses were detected in areas with high female densities that allowed groups of males to successfully defend groups of females, it was identified as being of particular interest to know if male (slender mongoose) coalitions form only in areas with high female densities and not in areas where females are more highly dispersed and therefore less defendable (Waser *et al.* 1994). In striped hyenas, defense of multiple females is not

necessary for male coalition formation: groups of related or unrelated males cooperate to defend a territory containing a single female. No explanation for this pattern has previously been needed, because it has not been described (or predicted) for any other carnivore species.

Males must optimize the trade-off between the number of females defended and the effectiveness of their defense. Constraints on this optimization problem occur when 1) the species' diet results in solitary foraging and feeding—males need to defend the entire territory and cannot simply employ a mate guarding strategy and 2) breeding is not seasonal—seasonal roaming is not a viable option and the ability of males to restrict defensive behaviors to short time periods is limited, particularly if the costs of establishing a defended territory are higher than maintaining them. Both of these conditions exist in striped hyenas.

Under these conditions, sharing a female with a coalition (particularly of relatives) may yield greater fitness than failing to defend mating access alone. Hypothetical male territories encompassing more than one female may not be economically defensible, particularly when female home-ranges are large (Brown 1964). Moreover, if males expand their territories to include multiple females, with increasingly poor defense of an increasing number of females, at some point these males would effectively become nomads. On the other hand, even one female territory may not be 100% defensible. If a resident male cannot effectively defend a female territory against encroachment by solitary bordering males on each of four sides, for example, he would be competing for mating opportunities with four males. However, if a coalition of two

males can maintain exclusive use of the territory (which is the same as defending one female), each resident male is only competing with one other resident for mating. Consequently, the way in which the diet of striped hyenas affects female territory size and foraging behavior may result in an only marginal ability of solitary males to effectively guard females. This then could favor males who tolerate additional males guarding the same female territory, where one solitary male cannot.

Although this mechanism does require indirect cooperation (independent defense of the same territory) among group-living males for mate defense, these males will still be competing for mating opportunities and we would not expect the degree of toleration to be high. Reproductive success among unrelated group-living males is expected to be more evenly distributed than reproductive success among related males, because the latter accrue the benefits of inclusive fitness: relatives are always more exploitable than nonrelatives (Hamilton 1963, Vehrencamp 1983, Creel & Waser 1991, Packer *et al.* 1991, Keller & Reeve 1994). If reproductive success is skewed because one male is able to dominate other group-mates, coalitions may be unstable and subordinate males should be more likely to disperse than dominants. However, non-mating males could still accrue benefits by remaining for some time with unrelated males: group-living could provide a relatively safe haven compared to roaming, and subordinate males could wait for opportunities to inherit territories or observe and capitalize on vacancies in neighboring territories. These 'make-the-best-of-a-bad-lot' benefits are well-established for subordinates in many cooperatively breeding species (Brown 1987, Stacey & Koenig

1990, Koenig & Dickinson 2004), including some carnivores (Creel & Waser 1994, Waser 1996, Clutton-Brock *et al.* 2002).

Overall, striped hyena males should prefer to share territorial defense of females with relatives, but sharing with non-relatives could also be favored, if no adult male relatives were available. To resolve if this occurs in striped hyenas would require better data on the effectiveness of territorial defense in maintaining paternity, the distribution of reproductive success within male coalitions, and patterns of relatedness within male spatial groups. For now, logic suggests that coalitions of relatives and non-relatives could both occur through this mechanism.

Why should male ranges overlap completely with one female and not partially with several females, as in felids and mustelids? The latter strategy offers less protection of mating opportunities from intrusion by nomadic males (common in the aardwolf and brown hyena). In essence, trade-offs between the extent and effectiveness of defense appears to favor localized defense, in striped hyenas. In addition, females under this strategy would receive less benefit from the presence of males and consequently would be less likely to tolerate their presence (see Female Tolerance of Multiple Males below).

Relatedness Across Social Distances

Although frequent low levels of relatedness between group-mate males (Fig. 3.8) indicates that group formation in this species is not strictly a result of lack of dispersal, or co-dispersal with relatives, we did not observe natal dispersal (or philopatry) in this population. Consequently, we only discuss factors that could promote the static, observed patterns and, accordingly, interpret the patterns of relatedness across distances

(and within groups) as reflecting strategies of *dispersion* and not, explicitly, *dispersal* (see Box 3.1). Although closely related, the former considers the distribution of individuals in relation to each other (and why they remain there), whereas the latter considers movement between ranges and is the means of immigration and emigration. As with other aspects of a species' ecology, descriptions of these patterns are only 'caricatures' of intra-specific variability and we can only speak of species typical patterns (see Bekoff 1989, Waser 1996).

The pattern of relatedness across distance observed in striped hyenas is unusual. Related male striped hyenas have a significantly higher probability of living in non-adjacent than in adjacent areas. Similarly, related females have a marginally higher probability living at a distance than of living in adjacent groups. Relatedness in spotted hyenas is also lower in adjacent clans than clans separated by one territory. However, in contrast to striped hyenas (Fig. 3.7), relatedness in *Crocuta* drops significantly at greater distances and is significantly negatively correlated with distance (as measured by the number of clan borders between) (Van Horn *et al.* 2004). Because the observed *Hyaena* pattern has not been reported in other carnivores, it is interesting to ask what prior explanations for spatial or social grouping may pertain to *Hyaena* and how this striped hyena social structure may reflect individual strategies to maximize fitness in males and females.

The low level of relatedness observed between males living in adjacent groups can be accounted for if male striped hyenas that do not form coalitions with their relatives tend to live more than one home-range away from relatives. There are four possible

explanations worth considering for this apparent preference. First, given the very low degree of home-range overlap between non-group-mate males (or between females), it is likely that both sexes of this species exclude same-sex neighbors due to resource competition. As demonstrated in spotted hyenas (Van Horn *et al.* 2004), there is a hidden potential cost to dispersing to neighboring territories: during border interactions and aggressive encounters in territorial disputes, non-related group members fight together against neighboring relatives. Inclusive fitness theory generally predicts that cooperation with relatives against non-relatives would be more beneficial, so kin selection should favour mechanisms to avoid such conflicts (see Van Horn *et al.* 2004). If border confrontations are inevitable, dispersion strategies that maintain buffer territories between relatives would alleviate this potential problem (provided that the costs of dispersing that extra distance are not excessive). In a limited test of this hypothesis, reactions by lions to simulated territorial incursions were not affected by relatedness to the intruders (Spong & Creel 2004). This result suggests that the benefits of maintaining an exclusive territory are large enough to be favored, even when the intrusion is by related neighbors. Given this, relatives might be selected to avoid territorial disputes with one another by settling in non-adjacent territories.

Second, for males, if the number of overlapping and bordering females is a measure of the number of potential mates, males who settled in a non-adjacent manner would reduce competition with relatives for mating opportunities. Kin selection to avoid competition with relatives could operate on this component of fitness in the manner just described.

Third, familiarity among neighboring hyenas, and experiences that have bred distrust and dislike between them, could play some role. Past antagonistic encounters with neighbors may effectively deter immigrants from attempting to disperse into their neighbor's territory. Similarly, whereas the intentions of a previously unencountered individual may be less likely to be interpreted as overtly threatening, a developed lack of tolerance of known individuals may mean that any attempted immigration by a hyena from a neighboring territory is immediately identified as a threat to the resident. In this way, males may be less likely to try, and less likely to succeed in, dispersing to neighboring territories than to non-adjacent territories that contain males with which they have no antagonistic history.

Finally, in 2003, M11 secondarily dispersed to a home-range adjacent to the range he shared with a close relative (M10, who remained on the original range). Secondary dispersal, due to the death of a mate, eviction by new immigrants, to avoid inbreeding, or to increase mate access, is reported for male dwarf mongooses, lions, and spotted hyenas (Waser 1996). If secondary and tertiary dispersal, in combination with coalition fission, is common in striped hyenas, it could lead to a pattern of spatial separation developing between related males over time. Unlike the three other points above, this does not identify a selection pressure that may affect patterns of settlement, it simply notes that repeated movements will tend to increase separation (as in diffusion models of dispersal).

Overall, the spatial and genetic patterns in *Hyaena* can be well explained by:

1. Spatial group formation by males to defend access to females.

2. Past experiences leading to a lack of tolerance between neighbors that reduces the likelihood of attempting to disperse into a neighboring range, or succeeding that attempt.
3. Possible reinforcing selection to settle in a non-adjacent manner to reduce competition with relatives for resources (both sexes), or mates (males).

Female Tolerance of Multiple Males

Unlike striped hyenas, male white-tailed mongoose home-ranges are one and a half times the size of female home-ranges, resulting in a mosaic of exclusive male home-ranges overlapping a separate mosaic of exclusive female home-ranges (Waser & Waser 1985, Admasu *et al.* 2004). This pattern is typical of most mustelids (e.g. European pine martins: *Martes martes* and fishers *Martes pennanti*: Powell 1994) and felids (e.g. caracal *Felis carcal*: Avenant & Nel 1998). Such systems can persist without strong inter-sexual competition over food resources because female territories need only support a fraction of each additional (male) user, while males can effectively maximize mating ranges (Carr & Macdonald 1986). In the case of striped hyenas, female ranges must support the whole of each additional male user.

The RDH predicts that changes in group size do not necessitate changes in territory size. However, when resources become depleted (by mechanisms other than group size), primary territory holders will expand their territory size to compensate (Johnson *et al.* 2003). Striped hyenas offer one example of a possible interaction between male and female group sizes and territory sizes. In striped hyenas, it is reasonable to consider only the female (and her offspring) as the primary territory holder

(following the logic that resources determine female distribution, which in turn determines male distribution). Alternatively, one could also consider the first male on a territory a primary resident. The immigration of additional males is likely to reduce the resources available to the primary resident(s). Consequently, female territory size would need to increase to compensate, unless female-defended food resources can support several males in addition to herself and her offspring, without cost. For males, an equilibrium will be reached where increasing within-group competition for resources and mates is offset by the benefit of excluding non-group males more effectively. The equilibrium point might differ for females, because mate-defense is not a benefit to females, and this creates the possibility of inter-sexual conflict over group structure.

If female striped hyenas are ‘forced’ to maintain larger home-ranges because of the number of males within their territories, females should be intolerant of resident males, and more accepting of non-resident males as mates, unless resident males provide some offsetting fitness payoff to females. In terms of excluding competitors for resources, resident females are unlikely to accrue benefits from tolerating multiple males, because males are unlikely to exclude additional encroaching females. It remains possible, however, that stability in resident males may reduce the chances of infanticide by immigrating males, as it does in lions (Whitman *et al.* 2004), and perhaps brown bears *Ursus arctos* (Swenson *et al.* 1997), but infanticide has not been reported for this species in captivity or the wild. Females could also benefit from the presence of males if males acted as ‘hired-guns’ and protected females from harassment by non-resident males (Wittenberger & Tilson 1980), a hypothesis supported by the severity of male mobbing of

estrous females in other hyenids (e.g. *Crocuta*). However, this hypothesis does not fully explain female tolerance of resident males outside of breeding periods, when harassment is unlikely.

When one sex invests more in the care of offspring, the other sex will compete for the first (e.g. males typically will compete for females in the absence of paternal care) (Trivers 1972). For instance, the benefits to female dwarf and banded mongooses of tolerating the presence of multiple males include the opportunity for mate choice and increased paternal care. Females having access to multiple resident and non-resident males, as they do in striped hyenas, can allow for female mate choice and the demanding of rewards or pay-offs in exchange for tolerance and mating opportunities (Waser & Waser 1985). In studies of primate social evolution, this is known as the ‘food for sex’ hypothesis. Male payoffs could take the form of feeding or guarding offspring, but striped hyena males do not spend significant periods of time at den sites (Davidar 1990, pers. obs.). Consequently, there appears to be both the conditions and the opportunities for males to contribute to offspring care in striped hyenas, but there is no evidence that common forms of paternal care occur, beyond territorial defense.

However, being submissive to females (and their offspring) at feeding sites would be one simple mechanism by which males could provide pay-offs for tolerance and mating opportunities. Such a compensatory mechanism would be less costly to males than paternal care through offspring provisioning or guarding and could develop more readily than those alternatives because it is far less complex. We did not detect male submission in this study, and had little scope to detect it if it exists, but this is an

intriguing possibility because it could be an evolutionary origin leading to the pronounced female dominance system found in spotted hyenas (see Chapter 1, Fig. 1.2). Although the social structures in striped and spotted hyenas are very different, female mate choice may be even more important in determining paternity in spotted hyenas than male-male competition for dominating mate access (Engh *et al.* 2002).

Resources and Group Formation

Based on conventional logic, if we accept the premise that solitary female distributions reflect a lack of permissive and promoting conditions for group formation (e.g. uniformly distributed and small prey items comprising a substantial proportion of diet), our data support the generally accepted ideas that male distributions will be determined by female distributions. However, the way in which males respond to females (multiple males monopolizing single females) appears both unique and surprising.

Striped hyena spatial groups consist of one adult female and multiple males. If the distribution of females drives the distribution of males, the RDH predicts that males will be solitary in response to female over-dispersion. If the distribution of females is a response to the distribution of food, then this conclusion is reinforced, because the distributions of both food and females would predict that males should be solitary. Similarly, male group formation in coatis occurs in the absence of apparent reproductive or foraging advantages (Gompper & Krinsley 1992). A counter argument might be that the dispersion of females determines male range sizes, but the ‘value’ of a female determines the number of males using her range. However, given that the number of

striped hyena females per range is always one, this seems to be a weak argument. Nevertheless, it remains possible that variation among females in reproductive value could be great enough to offset the costs of shared paternity when multiple males share a territory. To be influential, that mechanism only requires an ability in males to assess female age or other indicators of female reproductive value.

Conventional explanations for group formation and social evolution have probably correctly identified the influential factors involved, but the interaction between those factors has resulted in unexpected and unanticipated patterns of association in this species. Overall, the relationships between resources, spatial patterns, and grouping in striped hyenas are best explained by diet determining group size and spacing patterns of females, the number of males neighboring a females' territory determining male group size, the number of guarding males determining female territory size, and female territory size determining male territory size.

Conclusion

For most carnivores, the benefits of grouping do not outweigh the associated costs. Consequently, 80-95% of carnivore species are solitary (Bekoff *et al.* 1984). For this majority, solitary foraging is more efficient than cooperative hunting and there is little or no benefit to cooperation in raising young. However, a disproportionately small effort has been devoted to studying these species and it is unlikely that their full value in unraveling factors that may influence the evolution of social systems has been realized.

The realized benefits of sociality can confound efforts to identify those factors that may cause or allow initial development of grouping. This results in an interesting paradox: studies of incipiently social species might tell us more about the mechanisms of, and constraints on, group formation and, consequently, social evolution (Johnson *et al.* 2001) than studies of social species (Waser 1981; Waser & Jones 1983). This study further illustrates that point by describing a unique, behaviorally solitary species that forms stable, polyandrous spatial groups by mechanisms not uniformly conforming to perceived mammalian norms.

Text Boxes

Box 3.1. Definition of terms, as used.*

areas of use	geographic areas that individuals use, with no connotation of exclusive use (synonymous with home-range)
associating	being in direct physical contact or in close proximity to one another; as applied in this study, being within 50m or 200m for day/night observations (see Methods)
breeding group	forming a reproductive unit (breeding pair)
cooperation	active collaboration; typically occurs within stable social groups
dispersal	means of immigration and emigration; movement between one home-range and another, usually permanent; 'natal' dispersal is dispersal from the range of birth; any subsequent dispersal is 'secondary'
dispersion	the distribution of objects, usually individuals or resources, but may also refer to home-ranges themselves; dispersion ranges from clumped (patchy, under-dispersed) to random to uniform (regular, over-dispersed)

Box 3.1 (continued).

exclusive home-range	home-range in which patterns consistent with exclusion of others have been observed, but territorial or active exclusion behaviors have not been directly observed; synonymous with 'ecological territory' but not with 'behavioral territory'
feeding group	grouping specifically while eating, without implying that the same group also foraged or hunted together
foraging group	grouping specifically while locating and catching prey or other resources (e.g. carcasses)
group	population subset sharing defended space; may be temporary
home-range	calculated area of space use in which an animal has a specified probability of being located
philopatry	remaining in natal territory
resource dispersion	the way resources (e.g. food, mates) are distributed in space (across a landscape) and time
social	descriptor of population subsets that demonstrate sociality
sociality	relatively long-lived form of grouping involving cooperative interactions and high levels of association; may be very complex
social evolution	process by which sociality develops beyond grouping as adaptive responses to benefits of cooperative behaviors
space-used	geographic areas in which utilization has been measured/detected
space-use polygon	physical representation of space-use on a map
spatial group	sharing a common home-range
spatial organization	the way in which a population arranges itself across space, time, and scales
territory	an area of use in which territoriality (active defense and exclusion of others) has been demonstrated
tolerance	tacit acceptance of the presence and/or behaviors of others occurring in the absence of significant costs or benefits of cooperation; considered the underlying condition permitting grouping

*Other definitions provided in Chapter 1, Box 1.1.

Box 3.2. Hypotheses of group formation.

HYPOTHESES / FACTORS	PERMITTING OR PROMOTING MECHANISMS	TOLERANCE OR COOPERATION	DEVELOPED IN AND APPLIED TO (AS IN TEXT):
RESOURCE DISPERSION	PERMITTING: variation in prey availability in space and time such that defense of one resource patch may allow for group formation without interference	TOLERANCE PERMITTING GROUP FORMATION	Macdonald 1983: European badgers-Kruuk 1978; kinkajous-Kays & Gittleman 2001; brown hyenas-Mills 1982; red fox-Macdonald 1983, Carr & Macdonald 1986
RESOURCE RENEWAL	PERMITTING: rapid renewal of prey resources such that several individuals may feed from the same resources within an area without interference due to resource depletion	TOLERANCE PERMITTING GROUP FORMATION	Waser 1981: white-tailed, dwarf, and banded mongooses, bat-eared fox-Waser 1981
TERRITORY INHERITANCE (LOW COST OF PHILOPATRY)	PERMITTING: low cost of natal philopatry such that offspring may remain without resource depletion	TOLERANCE PERMITTING GROUP FORMATION	Lindstrom 1986: Ethiopian wolves-Sillero-Zubiri 1994
TERRITORY INHERITANCE (HIGH COST OF DISPERSAL)	PROMOTING: costs of philopatry are low relative to high costs of dispersal such that philopatry is favored	TOLERANCE PROMOTING GROUP FORMATION	Lindstrom 1986: Ethiopian wolves-Sillero-Zubiri 1994; lions-Owens & Owens 1984, Packer 1986
FEMALES AS A RESOURCE	PROMOTING: coalition formation is favored to increase mate access via male group defense	TOLERANCE PROMOTING GROUP FORMATION	Macdonald 1983: cheetahs-Caro & Collins 1987; slender mongooses-Waser <i>et al.</i> 1994; see also Jarman 1974; Macdonald 1983, Johnson 2002
PREY DETECTION	PROMOTING: group formation favored because of improved ability to detect prey in foraging groups	COOPERATION PROMOTING SOCIAL DEVELOPMENT	lions-Packer <i>et al.</i> 1991
PREY DIVERSITY	PROMOTING: foraging/feeding group formation favored by benefits of expanding food types available	COOPERATION PROMOTING SOCIAL DEVELOPMENT	spotted hyenas-Kruuk 1972, Mills 1989; African wild dogs-Creel & Creel 1995
PREY DEFENSE	PROMOTING: feeding group formation favored by benefits of defending prey against competitors	COOPERATION PROMOTING SOCIAL DEVELOPMENT	coatis-Gompper 1996; lions and spotted hyenas-Owens & Owens 1984, Packer 1986
FORAGING SUCCESS	PROMOTING: foraging group formation favored by increased success of hunting	COOPERATION PROMOTING SOCIAL DEVELOPMENT	black-backed jackals-Moehlman 1989
TERRITORIAL DEFENSE	PROMOTING: spatial group formation favored by increased ability to defend territorial resources (food or mates)	TOLERANCE PROMOTING GROUP FORMATION	black-backed jackals-Moehlman 1989
ANTI-PREDATOR DEFENSE	PROMOTING: group formation favored by increased ability to detect or defend against predators	COOPERATION PROMOTING SOCIAL DEVELOPMENT	dwarf mongooses-Rasa 1986, Rood 1986, Waser & Waser 1985, Creel 1996

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TRANSIENT GENITAL ABNORMALITIES IN STRIPED HYENAS

Abstract

Unique among mammals, female spotted hyenas, *Crocuta crocuta*, have highly masculinized genitalia: they have no external vagina so urination, intromission, and parturition take place through the clitoris, which is the size of the male's penis and is fully erectile. Females secrete high levels of androstenedione during gestation and the placenta promotes enzymatic conversion of androstenedione to testosterone (Licht *et al.* 1992, Yalcinkaya *et al.* 1993). Fetal exposure to maternal androgens became the leading mechanistic explanation for female masculinization. Among Hyaenids, virilization of external female genitalia has previously been observed only in *Crocuta*, so functional explanations of masculinization have focused on aspects of social ecology unique to *Crocuta*. Here we describe unique genital structures occurring in a close relative, the striped hyena (*Hyaena hyaena*). Striped hyenas also have unusual androgen profiles. The existence of these traits in *Hyaena*, which is at the base of the Hyaenid clade, supports only those evolutionary and mechanistic models of masculinization in *Crocuta* which may now be extended to *Hyaena* and suggests a precursor or exaptive condition that may provide clues to the development of the syndrome seen in *Crocuta*.

Introduction

Existing models of female dominance and masculinization in *Crocuta* assume that ecological characteristics unique to the species have driven the evolution of

masculinization (Frank 1996; see Chapter 1, Figure 1.2). The spotted hyena is a communal hunter of large mammals that lives in large matrilineal, territorial social groups (Kruuk 1972, Frank 1986a & 1986b). Females are always dominant to males, and social rank affects access to carcasses (Frank *et al.* 1995) and reproductive success (which is 2.52-2.76 times higher for alpha females than for other clan members: Frank 1997). Masculinization and the correlated modifications to the reproductive tract are costly, as over 60% of cubs of first-time mothers are stillborn, and deaths of mothers while whelping are common (Frank *et al.* 1995). Early adaptive hypotheses (Kruuk 1972, East *et al.* 1993) for the evolution of masculinization have been criticized for being unlikely to compensate for the associated reproductive costs and for lack of adequate empirical support (Muller & Wrangham 2002). However, if high-ranking females commonly kill the female offspring of lower-ranking females, sexual mimicry through masculinization as a defensive adaptation (Muller & Wrangham 2002) remains a viable explanation. Intense feeding competition at carcasses is also hypothesized to have driven intense selection for female aggression that produced female masculinization as a non-adaptive consequence (East *et al.* 1993). Non-adaptive hypotheses argue that masculinization is an exaptive (Gould & Vrba 1982) consequence of high fetal androgen exposure facilitating aggression in adult females (Racey & Skinner 1979) or siblicide in same sex litters (Frank *et al.* 1991, Hofer & East 1995). With the exception of the spotted hyena and fossa (Hawkins *et al.* 2002), *Cryptoprocta ferox*, genital masculinization is not reported in other carnivores.

In normal mammalian sexual differentiation, chromosomal sex directs the development of embryonic ovaries or testes. Fetal androgen secretion then induces male phenotypic development or, absent fetal androgens, the female phenotype develops (Jost 1972). Unusually high levels of maternal androgens and an enhanced ability to pass those androgens to the fetus have been proposed as the proximal causes of virilization of female genitalia in *Crocuta* (Yalcinkaya *et al.* 1993, Glickman *et al.* 1987). In *Crocuta*, circulating androstenedione levels are higher in females than in males within all age classes (Glickman *et al.* 1987). Circulating testosterone is also unusually high in females and, during latter stages of pregnancy, surpasses the levels in males (Frank 1996).

Placental aromatase activity is low ($\frac{1}{20}$ th to $\frac{1}{40}$ th human levels) (Licht *et al.* 1992), while 17- β hydroxysteroid dehydrogenase activity in response to androstenedione is twice the level found in humans (Yalcinkaya 1993). These patterns promote the conversion of large amounts of maternal androstenedione to testosterone. However, treatment of pregnant *Crocuta* females with anti-androgens still produced female offspring with unusually masculine genitalia (although genitalia were more “feminized” in both sexes) (Drea 1998), leading to an alternative hypothesis that masculinization may be controlled by genetic mechanisms independent of hormonal activity (Place & Glickman 2005).

Methods

We captured striped hyenas in Laikipia District, Kenya, immobilized, sexed, and assigned each to an age class based on known dates of birth or estimates from body measurements, weight, and tooth wear (cub: < 6 months; juvenile: 6mos to 1year; young

adult: 1 to 3 yrs; adult: 3+ yrs). Blood was drawn and serum and plasma were subsequently isolated. Plasma taken from 63 individuals at 79 capture events were assayed (following methods described in Glickman *et al.* 1992) for circulating testosterone (cubs: females $n=2$; juveniles: females $n=3$, males $n=6$; young adults: females $n=11$, males $n=6$; adults: females $n=19$, males $n=32$). Samples from two separate capture events were assayed for 12 individuals (1 female cub; 1 male and 2 female young adults; 5 male and 3 female adults) and from three capture events for 1 male and 1 female adult. For each of these individuals, we used the average androgen concentration in the analyses. Testosterone measurements were log-transformed and analyzed by factorial ANOVA to test for effects of sex and age class.

Results

In young *Hyaena* between one and 18 months of age (females $n=4$, males $n=6$), we found a marked convergence in the appearance of genitalia in BOTH sexes (Fig. 4.1), traits not reported for *Crocuta* or any other carnivore. Young females develop prominent swellings anterior to the vagina with an appearance reminiscent of a scrotum, despite their placement, and a pronounced protrusion around the uro-genital opening. Young males exhibit genital swellings between the scrotum and penis similar in appearance to labial folds. This convergence in genital appearance is transient and does not appear in adults or newborns (newborns: females $n=1$, males $n=3$; adults: females $n=8$, males $n=7$), but external genitalia in the young of both sexes have unusual traits mimicking characteristics typical of the opposite sex. These genital anomalies were consistently

found in all sexually immature individuals older than a few weeks and minor residual marks were apparent in all sexually mature adults, which otherwise had typical mammalian genitalia.

Testosterone measurements were repeatable across duplicate assays run on 27 samples ($r^2=0.777$, $F=76.7$, $p<0.001$). Testosterone levels (Fig. 4.2) were higher in males than in females ($F=22.37$, $p<0.001$, $df=1$), with no detectable effect of age class ($F=0.65$, $p=0.525$, $df=2$) and no detectable sex-age interaction ($F=0.66$, $p=0.520$, $df=2$).

Backtransformed mean circulating testosterone in females was 0.29 ng/ml (95% CI=0.24-0.36, $se=0.03$, $n=26$) and 0.59 ng/ml in males (95% CI=0.48-0.72, $se=0.04$, $n=33$).

Testosterone levels in normal mammals (Table 4.1) are typically 5 to 100-times higher in males than females, but the observed male:female ratio for testosterone in *Hyaena* was only 2.03 (Fig. 4.2), well below mammalian norms. The backtransformed 95% CI for the difference between male and female mean testosterone levels was 0.48-2.09 ($df=57$).

That is, the ratio of testosterone levels in *Hyaena* males to females is 2.09 or less with =95% confidence.

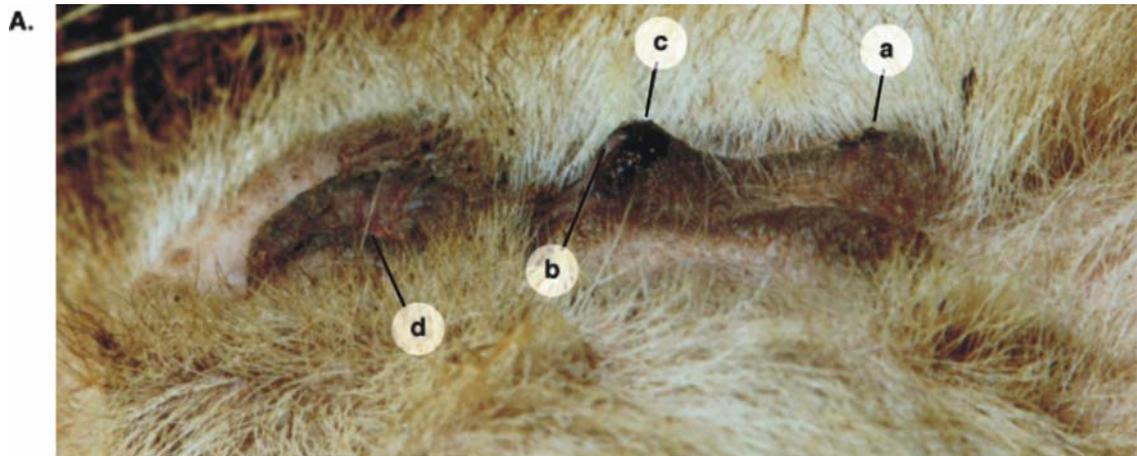
Figure 4.1. Convergent genital characteristics of young male and female striped hyenas. Major structures in young striped hyena genitalia are ambiguous with respect to gender, compared to typical mammals. Photographic angles were chosen to best illustrate the typical characteristics of each sex.

A) Young females:

(a) prominent, dark, hairless labia-like folds develop anterior to the vagina (b); (c) prominent labia-like protrusion with the uro-genital opening on the posterior side; (d) anus. In adult females (not shown), the labia-like protrusion becomes far less prominent, the opening of the uro-genital canal moves perpendicular to the body, and the swelling of the labial folds diminishes completely, with only a patch of dark skin remaining.

B) Young males:

(a) smooth, hairless, pre-scrotal skin folds apparent lateral to the raphae at the junction between the scrotum (b) and penis (c); (d) anus. In adult males (not shown), the swelling of the skin folds is reduced and becomes partially integrated into the raphae at the base of the testes.



POSTERIOR  ANTERIOR

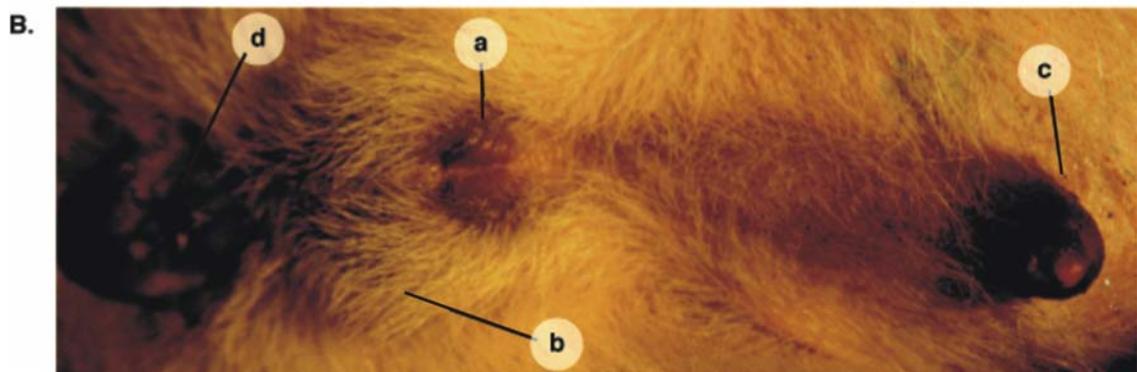


Figure 4.2. Backtransformed mean testosterone concentrations in *Hyaena* by sex*age class. Vertical bars denote \pm 95% confidence intervals. Two females were found to be pregnant by ELISA of serum progesterone (11,054 & 22,019 pg/ml compared to mean adult female progesterone levels of $5,835 \pm 1,062$ pg/ml), but corresponding testosterone levels were not outside the range for other females. One young adult and two adult males had testosterone concentrations 11, 13, and 21 times the male mean. High testosterone concentrations were not repeatable in duplicate assays and these values were removed from the final analyses. Inclusion or removal of the pregnant females and the three males with high testosterone concentrations did not affect significance (at $\alpha < 0.05$) of any statistical test. Plasma was available for only one cub, a female from two capture events. Her testosterone concentrations were not included in the analyses, but are included here for comparison: 0.21 & 0.15 ng/ml.

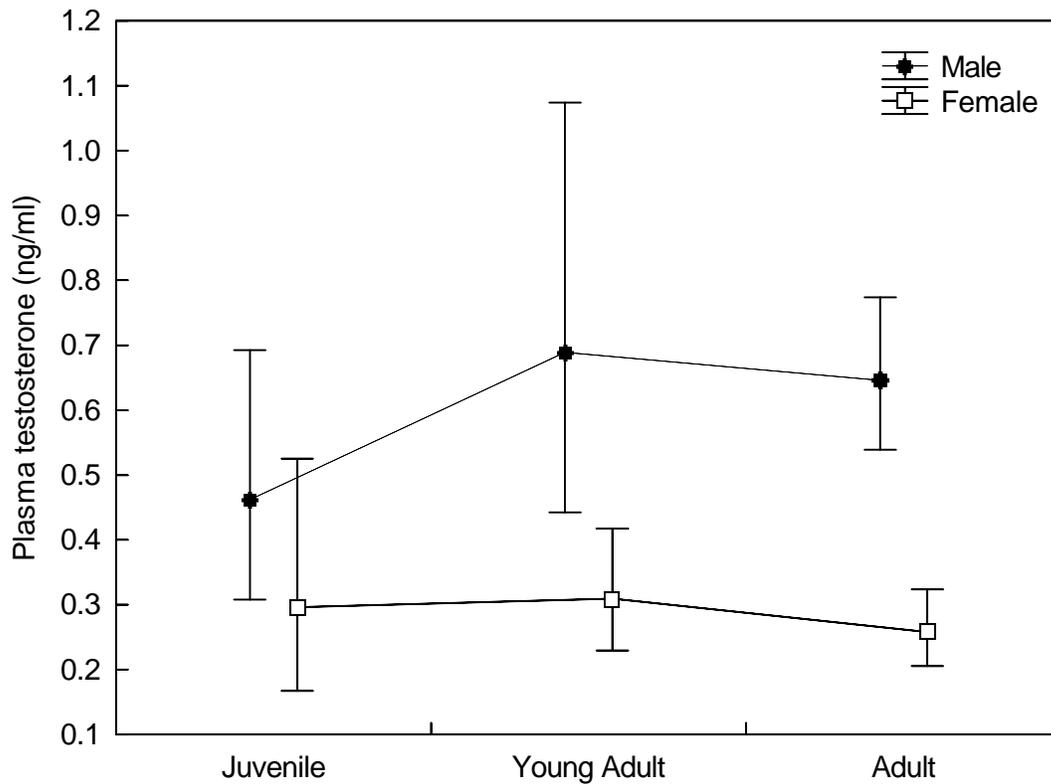


Table 4.1. Circulating testosterone concentrations in adult male and female mammals in species with genital masculinization of females and in normal species (lacking masculinization). Concentrations reported here in ng/ml. Superscripts indicate published source.* Values for *Crocuta* are for pre-dispersal adult males / post-dispersal adult males and non-pregnant adult females. Male *Talpa* values are from outside / inside the breeding season. In *Cryptoprocta* and *Hyaena*, only young females are masculinized, but there is no significant difference in testosterone concentrations between young and adult age classes. Male mean for *Cryptoprocta* was estimated from a published figure.

	Concentrations		
	Male	Female	M:F Ratio (min - max)
“Normal” Species:			
Human	4.62 - 6.46 ¹	0.20 - 0.60 ¹	7.70 - 32.30
Chimpanzees (<i>Pan troglodytes</i>)	4.95 ¹	0.41 - 0.71 ²	6.97 - 12.07
Rhesus macaque (<i>Macaca mulatta</i>)	4.57 - 17.76 ¹	0.20 - 0.84 ^{1,3}	5.44 - 25.12
Rats	2.15 - 3.70 ¹	0.17 ¹	12.65 - 21.77
Natal rat (<i>Praomys natalensis</i>)	4.2 ³	0.4 ³	10.50
Rabbits	0.5 - 5 ³	0.05 - 0.1 ³	5 - 100
Brown hyena (<i>Parahyaena brunnea</i>)	15 ⁴	0.52 ⁴	28.85
Masculinized Species:			
Spotted hyena (<i>Crocuta crocuta</i>)	0.51 / 3.99 ⁵	0.29 ⁵	1.76 / 13.76
	1.5 / 10.5 ⁶	1.2 ⁶	1.25 / 8.75
European mole (<i>Talpa europaea</i>)	1.75 / 7.47 ⁷	0.79 - 1.44 ⁷	1.22 - 2.22 / 5.19 - 9.46
Fossa (<i>Cryptoprocta ferox</i>)	~1.8 ⁸	1.1 ⁸	1.64
Striped hyena (<i>Hyaena hyaena</i>)	0.59	0.29	2.03

*¹Overpeck *et al.* 1978; ²Nadler *et al.* 1985; ³Feder 1985; ⁴Racey & Skinner 1979; ⁵Goymann *et al.* 2001; ⁶VanJaarsveld & Skinner 1991; ⁷Whitworth *et al.* 1999; ⁸Hawkins *et al.* 2002

Discussion

The unusually low relative difference in testosterone levels between sexes found in *Hyaena* is a pattern shared by other masculinized species outside of reproduction-related phases (Racey & Skinner 1979, Overpeck *et al.* 1978, Nadler *et al.* 1985, Feder 1985) (Table 4.1). As stated in other studies of female genital masculinization in mammals (Hawkins *et al.* 2002, Van Jaarsveld 1991, Whitworth *et al.* 1999) and suggested by the results of recent studies of *Crocuta* (Drea *et al.* 1998), masculinization may not be due to qualitatively unique mechanisms, but to modifications in genital androgen receptors or the timing and degree of androgen secretion. Given the lack of support for a clear link between masculinization and high maternal androgen levels, it is important to note that no data have directly tested hypotheses that the gonads have unusual patterns of steroid secretion, sensitivity, or timing during development.

Our findings reveal that unusual patterns of genital development are not unique to spotted hyenas within the Hyaenidae. However, the timing and incomplete nature of the convergence in genital appearance in *Hyaena* make identification of a clear adaptive function difficult. *Hyaena* share few features of social ecology with *Crocuta* and the selection pressures identified for the evolution of masculinization in *Crocuta* are probably weak or absent in *Hyaena*. Striped hyenas are solitary foragers that live in small territorial groups that include only one adult female (Wagner *In press*). Direct interactions when feeding are rare, so *Hyaena* experience little aggression in foraging situations. Additionally, their social structure does not promote female infanticide, there is no indication that females show higher levels of aggressive behavior than males, and

no evidence of siblicide or neonatal aggression from captive studies (Rieger 1978). Existing evolutionary models would predict normal genitalia in *Hyaena* in both sexes, when viewed as explanations for the ancestral development of masculinization. The genital feminization we have seen in young males is not directly addressed by any hypothesis. Further, if mimicry were solely an adaptive trait to reduce female infanticide, feminization would be a costly trait in male *Hyaena*. However, the mimicry hypothesis was developed to explain masculinization as found in *Crocota* and its central theorem predicts maximum convergence in genital appearance immediately after birth, when *Crocota* are most vulnerable to infanticide (East *et al.* 1993). This explanation fits well with what is known about *Crocota* ecology and with some modification, sexual mimicry or ambiguity remains a viable adaptive explanation for the genital development observed in *Hyaena*. The specifics of *Hyaena* ecology are not yet well known, but ambiguous genital appearance would be mutually advantageous for young males and females if both are more vulnerable to aggression at these ages. For example, increasing foraging forays could result in increased aggressive encounters with conspecifics of the same sex from outside of their natal groups.

Explanations for the evolution of masculinization as a non-adaptive trait in the Hyaenidae also cannot be ruled out. *Hyaena* are at the base of the Hyaenid clade and the transient genital anomalies observed suggest that the Hyaenidae may simply harbour preadaptations for unusual genital development that have been elaborated in *Crocota*. Under this scenario, masculinization did not evolve in response to unique aspects of *Crocota* ecology, but instead represents an exaptive trait that, once fixed, could not be

lost. In addition to the new evidence from *Hyaena*, the high reproductive cost of masculinization in *Crocuta* (Frank *et al.* 1995), the inability of male offspring to breed if maternal androgens are blocked (Drea *et al.* 2002), and the secondary exaptive benefits identified within adaptive hypotheses (Kruuk 1972, Frank *et al.* 1991), support this non-adaptive concept (see Chapter 1, Fig. 1.2). Identification of traits common to *Hyaena* and *Crocuta* that differ from other mammals may help to explain the evolution of masculinization. The most obvious commonality is that they are both Hyaenids, supporting the interpretation of masculinization as an exaptation. Aspects of the species' ecology that differ from one another may help to explain differences in the degree, appearance, and function of masculinization. The existence of genital abnormalities in both Hyaenids does indicate that investigations into the pathways underlying this phenomenon should be broadened beyond *Crocuta* in order to resolve the proximal or functional cause of masculinization. The discovery of these traits in *Hyaena* also supports the conceptual underpinnings of some existing non-adaptive and adaptive hypotheses originally developed to address masculinization in *Crocuta*.

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CONCLUSIONS

Striped Hyena Ecology and Species Account

Taxonomy

Pocock (1934a & 1934b) condensed the number of subspecies across the entire species range from the 28 defined by the beginning of the 20th century to five, based on cranial measurements and pelage characteristics: *H. h. barbara* from northwest Africa, *H. h. dubbah* from northeast Africa, *H. h. syriaca* from Syria, Asia Minor and the Caucasus, *H. h. hyaena* from India, and *H. h. sultana* from Arabia (although see Coetzee [1977] who regarded *vulgaris* as a distinct subspecies). Rieger (1979a) suggested integrating these into a smaller, north-east African-Arabian group composed of *H. h. dubbah* and *H. h. sultana* and a larger, north-west African-Asian group composed of *H. h. barbara*, *H. h. syriaca*, and *H. h. hyaena*. However, Jenks & Werdelin (1998) noted the inadequacy of available morphological data on variability to characterize each subspecies and that any classification was provisional.

The diploid chromosome number is 40 with 32 metacentric-submetacentric and 6 acrocentric autosomes, a metacentric X, and an acrocentric Y (Berube-Genest *et al.* 1987).

Description

The striped hyena is a medium-sized carnivore with overall appearance reminiscent of a dog. Muzzle pointed and head broad with long, pointed ears. Back slopes downward from the head to the tail. Coat has black vertical stripes on the side,

horizontal stripes on the legs, and a distinctive dark patch (or broad, dark “stripes”) on the throat. Underfur coloration is pale grey or beige; however, some individuals may appear whiter. Pelage coloration varies by region and may vary seasonally in the colder parts of its range (Pocock 1934a, Rosevear 1974, Ilani 1975). Ground colour of the pelt is typically grey to light brown, but may appear strikingly white even within the same population. Cub ground colour appears very white and the contrast between the ground colour and the black striation patterns is much more apparent (Rosevear 1974, Rieger 1978, A.P. Wagner *pers. obs.*). Longest hairs are up to 200 mm long (Rosevear 1974) and fall along the mid-dorsal line. The black dorsal mane may be held erect, significantly increasing the apparent size of the animal (Schneider 1926, Pocock 1934a, Kruuk 1976, Rieger 1978). Legs appear thin relative to their length and the hindlegs are shorter than the forelegs. Feet have four toes with four short, non-retractable claws (Pocock 1916). The tail is long with long coarse hairs. Striped hyenas have a well-developed anal pouch, a slit-like glandular orifice over-arching the anus from either side. The anal pouch may be inverted and thus be apparent while pasting or presenting during social encounters (Holzapfel 1939, Fox 1971, Kruuk 1976, Rieger 1977, 1978). In juveniles, there is an unusual convergence in genital appearance between sexes. Juvenile females have well-defined labia-like folds anterior to the vagina. These ridges are hairless and darker than the surrounding tissue. Juvenile males have smaller, smooth, hairless pre-scrotal skin folds along the middle septum close to, but anterior to, the scrotum (A.P. Wagner *this study*). Unlike spotted hyenas, *Crocuta crocuta*, these genital characteristics are not enough to confuse the sexing of juveniles and adult genitalia appear normal. There is no

apparent sexual dimorphism in body measurements and weight (Mendelssohn and Yom-Tov 1988, A.P. Wagner *unpublished data*). From the Kenya study, mean (95% CI, *n*) body measurements (cm or kg) were: head-body=108.3 (98-118, 55); tail=29.4 (26-36, 55); hind-foot=21.6 (19.4-24.5, 54); ear=14.7 (12.6-16, 51); weight=30.0 (23-35, 35)

The skull differs from that of *Crocuta* in being slightly smaller in size and less massive in build (Rosevear 1974). Permanent dentition is distinctly carnassial and the dental formula is $i\ 3/3, c\ 1/1, p\ 4/3, m\ 1/1=34$. Compared with *Crocuta*, the upper molar is much larger, as much as twice or more the size of the first premolar (Rosevear 1974, Coetzee 1977).

Geographic Variation

Existing subspecies descriptions are based on limited data except for *H. h. syriaca* in Israel and *H. h. dubbah* in Kenya (Mendelssohn & Yom-Tov 1988, A.P. Wagner *this study*). Differences in pelage across the species range appear minimal, although the Lebanese population is reported to have a reddish base coat colour (Lewis *et al.* 1968) and hyenas on the Arabian peninsula are described as having a yellow mark below the eyes (Gasperetti *et al.* 1985) and the dorsal crest is mixed grey and black rather than predominantly black (Mendelssohn & Yom-Tov 1988). Given the comments of Jenks & Werdelin (1998), no subspecies classifications are listed here.

Similar Species

Crocuta crocuta. Sympatric across much of the range. Spotted pelage, and back not maned; relatively short tail; short, rounded ears.

Proteles cristatus. Sympatric across much of the range. Marked difference in body size (about 9 kg) with more gracile build and large pointed ears; finer coat hairs appear glossier than the coarse coat of the striped hyena; cheek-teeth rudimentary.

Parahyaena brunnea. Southern Africa only. Stripes on legs only, with characteristic dark, long-haired, shaggy coat and lacking a distinct mane.

Distribution

The striped hyena has a very large range extending from Africa, north of and including the Sahel, and including much of East and North-east Africa, through the Middle East, Caucasus region, Central Asia, and the Indian subcontinent. Across their wide range, current distribution is patchy and most populations are likely composed of isolated small populations.

The current distribution of the species was reviewed extensively by Hofer & Mills (1998). In North Africa, their distribution extends from southern Morocco eastward along the extent of the North African coast to Egypt. They are absent from the central Sahara, their distribution in West Africa extending from Senegal in the drier Sahelian zone through Mauritania and Mali to Burkina Faso, Niger, northern Nigeria, southern Chad, and northern Cameroon. In East and North-east Africa, their range extends from Egypt south throughout much of the Horn to northern and central Tanzania. Although historically present, there are no reliable recent records of occurrence in Tunisia, Sudan, Eritrea, and Somalia (Hofer & Mills 1998), although they are still present in Djibouti (Kunzel *et al.* 2000). There are no records from the Central African Republic, though they may occur in the northern savannahs.

Habitat

Generally favours open or thorn bush country in arid to semi-arid environments (Prater 1948, Rosevear 1974, Kruuk 1976, Rieger 1978, Leakey *et al.* 1999, A.P. Wagner *unpublished data*) where water is available within 10 km (Rieger 1979a). Striped hyenas appear to avoid open desert and dense thickets and forests (Rosevear 1974, Rieger 1979a, Heptner & Sludskii 1980) and have been found to altitudes of 3,300 m in Pakistan (Roberts 1977, Rieger 1979a) and at least to 2,300 m in the Ethiopian Highlands (Yalden *et al.* 1996). While active, the striped hyena may cross more open areas, but they actively seek out relatively heavy vegetative cover or rocky depressions, particularly large caves, for resting (Rosevear 1974, Kruuk 1976, Rieger 1979a, Leakey *et al.* 1999, A.P. Wagner *unpublished data*). Where larger caves are not available, the resting sites used by striped hyenas are generally not revisited, although they frequently choose sites very close to those used previously (Kruuk 1976, A.P. Wagner *unpublished data*). Striped hyenas may remain active in areas frequented by humans, while avoiding them on a temporal scale (Rosevear 1974, Kruuk 1976, Mendelsohn & Yom-Tov 1988, A.P. Wagner *this study*).

Abundance

A total African population estimate of 2,450 to 7,850 individuals (Hofer & Mills 1998) represents roughly half of the total worldwide estimated population. Only Burkina Faso, Cameroon, Egypt, Kenya, Morocco, and Niger have populations estimated above 100 individuals, and, of those, only Egypt and Kenya have estimated populations over 1,000 (accounting for 51% of the maximum African population estimate and 82% of the minimum estimate!).

Throughout its range, the striped hyena occurs at low densities, but is distributed broadly across the landscape. Estimates of striped hyena abundance are complicated by the remarkably limited amount of information available on the species. This is undoubtedly due to its shy, nocturnal, mostly solitary nature, its apparent affinity for rugged terrain, its generally negative reputation, and frequent confusion with, or lack of differentiation from, spotted hyenas where the species overlap. There have been only two estimates of striped hyena density in Africa: in Serengeti National Park, Tanzania, an estimate based on observations of a limited number of individuals was given as greater than 0.02 per km² (Kruuk 1976), while from a larger study in Laikipia District, central Kenya, estimated the minimum regional density at 0.03 adults per km² (A.P. Wagner *this study*).

Adaptations

The dark throat patch has thickened skin and denser fur (Harrison 1968). Deliberate exposure of its black or brown throat by one striped hyena to another has been construed as a conciliatory gesture or signal of trust (Kingdon 1977). The exposure of dark and light areas during elaborate behaviour rituals has been interpreted as components of a signal code that might help regulate social interactions. Thus, during ritual or play-fights the pale cheeks and upper sides of the neck become targets for nipping with half-open mouth (Fox 1971, Kingdon 1977). In contradiction to these suggestions, the dark throat patch has been proposed as a mechanism to orient bites during agonistic interactions (Rieger 1978). However, further observations from captivity and of bite marks in wild hyenas indicate that bites are routinely directed at the

back of the head and upper neck (Kingdon 1977, A.P. Wagner *pers. obs.*, A. Roberts *pers. comm.*).

Large ducts from the anal glands open into an anal pouch dorsal to the anus. The pouch is inverted during scent marking (pasting) and greeting behaviour, and produces a pungent yellow to beige coloured paste which is deposited on grass stems and/or sticks (Brehm 1927, Holzapfel 1939, Fox 1971, Kruuk 1976, Rieger 1977, 1978, 1981). Rarely observed, it is unclear if this behaviour is used to mark territories, although it is presumed that this is the case. Pasting has also been observed near large carcasses and, in captivity, on food bowls (A.P. Wagner *pers. obs.*, A. Roberts *pers. comm.*).

The transient genital anomalies found in juveniles have only recently been discovered (A.P. Wagner *this study*) and their function is unclear. However, given the extraordinarily masculinized genitalia of female spotted hyenas, *Crocuta crocuta*, this author suggests that this may represent a genetic predisposition for genital masculinization from a common ancestral hyena species.

Cubs are raised in dens, which may be either holes dug by the mother, holes formed and abandoned by other species (Prater 1948, Roberts 1977, A.P. Wagner *pers. obs.*), deep, natural, and sometimes complex, caves (Heptner & Sludskii 1980, Kerbis-Peterhans & Horwitz 1992, Leakey *et al.* 1999), or simple rock depressions less than a meter deep (A.P. Wagner *unpublished data*). When large caves are not available, mothers may relocate cubs to a new den within the first few days after birth and do not reuse the same dens for subsequent litters (A.P. Wagner *pers. obs.*).

As is the case with *Crocota* and *Parahyaena*, the development of crushing teeth and an ability to extract nourishment, even from old sun-dried bones, is one of the primary adaptations of striped hyenas (Kingdon 1977). The structure of the head-neck-shoulder region is noticeably powerful. The high sagittal crest of the skull increases the area of origin for the powerful temporal muscles and the well-developed masticatory muscles facilitate seizing and crushing of prey (Buckland-Wright 1969).

Foraging and Food

The diet of the striped hyena is still a matter of some debate. However, it has been reported to consume a wide variety of vertebrates, invertebrates, vegetables, fruit, and human originated organic wastes (Flower 1932, Novikov 1962, Harrison 1968, Ilani 1975, Kruuk 1976, Macdonald 1978, Leakey *et al.* 1999, A.P. Wagner *this study*). It is known to scavenge off lion, *Panthera leo*, and spotted hyena kills (Kruuk 1976, A.P. Wagner *unpublished data*) as well as discarded livestock carcasses (Leakey *et al.* 1999, A.P. Wagner *this study*). In many areas, striped hyenas have also been described as raiding human graves and carrying away bones (Rosevear 1974, Horwitz & Smith 1988, Leakey *et al.* 1999), and fruit and vegetable crop raiding is considered a serious problem in Israel (Kruuk 1976).

The overall reputation of the species, therefore, is that of an omnivorous scavenger. However, in central Kenya analysis of bone fragments and hairs from faecal samples indicate that hyenas regularly consume smaller mammals and birds that are unlikely to be scavenged (A.P. Wagner *this study*). This is in accordance with Kruuk's (1976) observations, but previous interpretations of the limited data available on striped

hyena diet (Rosevear 1974, Skinner & Ilani 1979, Leakey *et al.* 1999) often under-emphasized the evidence for active hunting. Striped hyenas have been reported chasing hares, porcupines, bat-eared foxes, domestic cats, cheetah cubs, dikdik, reedbuck, and young gazelles (Kruuk 1976, Skinner & Ilani 1979). Further, there is strong evidence that small livestock (goats and sheep) and dogs are often killed by striped hyenas (Rosevear 1974, Leakey *et al.* 1999, Kuhn 2005, A.P. Wagner *unpublished data*).

Several studies have inferred diet by combining data from bone collections and faecal samples (Kerbis-Peterhans & Horwitz 1992, Leakey *et al.* 1999), while others have inferred diet from den bone collections alone (Skinner & Ilani 1979, Kuhn 2005). Bone collections are common at den sites for the species, although the degree to which these collections represent scavenged vs. killed prey and the nutritional role of the bones collected is unclear. Bone contains organic matter, mostly collagen, at up to 40% in weight (Kruuk 1972). The spotted hyena is able to digest and absorb this organic matter and only the inorganic material is excreted. Striped hyenas likely have a similar ability to draw nutrients from digestion of bones. Following a very severe drought in 1962, Kingdon (1977) examined the stomach of one striped hyena that contained nothing but 3 kg of large splintered bones and the rest of the digestive tract showed that the animal was subsisting habitually on similar old bones and the digestion was successfully breaking such bones down into congealed white faeces. In central Kenya, den bone collections examined represented a much broader range of prey than did scat analysis and significant portions of bone assemblages were very old bones unlikely to represent scavenging from fresh kills (A.P. Wagner *this study*). From faecal analysis alone, Kruuk (1976),

Macdonald (1978), Bouskila (1984), Leakey *et al.* (1999), and A.P. Wagner (*this study*) all found remains of prey items that are more likely to be scavenged. In addition, larger mammals were represented far less often in the analysis of hairs by A.P. Wagner (*this study*) than would have been expected based on den bone collections alone.

Although sometimes found in small groups of up to four individuals while resting, striped hyenas appear to be strictly solitary foragers in Africa (Kruuk 1976, A.P. Wagner *this study*). In Israel, however, groups of hyenas do converge at feeding sites (Kruuk 1976, Macdonald 1978, Skinner & Ilani 1979, Bouskila 1984), but there is no clear indication of cooperative foraging and relatedness of observed groups has not been investigated. Foraging activity in Kenya and Tanzania was restricted entirely to night-time except during rain and/or unusually dark and cloudy weather (Kruuk 1976, A.P. Wagner *unpublished data*). Under those weather conditions, striped hyenas may return to previously visited kills or carcasses, but do not embark on full foraging forays (A.P. Wagner *unpublished data*). Age specific foraging data are extremely limited, but cubs have been observed accompanying their mothers on foraging forays by 6-12 months of age (Kruuk 1976, A.P. Wagner *unpublished data*).

Kruuk (1976) described foraging behaviour in which striped hyenas zigzagged across the landscape and did not follow set routes, even when returning to the same food source on multiple nights. Striped hyenas spent long periods of time sniffing at the base of trees and bushes, but the head was generally held vertically while travelling. Caching of food under bushes was also observed. Minimum mean distance travelled per night was 19 km at speeds of 2-4 km/h, occasionally trotting at speeds of up to 8 km/h, or

running at a maximum of 50 km/h. From observations made at feeding stations in Israel, it was estimated that an adult striped hyena consumes 7-8 kg per night (Skinner & Ilani 1979).

Overall, the evidence indicates striped hyenas in Africa are solitary nocturnal foragers for which fruit and vegetable matter, where available, may play a significant part. Striped hyenas also regularly consume insects, invertebrates, small vertebrates, and actively hunt small mammals and ground-nesting and/or ground-feeding birds. In addition, they scavenge off carcasses of larger mammals and this activity appears to account for a significant portion of the bones collected at den sites.

Social and Reproductive Behaviour

Almost invariably described as solitary in Africa, research in Kenya has shown that striped hyenas routinely rest in pairs and occasionally in groups of up to four individuals (A.P. Wagner *this study*). These groups never include more than one adult female. Adults within a group are typically unrelated or distantly related. However, examples of full-sibling brother-sister and full-sibling brother-brother pairs were observed within groups. While group-living males appear to father the majority of cubs, multiple paternity of litters by those males has been detected through parentage analysis of genotype data (A.P. Wagner *this study*).

Very little has been recorded regarding direct social interactions outside of captive situations. Kruuk (1976) did note the meeting ceremony between greeting pairs, which involved mutual sniffing of the face, neck, and anal regions. The anal pouch was protruded during sniffing and either both hyenas were standing or one would lie down

while exposing the anal region. Observations in captivity (Rieger 1978) match these field observations and Fox (1971) observed anal protrusion displays in cubs at eight weeks of age. The dorsal mane is also erected by striped hyenas in a defensive posture or when threatened and, when aggressive, both the mane and the tail hairs are erected (Schneider 1926, Kruuk 1976, Rieger 1978, A.P. Wagner *pers. obs.*). Erect or limp dorsal manes have been interpreted as signals that, respectively, indicate dominant or subordinate status to other hyenas (Kingdon 1977).

Home range sizes in Serengeti were reported for one male and one female at 44 and 72 km², respectively (Kruuk 1976). In Kenya, mean home range size for four males and six females was 68.9 km² (SE=7.8) (A.P. Wagner *this study*), with no significant difference in home range size between sexes. No two adult females were found to have significant home range overlaps, although groups of up to three males and one adult female had almost complete overlap in home ranges.

Descriptions of mating behavior come from observations in captivity. Males will follow an oestrus female for several days before being allowed to mate (Bothma & Walker 1999). Copulation lasts only one day and consists of up to five 15-45 minute mating attempts, with intervals between mating attempts of about 20 minutes. No pelvic thrusting has been observed (Rieger 1979a).

Cubs are reared in dens (described above) and intense digging behaviour in the females announces parturition (Rieger 1979a). Mothers carry food back to the den for their cubs (Kruuk 1976, Davidar 1985, 1990) and prepare meat for cubs by biting off pieces (Rieger 1979a). There is some evidence of helpers being present to raise cubs. In

Kenya, two sub-adult females were frequently found at a den site with two new-born cubs and the mother (A.P. Wagner *this study*). Analysis of genotypes confirmed that the two sub-adults were full-sibling sisters and were daughters of the mother of the younger cubs.

Striped hyenas are considerably quieter than the spotted hyena (Rosevear 1974) in terms of both volume and frequency of vocalizations, and are generally silent. Kruuk (1976), however, noted that striped hyenas are more vocal in Israel and their relative silence in East Africa may reflect a behavioural response to avoid dominant carnivores in the region. Vocalizations are similar to those of the spotted hyena and include *whining* by cubs before suckling, *giggling* when frightened, *yelling* when being chased by conspecifics, *lowing* in a defensive position, *growling* when play or food-fighting, and a call by the mother to her cub(s) (Kruuk 1976, Rieger 1981, A.P. Wagner *pers. obs.*).

Reproduction and Population Structure

Parturition is aseasonal and gestation lasts 90-91 days (Pocock 1941, Ronnefeldt 1969, Skinner & Ilani 1979, Heptner & Sludskii 1980). Litter sizes in the wild range from 1-4 cubs and from 1-5 in captivity (Skinner & Ilani 1979, Rieger 1981, A.P. Wagner *this study*). In captivity, newborn cubs weigh 660-700 g (Skinner & Ilani 1979, Rieger 1981) and reach mean weights of 796 g at one week, 1.5 kg at four weeks, 2.8 kg at eight weeks, and 5.6 kg by 28 weeks (Skinner & Ilani 1979). In the wild, cub weights were reported at 2 kg at two to three weeks of age (A.P. Wagner *unpublished data*). Cubs are born blind with white to grey fur and clear black stripes. Eyes open at 7-8 days, and teeth erupt at 21 days; cubs start to eat meat after the first month (Rieger 1981).

Weaning in captivity takes place after eight weeks, but in the wild cubs have been observed suckling at 4-5 months (Ilani 1975) and 10-12 months (Kruuk 1976). Sexual maturity is reached at 2-3 years (Rieger 1979b), although one female in captivity gave birth at the early age of 18 months (Rieger 1979b). In captivity, post-partum oestrus follows 20-21 days after birth and oestrous cycles last 40-50 days (Rieger 1979a & 1979b). Bothma & Walker (1999) reported that males will remain with the female until the cubs have been raised, but given the gregarious nature of groups when no cubs are present, it is not clear if males are exceptionally attentive during cub rearing.

Little has been reported regarding sex ratios in the wild. However, in Kenya the two litters sexed had three males in one litter and one male and one female cub in the other (A.P. Wagner *this study*). The well-sampled adult population had a sex ratio of 0.8:1 adult females to males. As there are no long-term studies of the species in the wild, longevity has only been reported in captivity at 23 to 24 years (Rieger 1979a). From the Laikipia study, non-age specific probabilities of survival for adult hyenas (those over two years of age) were estimated for the duration following the time each hyena was first identified. Survival to six months post-identification was estimated at 0.96 (n=27), 0.89 for survival to one year post-identification (n=27), 0.62 for two years (n=21), and the probability of surviving three years after a hyena was first identified was 0.47 (n=17) (A.P. Wagner *this study*). Of the nine cubs identified before six months of age (three female, four male, two unknown sex), at least four (two female, two male) had survived to at least two years. This is a minimal survival rate as the cubs were not radio-collared and survival could only be confirmed if they were resighted.

Predators, Parasites, and Diseases

Interactions with other carnivores are best considered in terms of dominance and competition rather than predation. The striped hyena is subordinate to lions and spotted hyenas, although Kruuk (1976) described a mutual ‘attraction’ between the two Hyenids. There is also suggestive evidence that competition with spotted hyenas may be important in circumscribing the habitat used by striped hyenas (H. Kruuk pers. comm.). Outcomes of encounters with cheetahs, *Acinonyx jubatus*, and leopards, *Panthera pardus*, are not as predictable, but adults of those species are likely to dominate striped hyenas. Skinner & Ilani (1979) reported that a caracal, *Caracal caracal*, dominated three subadult striped hyenas of about 29 kg each.

The importance of the striped hyena as a reservoir or vector for domestic animal and human disease has not been evaluated. The spatial overlap with humans and domestic animals in many parts of the striped hyena range may be significant in this regard.

Conservation

IUCN - Lower Risk/Near Threatened. CITES – not listed.

Humans are consistently indicated as the major source of mortality throughout the evaluated range (Hofer 1998) and were responsible for 50% of recorded deaths in central Kenya (A.P. Wagner *unpublished data*). Negative perceptions of the species persist throughout its range and collection of human remains (“grave robbing”) and incidents of damage to agriculture and livestock perpetuate negative attitudes. In North Africa, the brain is used as an aphrodisiac and hairs are used as talisman (Ronnefeld 1969, Rieger

1979a, Osborn & Helmy 1980). Poisoning at oases in Egypt has been cited as a cause of population declines in addition to hunting for utilization of the whiskers and eyeballs as protection from the evil eye and the heart for courage (Prater 1948, Osborn & Helmy 1980). In Ethiopia, the species is protected; however, hunting under a special permit is allowed (Hofer & Mills 1998). Habitat destruction is viewed as a threat in Kenya and effective protection is absent as hyenas are viewed with contempt. In this region, due to the lack of differentiation between the species, striped hyenas are often killed when spotted hyenas are the intended target (A.P. Wagner *pers. obs.*). The Moroccan population has declined drastically and the remaining population has withdrawn into the southern mountainous regions (Hofer & Mills 1998). In Niger, the population is declining as a result of officially sanctioned eradication or poisoning and by habitat destruction. The main source of recorded mortality in Tanzania is road kills.

Striped hyenas are present in numerous protected areas, including: Algeria (Djurdjura N.P., El Kala N.P., Mergueb N.R., Béni-Salah N.R.), Burkina Faso (“W” N.P., Arly N.P., Kabore Tambi N.P.), Cameroon (Waza N.P.), Ethiopia (Awash N.P., Mago N.P., Omo N.P., Yabello Sanctuary), Kenya (Masai Mara N.R., Tsavo East & West N.P., Sibiloi N.P., Samburu N.R., Buffalo Springs N.R., Lake Nakuru N.P., private reserves in Laikipia District [A.P. Wagner *this study*]), Libya (Boucle du Baoulé N.P.), Morocco (Reserve integrale de Missou, Tazeka N.P., Parc National de l’Oriental, Iriki Hunting Reserve), Senegal (Boundou, Ferlo Nord, Ferlo Sud, Oualo, Cayor Fuanal Reserves), and Tanzania (Serengeti N.P., Tarangire N.P., Ngorongoro Conservation Area, Mkomazi G.R.) (Hofer & Mills 1998).

Recent records also indicate the species occurs outside of protected areas in a number of regions, including, for example, in Egypt, in the Nile valley, and near oases in the west, Mediterranean and Red Sea coastal areas, and, in Kenya, in Masailand, Lake Natron, the central Highlands including Laikipia and Samburu Districts, Lake Turkana region, and parts of Kajiado District [A.P. Wagner *this study*] (Hofer & Mills 1998). Because they exist outside of formally protected areas in regions where pastoralism is the norm and the potential for human-carnivore conflict is very high, populations in Egypt and Kenya are exceptionally vulnerable to human population growth, habitat destruction, and poisoning. Particular attention should be paid to ensuring the survival of the species in pastoral areas by identifying ways to reduce human-carnivore conflict through promotion of methods that ensure adequate numbers of prey persist and/or methods that reduce livestock killing by carnivores.

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