A GENETIC APPROACH TO UNDERSTANDING THE EVOLUTIONARY ECOLOGY OF THE FAMILY HYAENIDAE

By

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ABSTRACT

A GENETIC APPROACH TO UNDERSTANDING THE EVOLUTIONARY ECOLOGY OF THE FAMILY HYAENIDAE

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My dissertation combines molecular and behavioral data to examine the evolutionary ecology of the family Hyaenidae. More specifically, I focus on the evolution of adaptive genetic variation in hyenas, the influence of this variation on fitness, and the genetic and ecological causes and consequences of behavior. My dissertation consists of four chapters following the introductory chapter. The first of these research chapters, Chapter 2, characterizes the diversity of three genes located within the major histocompatibility complex (MHC), and are critical to the adaptive immune system. In addition to offering the first characterization of these loci in spotted (*Crocuta crocuta*) and striped (*Hyaenidae hyaenidae*) hyenas, this chapter describes the evolutionary history of alleles at each locus and documents the presence of positive selection acting to increase diversity at two out of three loci. The results of these analyses are consistent with the hypothesis that some shared selection pressure, rather than differing degrees of sociality, is the predominant force shaping MHC variation within the family Hyaenidae. Chapter 3 documents correlations between MHC variation and individual measures of fitness in spotted hyenas, and suggest the importance of specific MHC alleles to longevity and immune system function. Chapter 4 investigates whether captive female spotted hyenas exhibit differences in their behavioral responses to odors originating from unknown males that vary in degree of MHC similarity to the female. I found a significant correlation between average pairwise relatedness and time spent sniffing, such that females spent significantly

longer times investigating odors from males that were more closely related to them and from males that were MHC-dissimilar to them. These data suggest that genetic variation may influence hyena behavior, and that female spotted hyenas prefer the odor of males that contain dissimilar genotypes from them at functional (i.e. MHC) loci. Chapter Five presents neutral microsatellite marker data from two populations of striped hyenas and examines how space use and genetic relatedness are influenced by local population ecology. These data suggest differences in the relationship between relatedness and space use between these two populations. Further, I show that the area occupied by the population inhabiting Shompole, Kenya has more than triple the ungulate (i.e. prey) density than does the area inhabited by the Laikipia, Kenya, population, whereas both populations exhibit the same hyena density. Female home range sizes in Shompole were significantly smaller in Shompole than in Laikipia, suggesting that the ecological differences in prey availability influence the amount of area needed by females to meet their food requirements.

My dissertation utilizes both neutral and adaptive molecular marker data, in combination with behavioral data, as tools to address questions pertaining to the evolutionary ecology of hyenas. The results of my analyses demonstrate positive selection acting to increase variation at functional loci, and show that this variation may indeed be integral to individual fitness in spotted hyenas. Further, I show that genetic variation may influence behavior in hyenas by affecting odor preference, and that hyenas may alter their space use patterns in response to local ecology. Overall, my dissertation offers molecular, behavioral, and ecological data rarely available for large mammalian (non-model) species and contributes to the body of knowledge regarding extant members of the family Hyaenidae.

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CHAPTER ONE

GENERAL INTRODUCTION

Wild populations of large mammals are becoming increasingly rare, as their habitat is becoming degraded and eliminated by human alterations; these alterations are also causing wild populations to become fragmented (Frankham et al. 2002). Large mammalian carnivores in particular are vulnerable to habitat loss as they require enormous areas of undisturbed land in which to roam to acquire sufficient resources and cope naturally with their environment. As their population sizes decrease, so does the pool of standing genetic variation. This reduced genetic diversity can severely limit animals' abilities to adapt to change in their social and physical environments and can drastically decrease population viability and increase extinction risk (Gilpin and Soulé 1986; Newman and Pilson 1997; Westmeier et al. 1998).

Investigating patterns of variation in population genetic structure can offer insights into the evolutionary mechanisms influencing fitness in wild populations, and many studies to date have confirmed a fundamental relationship between genetic diversity and the fitness of animal populations (Hansson and Westerberg 2002; Altizer et al 2003; Reed and Frankham 2003). Specifically, it is well – documented that host genetic diversity plays an important role in countering immune risks imposed by parasites and pathogens (Cassinello et al. 2001; Keller and Waller 2002; Spielman et al. 2004; Keesing et al. 2010), and increased diversity can reduce an individual's susceptibility to infectious disease (O'Brien and Evermann 1988; Siddle et al. 2007; Altermatt and Ebert 2008). As a consequence of the intricate relationship between disease and genetic diversity, disease plays a major role in shaping genetic population structure in the wild

(e.g. Keyghobadi et al. 2006; Foster et al. 2007; Blanchong et al. 2008). Theory and empirical evidence show the significant effects that parasites and diseases can have on individual reproduction and survival (e.g. Anderson and May 1979). Characterization of this genetic diversity, particularly of functionally relevant immune system genes, can contribute vital components to a comprehensive understanding of the complex relationship between genetic diversity and fitness in wild mammals, by elucidating ways in which disease influences selection upon varying phenotypes.

Until recently, empirical assessment of genetic diversity has often focused on neutral (not under selection), rather than adaptive (functional, protein – coding) markers (Sunnucks 2000; Coltman & Slate 2003; DeWoody and DeWoody 2005) to characterize overall genetic diversity in individuals or populations. However, although neutral markers provide vital information about population parameters such as relatedness and dispersal patterns, they are limited in their ability to detect evolutionarily relevant adaptive variation (Bekessy et al. 2003; Luikart et al. 2003; Waples and Gaggiotti 2006; de Guia and Saitoh 2007; Vali et al. 2008). Further, patterns of diversity at neutral loci offer an incomplete picture of overall genome-wide diversity, as natural selection sometimes acts to maintain higher diversity at functional loci compared to what is seen throughout the entire genome (Aguilar et al. 2004). It is the genetic diversity underlying phenotypic traits that allows for evolutionary adaptation in response to environmental change, often referred to as a population's 'evolutionary potential' (Endler 1986; Reusch and Wood 2007).

The importance of integrating information at adaptive loci into molecular and conservation studies based on neutral markers is becoming increasingly evident (e.g. Reed and

Frankham 2001; Holderegger et al. 2006; Gebremedhin et al. 2009; Kirk and Freeland 2011; Marsden et al. 2013), as well as increasingly feasible in non-model species (e.g. Langefors 2005; Schwensow et al. 2007; Ouborg et al. 2010). Adaptive variation is now consistently recognized as a critical component that must be considered in the fields of molecular ecology and conservation.

The aim of my dissertation is therefore to utilize both neutral and adaptive molecular marker data, in combination with behavioral data, as tools to address questions pertaining to the conservation genetics of species belonging to a behaviorally diverse family of carnivores, the family Hyaenidae. I examine data from a population of free-living spotted hyenas (*Crocuta crocuta*) that has been studied continuously in the Masai Mara National Reserve in Kenya, East Africa, since 1988 by personnel associated with the Mara Hyena Project. In addition, I analyze data from a captive population of spotted hyenas maintained at the Field Station for Behavioral Research at the University of California, Berkeley (UCB). I also utilize data collected from two wild populations of striped hyenas (*Hyaena hyaena*), located in Laikipia District, Kenya and in Shompole, Kenya, approximately 300 kilometers apart from each other.

To study functionally – relevant adaptive markers in the two hyena species, I examine and compare genetic diversity at three loci (*DRB, DQB,* and *DQA*) of the major histocompatibility complex (MHC) in both species in Chapter Two. The MHC is a gene-dense region present in all jawed vertebrates, and codes for cell-surface proteins involved in adaptive and innate immunity. The genes in this complex are among the most diverse genes of the vertebrate genome, and research indicates that diversity at specific MHC genes has important consequences for reproduction and survival in a wide range of species (Hughes 1991; Hughes

and Nei 1992). Proteins encoded by the MHC genes analyzed here are found on specialized immune cells (e.g. macrophages) that bind antigens derived from extracellular pathogens or parasites and initiate an adaptive immune response (Klein 1986). Recognition and binding of these antigens is highly specific, such that an individual who possesses a larger number of different binding proteins, resulting from a higher diversity of the underlying loci, is capable of recognizing and binding a wider array of pathogens to mount an immune response against.

There are two well – supported, non – mutually exclusive, hypotheses to explain the maintenance of the high observed variation at MHC loci. Several lines of evidence suggest that forms of pathogen-driven balancing selection generate and maintain these high levels of diversity via heterozygote advantage or rare allele advantage (Hughes and Nei 1988, 1989; Potts and Slev 1995; Hughes and Yeager 1998; Paterson 1998; Hedrick 1998; Binz *et al.* 2001; Garrigan and Hedrick 2003; Piertney and Oliver 2006). Many studies have also presented evidence to support the hypothesis that sexual selection is acting to favor disassortative mating based on MHC diversity, mediated via MHC – based odor preferences (Yamazaki et al. 1976; Wedekind & Furi 1997; Sauermann 2001; Penn 2002; Wysocki et al. 2004; Consuegra and de Leaniz 2008; Radwan *et al*. 2008).

In Chapter Three, I examine MHC diversity further in wild living spotted hyenas to assess the implications of this adaptive genetic variation on individual fitness measures related to both of the above hypotheses. In the fourth chapter, I further investigate MHC – based disassortative mating by asking whether odor preference in captive spotted hyenas is related to underlying adaptive genetic diversity at the MHC *DRB* locus. In Chapter Five, I utilize neutral microsatellite marker data to describe basic population level parameters and the relationship

between genetic relatedness and spatial distance in two ecologically differentiated populations a species about which little is known: the near – threatened, possibly endangered, striped hyena (*Hyaena hyaena*).

Overview of Chapters

In Chapter Two, I examine the evolutionary history of MHC genes in hyenas by comparing patterns of variation at three class II loci between spotted and striped hyenas. I found high levels of variation and evidence of positive selection at two out of three loci in both species, but found no discernible differences in levels of MHC variation between spotted and striped hyenas. If differences in sociality were influencing the strength of selection acting on MHC loci, as has been shown in some rodent species (Hambuch and Lacey 2002; Kundu and Faulkes 2003), then I predicted I would see greater MHC diversity, and evidence of stronger selection, in the gregarious spotted hyena than in the solitary striped hyena. Greater degrees of sociality have been shown to influence rates of parasite transmission, and subsequent selection acting on disease resistance, in several host species (Altizer et al. 2003; Bull 1994; Frank 1996; Møller et al. 2001; Nunn et al. 2008). However, in addition to finding no strong differences in the level of diversity at these loci between the two hyaenid species, I also found multiple cases of trans-species alleles, indicating that alleles have been maintained in each species since these two species last shared a common ancestor (approximately 8.6 million years ago; Koepfli et al. 2006). These results led me to conclude that some shared selection pressure, rather than different forms of sociality, is the predominant force shaping variation at MHC loci within the family Hyaenidae.

In the third chapter, I investigate how individual MHC variation influences measures of fitness in the spotted hyena. More specifically, I use pedigree data from the Masai Mara Talek hyena population to ask whether MHC similarity influences the survival of a female's offspring, an individuals' longevity, its endoparasite load, or its circulating concentrations of antibodies. I also inquire whether male MHC diversity predicts male reproductive success, testing hypotheses regarding the genetic basis of mate choice. My small sample sizes in this chapter prevented me from conducting more rigorous statistical analyses, and may have led to spurious significant effects. However, this remains one of few studies in a non – model organism in nature to address fitness effects of adaptive immune loci in the wild. My results serve as information to guide future research, as these data indicate there may be allele-specific effects on fitness in spotted hyenas, with particular focus on functional binding sites in amino acid sequences, which recognize foreign pathogens and initiate the adaptive immune response.

In Chapter Four, I inquire whether captive spotted hyenas demonstrate MHC – based odor preferences. I found that, in concordance with previous studies, females spent significantly more time investigating straw piles scented with odiferous secretions (hereafter referred to as 'paste') deposited on them by hyenas when compared to the piles without paste. Relevant to the hypothesis that sexual selection is operating on MHC diversity in spotted hyenas, I found that females spent significantly more time sniffing a pile with paste from an MHC – dissimilar male than she did if the paste was from an MHC – similar male. Finally, while I found no relationship between pairwise relatedness based on microsatellites and pairwise MHC similarity, I found a significant correlation between the average pairwise relatedness and time spent sniffing, such that females spent significantly longer times investigating odors from males

that were more closely related to them. This suggests that perhaps females prefer the paste of individuals that are closer in overall genetic relatedness to them, but are dissimilar at specific functional (i.e. MHC) loci.

In Chapter Five, I examine the behavioral plasticity and resulting patterns of genetic relatedness and space use in two wild populations of striped hyenas. These two populations vary in many aspects of their ecology, but the salient data in Chapter Five are that the area occupied by the population inhabiting Shompole, Kenya has more than triple the ungulate (i.e. prey) density than does the area inhabited by the population in Laikipia, Kenya. Striped hyenas are largely solitary foragers, and feed primarily on small, rare pieces of carrion or young gazelles. Wagner et al. (2007) postulated that limited availability of these small food items in Laikipia explains why they observed the unusual pattern in the Laikipia population, in that female pairwise genetic relatedness increased with spatial distance. This pattern indicated that females of the Laikipia population are preferentially moving away from other female kin, and Wagner et al. (2007) hypothesized that this occurs in order to limit kin competition over nutritional resources. We found the opposite pattern in the Shompole population, where striped hyenas behave in a more typically mammalian fashion, with females exhibiting philopatry, and female kin being found in closer proximity to one another than they are to less related individuals. In addition to higher prey availability, we found significantly smaller female home range sizes in Shompole, suggesting that the area females need to meet their food requirements is smaller due to the increased prey density. It remains unclear what drives male space use patterns in this species, as there were no significant differences in home range size or overlap among males between these two populations. More data, likely collected over longer

time periods and in several populations, are needed to understand what behavioral mechanisms are driving the relatedness patterns seen in these two disparate populations.

The research I presented in my dissertation was the result of collaborative effort, and because each chapter was prepared in manuscript form, I hereafter use the term "we," rather than "I." Chapter Two is currently in press at the *Journal of Mammalogy*, and I plan to submit Chapter Three to either *Molecular Ecology* or *Immunogenetics*, Chapter Four to either *Immunogenetics* or *Ethology*, and Chapter Five to *Animal Behaviour* or *Behavioral Ecology*.

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CHAPTER TWO

FORCES SHAPING MHC EVOLUTION IN TWO HYENA SPECIES

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ABSTRACT

Genes of the mammalian major histocompatibility complex (MHC) are central to adaptive immunity. High levels of observed polymorphism at MHC loci have been hypothesized to be maintained by natural selection acting to preserve alleles for pathogen resistance. Here we examined patterns of multilocus MHC diversity in natural populations of 2 closely related carnivore species: spotted (*Crocuta crocuta*) and striped (*Hyaena hyaena*) hyenas. We also tested hypotheses suggesting specific selection pressures favoring MHC diversity in these hyena species. We found several lines of evidence consistent with positive selection acting at multiple MHC loci in both species. These included high allelic variation, pervasive gene duplication, trans-species segregation of alleles, and codons evolving under positive selection that disproportionately map to known antigen binding regions. Despite striking behavioral differences between these 2 hyaenids with respect to their mating systems and social behavior, we found no qualitative species differences in MHC loci, nor did we detect differences in the strength of natural selection. Our findings suggest that ancient shared selection pressures, including a common ancestral pattern of carrion-feeding, has influenced MHC diversity more strongly in these hyena species than have selection pressures imposed relatively recently by sociality or sexual selection.

Key words: carrion feeding, hyenas, major histocompatibility complex, molecular evolution, population genetics

INTRODUCTION

Variation among free-living mammals in their susceptibility to pathogens remains poorly understood. Among all mammals, carnivores are arguably the most threatened by pathogens (Pedersen 2007), due in large part to anthropogenic disturbance. Characterization of the diversity of functionally relevant immune system genes can shed considerable light on both disease ecology in carnivores, and the relationship between their genetic diversity and immune function.

The major histocompatibility complex (MHC) is composed of functionally related and physically linked genes that are critical to the adaptive immune system. The subset of MHC genes termed "class II" present extracellular antigens to T cells in order to initiate an immune response, and are among the most diverse genes in the mammalian genome (Garrigan and Hedrick 2003; Gaudieri et al. 2000; Klein 1986). The high diversity observed at these loci is thought to represent an adaptation for recognizing and mounting an adaptive immune response to a wide array of rapidly evolving pathogens (reviewed in Garrigan and Hedrick 2003; Piertney and Oliver 2006; Sommer 2005).

Due to the integral role of these genes in the adaptive immune response, MHC diversity has been linked in various mammals to individual fitness and long-term survival of populations (e.g. Hughes 1991; Paterson 1998; Penn 2002; Thoss et al. 2011). In fact, MHC has become a

popular model for examining patterns of adaptive variation in vertebrates (reviewed in Bernatchez and Landry 2003; Piertney and Oliver 2006; Sommer 2005). Analyses of MHC polymorphism in non-model organisms offers opportunities to learn about immune system evolution in the wild, and provide insights into the role of genetic variation in long-term population viability. Here we characterize MHC diversity in 2 hyena species, spotted (*Crocuta crocuta)* and striped hyenas (*Hyaena hyaena*), and test hypotheses suggesting selective forces that may have shaped the evolution of this genetic diversity.

Several types of evidence support the hypothesis that MHC diversity is selectively maintained (e.g. Axtner and Sommer 2007; Bernatchez and Landry 2003; Bryja et al. 2006; Edwards and Hedrick 1998; Hughes and Nei 1992; Klein 1986; Richman 2000; Takahata 1995). First, selective maintenance of MHC diversity is suggested by high allelic variation. Second, selective maintenance of MHC diversity is indicated by substantial trans-species polymorphism, where allelic lineages are maintained over time scales that span speciation events; as a result, more closely related alleles are found among, rather than within, species (Figueroa et al. 1988; Klein et al. 1998). Selective maintenance of MHC diversity is also indicated by higher rates of non-synonymous (d_N) than synonymous (d_S) nucleotide substitutions at codons responsible for antigen binding (antigen binding sites, or ABS—Hughes and Nei 1988, 1989; Klein 1986; Takahata and Nei 1990). Here we examine these phenomena to determine whether or not MHC diversity is maintained by selection in hyenas.

We characterized MHC diversity at 3 class II loci (*DRB, DQB,* and *DQA*) in spotted and striped hyenas, and compared the patterns of diversity at these MHC loci within and between these species to test for evidence of positive selection. We also addressed non-mutually

exclusive hypotheses concerning selection pressures favoring MHC diversity in hyenas. High diversity at MHC loci is hypothesized to be maintained by pathogen-driven positive selection, acting through overdominance (heterozygote advantage) and/or negative frequency dependence (rare allele advantage—Doherty and Zinkernagel 1975; Hedrick 1998; Hughes and Nei 1988, 1989; Hughes and Yeager 1998; Jeffery and Bangham 2000; Piertney and Oliver 2006; Potts and Slev 1995). Sexual selection is also thought to play a role in maintaining this diversity, by favoring MHC-based disassortative mating preferences via odor based discrimination of MHC genotypes, or via selective abortion due to maternal-fetal incompatibility (Edwards and Hedrick 1998; Fernandez et al. 1999; Milinski 2006; Ober 1992; Penn and Potts 1998a, 1999; Wedekind et al. 1995; Wedekind and Penn 2000; Yamazaki et al. 1999). In addition, sociality has been found to influence strength of selection at MHC loci, with evidence of stronger selection in more gregarious species imposed by higher rates of contact among conspecifics (Hambuch and Lacey 2002; Kundu and Faulkes 2003). Previous studies have also demonstrated a strong link between degree of sociality and rates of parasite transmission, and subsequent selection for increased disease resistance in gregarious host species (Altizer et al. 2003; Bull 1994; Frank 1996; Møller et al. 2001; Nunn et al. 2008). Here, we consider these possibilities in regard to hyenas.

Spotted and striped hyenas belong to a clade morphologically specialized for feeding on carrion, with strong jaws and massive crania adapted for durophagy (Tanner et al. 2008; Werdelin and Solounias 1991). Whereas all carnivores are exposed to pathogens through their prey, hyenas are potentially exposed to higher pathogen concentrations, and to a greater diversity of pathogens, than sympatric carnivores, due to their regular consumption of carrion

(e.g. Boone et al. 2009; Getz 2011; Gortazar et al. 2010; Holekamp and Dloniak 2010; Jennelle et al. 2009; Reperant et al 2008; Wilson and Wolkovich 2011). This is the 1st of several reasons why analysis of MHC diversity among Hyaenids is particularly interesting.

Second, whereas non-Hyaenid carnivores in Africa are known to suffer high mortality rates from various infectious diseases (e.g. rabies— Kat et al. 1995; Maas 1993; canine distemper virus—Carpenter et al. 1998; Roelke-Parker et al. 1996; van de Bildt et al. 2002), spotted hyenas seldom exhibit symptoms of infection, and disease-induced mortality is surprisingly rare, despite evidence for infection rates comparable to those documented in sympatric carnivores (East et al. 2001; Haas et al. 1996; Murray et al. 1999; but see Mills 1990). Natural populations of spotted hyenas host a wide array of pathogens, ranging from viruses to macroparasites (East et al. 2001; Engh et al. 2003; Haas et al. 1996; Harrison et al. 2004), and striped hyenas are known to be affected by at least a subset of the same infectious agents (e.g. Samuel et al. 2001). Little is known about mortality sources in striped hyenas, but no evidence exists of massive disease mortality among them. Low disease mortality and carrion feeding suggest that immune function in bone-cracking hyenas might be unusually robust. If pathogens encountered during carrion feeding represent an important source of selection driving diversity in MHC loci in hyena species, then we would expect spotted and striped hyenas to exhibit similar patterns of diversity at MHC loci, as they are both recently descended from carrion feeding ancestors (Werdelin and Solounias 1991) and both species regularly eat carrion (Kruuk 1972, 1976).

Although both hyena species examined here consume substantial amounts of carrion, other aspects of their behavioral ecology differ dramatically. Spotted hyenas live in large

fission-fusion groups, called 'clans,' that contain up to 98 individuals; within clans there is intense direct competition for food at kills and clan members frequently cooperate to defend carcasses against sympatric carnivores (Boydston et al. 2001; Holekamp et al. 2012; Smith et al. 2008). In contrast, striped hyenas live alone or in small family groups, feed solitarily, and avoid close contact with sympatric carnivores (Kruuk 1976; Macdonald 1978; Wagner et al. 2008).

Chemical communication via scent marking is well documented in both hyena species, and earlier work indicates that these scent cues convey information about relatedness and individual identity (e.g. Burgener et al. 2009; Drea et al. 2002; Kruuk 1976; Mills 1990, Theis 2008). Given the significant role of chemical communication in both species, it is possible that MHC-mediated odor cues are used by hyenas in mate choice decisions. If MHC diversity in offspring influences the fitness of their mothers, then females should choose mates that offer optimal MHC diversity for their offspring. However, mating systems differ markedly between the 2 hyena species. The spotted hyena is unique among mammals in that many aspects of the female's morphology and behavior are heavily 'masculinized.' Female spotted hyenas are socially dominant to all adult immigrant males within a clan, and possess masculinized genitalia that preclude forced copulation (East et al. 1993; Frank et al. 1995; Holekamp et al. 2012; Kruuk 1972). These traits suggest that female mate choice is absolute in the spotted hyena. In contrast to spotted hyenas, striped hyenas show neither behavioral sex-role reversals, nor any unusual traits expressed in the adult genitalia (Holekamp and Kolowski 2009; Wagner et al. 2007a). The unique "role-reversed" traits of female spotted hyenas, together with larger group sizes and higher interaction rates with conspecifics, indicate that intersexual selection is likely to be stronger in spotted than striped hyenas. If intersexual selection affects MHC diversity, we

would therefore expect to see differences between these two species in degree of MHC polymorphism.

If pathogen pressure mediated by social behavior in hyenas represents a stronger selective force acting on MHC loci in hyenas than that imposed by shared ancestry, we would expect to see greater MHC diversity, and evidence of stronger selection, in spotted than striped hyenas. If genetic drift is the predominant force shaping levels of genetic diversity, we would also expect to see higher levels of genetic diversity in spotted than striped hyenas, because the gregarious spotted hyena has a much larger effective population size than does the solitary striped hyena (Mills and Hofer 1998).

Currently, no information exists regarding MHC sequence variation among hyaenids. Here we describe variation at 3 MHC loci in spotted and striped hyenas using genetic material collected from free-living animals in Kenya. We investigate the evolution of these genes by testing for signatures of historical positive selection. We also inquire whether observed patterns of MHC diversity in spotted and striped hyenas are more consistent with selection pressures imposed by pathogen exposure via a shared evolutionary history or by recently evolved differences between these two species with respect to their social systems and reproductive biology.

METHODS

Study subjects

All sampled spotted hyenas were wild animals living in and around the Maasai Mara Game Reserve in Nark District, Kenya, and all sampled striped hyenas were wild animals living

in Laikipia District, Kenya. All sampling procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (AUF 07/08-099-00) and met guidelines approved by the American Society of Mammalogists (Sikes et al. 2011). All samples were collected from both sites between 1996 and 2009. Methods used to capture animals and collect blood samples used to extract DNA are described in detail in Engh et al. (2002) and Wagner et al. (2007b). In order to obtain a representative sample of alleles for a population from each species, we characterized diversity in a sample of 20 individuals of each species at 3 loci and sequenced individual clones to identify alleles (see below).

To evaluate relatedness within the sample set for each species, all samples were genotyped at 10 (in spotted hyenas) and 8 (in striped hyenas) microsatellite loci as previously described (Van Horn et al. 2004; Wagner et al. 2007b; Watts et al. 2011), and pairwise relatedness values (*R*) were estimated using the program RELATEDNESS (Queller and Goodnight 1989).

Characterizing MHC variation

We focused on regions of functional class II MHC loci that have previously been reported to contain antigen binding regions (ABS); these are highly polymorphic sites known to be involved in antigen presentation (Hughes and Nei 1988, 1989; Klein 1986). We PCR-amplified loci of 198 bp, 195 bp, and 222 bp in length from *DRB* exon 2, *DQB*, and *DQA*, respectively. DNA was extracted from blood and tissue samples using Gentra Puregene kits (Gentra systems Cat# D-5000, Minneapolis, Minnesota) or Qiagen DNeasy Blood and Tissue kits (Qiagen Cat# 69506, Qiagen, Valencia, California). Each DNA extract was amplified via PCR using the primers Msp*DRB*F, Msp*DRB*R, Msp*DQB*F, Msp*DQB*R, Msp*DQA*F, and Msp*DQA*R, which

were designed from a multispecies alignment (Table 1). All putative alleles were subjected to BLASTP and TBLASTN similarity searches in vertebrates, which yielded reciprocal best matches that correctly differentiated alleles among the putative targeted loci of *DRB*, *DQB*, and *DQA*. These results support gene orthology among the 3 loci and we therefore refer to these regions as *DRB*, *DQB*, and *DQA*, respectively. All sequences were deposited in GenBank (accession numbers HQ230582-3; HQ230586-7; HQ230598; HQ230640; HQ230642-3; HQ230647; HQ230655; HQ230671-2; HQ230684; HQ230726; HQ230734; HQ230742; HQ230750; HQ230766; JN985739-JN985781).

Sequencing indicated multiple alleles at all loci and putative gene duplication at all 3 loci; accordingly we sequenced multiple clones from each individual at all loci. We recovered more than 2 alleles per individual at all MHC loci sampled and therefore assume the existence of paralogs for each locus. Importantly, assignment of alleles to each of these loci was unequivocal. We were able to recover singleton alleles by random re-sequencing of independent PCR and cloning reactions from a subset of individuals, indicating that rare alleles were not due to PCR error. However, to be conservative, all singleton alleles were removed from all intraspecific analyses presented here. Alleles that were shared by both species (i.e., no longer singletons) were included in interspecific analyses. The criteria used to define a sequence as a true allele were based on its occurrence in at least 2 independent PCR reactions derived from different individuals, or at least 3 independent PCR reactions derived from the same individual. This cloning approach and pervasive duplication prevented us from estimating allele frequencies, but did allow us to determine minimum levels of diversity and assess historical relationships among the sampled alleles.

For each individual sampled, 3 independent PCR products per locus were combined for subsequent cloning and sequencing analyses. PCR reactions were prepared in 20 µL volumes including 375 µM each dNTP, 10 pmol of each primer, and 1 U of Go Taq DNA polymerase (Promega Corporation, Madison, Wisconsin). Thermal cycling conditions were: 95°C for 2 min; 40 cycles of 95°C for 5 s, T_a for 5 s, 72°C for 5 s; and 72°C for 10 min (T_a given in Table 1). Mixed PCR products were cloned using the pGEM-T Easy kit (Promega Corporation, Madison, Wisconsin) following manufacturers' recommendations. Using an automated sequencer at the Research Technology Support Facility at Michigan State University, we sequenced an average of 9.6 (± 0.2) clones per locus in each individual animal (means of 9.9 ± 0.3 in spotted hyenas, 9.3 ± 0.2 in striped hyenas) to provide a minimum estimate of allelic diversity.

Population genetics, phylogenetic analyses, and codon models of selection

We calculated rates of non-synonymous (d_N) and synonymous (d_S) substitutions using distance-based methods in DnaSP v5 (Librado and Rozas 2009). However, since inferring selection from distance based measures of d_N/d_S can be problematic (e.g. Kryazhimsky and Plotkin 2008), here we utilize Bayesian methods implemented in codeML within the PAML 4 software package (Yang 2007). Using these methods, we obtained measures of the selection parameter ω, which measures the ratio between non-synonymous (d_N) and synonymous substitutions (d_S) per site. A ratio of $\omega > 1$ (or an excess of nonsynonymous substitutions relative to synonymous substitutions) indicates positive selection, whereas a ratio of $\omega < 1$ indicates purifying selection and a ratio of $\omega = 0$ results in a failure to reject neutrality at the

codons in question. Maximum-likelihood methods have been widely used to test for the presence of codons affected by positive selection and to identify those sites. We compared 5 codon-based models of sequence evolution (M0, M1a, M2a, M7, and M8). The M0 model assumes 1 ω (d_N/d_S) ratio for all sites; the nearly neutral model, M1a, assumes a proportion (p_0) of sites evolving at $\omega < 1$ and the rest (p_1) at $\omega_1 = 1$; the selection model, M2a, adds an additional class of sites to the M1a model that are evolving at $\omega_2 > 1$, where ω_2 is estimated from the data. M7 and M8 are extensions of M1a and M2a that include variation in ω according to a beta distributed pattern of substitution rates. The best fitting model was chosen on the basis of likelihood ratio tests and Akaike's Information Criterion (AIC—Posada and Buckley 2004; Sullivan and Joyce 2005). Both methods agreed on the choice of the best fitting model in all cases. Positively selected codons were identified through the Bayes Empirical Bayes procedure (Zhang et al. 2005).

As recombination within sequences can lead to false identification of positive selection (Anisomova et al. 2003), we screened for the presence of recombination using the program 'permute' to calculate 3 common measures of linkage disequilibrium (LD): r^2, D', and G4 (Wilson and McVean 2006). Given evidence indicating the presence of recombination, we then used omegaMap version 5.0 to perform a Bayesian inference of codon specific ω, which may alleviate potential biases in the calculation of ω with the presence of recombination (Wilson and McVean 2006). Two independent analyses were run for 2 sets of uninformative priors (improper inverse and inverse), which follows suggestions contained in the program documentation. We ran each prior with 2 sets of codon frequencies, 1 assuming equal

frequencies and 1 using codon frequencies estimated from genomic feline and canine codon frequencies, which were similar to each other and represented the sequenced genomes that are most closely related to hyenas. For each codon frequency, we ran 2 sets of starting values, and 2 replications of each of these starting values to validate convergence. Two Markov chain Monte Carlo (MCMC) runs were performed, each with 500,000 iterations. The first 10% of iterations were discarded as burn-in. These results were compared to results obtained from PAML. All omegaMap input parameters are given in Appendix A and Appendix B.

Phylogenies were constructed for all 3 loci using likelihood based methods in PhyML 3.0 (Guindon and Gascuel 2003), and the Bayesian approach implemented in MRBAYES 3.1 (Huelsenbeck and Ronquist 2001). Topologies estimated from all methods of analysis were highly concordant. The likelihood based model used for phylogenetic reconstruction was the "GTR + Γ + gamma distributed rates" model, and all parameter values were estimated from the data. Tree search algorithms utilized SPR plus NNI branch swapping and branch support was determined with 1000 bootstrap replicates. For Bayesian analyses, we again used the same substitution model as used for likelihood, as well as default prior values. Two independent runs of 4 Metropolis coupled Markov chain Monte Carlo simulations (3 of them "heated") were each run for 2×10^7 generations and sampled every 100 generations. Convergence was assessed by checking that the average standard deviation of split frequencies in MRBAYES was less than 0.01 early in each run, by verifying a lack of pattern in the residuals of the parameter estimates over generations, and because 2 independent runs were compared and found to have identical topologies. The first 4×10^5 trees were discarded as burn-in, resulting in 1.2×10^6 sampled

trees. To calculate the posterior probability of each bipartition, the majority-rule consensus tree was computed from the sampled trees.

RESULTS

Pairwise relatedness and allelic diversity

Analyses of microsatellite data revealed low average pairwise relatedness (*R*) values among both spotted and striped hyenas (*R* = 0.03 ±0.03 and *R* = 0.05 ±0.02, respectively). No differences were observed between species in *R* (*t* = -0.033, $p > 0.1$), mean number of alleles at microsatellite loci (spotted= 4.75 ±0.65; striped= 4.80 ±0.59; *t* = 0.057; $p > 0.1$), or average microsatellite heterozygosity (H_O; spotted = 0.59 ±0.06; striped = 0.51 ±0.08; *t* = 0.822; $p > 0.1$).

We observed high allelic and nucleotide diversity at all 3 MHC loci in both hyena species (Table 2). Alleles that had been discarded as singletons for intra-species analysis were re-inserted into the data set used for inter-species analysis when these alleles were shared between the 2 species (i.e were no longer singletons). Four alleles (2 spotted *DRB* alleles, 1 striped *DQB* allele, and 1 striped hyena *DQA* allele) identified in our initial survey showed signs of non-functionality, containing premature stop codons. Some of these were the result of frameshift mutations, and we concluded that these alleles represent putative pseudogenes. All 4 of these alleles were eliminated from subsequent analyses. Nucleotide diversity, as estimated from the parameter pi (π—Nei and Li 1979), ranged from 0.003 at the *DQA* locus in striped hyenas to 0.135 at the *DQB* locus in striped hyenas (Table 2). The values of nucleotide diversity reported in Table 2 did not differ significantly between spotted and striped hyenas at any of the 3 loci examined (χ^2: for all loci, $p > 0.05$). For intraspecific comparisons among

alleles, we observed average, distance-based, ratios of non-synonymous (d_N) to synonymous (d_S) substitution rates among alleles at each locus that exceeded 1.0. These values did not differ significantly between the 2 species at any locus (Wilcoxon rank sum test: for all loci, $p > 0.05$). Thus, all our estimates of d_N/d_S were consistent with the action of positive selection (Table 2).

Gene duplication

Our method of sequencing multiple clones from independent PCR reactions led to the discovery of more than 2 alleles per locus in 70% of individuals examined (97.5% at *DRB*; 82.5% at *DQB*, and 30% at *DQA*; after removing singleton alleles). Multiple gene copies for MHC loci have been verified in several mammals for which relatively thorough genomic assemblies and gene annotations are available (e.g. Axtner and Sommer et al. 2007; Barbisan et al. 2009; Bryja et al. 2006; Yang and Yu 2000), although population-based studies have tended to focus on loci that segregate in a bi-allelic fashion. In the most extreme cases observed here, 7 and 8 *DRB* alleles were identified in individual spotted and striped hyenas, respectively. If we conservatively assume heterozygosity at all loci, this indicates a minimum of 4 paralogs at the *DRB* locus in both hyena species.

Trans-species allelism

Given the high numbers of alleles found per individual, and evidence of paralogs at each of the 3 loci, we next inquired whether the alleles were recently evolved (monophyletic clades of alleles for each species), or whether alleles have been segregating for much longer periods of time (polyphyletic clades of alleles among species). Bayesian and likelihood based phylogenies

of alleles from both species were polyphyletic for each of the 3 loci (Figure 1), demonstrating trans-species polymorphism. Maximum likelihood and Bayesian approaches yielded concordant results, but for simplicity, only results from Bayesian phylogenetic analyses are shown. We identified shared, or trans-species polymorphisms, which indicate that some of these alleles have been maintained since before striped and spotted hyenas last shared a common ancestor. As we found no clear evolutionary delineation between these 2 species at any locus, data for both species were combined for subsequent analysis. Average branch lengths (*DRB*: spotted: 0.09 ± 0.02, striped: 0.08 ± 0.02; *DQB*: spotted: 0.03 ± 0.01, striped: 0.05 ± 0.01; DQA: spotted: 0.1 ± 0.001, striped: 0.1 ± 0.001) of tree topology did not change significantly when the species were analyzed separately (Wilcoxon rank-sum test to compare average branch lengths: for all loci, $p > 0.1$), indicating that the shape of tree topologies were not significantly different between species.

Positive selection and rapid evolution

In order to test more formally whether rates of evolution were consistent with positive selection, we performed sites-based tests of selection using codon models of evolution as implemented in PAML 4 (Yang 2007). Likelihood ratio tests and AIC values revealed that, for the *DRB* and *DQB* loci, codon evolution models that incorporate positive selection fit the data significantly better than models that did not (Table 3). M2a and M8 had similar likelihood scores, but since M8 has more parameters than M2a, M2a (the selection model) was the most parsimonious model explaining our data. High proportions of sites were identified as evolving rapidly at 2 loci: 14.6% of sites within *DRB* evolving at ω=5.36, and 13.9% of sites within *DQB* evolving at ω=4.89 (Table 3). The majority of the remaining sites at each locus (76.2% at *DRB*

and 68.7% at *DQB*) were identified as evolving under purifying selection (ω < 1). For the DQA locus, one rate of evolution was sufficient to explain the observed pattern within hyenas, as adding more than one rate did not improve the fit of the model. Using the Bayes Empirical Bayes approach implemented in PAML, 8 codons within *DRB* and 3 codons within *DQB* were identified with high posterior probability (probability ≥ 0.95) as evolving under positive selection (Figure 2). We determined putative antigen binding sites (ABS) for *DRB* and *DQB* based on homology with previously identified functional sites (Brown et al. 1993; Mikko et al. 1999; Yuhki et al. 2008). Of the 8 sites predicted to be evolving under positive selection within *DRB*, 5 occurred within putative ABS (Figure 2; Yuhki et al. 2008). Within *DQB*, 6 codons were identified as being subject to positive selection, and 4 of these occurred at ABS (Figure 2; Brown et al. 1993; Mikko et al. 1999). When alleles from each species were analyzed separately, a high proportion of sites were still predicted to be under positive selection at *DRB* and *DQB*, and the location of sites predicted to be under selection did not differ between species (data not shown).

We found evidence for intragenic recombination at 2 loci in both hyena species (*DRB*: r^2, D', G4, all $p < 0.001$; *DQB*: r^2, D', G4, all $p < 0.01$). The omegaMap results were largely consistent with the PAML results (Figure 2). Here we present results from the improper inverse prior, using codon frequencies estimated from felids; we chose to report these results because the improper inverse does not force initial parameter values to be defined, as does the inverse prior, and based on limited sequence data, hyenas exhibit codon bias similar to that in other carnivores. Summaries of all analyses are available online (Supporting Information Appendix A

(S1 online) and Appendix B (S2 online)). Eight sites at *DRB* and 9 sites at *DQB* were predicted to be under selection with a 95% or higher posterior probability by omegaMap, compared with 8 and 6 sites for *DRB* and *DQB*, respectively, called by PAML. Of the sites predicted to be under positive selection by omegaMap, 6 out of 8 (75%) *DRB* sites, and 5 out of 9 (55.6%) *DQB* sites, corresponded to putative ABS (Figure 2). There was 62.5% and 55.6% agreement of the identity of these positively selected sites between PAML and omegaMap at *DRB* and at *DQB*, respectively. Of the sites predicted to be under positive selection by both omegaMap and PAML, 60% of sites (3 out of 5) sites at both loci overlap with putative ABS (Figure 2).

DISCUSSION

Our study is among the 1st analyses of MHC diversity in wild populations of carnivores (Hedrick et al. 2003; Siddle et al. 2007; Soll et al. 2005; Yoshiki et al. 2010). We have reported several lines of evidence consistent with positive selection acting on MHC loci in both species of hyenas: high allelic variation, pervasive gene duplication, trans-species polymorphisms, and d_N/d_S values greater than 1.0 at codons that correspond to known functional sites. In contrast to previous comparative studies in other taxa, despite the marked differences in mating system and sociality between the 2 species, here we found no strong differences in the extent or evolutionary patterns of MHC diversity between spotted and striped hyenas. Our data suggest that a shared evolutionary history, rather than behavioral and ecological differences between species, may have been the primary selection pressure leading to the observed patterns of MHC variation in hyenas.

Patterns of allelic variation and evidence for selection

The high levels of diversity we found in both hyena species are consistent with previous studies of MHC loci in mammals (e.g. Kennedy et al. 2005; O'Brien and Yuhki 1999; Schwensow et al. 2010; van Haeringen et al. 1999; Worley et al. 2006). High MHC diversity is often interpreted as resulting from selection acting to increase variation (e.g. Apanius et al. 1997; Blais et al. 2007; Hughes and Yeager 1998; Ohta 1991). Most MHC-related studies in non-model species face the problem of lacking detailed genomic information, as we do here, and this has precluded accurate assignments of the observed sequences to specific paralogous loci. Whereas we could not discriminate between orthology and paralogy within each locus, we could unequivocally ascribe each allele to the appropriate gene (*DRB* versus *DQB* versus *DQA*). Despite multiple paralogs for each of the 3 genes, we were able to infer that positive selection has been acting, on average, at functionally important codons over the history of these genes. Large proportions of sites corresponding to known ABS were predicted to be under positive selection (Figure 2; Table 3; 14.6% at *DRB*; 13.9% at *DQB*), with a substantial remainder of sites under negative, or purifying, selection ($\omega < 1$; 76.2% at *DRB*; 68.7% at *DQB*). Our approach has allowed us to estimate a minimum number of alleles for each of the 3 genes, but we most likely failed to recover all rare alleles. Thus, we suspect that the number of alleles reported here represents a conservative estimate.

There have been few MHC studies conducted in free ranging carnivore populations, and none previously in hyaenids, which makes inferences from comparative biology difficult. However, other carnivores are known to have lower numbers of paralogs per MHC locus than observed here in either hyena species, suggesting that the high gene copy number found here

may have originated in the hyena lineage (e.g. Drake et al. 2004; Hedrick et al. 2000, 2002; O'Brien and Yuhki 1999; Wang et al. 2009). Further, both hyena species appear to show high levels of MHC variation when compared with other mammals (e.g. domestic cat—O'Brien and Yuhki 1999; coyote and wolves—Hedrick et al. 2000; Hedrick 2002; pinnipeds—Lehman et al. 2004; domestic dog—Kennedy et al. 2005; 3 vole species—Bryja et al. 2006; baboons—Huchard et al. 2010; Table 2).

Balancing selection can act to maintain allelic lineages over long evolutionary time scales (Klein et al. 1998; Takahata and Nei 1990). Genes that demonstrate long-term balanced polymorphisms, in the case of the MHC, often have coalescence times that predate speciation events (Figueroa et al. 1988; Takahata 1990; Takahata and Nei 1990). Our phylogenetic analyses revealed multiple monophyletic clades of alleles among spotted and striped hyenas, as well as cases of trans-species alleles (shared alleles between species). Since no contemporary gene flow exists between these species, we have ruled out hybridization and introgression as the source of shared alleles, suggesting that these alleles were segregating in an ancestral species. The allelic lineages examined here have likely been maintained over extremely long time periods, as these 2 hyena species are estimated to have diverged roughly 8.6 million years ago (Koepfli et al. 2006).

Whereas some of the alleles shared between striped and spotted hyenas are clearly old (related to each other by deeper branches), other monophyletic clades of intra-specific alleles exhibited shorter branch lengths. These more recent bursts of allelic diversification within some clades of alleles may represent functional diversification, given that many of the substitutions were non-synonymous (Table 2). Further, if all allelic diversification was

exclusively ancient, we would expect to see greater average sequence divergence among alleles than is observed here. Due to the high similarity among some allelic sequences, we hypothesize that allelic diversification is an ongoing process at these loci in hyenas.

Explicit tests for positive selection were highly significant for 2 out of 3 loci, even when we incorporated conservative methods that account for the presence of recombination. With respect to these loci (*DRB* and *DQB*), the proportion of sites evolving under positive selection is remarkably high, averaging 14% (Table 3). Further, the rate at which these sites are evolving was estimated to be at least 4 times higher than the neutral rate of evolution (*DRB*: $\omega = 5.36$; *DQB*: $\omega = 4.89$; Alba and Castresana 2005; Gibbs et al. 2007; Toll-Riera et al. 2008; Yang and Nielsen 1998). Interestingly, a disproportionately large number of the codons predicted to be evolving under positive selection correspond to known antigen binding sites (Figure 2; 64% total for both loci together in PAML; 65% for both loci together in omegaMap). The fact that rapidly evolving sites correspond to functional sites, even in the presence of recombination, provides further compelling evidence for functional diversification by natural selection at codons that are important for the recognition of pathogens. We were unable to reject neutrality in the *DQA* locus, so further work will be required to draw firm conclusions about mechanisms of evolution at this locus.

Limitations of inferring selection in the presence of gene duplication

Clearly, no single conceptual model of MHC evolution can explain all of the patterns found in our data. Selective forces change over evolutionary time, and may not always be mutually exclusive. The conclusions we can draw here are limited by virtue of the fact that we observed considerable gene duplication. Although our data indicated that, on average, positive

selection acted on a subset of functionally important codons and negative selection acted on a majority of the remaining of codons at MHC loci in hyenas, we cannot rule out the possibility that both forces have also acted in a more complex manner. For example, it is possible that the signature of positive selection may be due to a subset of rapidly evolving alleles for one of the duplicate copies of *DRB*, whereas the remainder of *DRB* alleles experienced negative selection.

If a subset of alleles were responsible for the signature of positive selection, we should have observed non-synonymous substitutions (d_N) that localized to that subset of closely related alleles on the gene tree, but we did not see this. Instead, for each codon predicted to have evolved under positive selection, we found that non-synonymous mutations (d_N) mapped to multiple branches on all plausible trees, and were not exclusive to tip or interior branches. Therefore, although there was no strong qualitative pattern consistent with positive selection acting on only a subset of alleles or gene duplicates, it is important to acknowledge that we cannot rule out more complex patterns of evolution. To address whether particular gene duplicates have unique patterns of evolution, future work will be required to annotate alleles to specific loci. To further validate the selective value of MHC alleles in hyenas, future work in the realm of immunology will also be required to determine whether functional diversification has taken place among these alleles, and to identify the specific immunological challenges influencing selection on these loci in natural populations.

Inter-specific comparisons

Given the number of behavioral differences known to exist between spotted and striped hyenas, we found it surprising that the patterns of molecular evolution we observed were

comparable between species. Spotted and striped hyenas did not differ in the identities of codons predicted to be under selection, nor the proportions of sites identified as neutral or under purifying selection pressure. Although we do not have the statistical power to detect small differences in diversity, our data provide strong evidence of shared selection pressure and a shared evolutionary history of alleles between species. In particular, the occurrence of shared alleles between species, and sites under positive selection over the history of those alleles that corresponded to known antigen binding sites, suggest that forces other than sociality were likely important in shaping the evolution of MHC genes in hyenas.

There are many costs associated with sociality, including increased disease and parasite transmission resulting from more contact with conspecifics (e.g. Altizer et al. 2003; Arnold 1990; Bordes et al. 2007; Brown and Brown 1986; Côté and Poulin 1995; Hoogland 1979; Majolo et al. 2008; Molvar and Bowyer 1994). Due to this greater pathogen pressure, and to larger effective population size in spotted than striped hyenas (Mills and Hofer 1998), we expected to find much higher MHC diversity in spotted than striped hyenas. Previous work has demonstrated greater MHC diversity in social species than in closely related but solitary-living species (e.g. Hambuch and Lacey 2002; Kundu and Faulkes 2003). However, our data suggest that sociality is not the strongest selection pressure driving diversity in MHC genes in spotted and striped hyenas.

Regardless of other behavioral differences, direct immune challenges stemming from the shared ancestral habit of carrion feeding may be similar between spotted and striped hyenas; we postulate that these may be important determinants of MHC variation. Although more work and larger sample sizes are needed to confirm this, our data are consistent with the

idea that MHC diversity is driven more strongly by shared ancestry than by contrasting patterns of sociality or mate preference. The hypothesis that the ancestral habit of carrion feeding selects for high MHC diversity predicts that other carrion feeding species (e.g. vultures) may have independently experienced strong selection for pathogen resistance, resulting in relatively high MHC diversity. Furthermore, it predicts that MHC diversity in aardwolves (*Proteles cristata*), which are also members of the family Hyaenidae but never feed on carrion, should be lower than in the carrion-feeding hyenas.

ACKNOWLEDGMENTS

We thank the Kenyan Ministry of Education, Science and Technology, the Kenya Wildlife Service, Narok County Council, the Senior Warden of the Masai Mara National Reserve, Loisaba, Mpala and Kisima Ranches, and the Laikipia Predator Project. Our research, described in Animal Research Protocol AUF 07/08-099-00, was approved most recently on 4 June 2010 by the Institutional Animal Care and Use Committee at MSU and complies with Kenyan law. This work was supported by NSF IOB 0618022, IOS 0819437, and IOS 1121474 to K.E.H., and graduate research fellowships from NSF, MSU, and Sigma Xi to K.J.C.

DATA ARCHIVING

All sequences generated by this research have been submitted to GenBank, the NIH genetic sequence database (accession numbers HQ230582-3; HQ230586-7; HQ230598; HQ230640; HQ230642-3; HQ230647; HQ230655; HQ230671-2; HQ230684; HQ2307226; HQ230734; HQ230742; HQ230750; HQ230766; JN985739-JN985781).

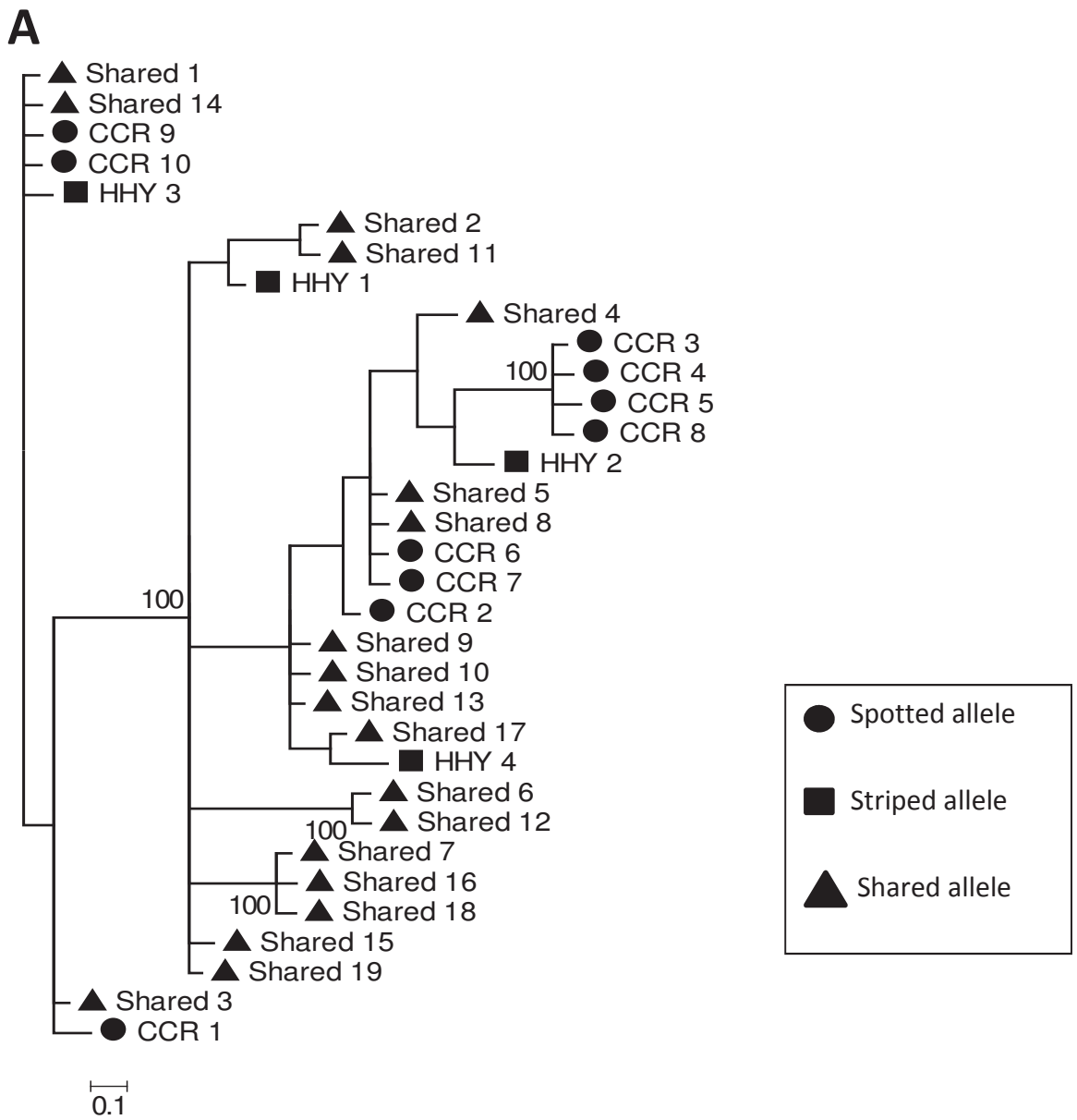


Figure 1. Unrooted phylograms based on nucleotide sequences of spotted and striped hyena MHC loci A) *DRB*, B) *DQB* and C) *DQA*. Bayesian posterior probabilities above 90% are shown along their respective branches. Circles represent spotted hyena (CCR) alleles; squares represent striped hyena (HHY) alleles, and triangles represent alleles present in both hyena species (Shared). The scale bar accompanying each phylogram indicates the rate of substitutions per site.

Figure 1 (cont'd)

B

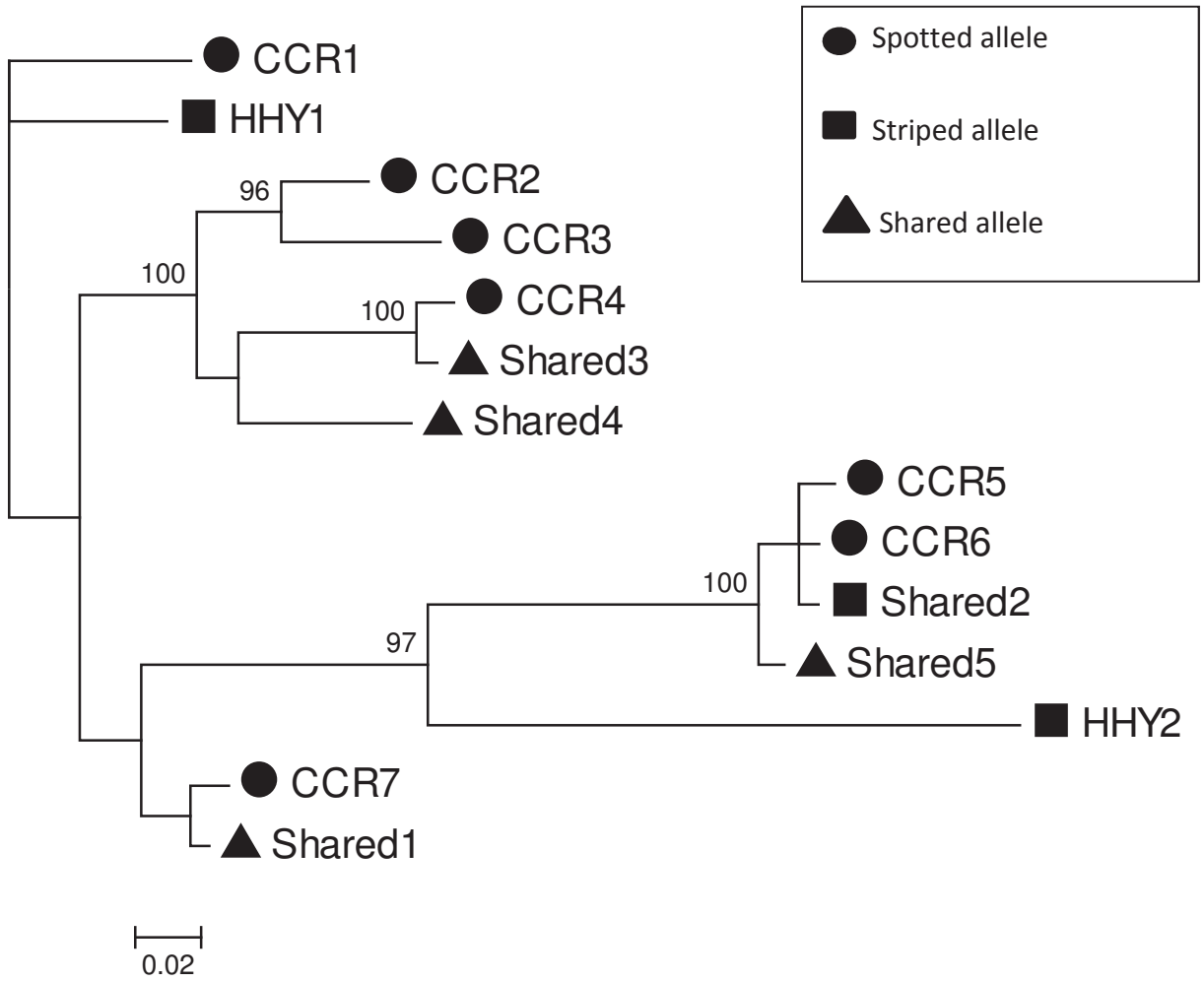
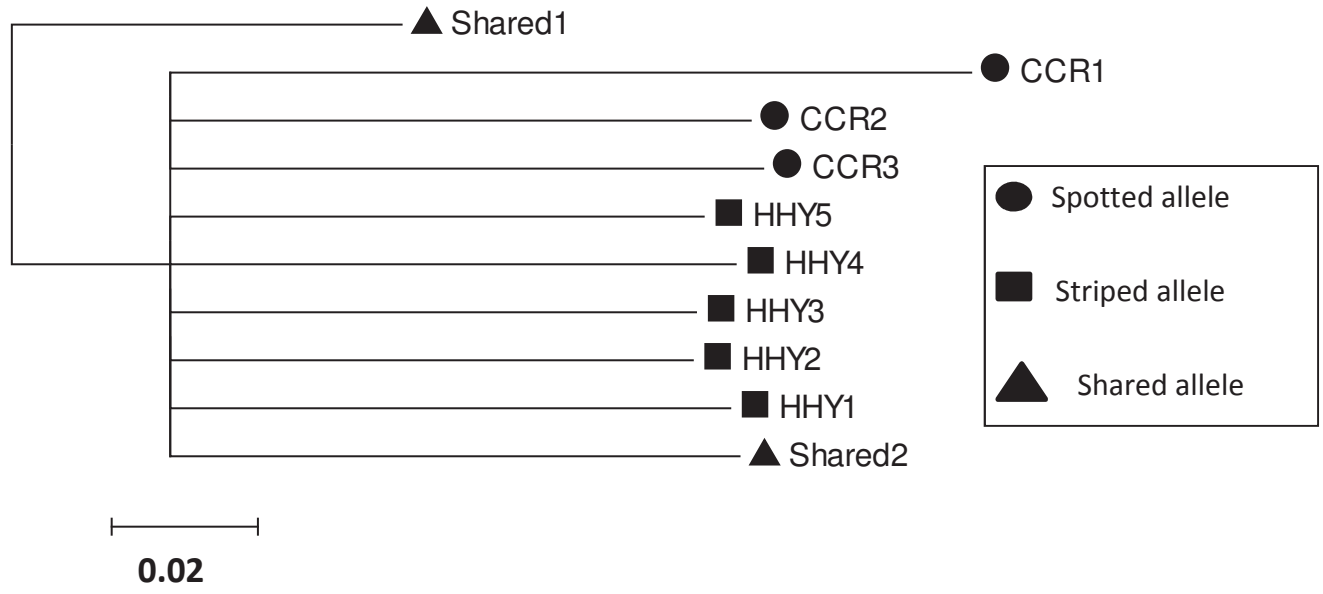


Figure 1 (cont'd)

C



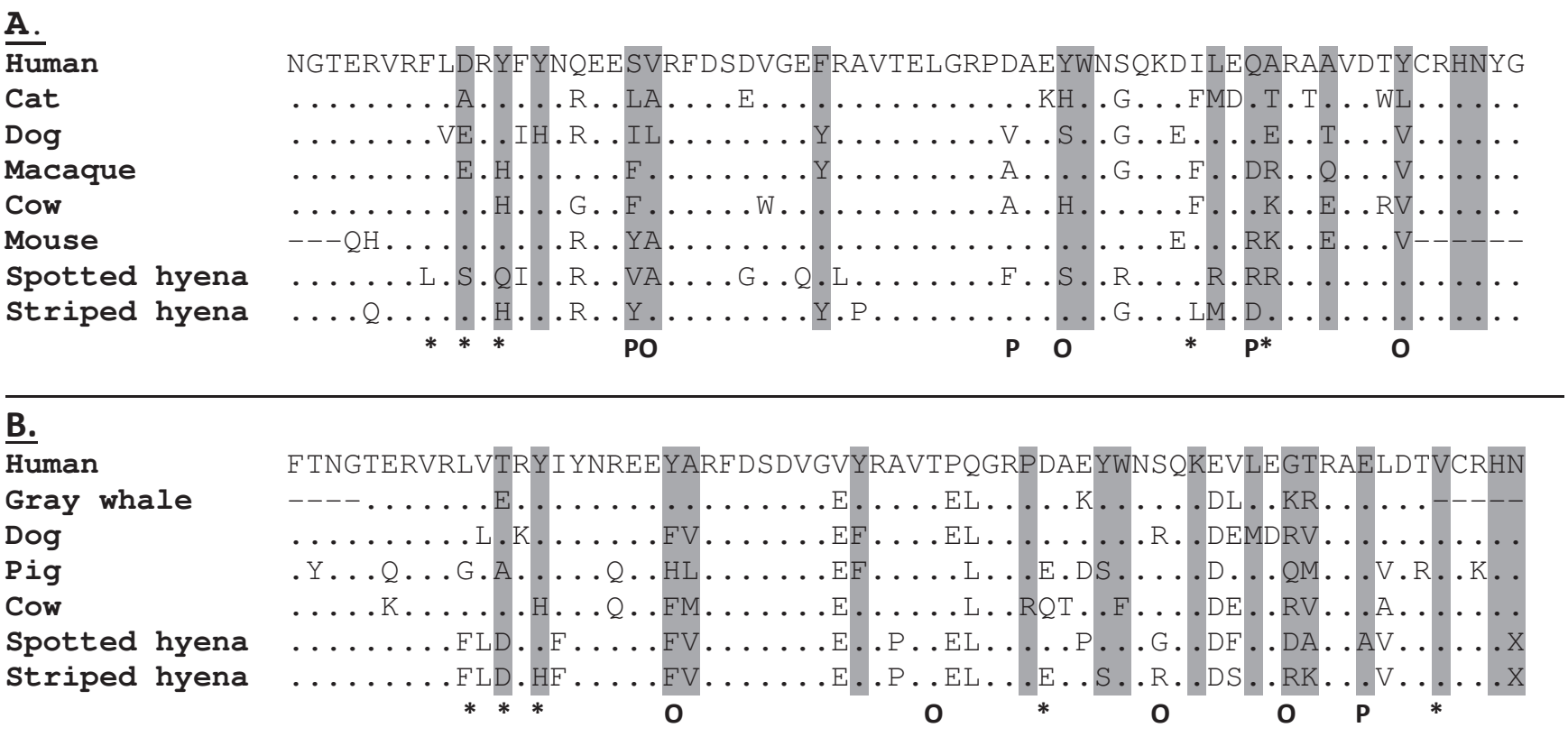


Figure 2. Multiple sequence alignment of A) DRB and B) DQB. Sites predicted to be under positive selection (probability ≥ 0.95) in both hyena species identified by the Bayes empirical Bayes (BEB) procedure of the PAML package are indicated by asterisks below the sequence alignment. Putative antigen recognition sites (from Yuhki et al. 2008 (DRB) and after Brown et al. 1993 as shown by Mikko et al. 1999 (DQB)) are shaded in gray.

Table 1. Primer sets, sequences and annealing temperatures used in this study

Primer name	Primer sequence (5' to 3')	T_a (C°)
Msp*DRB*F	AAC GGG ACR GAG CRG GTG CG	47
Msp*DRB*R	ACA CCG TAG TTG TGT CTG CA	47
Msp*DQB*F	YTT CAC CAA CGG GAC GGA GCG	56
Msp*DQB*R	TTG TGT CTG CAC ACC GTG TCC A	56
Msp*DQA*F	TGG CCA GTA CAC CCA TGA ATT TGA TG	49
Msp*DQA*R	AAG AGA GGC AGA ATG GTR RAC	49

Table 2. Allelic diversity in spotted and striped hyenas at three MHC loci

	DRB	*DQB*	*DQA*
Total # allele			
Spotted*	26 (0.054)	9 (0.098)	4 (0.006)
Striped	18 (0.053)	7 (0.135)	5 (0.003)
Both Species[§]	33 (0.055)	14 (0.104)	10 (0.008)
Average d_N/d_S[†]			
Spotted	1.4	1.5	1.5
Striped	1.7	1.5	1.5
Average # alleles/individual			
Spotted	4.7	2.4	2.9
Striped	6.1	2.1	5.1

*n= 20 individuals sampled for both species at all loci; nucleotide diversity (π) given in parentheses after allele numbers; [†] Distance based estimates of d_N/d_S among all alleles within each species; [§] Singleton alleles added back to analysis when they occurred in both species (i.e. no longer singletons).

Table 3. Evaluation of codon based evolution models indicates positive selection acting at three MHC loci in hyenas

Model[†]		ln *L*[§]	ΔAIC	Parameters[ϕ]
		DRB all unique alleles; both species		
M0 – one ω		-1376.3	513.86	ω=0.783
M1a – nearly neutral	(ω_0<1, ω_1=1)	-1157.5	78.39	p_0=0.769, ω_0=0.004
M2a – positive selection	(ω_0<1, ω_1=1, ω_2>1)	-1116.3	best*	p_0=0.762, p_2=0.146, ω_0=0.007, ω_2=5.36
M7 – beta	(p, q)	-1158.1	79.44	p=0.008, q=0.028
M8 – beta & ω	(p, q, ω_s>1)	-1116.6	0.56	p_0=0.853, (p_1=0.147), p=0.009, q=0.064, ω=5.37
		DQB all unique alleles; both species		
M0 – one ω		-1148.4	292.8	0.410
M1a – nearly neutral	(ω_0<1, ω_1=1)	-1021.8	41.6	p_0=0.701, ω_0=0.02
M2a – positive selection	(ω_0<1, ω_1=1, ω_2>1)	-999.03	best*	p_0=0.687, p_2=0.139, ω_0=0.03, ω_2=4.89
M7 – beta	(p, q)	-1022.1	42.2	p=0.082, q=0.209
M8 – beta & ω	(p, q, ω_s>1)	-1000.3	2.7	p_0=0.855, (p_1=0.145), p=0.09, q=0.343, ω=4.46
		DQA all unique alleles; both species		
M0 – one ω		-356.3	35.8	4.575
M1a – nearly neutral	(ω_0<1, ω_1=1)	-357.2	39.6	p_0=0.499, ω_0=0
M2a – positive selection	(ω_0<1, ω_1=1, ω_2>1)	na	na	na

Estimated proportions of sites (p_x) evolving at corresponding estimated rates ($\omega_x = d_N/d_S$) are given in the parameters column.

[†] Alternative sites based models of codon evolution from PAML 4 (Yang 2007)

[§] Log likelihood score

[ϕ] Proportion of sites (p) evolving at corresponding rate (ω)

* Model of best fit (p<0.001)

APPENDIX

Table A1. Key for terms for summaries for all omegaMap analyses for spotted (Crocuta crocuta) and striped (Hyaena hyaena) hyena MHC DRB sequences.

Prior distributions used	
impinv =	improper inverse
inv =	inverse
Codon frequency	
cat =	Codon frequencies from domestic cat (*Felis catus*)
equal =	Equal codon frequencies
Start1 or Start2 = 2 different sets of starting values of each following parameter:	
Mu	
Kappa	
Indel	
omega	
rho	
Rep1 or Rep2 = repetition of each set of starting values	

Table A1 (cont'd)

Impinv_cat_start1_rep1

	Synonymous theta			kappa			phi	
Lower 95% HPD	Point estimate	Higher 95% HPD	Lower 95% HPD	Point estimate	Higher 95% HPD	Lower 95% HPD	Point estimate	Higher 95% HPD
6.70E-06	3.52E-05	0.000177788	0.423284	1.01991	2.78422	0	0	0

		omega		Posterior		rho	
Site	Lower 95% HPD	Point estimate	Higher 95% HPD	prob of +ve selection	Lower 95% HPD	Point estimate	Higher 95% HPD
0	0.0710459	27.6119	24355.4	0.779	0.000277677	0.733498	238.559
1	0.127458	66.8978	9283.87	0.865	0.000128708	0.353299	1605.56
2	9.80E-07	0.000125028	0.0380314	0	260014	1.01E+10	1.88E+13
3	9.58E-08	1.54E-05	0.0332876	0	2.78E-15	2.29E-11	0.000112747
4	0.000683495	70.2087	6844.8	0.828	5.26672	2514.05	4.38E+06
5	4.58674	117.064	3422.43	1	0.549857	333.339	684019
6	0.000131966	0.00937958	0.310164	0.021	0.00204753	0.545131	6041.19
7	410.642	4103.82	39314	1	7.61E-07	0.00108108	2.53774
8	0.261121	28.2706	2032.19	0.863	8.08E-05	0.0081824	2.30775
9	295.562	4520.1	29302.9	1	0.0182168	1.89057	36049.9
10	3.87E-08	0.000952703	13.2077	0.098	3.18E-06	0.00515017	1.02822
11	47.2279	1259.64	22806.5	1	7.38E-06	0.0171533	76.9172
12	2.61E-06	0.000815911	0.197563	0	0.000430922	0.0628517	2.24585
13	2.3475	181.248	5443.44	0.996	0.000503871	0.13939	12.1242

Table A1 (cont'd)

14	0.000109168	0.269645	2095.03	0.391	1.01E-05	0.00221788	0.343893
15	0.000660273	3.40875	18628	0.53	0.0408948	2.42944	334.374
16	3.54E-08	1.43E-05	0.00427485	0	5.35E-13	9.78E-06	193.775
17	1.30E-08	1.30E-05	0.00859141	0	0.000175193	0.202174	1148.3
18	166.969	3450.34	39675.1	1	5.71E-06	0.000536713	0.933553
19	326.483	3275.21	42839.9	1	4.58751	4.85E+07	5.03E+14
20	4.60E-06	0.000511869	0.197228	0.001	0.00375028	4.01472	5472.18
21	0.119344	36.5769	1802.58	0.858	0.129053	32.9062	154063
22	2.95E-05	0.00122361	0.0886605	0	3599.84	282452	8.35E+07
23	3.22E-05	0.00733559	11.9839	0.104	1.95E-07	0.00658481	6.45163
24	0.790976	68.1149	2650.21	0.95	1.96E-07	0.0434153	842.184
25	5.32E-07	0.00186635	0.678322	0	6.94E-07	0.000173927	0.0145714
26	0.00502966	5.41779	1082.77	0.613	5.02E-06	0.000210326	0.0212714
27	0.000782157	4.32027	2289.09	0.622	7.09E-10	8.93E-06	0.280063
28	1.01E-08	2.54E-06	0.000575055	0	6333.89	262998	7.33E+06
29	0.429789	106.235	30831	0.896	35.584	1858.22	779792
30	0.000945232	0.395579	566.097	0.389	8.76E-09	1.22E-06	0.000115136
31	0.000442374	0.0531043	27.0505	0.213	152.842	3.85E+06	7.93E+10
32	0.119142	14.6938	31662.6	0.742	105.891	20374.9	1.16E+07
33	1.21E-12	6.43E-08	0.00984537	0	3.45E-06	0.00820346	77.8555
34	0.00343114	7.76751	1056.84	0.725	3.12E-06	0.00219469	0.660434
35	2.95E-10	2.06E-06	0.0161592	0	0.00773568	2.97429	4657.31
36	3.05E-07	0.00396539	25.9718	0.166	2.54E-08	1.34E-05	0.00268616
37	0.00627943	0.209802	43.1185	0.26	0.000439057	0.0271554	2.66189
38	0.0352878	167.85	13654.6	0.813	0.00216644	0.201022	3.56188
39	0.328007	487.633	146600	0.892	0.00150522	0.17362	16335.9
40	0.000208864	0.0174561	0.196747	0	2.38E-08	1.22E-05	0.0690295

Table A1 (cont'd)

41	35.4568	714.514	14170.2	1	0.000389326	1.16641	135986
42	0.671643	76.7138	10680.2	0.939	0.42483	1150.95	1.84E+06
43	7.50511	342.825	6299.36	1	0.000372126	1.01685	1323.83
44	0.0784071	156.4	10067.9	0.845	1.54E-06	9.80E-05	0.00447044
45	0.00277889	6.5682	1025.85	0.692	1.42E-05	0.128736	191542
46	0.0220798	6.69489	3442.69	0.731	2.95207	1688.38	709804
47	0.000123348	0.0612822	22.1692	0.174	1.95E-11	8.00E-07	0.00042429
48	98.3503	2808.01	63795	1	5.26E-06	0.000904153	0.106569
49	128.967	1225.79	23006.6	1	54.1186	43708.1	2.73E+07
50	0.1483	28.9581	7858.02	0.79	12616.6	7.30E+06	2.89E+10
51	757.395	6249.13	42008.5	1	0.00182201	0.151015	230.069
52	1762.57	8335.41	82367.9	1	8.34E-05	0.00797837	21.938
53	8.81E-05	0.0166762	19.7622	0.164	2.08E-05	0.0422854	8.90707
54	0.0133062	0.341701	8.95226	0.32	0.00802477	18758.6	2.02E+09
55	841.912	7543.08	58656	1	0.000113256	0.176623	12.2208
56	0.0349824	13.1669	493.02	0.795	18328.8	4.11E+07	2.65E+09
57	2.18E-06	0.000101206	0.0276392	0	0.0421618	1.61806	101.139
58	152.26	3291.4	32842.2	1	3.17E-07	0.000227042	0.17727
59	552.206	4457.24	23786.4	1	12.5412	464438	1.20E+09
60	0.000583143	22.2761	9808.7	0.791	0.000100441	0.011381	1.97005
61	7.35E-06	0.00228247	0.689358	0.014	0.74338	56.5965	97997.2
62	0.00149481	0.568781	422.58	0.383	857.225	175299	4.24E+07
63	1.48E-08	2.90E-06	0.000161893	0	2.64E-05	0.615128	64983.9
64	5.80E-05	0.0156296	5.80392	0.127	0.00291814	34.8128	4.91E+08
65	0.417163	22.1738	6978.27	0.889			

Table A1 (cont'd)

Impinv_cat_start1_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
6.70E-06	3.52E-05	0.000177788	0.423284	1.01991	2.78422	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.0710459	27.6119	24355.4	0.779	0.000277677	0.733498	238.559
1	0.127458	66.8978	9283.87	0.865	0.000128708	0.353299	1605.56
2	9.80E-07	0.000125028	0.0380314	0	260014	1.01E+10	1.88E+13
3	9.58E-08	1.54E-05	0.0332876	0	2.78E-15	2.29E-11	0.000112747
4	0.000683495	70.2087	6844.8	0.828	5.26672	2514.05	4.38E+06
5	4.58674	117.064	3422.43	1	0.549857	333.339	684019
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12	2.61E-06	0.000815911	0.197563	0	0.000430922	0.0628517	2.24585

Table A1 (cont'd)

13	2.3475	181.248	5443.44	0.996	0.000503871	0.13939	12.1242
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15	0.000660273	3.40875	18628	0.53	0.0408948	2.42944	334.374
16	3.54E-08	1.43E-05	0.00427485	0	5.35E-13	9.78E-06	193.775
17	1.30E-08	1.30E-05	0.00859141	0	0.000175193	0.202174	1148.3
18	166.969	3450.34	39675.1	1	5.71E-06	0.000536713	0.933553
19	326.483	3275.21	42839.9	1	4.58751	4.85E+07	5.03E+14
20	4.60E-06	0.000511869	0.197228	0.001	0.00375028	4.01472	5472.18
21	0.119344	36.5769	1802.58	0.858	0.129053	32.9062	154063
22	2.95E-05	0.00122361	0.0886605	0	3599.84	282452	8.35E+07
23	3.22E-05	0.00733559	11.9839	0.104	1.95E-07	0.00658481	6.45163
24	0.790976	68.1149	2650.21	0.95	1.96E-07	0.0434153	842.184
25	5.32E-07	0.00186635	0.678322	0	6.94E-07	0.000173927	0.0145714
26	0.00502966	5.41779	1082.77	0.613	5.02E-06	0.000210326	0.0212714
27	0.000782157	4.32027	2289.09	0.622	7.09E-10	8.93E-06	0.280063
28	1.01E-08	2.54E-06	0.000575055	0	6333.89	262998	7.33E+06
29	0.429789	106.235	30831	0.896	35.584	1858.22	779792
30	0.000945232	0.395579	566.097	0.389	8.76E-09	1.22E-06	0.000115136
31	0.000442374	0.0531043	27.0505	0.213	152.842	3.85E+06	7.93E+10
32	0.119142	14.6938	31662.6	0.742	105.891	20374.9	1.16E+07
33	1.21E-12	6.43E-08	0.00984537	0	3.45E-06	0.00820346	77.8555
34	0.00343114	7.76751	1056.84	0.725	3.12E-06	0.00219469	0.660434
35	2.95E-10	2.06E-06	0.0161592	0	0.00773568	2.97429	4657.31
36	3.05E-07	0.00396539	25.9718	0.166	2.54E-08	1.34E-05	0.00268616
37	0.00627943	0.209802	43.1185	0.26	0.000439057	0.0271554	2.66189
38	0.0352878	167.85	13654.6	0.813	0.00216644	0.201022	3.56188
39	0.328007	487.633	146600	0.892	0.00150522	0.17362	16335.9
40	0.000208864	0.0174561	0.196747	0	2.38E-08	1.22E-05	0.0690295

Table A1 (cont'd)

41	35.4568	714.514	14170.2	1	0.000389326	1.16641	135986
42	0.671643	76.7138	10680.2	0.939	0.42483	1150.95	1.84E+06
43	7.50511	342.825	6299.36	1	0.000372126	1.01685	1323.83
44	0.0784071	156.4	10067.9	0.845	1.54E-06	9.80E-05	0.00447044
45	0.00277889	6.5682	1025.85	0.692	1.42E-05	0.128736	191542
46	0.0220798	6.69489	3442.69	0.731	2.95207	1688.38	709804
47	0.000123348	0.0612822	22.1692	0.174	1.95E-11	8.00E-07	0.00042429
48	98.3503	2808.01	63795	1	5.26E-06	0.000904153	0.106569
49	128.967	1225.79	23006.6	1	54.1186	43708.1	2.73E+07
50	0.1483	28.9581	7858.02	0.79	12616.6	7.30E+06	2.89E+10
51	757.395	6249.13	42008.5	1	0.00182201	0.151015	230.069
52	1762.57	8335.41	82367.9	1	8.34E-05	0.00797837	21.938
53	8.81E-05	0.0166762	19.7622	0.164	2.08E-05	0.0422854	8.90707
54	0.0133062	0.341701	8.95226	0.32	0.00802477	18758.6	2.02E+09
55	841.912	7543.08	58656	1	0.000113256	0.176623	12.2208
56	0.0349824	13.1669	493.02	0.795	18328.8	4.11E+07	2.65E+09
57	2.18E-06	0.000101206	0.0276392	0	0.0421618	1.61806	101.139
58	152.26	3291.4	32842.2	1	3.17E-07	0.000227042	0.17727
59	552.206	4457.24	23786.4	1	12.5412	464438	1.20E+09
60	0.000583143	22.2761	9808.7	0.791	0.000100441	0.011381	1.97005
61	7.35E-06	0.00228247	0.689358	0.014	0.74338	56.5965	97997.2
62	0.00149481	0.568781	422.58	0.383	857.225	175299	4.24E+07
63	1.48E-08	2.90E-06	0.000161893	0	2.64E-05	0.615128	64983.9
64	5.80E-05	0.0156296	5.80392	0.127	0.00291814	34.8128	4.91E+08
65	0.417163	22.1738	6978.27	0.889			

Table A1 (cont'd)

Impinv_cat_start2_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
1.25E-05	0.00109349	0.010136	0.530607	1.32579	3.34337	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	3.19E-08	0.0125289	463.619	0.421	3.54E-05	0.0923407	110.788
1	0.00029196	0.0158681	38.6693	0.137	5.81E-15	5.93E-11	5.98E-07
2	0.00474811	3.46586	266989	0.549	0.000560824	5.10408	913.062
3	0.0904547	6.03602	537.541	0.778	1107.7	1.37E+10	2.70E+15
4	0.000188059	5.82144	314.789	0.794	17376.2	1.50E+08	6.70E+10
5	7.87E-06	0.28991	488.842	0.561	4.32E-06	0.000149201	0.0205475
6	0.046674	10.996	12868.1	0.728	0.101313	41.6642	2260.86
7	20.7999	112.47	2946.62	1	1802.95	314478	9.84E+07
8	8.26E-09	0.000110957	1.1237	0.04	4.75E-05	0.235909	9952.14
9	8.01996	173.364	143557	1	8.00E-05	0.122414	21.3792
10	1.40E-10	0.000611975	2394.98	0.237	0.00111328	0.150778	9.31529
11	2.59268	64.2714	1367.5	0.979	9078.7	8.05E+07	9.35E+10
12	5.44E-07	6.53E-05	0.0228513	0	5.60E-07	0.000309607	0.148833

Table A1 (cont'd)

13	0.0151084	3.83117	2736.57	0.565	0.00546179	48.9821	26156
14	5.59E-09	1.49E-06	7.15E-05	0	6.05E-13	6.02E-09	1.66E-05
15	2.49E-11	5.91E-08	1.23E-05	0	272.093	37842	5.67E+06
16	0.000319706	0.00970687	33.1075	0.109	0.00181338	0.144369	8.80938
17	1.39E-11	5.92E-08	7.27E-05	0	4.51E-17	4.08E-10	0.0139444
18	0.0099446	19.0584	497.524	0.854	5543.87	4.85E+08	3.95E+11
19	11.6644	180.449	26380.2	1	0.386134	29.4017	2855.01
20	0.000152548	0.392368	173.193	0.495	5.88636	7580.85	3.68E+06
21	2.89E-09	5.48E-07	0.000242631	0	8.32E-06	0.00649296	1.47537
22	1.12E-07	0.00863392	184.305	0.44	0.00171486	0.101311	9.69815
23	8.30E-05	0.0220597	4.86816	0.165	53.4515	1345.75	601274
24	8.60E-11	7.65E-05	425.072	0.291	5.50E-08	2.88E-06	0.000103133
25	8.66E-07	0.00342013	16.0014	0.239	2.63E-05	0.218973	67.3108
26	1.68E-08	8.10E-06	0.00424247	0	5.10E-05	0.00966753	4.09716
27	0.0108003	2.62632	114.372	0.746	1.95E-13	2.39E-09	3.56E-05
28	0.0357036	2.08894	7564.79	0.549	0.344561	35.9617	5699.03
29	0.000143044	1.41029	17848.3	0.582	0.12239	14.0311	1650.34
30	9.78E-08	7.25E-05	0.0400519	0	1.54E-12	1.55E-05	0.0489557
31	1.99E-05	0.0963162	1497.64	0.329	10595.3	869140	4.20E+08
32	0.46618	18.8011	30212.1	0.943	3.69E-07	0.000179211	0.0670731
33	1.45E-10	4.45E-06	0.107581	0	5.00E-07	3.17E-05	0.003018
34	0.114497	5.36729	5378.07	0.751	0.30409	553.687	840494
35	1.18E-05	0.000789327	0.232261	0	1.14E-08	1.20E-05	0.0149424
36	0.00245492	0.606642	68.7025	0.434	8147.52	1.28E+07	1.08E+12
37	2.00E-11	4.16E-08	0.000442333	0	5.36E-11	5.68E-09	2.42E-05
38	7.97947	64.1556	316.184	1	7.49E-07	0.00932279	14814.4
39	2.87E-06	0.0877293	248.41	0.344	0.0109178	0.59036	111.811
40	6.41862	70.7373	681.148	1	4.11E-08	6.56E-06	0.000303793

Table A1 (cont'd)

41	1.79831	48.3064	738.72	0.997	3.94E-08	1.61E-05	0.0976043
42	0.00778478	0.265365	6.17927	0.328	0.000695266	0.341403	15.4007
43	0.00425626	0.364884	20.9957	0.313	0.00201845	1.09951	50.4376
44	0.77748	15.708	635.12	0.926	7.51E-06	0.00673779	0.637685
45	0.000146865	0.793333	167.467	0.563	0.000140231	0.398723	31.0156
46	8.31E-11	2.61E-07	0.00381873	0	0.0246572	1.59334	20.9732
47	0.000241938	0.937155	13229.2	0.4	1.02E-08	0.000110058	7.82251
48	12.964	171.962	7705.18	1	0.000257213	0.0480059	108.089
49	0.349927	36.5735	776.783	0.946	132.658	268597	1.22E+08
50	0.000912474	0.0976927	8.59632	0.26	8.92E-06	0.19705	269.552
51	0.0252165	46.7272	435.122	0.892	1.73E-06	0.000337735	0.0285192
52	2.5683	108.11	1302.2	0.967	0.00136417	0.210743	5.68736
53	1.48E-08	0.00185116	20.2851	0.273	0.00787093	2.42232	708.518
54	7.48E-12	7.89E-07	0.00115782	0	0.001064	0.1315	15.4385
55	0.0366539	4.37817	239.02	0.668	6.27E-07	0.00323739	13.447
56	2.01E-06	0.00207672	1.30123	0.063	4.74E-06	0.0113194	4.33856
57	0.00593851	2.8332	166.629	0.702	4.99E-11	1.09E-08	2.05E-06
58	0.0845357	3.0369	58.1986	0.76	1851.27	82571.2	6.10E+06
59	1.93E-07	1.77E-06	2.26E-05	0	0.00395676	0.752603	74.1896
60	4.11E-09	8.28E-07	0.00127598	0	5.37E-06	0.545636	544.813
61	0.000708273	0.274218	13208.2	0.37	30.9036	8.99E+06	2.61E+11
62	0.0641964	5.30172	12460.6	0.635	2.01E-07	0.00192762	79.2848
63	0.000414035	0.0180937	0.718084	0.031	0.0729169	62.5096	28905
64	0.000461866	0.0549637	2.05232	0.117	74.3557	13303.6	2.99E+07
65	1.24E-05	0.0226097	80.4761	0.228			

Table A1 (cont'd)

Impinv_cat_start2_rep2

	Synonymous theta			kappa			phi	
Lower 95% HPD	Point estimate	Higher 95% HPD	Lower 95% HPD	Point estimate	Higher 95% HPD	Lower 95% HPD	Point estimate	Higher 95% HPD
1.25E-05	0.00109349	0.010136	0.530607	1.32579	3.34337	0	0	0

		omega		Posterior		rho	
Site	Lower 95% HPD	Point estimate	Higher 95% HPD	prob of +ve selection	Lower 95% HPD	Point estimate	Higher 95% HPD
0	3.19E-08	0.0125289	463.619	0.421	3.54E-05	0.0923407	110.788
1	0.00029196	0.0158681	38.6693	0.137	5.81E-15	5.93E-11	5.98E-07
2	0.00474811	3.46586	266989	0.549	0.000560824	5.10408	913.062
3	0.0904547	6.03602	537.541	0.778	1107.7	1.37E+10	2.70E+15
4	0.000188059	5.82144	314.789	0.794	17376.2	1.50E+08	6.70E+10
5	7.87E-06	0.28991	488.842	0.561	4.32E-06	0.000149201	0.0205475
6	0.046674	10.996	12868.1	0.728	0.101313	41.6642	2260.86
7	20.7999	112.47	2946.62	1	1802.95	314478	9.84E+07
8	8.26E-09	0.000110957	1.1237	0.04	4.75E-05	0.235909	9952.14
9	8.01996	173.364	143557	1	8.00E-05	0.122414	21.3792
10	1.40E-10	0.000611975	2394.98	0.237	0.00111328	0.150778	9.31529
11	2.59268	64.2714	1367.5	0.979	9078.7	8.05E+07	9.35E+10
12	5.44E-07	6.53E-05	0.0228513	0	5.60E-07	0.000309607	0.148833

Table A1 (cont'd)

13	0.0151084	3.83117	2736.57	0.565	0.00546179	48.9821	26156
14	5.59E-09	1.49E-06	7.15E-05	0	6.05E-13	6.02E-09	1.66E-05
15	2.49E-11	5.91E-08	1.23E-05	0	272.093	37842	5.67E+06
16	0.000319706	0.00970687	33.1075	0.109	0.00181338	0.144369	8.80938
17	1.39E-11	5.92E-08	7.27E-05	0	4.51E-17	4.08E-10	0.0139444
18	0.0099446	19.0584	497.524	0.854	5543.87	4.85E+08	3.95E+11
19	11.6644	180.449	26380.2	1	0.386134	29.4017	2855.01
20	0.000152548	0.392368	173.193	0.495	5.88636	7580.85	3.68E+06
21	2.89E-09	5.48E-07	0.000242631	0	8.32E-06	0.00649296	1.47537
22	1.12E-07	0.00863392	184.305	0.44	0.00171486	0.101311	9.69815
23	8.30E-05	0.0220597	4.86816	0.165	53.4515	1345.75	601274
24	8.60E-11	7.65E-05	425.072	0.291	5.50E-08	2.88E-06	0.000103133
25	8.66E-07	0.00342013	16.0014	0.239	2.63E-05	0.218973	67.3108
26	1.68E-08	8.10E-06	0.00424247	0	5.10E-05	0.00966753	4.09716
27	0.0108003	2.62632	114.372	0.746	1.95E-13	2.39E-09	3.56E-05
28	0.0357036	2.08894	7564.79	0.549	0.344561	35.9617	5699.03
29	0.000143044	1.41029	17848.3	0.582	0.12239	14.0311	1650.34
30	9.78E-08	7.25E-05	0.0400519	0	1.54E-12	1.55E-05	0.0489557
31	1.99E-05	0.0963162	1497.64	0.329	10595.3	869140	4.20E+08
32	0.46618	18.8011	30212.1	0.943	3.69E-07	0.000179211	0.0670731
33	1.45E-10	4.45E-06	0.107581	0	5.00E-07	3.17E-05	0.003018
34	0.114497	5.36729	5378.07	0.751	0.30409	553.687	840494
35	1.18E-05	0.000789327	0.232261	0	1.14E-08	1.20E-05	0.0149424
36	0.00245492	0.606642	68.7025	0.434	8147.52	1.28E+07	1.08E+12
37	2.00E-11	4.16E-08	0.000442333	0	5.36E-11	5.68E-09	2.42E-05
38	7.97947	64.1556	316.184	1	7.49E-07	0.00932279	14814.4
39	2.87E-06	0.0877293	248.41	0.344	0.0109178	0.59036	111.811
40	6.41862	70.7373	681.148	1	4.11E-08	6.56E-06	0.000303793

Table A1 (cont'd)

41	1.79831	48.3064	738.72	0.997	3.94E-08	1.61E-05	0.0976043
42	0.00778478	0.265365	6.17927	0.328	0.000695266	0.341403	15.4007
43	0.00425626	0.364884	20.9957	0.313	0.00201845	1.09951	50.4376
44	0.77748	15.708	635.12	0.926	7.51E-06	0.00673779	0.637685
45	0.000146865	0.793333	167.467	0.563	0.000140231	0.398723	31.0156
46	8.31E-11	2.61E-07	0.00381873	0	0.0246572	1.59334	20.9732
47	0.000241938	0.937155	13229.2	0.4	1.02E-08	0.000110058	7.82251
48	12.964	171.962	7705.18	1	0.000257213	0.0480059	108.089
49	0.349927	36.5735	776.783	0.946	132.658	268597	1.22E+08
50	0.000912474	0.0976927	8.59632	0.26	8.92E-06	0.19705	269.552
51	0.0252165	46.7272	435.122	0.892	1.73E-06	0.000337735	0.0285192
52	2.5683	108.11	1302.2	0.967	0.00136417	0.210743	5.68736
53	1.48E-08	0.00185116	20.2851	0.273	0.00787093	2.42232	708.518
54	7.48E-12	7.89E-07	0.00115782	0	0.001064	0.1315	15.4385
55	0.0366539	4.37817	239.02	0.668	6.27E-07	0.00323739	13.447
56	2.01E-06	0.00207672	1.30123	0.063	4.74E-06	0.0113194	4.33856
57	0.00593851	2.8332	166.629	0.702	4.99E-11	1.09E-08	2.05E-06
58	0.0845357	3.0369	58.1986	0.76	1851.27	82571.2	6.10E+06
59	1.93E-07	1.77E-06	2.26E-05	0	0.00395676	0.752603	74.1896
60	4.11E-09	8.28E-07	0.00127598	0	5.37E-06	0.545636	544.813
61	0.000708273	0.274218	13208.2	0.37	30.9036	8.99E+06	2.61E+11
62	0.0641964	5.30172	12460.6	0.635	2.01E-07	0.00192762	79.2848
63	0.000414035	0.0180937	0.718084	0.031	0.0729169	62.5096	28905
64	0.000461866	0.0549637	2.05232	0.117	74.3557	13303.6	2.99E+07
65	1.24E-05	0.0226097	80.4761	0.228			

Table A1 (cont'd)

Impinv_equal_start1_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
9.54E-06	0.000120284	0.00051575	0.319752	0.825712	1.9351	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.017238	2.02355	110.027	0.575	4.41E-05	0.00424826	42.1525
1	7.77E-06	0.000253438	0.0221913	0	1.83E-05	0.00829839	3.87958
2	1.57E-06	0.074841	1264.15	0.503	80.0537	7.76E+07	1.35E+12
3	1.97E-06	8.56E-05	0.00643236	0	624.778	6.60E+08	2.47E+12
4	4.4774	401.093	24705.7	1	1.33E-06	0.000344551	0.0195393
5	0.232204	36.3659	566.893	0.854	1.32E-08	2.79E-06	0.00144367
6	4.81E-09	1.01E-05	2.14513	0.069	0.790803	113.322	18864
7	215.298	1517.27	15902	1	4.54E-06	9.63E-05	0.00413202
8	0.000330076	1.66627	6199.83	0.582	1876.86	6.17E+08	3.11E+13
9	138.329	1391.82	8786.02	1	29438.7	3.31E+07	6.16E+10
10	0.319497	169.532	15308	0.967	0.000138181	0.018419	3.00447
11	41.0523	412.701	4313.43	1	0.10802	394.324	805691
12	0.00553493	0.792895	183.555	0.418	1.16E-05	0.0100668	12.1976
13	4.31E-06	0.0317868	493.966	0.361	0.601343	54.6872	2720.16

Table A1 (cont'd)

14	0.0580238	7.47052	339.511	0.803	3.58E-07	3.47E-05	0.423859
15	9.11E-08	0.00872151	106.215	0.313	2.13E-05	0.0038696	0.37565
16	0.0142758	42.811	15406	0.757	4.32521	545.181	161208
17	0.0258969	1.08864	153.381	0.419	0.0107825	35.0921	79124.2
18	6.35E-05	84.8853	5909.63	0.852	4.47E-07	0.00246927	2.30238
19	73.3055	980.386	13042.9	0.982	56.391	5214.49	147287
20	0.0542615	38.2452	2074.06	0.846	1.86E-07	9.78E-06	0.00158569
21	0.0813533	40.5472	2666.09	0.889	2.90E-05	0.0313214	89.4568
22	0.183893	5.73526	165.547	0.809	7.59E-08	9.82E-06	0.00148117
23	4.12E-05	0.0287913	225.027	0.323	1.55E-06	0.000524184	0.0821062
24	0.00121499	0.161997	19.9996	0.301	20.3252	3032.39	299689
25	0.000370773	0.0305598	4.38206	0.11	0.000295146	3.08204	2265.32
26	1.65324	63.7733	1098.12	0.98	211.159	238260	4.64E+08
27	0.683478	69.8315	1616.59	0.939	9.41E-06	0.000742238	0.166801
28	2.15E-05	0.0486307	21244.9	0.359	25.2822	19875.3	3.69E+06
29	0.173246	46.6343	2253.61	0.867	0.0140152	79.6241	49057
30	3.58E-11	3.75E-06	0.159672	0	0.209038	66.7746	12875.8
31	0.000760664	10.1716	36989.2	0.729	2.64419	130234	2.27E+07
32	4.65E-05	0.0131647	1.53892	0.039	0.0501286	7.55661	366.588
33	0.0470674	4.77753	236.442	0.742	3.31E-11	2.67E-08	0.000224513
34	1.27E-07	0.000873708	243.449	0.095	7.21E-07	0.00194646	13.4692
35	2.12E-05	0.000808655	0.045131	0	2.94E-09	4.59E-05	0.365595
36	1.06E-06	2.42E-05	0.00177801	0	2.26E-08	1.36E-05	0.642324
37	0.000228391	0.188669	493.998	0.408	2.97E-09	3.77E-07	0.00019382
38	0.0113259	192.989	4893.85	0.905	0.00365093	0.0561353	1.37944
39	3.42E-09	3.06E-06	0.00186696	0	0.00624614	15.1841	4.09E+06
40	460.361	3052.33	46102.8	1	0.000677212	0.0351514	14.8089
41	0.000265266	12.2703	2064.94	0.762	1.60E-05	3.58495	116448

Table A1 (cont'd)

42	0.0474949	4.73833	736.905	0.669	23.9931	1444.79	627577
43	4.50E-05	0.0281549	1121.61	0.167	6.27E-06	0.00325035	47.7739
44	139.272	731.729	5490.83	1	0.00869892	4.2415	31432.7
45	0.00223733	0.804936	19.3762	0.533	2.91E-08	9.87E-06	0.0895783
46	2.57E-05	0.145825	124.848	0.347	0.000339518	0.0928325	47.6187
47	0.144294	46.4569	13403.8	0.878	3.67E-08	5.48E-06	0.00309856
48	0.265641	394.887	6754.14	0.92	2.13E-07	2.30E-05	0.00403711
49	0.000819234	57.8162	3579.05	0.869	0.000356255	0.135405	7.41704
50	3.53904	115.21	20320.4	1	0.000126287	0.33891	9.87371
51	353.038	2049.94	8803.71	1	3.85E-11	1.93E-07	0.0132489
52	591.958	3160.11	18748.1	1	7.90E-07	0.0625803	1800.24
53	0.00101114	0.23228	75.3305	0.361	0.00466816	1.5978	33.2616
54	0.00156996	0.139572	6.61399	0.219	4.78E-06	0.00724173	36.2073
55	10.5166	752.579	9689.93	0.967	0.000160499	0.0663242	24.7665
56	1.17E-12	4.18E-10	5.32E-07	0	4.70E-05	0.00163877	0.0963197
57	0.22642	9.14476	2447.52	0.852	4.22E-08	1.65E-05	0.00408931
58	0.0274815	1.23097	43.2288	0.512	8.53E-05	0.00771528	0.952803
59	359.323	1983.75	26628.1	1	1.13E+07	6.62E+08	2.30E+10
60	0.132999	46.1654	16386.9	0.919	0.00152574	0.0792095	3.89972
61	0.00151077	4.69883	2306.23	0.641	2.87E-08	5.98E-05	0.0142794
62	0.242655	87.232	8216.62	0.934	1.72515	9813.42	5.83E+06
63	0.000775166	2.79806	226.388	0.684	184.439	4169.03	118446
64	0.000986942	5.45367	6960.88	0.72	0.478241	29.2735	1333.89
65	0.0012877	0.426598	63.9672	0.526			

Table A1 (cont'd)

Impinv_equal_start1_rep2

	Synonymous theta			kappa			phi	
Lower 95% HPD	Point estimate	Higher 95% HPD	Lower 95% HPD	Point estimate	Higher 95% HPD	Lower 95% HPD	Point estimate	Higher 95% HPD
9.54E-06	0.000120284	0.00051575	0.319752	0.825712	1.9351	0	0	0

		omega		Posterior		rho	
Site	Lower 95% HPD	Point estimate	Higher 95% HPD	prob of +ve selection	Lower 95% HPD	Point estimate	Higher 95% HPD
0	0.017238	2.02355	110.027	0.575	4.41E-05	0.00424826	42.1525
1	7.77E-06	0.000253438	0.0221913	0	1.83E-05	0.00829839	3.87958
2	1.57E-06	0.074841	1264.15	0.503	80.0537	7.76E+07	1.35E+12
3	1.97E-06	8.56E-05	0.00643236	0	624.778	6.60E+08	2.47E+12
4	4.4774	401.093	24705.7	1	1.33E-06	0.000344551	0.0195393
5	0.232204	36.3659	566.893	0.854	1.32E-08	2.79E-06	0.00144367
6	4.81E-09	1.01E-05	2.14513	0.069	0.790803	113.322	18864
7	215.298	1517.27	15902	1	4.54E-06	9.63E-05	0.00413202
8	0.000330076	1.66627	6199.83	0.582	1876.86	6.17E+08	3.11E+13
9	138.329	1391.82	8786.02	1	29438.7	3.31E+07	6.16E+10
10	0.319497	169.532	15308	0.967	0.000138181	0.018419	3.00447
11	41.0523	412.701	4313.43	1	0.10802	394.324	805691
12	0.00553493	0.792895	183.555	0.418	1.16E-05	0.0100668	12.1976
13	4.31E-06	0.0317868	493.966	0.361	0.601343	54.6872	2720.16

Table A1 (cont'd)

14	0.0580238	7.47052	339.511	0.803	3.58E-07	3.47E-05	0.423859
15	9.11E-08	0.00872151	106.215	0.313	2.13E-05	0.0038696	0.37565
16	0.0142758	42.811	15406	0.757	4.32521	545.181	161208
17	0.0258969	1.08864	153.381	0.419	0.0107825	35.0921	79124.2
18	6.35E-05	84.8853	5909.63	0.852	4.47E-07	0.00246927	2.30238
19	73.3055	980.386	13042.9	0.982	56.391	5214.49	147287
20	0.0542615	38.2452	2074.06	0.846	1.86E-07	9.78E-06	0.00158569
21	0.0813533	40.5472	2666.09	0.889	2.90E-05	0.0313214	89.4568
22	0.183893	5.73526	165.547	0.809	7.59E-08	9.82E-06	0.00148117
23	4.12E-05	0.0287913	225.027	0.323	1.55E-06	0.000524184	0.0821062
24	0.00121499	0.161997	19.9996	0.301	20.3252	3032.39	299689
25	0.000370773	0.0305598	4.38206	0.11	0.000295146	3.08204	2265.32
26	1.65324	63.7733	1098.12	0.98	211.159	238260	4.64E+08
27	0.683478	69.8315	1616.59	0.939	9.41E-06	0.000742238	0.166801
28	2.15E-05	0.0486307	21244.9	0.359	25.2822	19875.3	3.69E+06
29	0.173246	46.6343	2253.61	0.867	0.0140152	79.6241	49057
30	3.58E-11	3.75E-06	0.159672	0	0.209038	66.7746	12875.8
31	0.000760664	10.1716	36989.2	0.729	2.64419	130234	2.27E+07
32	4.65E-05	0.0131647	1.53892	0.039	0.0501286	7.55661	366.588
33	0.0470674	4.77753	236.442	0.742	3.31E-11	2.67E-08	0.000224513
34	1.27E-07	0.000873708	243.449	0.095	7.21E-07	0.00194646	13.4692
35	2.12E-05	0.000808655	0.045131	0	2.94E-09	4.59E-05	0.365595
36	1.06E-06	2.42E-05	0.00177801	0	2.26E-08	1.36E-05	0.642324
37	0.000228391	0.188669	493.998	0.408	2.97E-09	3.77E-07	0.00019382
38	0.0113259	192.989	4893.85	0.905	0.00365093	0.0561353	1.37944
39	3.42E-09	3.06E-06	0.00186696	0	0.00624614	15.1841	4.09E+06
40	460.361	3052.33	46102.8	1	0.000677212	0.0351514	14.8089
41	0.000265266	12.2703	2064.94	0.762	1.60E-05	3.58495	116448

Table A1 (cont'd)

42	0.0474949	4.73833	736.905	0.669	23.9931	1444.79	627577
43	4.50E-05	0.0281549	1121.61	0.167	6.27E-06	0.00325035	47.7739
44	139.272	731.729	5490.83	1	0.00869892	4.2415	31432.7
45	0.00223733	0.804936	19.3762	0.533	2.91E-08	9.87E-06	0.0895783
46	2.57E-05	0.145825	124.848	0.347	0.000339518	0.0928325	47.6187
47	0.144294	46.4569	13403.8	0.878	3.67E-08	5.48E-06	0.00309856
48	0.265641	394.887	6754.14	0.92	2.13E-07	2.30E-05	0.00403711
49	0.000819234	57.8162	3579.05	0.869	0.000356255	0.135405	7.41704
50	3.53904	115.21	20320.4	1	0.000126287	0.33891	9.87371
51	353.038	2049.94	8803.71	1	3.85E-11	1.93E-07	0.0132489
52	591.958	3160.11	18748.1	1	7.90E-07	0.0625803	1800.24
53	0.00101114	0.23228	75.3305	0.361	0.00466816	1.5978	33.2616
54	0.00156996	0.139572	6.61399	0.219	4.78E-06	0.00724173	36.2073
55	10.5166	752.579	9689.93	0.967	0.000160499	0.0663242	24.7665
56	1.17E-12	4.18E-10	5.32E-07	0	4.70E-05	0.00163877	0.0963197
57	0.22642	9.14476	2447.52	0.852	4.22E-08	1.65E-05	0.00408931
58	0.0274815	1.23097	43.2288	0.512	8.53E-05	0.00771528	0.952803
59	359.323	1983.75	26628.1	1	1.13E+07	6.62E+08	2.30E+10
60	0.132999	46.1654	16386.9	0.919	0.00152574	0.0792095	3.89972
61	0.00151077	4.69883	2306.23	0.641	2.87E-08	5.98E-05	0.0142794
62	0.242655	87.232	8216.62	0.934	1.72515	9813.42	5.83E+06
63	0.000775166	2.79806	226.388	0.684	184.439	4169.03	118446
64	0.000986942	5.45367	6960.88	0.72	0.478241	29.2735	1333.89
65	0.0012877	0.426598	63.9672	0.526			

Table A1 (cont'd)

Impinv_equal_start2_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
6.49E-06	2.24E-05	5.80E-05	0.283491	0.817718	2.27668	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	4.26E-05	0.0041198	0.15708	0	5.66E-08	0.00556366	164.16
1	0.00152342	1.55295	346.988	0.575	33401.7	4.95E+06	1.64E+08
2	0.109517	928.024	126092	0.867	21238.2	1.41E+09	2.04E+13
3	0.000915121	7.72912	7474.15	0.64	2737.23	3.21E+07	6.25E+11
4	0.409453	715.55	21811.7	0.949	0.0402234	154.767	18888.6
5	5.89E-08	0.208008	193.405	0.638	4.36065	59058.9	1.40E+08
6	0.00247299	0.218878	134169	0.166	34.3447	2029.36	1.53E+06
7	903.109	6125.05	27539.2	1	0.0316029	610.053	3.07E+06
8	3.54E-06	0.0247899	39.1522	0.299	2.13E-07	3.60E-05	0.0446796
9	1424.95	7957.54	50611.3	1	4.13E-07	0.0193216	5.02426
10	6.36E-09	0.00257363	23.5471	0.198	0.00394845	0.514066	246.437
11	94.0855	1415.2	28451.6	1	0.000519823	0.24496	9.48034
12	0.0389268	19.2811	1975.54	0.853	1.49E-08	3.30E-06	0.0010621
13	0.00196474	4.30063	710.277	0.769	6.28E-12	1.72E-07	0.000749879

Table A1 (cont'd)

14	1.33E-08	1.50E-06	0.0103021	0	1.25E-06	8.47E-05	0.0039169
15	0.0954021	33.1828	25520.6	0.795	0.0785869	4.31165	3480.47
16	6.57E-13	4.33E-08	0.00774425	0	9.49E-06	0.00311869	1.55361
17	1.47E-10	0.0318314	11010.2	0.552	1.31E-08	4.81E-05	0.684325
18	189.439	4650.61	56267.2	0.975	0.0118688	26.6008	3.61E+06
19	1336.11	8055.12	66220.4	1	0.000547449	0.0683606	72.6579
20	3.20E-05	0.200001	3179.08	0.363	7.06E-06	0.00923382	119.009
21	1.16058	449.031	9579.86	0.998	3.33E-08	0.000236612	135.78
22	1.71E-16	2.30E-10	2.24E-05	0	0.00102621	6.3916	168756
23	1.13E-06	0.0365354	65417.8	0.436	154983	1.10E+07	2.30E+09
24	5.61E-06	0.0280058	45.2989	0.4	0.198807	14866.1	2.82E+07
25	0.00549932	0.712066	4976.37	0.318	2.38E-06	0.0176413	1948.73
26	1.70E-12	3.24E-10	2.00E-07	0	209.536	51354.2	4.76E+06
27	0.234908	601.475	18347.4	0.909	9.37E-07	0.00275858	3709.51
28	6.00E-15	3.53E-10	5.79E-05	0	6197.53	1.42E+06	1.49E+08
29	4.73E-08	1.15E-05	0.0959056	0	1.50E-07	3.33E-05	0.00953038
30	0.000780617	0.705774	1147.72	0.36	0.114575	10534.5	2.52E+08
31	0.000652845	0.148086	837.619	0.286	3.96E-10	2.54E-07	0.00474554
32	1.26E-07	6.89E-05	0.0236836	0	8.36E-05	0.100196	1108.73
33	3.92426	110.172	18090.1	0.965	1.32E-10	8.41E-08	7.56E-06
34	6.34E-09	2.58E-07	8.16E-06	0	3.58E-10	6.10E-08	8.71E-06
35	0.000108454	0.0109581	1.091	0.028	3.38E-07	0.331351	5722.29
36	5.52E-07	2.50E-05	0.001941	0	26.353	6093.18	1.75E+07
37	6.17E-09	0.0824959	23400.3	0.418	5.81E-07	9.91E-05	0.0255201
38	0.00169969	592.279	74093.3	0.868	2.67E-07	9.13E-05	0.111575
39	0.0426146	34.0638	4476.03	0.865	7.70E-05	0.286049	2024.68
40	18.1296	8349.29	54332	1	5.39E-05	0.00837238	14.576
41	85.4653	3429.87	36263.1	1	1.68E-10	0.000127317	253.291

Table A1 (cont'd)

42	1.66729	190.027	3928.85	0.986	8.50E-07	0.000552005	1.34726
43	0.00468743	0.761852	74.1156	0.492	2.40357	1784.15	5.42E+06
44	0.00576022	25.0148	11539.1	0.655	0.422521	139.5	1.05E+06
45	3.19E-09	0.000386753	76.0596	0.247	37.5746	30639.4	5.86E+07
46	2.20027	103.331	5696.7	0.982	1317.6	8.17E+06	2.93E+11
47	0.22318	82.0018	32379	0.923	1.55785	44.347	2194.77
48	1011.81	9589.08	69499.7	1	46644.4	1.19E+06	8.35E+07
49	6.58779	1126.19	13380.5	1	0.000877996	0.290683	7975.61
50	0.000705215	0.0464397	2.45255	0.061	1.26E+06	1.38E+09	7.18E+12
51	1740.15	8165.46	39087.7	1	6.22E-09	4.31E-06	0.0078862
52	3769.54	16118	106536	1	2.01439	19245.8	8.44E+08
53	0.82394	323.136	14824	0.969	2.63491	222.045	18449.9
54	3.74E-09	8.21E-07	0.00796291	0	100.079	620402	3.32E+10
55	0.00140423	694.428	61445.5	0.856	4.56E-12	9.93E-10	2.12E-06
56	1.36E-05	0.027091	2329.86	0.234	4.13E-09	2.46E-05	0.00586414
57	2.42E-06	0.00231217	0.396687	0.008	2.21E-12	2.22E-09	1.39E-06
58	80.6217	4140.78	50680.4	1	0.000118154	0.0216444	2.49314
59	1228.49	8118.95	42103.6	1	4.47E-10	8.17E-07	0.00155913
60	0.000553843	0.0596396	2.05299	0.062	0.236855	1366.82	1.66E+09
61	0.000148177	0.0128018	5.00716	0.07	0.0055687	6069.49	4.04E+06
62	0.0148905	78.9258	7580.77	0.871	0.0140254	2.29534	905.189
63	0.00072371	0.0229855	1.04939	0.024	7.16E-05	0.0253539	4.6009
64	0.240667	65.0568	16049.5	0.921	0.616378	1095.38	1.06E+07
65	4.06E-06	0.0498607	847.924	0.34			

Table A1 (cont'd)

Impinv_equal_start2_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
2.33E-06	2.54E-05	0.00017724	0.25793	0.697569	1.8842	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	6.03E-11	1.30E-06	0.000205226	0	1.03E-07	6.89E-06	0.00430454
1	0.00866679	14.4688	26190.7	0.602	0.00923592	0.429008	70.5356
2	0.122592	40.8539	152863	0.82	3.43E-07	1.56E-05	0.000653639
3	1.16E-05	0.0224733	55.019	0.258	7.56553	1035.34	181387
4	134.385	2968.91	26968.9	1	1516.92	4.13E+06	1.65E+10
5	9.98E-05	0.00937193	5.27615	0.063	2.22E-06	0.000364174	0.0138776
6	0.582547	385.933	35855	0.966	3.05E-13	4.45E-10	8.35E-07
7	0.0505853	878.409	56538.5	0.877	0.00608896	0.155758	2.38336
8	1.32E-10	3.53E-07	0.000964291	0	1.84E-07	8.00E-06	0.00510632
9	1.31116	2029.62	34276.8	0.962	1499.18	4.14E+06	2.41E+11
10	0.104011	128.778	107857	0.788	1.82973	3274.53	220497
11	0.000462352	4.6641	7926.56	0.564	4.47335	905.692	155323
12	1.34E-05	0.123891	302.26	0.356	6.87E-06	0.00396074	10.5968
13	7.86E-05	0.397565	2667.05	0.426	1.79E-10	6.59E-08	4.70E-05

Table A1 (cont'd)

14	1.36E-06	0.000908491	0.0735519	0	0.00219424	0.522219	225.719
15	3.20E-06	0.000675478	1.15295	0.048	505022	8.28E+11	6.59E+19
16	0.00233758	2.58042	2662.36	0.626	1.29E-05	0.0137084	7.8284
17	0.104309	9.90225	27946.5	0.672	4.57E-07	0.00108041	10.2625
18	1083.75	8002.31	156355	1	0.000659064	0.162772	4.52719
19	792.801	9423.87	214934	1	0.186991	28.6765	8046.6
20	1.27E-11	1.57E-07	0.00191956	0	0.0123161	4.88718	103373
21	2.12E-07	5.83E-05	0.0106027	0	0.227231	1171.71	8.90E+06
22	0.00099793	13.2804	144548	0.634	0.00934237	0.636383	19.737
23	8.30E-06	0.00257201	11.7604	0.145	3157.5	633347	5.47E+07
24	1.51E-09	5.86E-06	1.82105	0.046	1.44E-06	0.0191939	3644.47
25	0.0184262	71.611	48308.4	0.851	4.05E-05	0.00211728	0.838954
26	0.739155	68.4173	3757.62	0.917	2.39391	17099.9	8.73E+08
27	132.324	1967.61	18237.7	1	2.98E-05	0.00176555	0.646654
28	6.18E-10	8.53E-08	1.33E-05	0	6.07416	1214.5	113734
29	9.16E-07	0.000371132	2.14856	0.065	0.000495665	0.936519	434.845
30	1.4272	263.28	614630	0.954	0.00101285	1.04698	260.833
31	0.675764	82.988	9309.22	0.942	0.00127316	0.046556	2.47001
32	7.07E-07	0.109009	14959.9	0.371	5.69E-07	0.00269459	8.90309
33	1.40E-05	2.17206	1363.16	0.725	14.8547	1635.16	359512
34	8.98E-09	3.84E-07	0.00245405	0	1.04E-10	2.45E-08	0.000162196
35	2.40E-06	0.000131202	0.00582944	0	8649.56	144333	3.04E+06
36	0.260425	41.7149	10083.9	0.897	5495.54	1.28E+06	8.46E+08
37	1.30E-05	0.0735062	111.331	0.463	1.21E-07	4.12E-05	0.00469732
38	582.902	4388	126583	1	0.000582459	0.0676534	4.09026
39	2.18782	733.052	367828	0.987	7.24E-14	5.21E-09	0.000178276
40	3141.07	13825.9	345949	1	1.12E-08	2.25E-05	0.0256174
41	4.63E-06	50.8033	26611.3	0.804	0.000436563	0.54442	68.689

Table A1 (cont'd)

42	0.0135243	9.79482	3037.03	0.745	7.31E-06	0.0572445	11.0168
43	6.32E-05	0.165516	2718.32	0.315	2.02E-08	4.67E-06	0.00172654
44	0.0244527	1431.07	85849.8	0.873	0.044576	0.774034	7.28627
45	8.87E-12	2.85E-09	1.36E-05	0	0.00208246	0.160411	7.97777
46	4.64E-13	6.20E-08	0.00848245	0	0.00993939	0.29068	16.8242
47	0.00256169	0.313489	134.181	0.356	0.00120556	0.110411	5.24946
48	0.00123871	386.968	45348.5	0.856	1.02E-07	7.79E-05	0.0679769
49	187.012	2938.08	58747.3	1	0.0090843	3.95167	13095.4
50	0.0203332	8.24791	713.734	0.764	4.16E-08	0.000317798	5.39311
51	4.86292	2615.97	30821.2	0.985	6.37E-06	0.000253648	0.00738335
52	1119.82	14583.1	240057	1	0.000163287	0.132666	49.1487
53	4.57E-07	2.65E-05	0.0029202	0	25.4029	8889.59	1.35E+07
54	1.29E-09	8.62E-05	0.065886	0	0.0107758	35.0023	37661.2
55	0.000370487	51.2264	20892.7	0.712	0.0125544	0.468503	5.96649
56	0.000830789	6.37075	101774	0.673	6.43E-06	0.0703926	6.69951
57	1.31E-05	0.0353132	280.042	0.359	2.09E-09	0.000122511	0.746251
58	3.22E-09	1.19E-05	0.023385	0	1969.15	9.16E+06	2.27E+09
59	856.518	7793.86	81390.5	1	2.82E-08	8.49E-05	0.0194132
60	0.939593	35.468	1207.38	0.959	2.24E-06	0.00276174	5.34826
61	8.21E-14	1.53E-10	5.53E-05	0	1.27E-07	1.86E-05	0.0110228
62	8.95E-06	0.000889196	3.54651	0.094	3.72E-08	1.99E-06	7.59E-05
63	0.000707445	11.7017	3412.31	0.74	686735	2.59E+08	3.52E+11
64	0.711384	37.8269	2491.01	0.944	7.15E-10	8.19E-06	0.0105724
65	1.96E-05	0.0471928	3889.81	0.315			

Table A1 (cont'd)

Inv_cat_star1_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.00342164	0.00728711	0.0241626	0.610263	1.3288	3.17303	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.00181299	0.121867	3.85354	0.272	0.142625	5.78367	89.6604
1	0.0010909	0.0177355	0.506778	0.018	0.0121757	0.555097	19.6731
2	0.00101259	0.0508921	1.27615	0.087	0.069519	5.38083	98.5899
4	0.823532	13.6683	91.8296	0.941	0.0101776	1.47295	70.5528
5	0.00998112	0.255521	12.6107	0.243	0.012125	4.37845	81.7217
6	0.00110351	0.0511951	3.975	0.1	0.0993796	4.39544	99.7257
7	0.913268	13.4345	92.9637	0.95	0.0131851	0.142299	1.81652
8	0.00115425	0.042399	2.84126	0.15	0.0152008	0.537231	8.85741
9	3.77924	22.3141	89.0469	0.986	0.0115384	0.146319	1.93031
10	0.00106494	0.0551596	1.21882	0.097	0.0101885	0.208074	5.32058
11	2.67011	16.9044	75.8997	1	0.0103928	0.110341	2.58997
12	0.00630865	0.283084	6.57675	0.306	0.0100768	0.136748	3.4056
13	0.001014	0.0193856	0.729388	0.028	0.0139119	0.203705	4.14294
14	0.0010329	0.045276	1.4804	0.075	0.0100567	0.0876343	1.92423
15	0.0010195	0.0621144	7.37697	0.149	0.0125916	0.0868786	1.39309
16	0.00912449	0.607462	25.4206	0.473	0.011314	0.170209	2.5863

Table A1 (cont'd)

17	0.00104665	0.00881212	0.0475781	0.016	0.0178126	0.500861	5.04394
18	3.12743	17.8509	84.5892	0.976	0.0107768	0.206101	1.4174
19	1.26141	14.0114	80.3473	0.962	0.0105136	0.257211	10.7266
20	0.00147337	0.282269	19.8352	0.383	0.0107419	1.35296	61.7734
21	0.00213447	0.101182	22.0691	0.17	0.0101953	0.130011	52.0159
22	0.00101734	0.186066	7.5487	0.276	0.0196512	0.475855	65.9004
23	0.00103848	0.074437	3.81214	0.219	0.0108562	0.361293	24.3381
24	0.0059236	0.0795232	1.0372	0.034	0.0102675	0.134447	2.99511
25	0.00129368	0.0583289	11.7448	0.212	0.0141156	0.391993	48.4711
26	0.00664653	0.576049	11.7444	0.44	0.0325457	0.658168	41.9985
27	0.143204	3.51561	27.3048	0.809	0.010703	0.0754141	0.738436
28	0.0349986	0.709269	11.6985	0.435	0.013667	0.908324	62.9319
29	0.0118702	0.667063	45.0991	0.442	0.0100882	0.097342	1.0531
30	0.00133265	0.131757	3.36193	0.196	0.011411	0.2841	65.2508
31	0.00922976	0.349318	12.0755	0.384	0.0179991	1.08485	94.3461
32	0.00102121	0.0355753	4.96954	0.138	0.0102917	0.243748	8.57882
33	0.00248119	0.105724	4.51953	0.169	0.0314827	1.60606	97.1258
34	0.00159136	0.051193	0.786604	0.019	0.0100042	0.135787	4.75178
35	0.00122135	0.0236622	1.14985	0.056	0.0126082	0.59017	29.061
36	0.0469063	1.71682	29.3577	0.588	0.0101605	0.100649	20.3088
37	0.00523683	0.178154	10.4742	0.272	0.0166186	1.24793	61.3122
38	1.75725	11.419	77.5375	0.987	0.0101372	0.185506	1.57772
39	0.00128719	0.18769	8.5916	0.297	0.0100913	0.145844	3.2951
40	3.12092	16.2076	97.7605	1	0.0101814	0.121181	1.1521
41	0.0555899	4.74447	33.7213	0.876	0.0130002	0.334044	6.108
42	0.00509662	1.79823	23.4698	0.751	0.0129544	0.733568	9.07615
43	0.00103107	0.27414	14.1857	0.467	0.01538	0.720736	9.05447
44	0.359604	6.58651	89.2345	0.861	0.012797	0.150829	4.69065

Table A1 (cont'd)

45	0.00103989	0.250336	5.80493	0.404	0.0102135	0.342409	3.35539
46	0.00100571	0.013905	0.254316	0	0.117195	0.588506	5.12427
47	0.00228417	0.104013	6.3084	0.21	0.0215692	0.430894	8.27551
48	0.00881941	7.16647	97.4323	0.845	0.0102415	0.272598	3.6066
49	0.0455811	2.58787	39.4706	0.776	0.0635992	0.722255	4.42511
50	0.00121242	0.0261104	1.89988	0.084	0.0108282	0.0994067	2.04092
51	1.04156	26.3628	97.8342	0.951	0.010035	0.085177	0.944589
52	0.259014	17.7892	96.7384	0.915	0.212781	3.89682	36.7663
53	0.00109992	0.175976	7.85845	0.353	0.0114521	0.0791608	6.19419
54	0.00106638	0.0127411	0.738731	0.037	0.010647	0.398842	14.5072
55	0.935553	9.91039	79.3592	0.944	0.0111859	0.107642	2.15009
56	0.00358495	0.152616	19.7619	0.249	0.0119534	0.197557	7.5933
57	0.0885022	1.39644	12.6	0.609	0.0104058	0.135643	2.49647
58	0.00295036	0.0782878	2.22259	0.102	0.0115396	0.294024	5.02726
59	6.31916	27.8977	91.8937	1	0.0105342	0.270306	4.15527
60	0.00102503	0.0221074	2.01368	0.075	0.0100438	0.165409	11.3708
61	0.00105251	0.0366453	0.991461	0.048	0.0104625	0.107564	11.4107
62	0.0041908	0.371508	15.7748	0.492	0.457678	6.1774	75.5385
63	0.00104766	0.0350078	5.12942	0.091	0.0160807	0.241223	10.5628
64	0.00101779	0.0309997	3.26204	0.14	0.0152245	1.1709	80.8248
65	0.00104377	0.10809	7.20282	0.342			

Table A1 (cont'd)

Inv_cat_start1_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.00331242	0.00707247	0.0216013	0.609589	1.3055	3.00527	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.00102308	0.0636664	6.41664	0.23	0.0103506	0.467908	35.4581
1	0.00301124	0.127991	3.56804	0.137	0.0777802	1.6097	74.7885
2	0.00107303	0.0323492	1.49824	0.104	0.0142226	0.624974	99.9031
3	0.00105199	0.0410748	3.66757	0.113	1.00917	26.2215	99.5396
4	0.0207747	7.29081	92.7086	0.875	0.010599	2.49805	88.1304
5	0.00102298	0.100713	4.98458	0.204	0.0107997	0.0706431	0.93725
6	0.00103204	0.241212	26.7015	0.418	0.073928	10.086	99.6167
7	6.22389	30.1157	98.3628	1	0.0104418	0.130256	1.98352
8	0.00106121	0.0333202	0.637116	0.025	0.0101724	0.0758131	2.26243
9	6.16465	22.0285	96.1771	1	0.0103012	0.170671	4.65845
10	0.00110552	0.12557	8.43934	0.364	0.0106862	0.329183	5.19719
11	0.838479	13.9923	95.3201	0.943	0.0102974	0.114682	2.58998
12	0.00116227	0.0267993	5.52128	0.124	0.0101828	0.288843	5.78505

Table A1 (cont'd)

13	0.00148717	0.357378	10.8903	0.337	0.0110914	0.202586	2.79459
14	0.00141249	0.176148	8.38673	0.34	0.0238116	0.401679	5.81716
15	0.00268524	0.174944	13.8792	0.312	0.0122865	0.296188	2.85462
16	0.00514781	0.390976	12.9312	0.411	0.0101242	0.134324	1.74467
17	0.00102311	0.129052	3.55474	0.265	0.0114116	0.214624	3.10896
18	0.122412	10.7342	97.4793	0.926	0.0102828	0.0880552	0.422514
19	0.640539	15.9872	73.5011	0.944	0.0109199	0.325593	25.1756
20	0.00105971	0.0711442	3.17151	0.197	0.0100181	0.139242	1.38806
21	0.00113949	0.0296244	1.53907	0.052	0.0102754	0.188708	6.90985
22	0.00100143	0.00889506	0.786548	0.048	0.0104233	0.0900585	1.55883
23	0.00201133	0.47376	11.0984	0.359	0.0104518	0.138419	19.0027
24	0.00119507	0.026487	2.46999	0.106	0.0117383	0.0703542	0.956839
25	0.00234563	0.155608	5.77373	0.264	0.0100026	0.169117	7.64491
26	0.00119603	0.0898021	5.68921	0.219	0.0122249	0.124758	1.92428
27	0.363888	4.06517	38.721	0.829	0.0125194	0.57829	7.30606
28	0.00113954	0.293662	29.9294	0.424	0.0105212	0.0732817	1.09558
29	0.00112276	0.0350527	2.46201	0.119	0.0150056	0.535927	17.0443
30	0.00102028	0.00942523	0.127481	0	0.0133661	0.158903	3.04587
31	0.00246815	0.210317	13.024	0.23	0.0100347	0.292862	19.1058
32	0.00121103	0.0397854	1.55373	0.088	0.0118226	0.575637	42.5138
33	0.00137942	0.26603	12.8829	0.431	0.0116016	0.181424	2.81215
34	0.00102795	0.0245402	0.714802	0.031	0.0132489	0.290572	5.34376
35	0.00107812	0.592311	21.566	0.47	0.0285115	0.772417	30.5028
36	0.00102181	0.143156	4.4756	0.23	0.0107157	0.13638	3.34689
37	0.00102615	0.119047	8.21346	0.267	0.0101515	0.101013	1.53218
38	0.98437	15.0677	94.9011	0.949	0.010097	0.0612356	0.90488
39	0.00100162	0.0199153	2.62781	0.088	0.0100568	0.0925811	0.69783
40	1.72801	11.7195	66.0636	0.975	0.0101308	0.116902	1.34229

Table A1 (cont'd)

41	0.293549	5.28917	45.2979	0.812	0.0101603	0.107524	3.91017
42	0.00193763	0.0755583	4.39454	0.135	0.0256317	1.02495	8.49246
43	0.00101168	0.0141286	0.418634	0.009	0.0878975	1.61113	12.2719
44	0.0898335	11.6343	96.7664	0.875	0.0100547	0.128241	1.80564
45	0.0272761	0.836982	10.4294	0.517	0.0131133	0.265478	6.85446
46	0.00113913	0.0337223	3.58297	0.125	0.0100064	0.212105	2.36543
47	0.010007	0.281479	8.76203	0.294	0.0100508	0.104433	0.968786
48	0.133792	16.9783	99.1723	0.938	0.0100081	0.221084	2.33161
49	0.0707242	4.30315	99.0951	0.866	0.013425	0.270032	3.55175
50	0.00106887	0.0261873	1.34753	0.067	0.0100793	0.0805579	1.41617
51	0.14043	23.2066	98.8128	0.941	0.0100498	0.0593397	2.61671
52	0.0195958	15.5946	93.0246	0.913	0.384872	4.38997	35.6097
53	0.0024821	0.333283	22.8542	0.39	0.0106347	0.522223	26.1618
54	0.00205824	0.0918013	21.8313	0.177	0.0525319	0.624474	8.6126
55	0.13752	8.52401	95.5606	0.922	0.0235445	0.441046	9.73256
56	0.00102357	0.0234488	1.6449	0.057	0.0146212	0.416289	13.8424
57	0.00277118	0.112922	4.88358	0.13	0.0108039	0.100695	1.09709
58	0.00101751	0.00605942	0.124439	0	0.016208	0.233922	4.89144
59	7.10326	24.1528	87.7875	1	0.0255546	1.42857	86.5337
60	0.00216129	0.0924286	11.2038	0.153	0.0199433	0.944577	64.7278
61	0.00103969	0.0259166	0.409311	0.02	0.0187098	1.30038	29.1439
62	0.00472691	0.185464	21.4486	0.335	0.0897611	5.32202	95.9734
63	0.00100806	0.0111838	0.159215	0	0.0103515	0.406061	68.7553
64	0.00627404	1.02543	18.1319	0.56	0.012706	1.34816	34.8475
65	0.00132849	0.138619	18.0073	0.304			

Table A1 (cont'd)

Inv_cat_start2_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.00312621	0.00577543	0.0151099	0.506194	1.20805	2.93847	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.00110643	0.0864679	4.45966	0.181	0.0216333	1.29805	83.307
1	0.00108372	0.0742702	3.99966	0.203	0.0339418	2.86398	81.99
2	0.00101947	0.023755	0.770855	0.042	0.0178939	4.45301	99.3676
3	0.00100765	0.022795	11.8643	0.178	0.0125134	0.282429	5.53409
4	0.329937	18.0216	98.3714	0.939	0.0191448	1.3054	97.4648
5	0.00101439	0.0723635	5.70937	0.149	0.0128347	4.90116	94.0968
6	0.00112921	0.157259	7.6312	0.283	0.118531	6.69605	98.8772
7	0.261335	22.4872	91.9781	0.93	0.0102314	0.0621616	0.663832
8	0.0010537	0.0568744	4.08677	0.185	0.01044	0.24661	2.66095
9	6.9833	24.6807	87.942	1	0.0110001	0.481066	7.18285
10	0.00116093	0.0720402	13.3389	0.213	0.0108886	0.281114	3.31137
11	0.0345062	11.5999	98.7143	0.876	0.0305094	0.612075	14.5328
12	0.00104783	0.039674	5.68064	0.156	0.0159446	0.126449	2.0175
13	0.00338419	0.189514	13.3088	0.26	0.0219233	0.735752	38.7017
14	0.00145082	0.0768303	15.28	0.259	0.0108038	0.104043	0.844186

Table A1 (cont'd)

15	0.0013755	0.0812573	4.06037	0.147	0.0100459	0.109699	14.7667
16	0.0012961	0.0355167	0.780111	0.026	0.0104538	0.203828	15.4748
17	0.00101012	0.0819725	2.5943	0.215	0.0101398	0.131719	1.2517
18	0.0555228	13.7421	88.0885	0.93	0.0145064	0.247818	5.74917
19	2.15915	14.0672	65.4523	0.974	0.160467	6.90895	96.8861
20	0.00122763	0.0542226	8.29114	0.22	0.0132339	0.254382	78.1354
21	0.00131855	0.0998875	5.62593	0.353	0.0366264	1.16688	76.3898
22	0.00113794	0.0218633	0.467352	0.001	0.0231952	1.40593	86.9974
23	0.0017304	0.320218	11.4804	0.396	0.189937	10.822	95.126
24	0.0198598	0.622141	11.199	0.477	0.0557385	1.13019	98.5855
25	0.00199446	0.0516679	2.45366	0.075	0.0101148	0.686096	45.1093
26	0.00106351	0.121864	15.5673	0.265	0.0100087	0.111722	21.4697
27	0.511976	5.05115	49.8746	0.891	0.103694	2.40321	98.1906
28	0.027171	0.832769	29.0695	0.433	0.0119861	0.740331	56.9939
29	0.11358	2.19335	70.7042	0.717	0.0103496	0.203181	28.2076
30	0.00106757	0.0703394	24.3034	0.221	0.0865436	4.06659	90.6359
31	0.0264176	1.00283	19.6945	0.55	0.0192043	2.15517	92.0251
32	0.00104956	0.0155447	0.344991	0.015	0.0688889	2.15979	89.307
33	0.00176577	0.150102	5.61285	0.239	0.0100361	0.827726	19.4888
34	0.00180725	0.242344	18.5412	0.306	0.0661696	1.55945	95.9353
35	0.00134443	0.139365	7.59813	0.284	0.0247436	0.85721	76.8028
36	0.00103057	0.0719675	6.31066	0.271	0.0109002	0.345227	55.175
37	0.0639698	2.2509	40.6944	0.736	0.0101229	0.422204	33.035
38	0.0645228	9.06415	70.8345	0.869	0.012024	0.0862727	0.894762
39	0.0291207	1.52767	14.4562	0.68	0.0114914	0.280678	2.32341
40	0.160228	9.31347	92.2421	0.87	0.0102028	0.199924	1.76645
41	0.298998	9.56076	85.2768	0.931	0.0145547	0.99425	20.0432
42	0.00112955	0.0196353	0.426709	0.007	0.0100358	0.362707	10.6586

Table A1 (cont'd)

43	0.0137906	0.588296	44.4801	0.479	0.010065	0.0945481	2.34592
44	3.23302	23.0276	98.0775	1	0.0102793	0.133812	1.98326
45	0.00113036	0.0852049	2.51341	0.162	0.0119137	0.306505	14.7693
46	0.126474	1.5963	47.3712	0.587	0.0153209	0.730067	11.3414
47	0.00192465	0.619864	16.5701	0.531	0.0160436	0.846115	18.7501
48	6.04782	26.4513	93.6323	1	0.0100461	0.129362	2.58852
49	0.0252095	3.75805	68.9828	0.813	0.0333781	0.407991	3.51922
50	0.00100218	0.0338201	0.819739	0.041	0.0189819	0.312811	3.38404
51	0.626292	23.5195	99.5643	0.947	0.0105316	0.0593055	0.635339
52	6.01567	31.2159	96.8146	0.989	0.049969	1.44588	28.6805
53	0.00124125	0.0523379	3.93176	0.197	0.0103963	0.243001	8.47808
54	0.0010053	0.0849652	12.7874	0.221	0.0103636	0.334965	6.12058
55	2.01891	15.1604	99.1152	0.996	0.013707	0.515926	17.6958
56	0.0261611	0.401595	14.1615	0.305	0.0150473	0.333481	42.2954
57	0.00431959	0.672525	25.3095	0.497	0.0100781	0.466869	28.6619
58	0.00122056	0.138703	10.3689	0.238	0.0122261	0.225096	2.36509
59	0.0962271	18.992	99.7648	0.938	0.0111916	0.397902	67.1111
60	0.00104039	0.0212285	0.603935	0.023	0.0103402	0.42391	26.7115
61	0.0364074	0.908547	22.7045	0.475	0.0117633	0.217161	26.0171
62	0.00142328	0.148069	10.3865	0.315	0.0204222	2.45249	60.9601
63	0.00109709	0.0504356	1.81429	0.094	0.0106551	0.320853	51.1438
64	0.00539981	0.51155	18.6911	0.493	0.0336102	1.21301	95.1661
65	0.0020293	0.321833	52.3833	0.384			

Table A1 (cont'd)

Inv_cat_start2_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.0034332	0.00849829	0.0204415	0.554912	1.26532	2.82281	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.00152386	0.220188	15.468	0.369	0.0491492	1.60304	85.6597
1	0.00138573	0.14264	10.2273	0.248	0.0101879	0.0890147	3.41235
2	0.00186851	0.0943291	7.88976	0.256	0.0101583	0.0530311	0.399315
3	0.00103498	0.0672241	3.69863	0.169	1.25742	17.674	93.6236
4	0.52509	9.98911	95.7907	0.938	0.0164398	4.99699	98.8654
5	0.00111928	0.0759971	2.01299	0.123	0.0103726	0.367337	48.4318
6	0.00104591	0.131509	13.6005	0.278	0.0446429	9.7014	98.1698
7	2.01962	15.2427	87.4701	0.963	0.0102279	0.234253	3.80757
8	0.00105154	0.0695551	17.5195	0.157	0.010285	0.110905	7.95389
9	0.2024	11.0911	88.1005	0.891	0.010034	0.156308	3.99417
10	0.00100127	0.0137939	1.45372	0.059	0.0102024	0.341501	4.77404
11	0.268896	8.8081	62.5425	0.857	0.01155	0.194738	25.5042
12	0.00111921	0.0816614	2.49335	0.106	0.0100121	0.306148	9.05699
13	0.00121758	1.31189	26.5761	0.673	0.0185603	0.276939	3.38455

Table A1 (cont'd)

14	0.00106137	0.0130268	0.15702	0.01	0.0106522	0.112759	3.22024
15	0.00175045	0.441409	12.5288	0.416	0.0193741	0.277795	4.11683
16	0.00330951	0.495089	7.39095	0.447	0.0100069	0.244994	11.0455
17	0.00101069	0.0864443	3.88804	0.207	0.0112352	0.361269	4.91732
18	0.0866955	8.39988	34.6819	0.942	0.0122967	0.0988531	1.04199
19	1.80893	14.2465	97.4854	0.973	0.0107429	0.158255	5.78946
20	0.0067068	0.270798	13.355	0.382	0.0100977	0.0749749	1.4412
21	0.00117563	0.0145377	0.319868	0.005	0.0102593	0.0728461	2.47404
22	0.00312781	0.201769	5.01545	0.277	0.0100196	0.147284	1.70525
23	0.00122925	0.148894	3.16493	0.243	0.0122897	0.178159	1.75302
24	0.00591646	0.15281	22.5453	0.174	0.0100557	0.212376	2.88576
25	0.00111574	0.0551556	3.84141	0.163	0.0101297	0.108862	2.58936
26	0.0717084	0.991772	15.1833	0.477	0.0310213	0.310399	9.62345
27	0.00393043	1.3888	23.5224	0.64	0.0100209	0.202801	3.29303
28	0.00129942	0.0621375	6.75361	0.191	0.0177499	0.444166	12.9194
29	0.00100305	0.0408312	4.39324	0.157	0.0193202	0.235613	3.14984
30	0.0220336	0.620087	11.1189	0.364	0.0113171	0.197423	2.82565
31	0.00130129	0.0294407	0.7931	0.044	0.0110255	0.129556	2.52219
32	0.00100975	0.00844004	0.695495	0.029	0.0103087	0.222433	3.25447
33	0.00163693	0.0461354	3.50879	0.148	0.0161088	0.297524	5.15226
34	0.00124027	0.0120488	0.168305	0	0.0114739	0.145227	2.78001
35	0.0011284	0.0472759	6.41829	0.2	0.0100535	0.0634921	2.12076
36	0.00539208	1.08446	36.5049	0.609	0.0101907	0.200242	2.11304
37	0.00454517	0.182768	18.3885	0.301	0.0121877	0.162251	2.3858
38	2.39171	13.2122	77.7214	0.996	0.0109354	0.10637	1.09914
39	0.00121737	0.0284913	5.1111	0.1	0.0112865	0.119341	1.77782
40	3.18045	13.6245	89.6183	1	0.0105737	0.158027	1.98658
41	0.0189136	3.22755	52.2552	0.826	0.0168618	0.231952	6.39017

Table A1 (cont'd)

42	0.00114745	0.0257635	2.9606	0.105	0.33253	2.22035	7.31994
43	0.00144806	0.0493398	2.42333	0.101	0.0104749	0.259447	5.16424
44	0.607509	7.69769	81.5916	0.93	0.010488	0.220991	6.26197
45	0.00100698	0.0244321	2.81649	0.114	0.0102987	0.0843298	1.21917
46	0.00116371	0.00925527	0.294452	0.018	0.0124639	0.190275	3.78835
47	0.00107101	0.0174461	0.668691	0.017	0.0191083	0.298841	5.62457
48	2.02406	17.2886	91.3103	0.995	0.0103911	0.0732019	1.00331
49	0.454987	4.55784	29.4371	0.882	0.0133958	0.245541	2.32834
50	0.00107028	0.0793068	3.78062	0.185	0.0130317	0.164851	2.87338
51	7.6802	25.1136	95.5975	1	0.0103115	0.11942	1.92761
52	0.113617	18.7076	96.8917	0.944	0.0113077	0.614238	29.0536
53	0.00106174	0.00717273	0.143393	0	0.0118458	0.101169	1.76658
54	0.0130396	0.690411	9.64158	0.496	0.0161216	1.2637	19.4156
55	0.0102298	4.57319	95.3098	0.817	0.0100629	0.0624683	0.470369
56	0.00929575	0.275072	11.2714	0.292	0.0385043	0.457238	17.0146
57	0.00220981	0.470761	13.0811	0.381	0.0113547	0.217706	18.012
58	0.00101707	0.0632852	4.40264	0.123	0.0109182	0.224619	6.03399
59	0.0123878	13.7802	93.7218	0.917	0.019679	4.53909	97.7908
60	0.00101129	0.0353844	9.62122	0.184	0.0108108	0.14294	5.19079
61	0.00123077	0.0308206	8.42973	0.155	0.0119484	0.962554	67.0487
62	0.0117957	0.34634	12.2049	0.374	0.422063	8.34055	85.7711
63	0.00135027	0.0347571	1.99443	0.063	0.0189508	4.9289	99.7917
64	0.00102545	0.0231742	0.179158	0	0.106772	5.27143	99.7908
65	0.0014295	0.377945	10.9316	0.424			

Table A1 (cont'd)

Inv_equal_start1_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.00429085	0.00814871	0.0165519	0.464392	1.12535	2.62491	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.00107358	0.0672008	2.18535	0.107	0.0118433	0.171546	3.16901
1	0.00115464	0.103706	9.79239	0.259	0.010524	0.191415	17.5957
2	0.00109465	0.0476075	1.02553	0.052	0.014311	2.01575	98.6791
3	0.00359115	0.239978	9.1481	0.325	0.0150307	1.54849	99.288
4	0.0415538	7.69939	99.5212	0.824	0.0113687	1.57283	73.039
5	0.00159489	0.406397	10.6334	0.406	0.026044	3.81395	99.7873
6	0.00144076	0.365742	29.7629	0.452	0.0170525	5.57641	98.0111
7	0.048045	8.24745	78.129	0.87	0.0140264	0.245141	2.32176
8	0.00290748	0.100539	3.14439	0.138	0.0116273	0.241577	3.17549

Table A1 (cont'd)

9	10.5821	28.9524	87.6141	1	0.0103396	0.115575	3.87064
10	0.00102652	0.0575762	5.36881	0.171	0.0118666	0.149495	1.98404
11	0.709663	10.3855	91.2342	0.943	0.0187928	0.396817	3.41929
12	0.00128364	0.0551253	6.94922	0.207	0.0135882	0.694249	5.7868
13	0.0024238	0.172664	7.18422	0.344	0.0141576	0.2479	2.77678
14	0.0346845	0.54394	11.1987	0.453	0.0103066	0.180043	3.18679
15	0.00149488	0.312055	15.7213	0.39	0.0100951	0.122764	2.53617
16	0.00140568	0.0634144	3.80806	0.134	0.0102183	0.167438	3.5529
17	0.00100053	0.0878458	5.47843	0.318	0.0100011	0.082309	1.40739
18	0.348927	12.1981	56.007	0.94	0.0158593	0.236722	3.08529
19	0.595656	17.8561	95.0055	0.95	0.0102099	0.347357	6.54535
20	0.00110333	0.0779215	3.75166	0.223	0.0101154	0.12902	11.8579
21	0.00121874	0.0569526	1.94653	0.087	0.0104783	0.706459	46.1475
22	0.002905	0.117063	3.67999	0.167	0.0100097	0.291622	21.243
23	0.00173153	0.0349418	1.59997	0.066	0.0356198	0.909628	64.6999
24	0.00238939	0.42448	10.8556	0.43	0.013761	1.33182	77.6376
25	0.00100049	0.200857	15.2594	0.405	0.0101976	0.0910493	2.09445
26	0.00970083	1.0128	42.953	0.526	0.0163518	0.515468	32.1026
27	0.45332	5.48744	85.8432	0.897	0.0100827	0.0882635	1.70795
28	0.0016905	0.102638	16.5388	0.243	0.0111095	0.3216	14.1004
29	0.00100984	0.0609031	8.63269	0.233	0.0270564	2.61351	95.7627
30	0.00216465	0.449413	10.0852	0.488	0.0147786	1.16506	76.4595
31	0.00103604	0.165479	10.0559	0.34	0.0130989	1.19262	54.3113
32	0.0299141	0.801809	24.7082	0.513	0.0137474	0.363058	51.2136

Table A1 (cont'd)

33	0.00105718	0.00900553	0.204708	0.009	0.0101735	0.08256	1.07678
34	0.00107665	0.0113612	1.76902	0.08	0.0401458	1.58506	98.2464
35	0.0293336	0.577641	35.1816	0.395	0.0100935	0.177192	4.44907
36	0.00146011	0.0992281	4.11786	0.154	0.0488952	1.23735	96.065
37	0.00912564	0.219182	6.9469	0.338	0.0105011	0.170799	6.81772
38	3.09873	12.6067	84.9727	0.993	0.0100884	0.0648546	0.903389
39	0.0109239	0.445367	26.8687	0.437	0.0110232	0.154428	1.9027
40	0.966772	13.3045	86.8221	0.952	0.0118236	0.151684	1.73035
41	0.0728722	6.22065	72.2239	0.888	0.0280085	0.860107	12.5571
42	0.0014084	0.092784	7.07505	0.203	0.0145855	0.310353	4.96732
43	0.00141693	0.0500063	3.90286	0.135	0.0128045	0.493365	18.3094
44	0.82232	11.3618	72.5632	0.951	0.0157014	0.369301	5.89094
45	0.0268748	0.742669	19.2151	0.481	0.0112383	0.246557	4.1851
46	0.00211702	0.223669	11.2576	0.273	0.0126551	0.215161	3.58237
47	0.00203893	0.137785	12.4469	0.325	0.0102896	0.106576	2.35768
48	4.95453	22.5518	90.4093	1	0.0100777	0.137399	1.38953
49	0.00148663	1.18311	56.6939	0.643	0.0100915	0.171503	1.6834
50	0.00317271	0.261143	12.0568	0.331	0.0113109	0.382652	5.19302
51	1.72725	22.1687	95.2644	0.957	0.0101856	0.0539913	0.391791
52	0.059796	15.8156	95.0705	0.898	0.0100123	0.404721	14.5652
53	0.00259436	0.163782	5.91354	0.226	0.0100606	0.183312	5.248
54	0.00118524	0.130771	3.93672	0.195	0.127487	2.23806	27.3971
55	0.705691	7.98596	79.9084	0.938	0.010067	0.123935	4.22486
56	0.00802773	0.307675	9.614	0.24	0.0100606	0.0740892	0.729794
57	0.00110441	0.126096	10.2324	0.211	0.0102512	0.208051	6.01757
58	0.00351798	0.119733	13.9159	0.243	0.0109491	0.503805	7.78118
59	8.72534	33.872	99.3735	1	0.0138498	0.509134	99.1181

Table A1 (cont'd)

60	0.00101412	0.0323851	1.75339	0.122	0.0100878	0.271542	9.8996
61	0.00113938	0.0162664	1.13158	0.054	0.0107197	0.731154	58.225
62	0.0160538	0.46358	20.2742	0.388	0.0137618	2.80167	96.0608
63	0.0010898	0.0161228	0.638709	0.025	0.0102554	0.167418	31.8535
64	0.124314	2.05671	28.4254	0.73	0.014112	2.77552	87.6245
65	0.00128957	0.121449	7.04478	0.297			

Inv_equal_start1_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.00417473	0.00795147	0.0174716	0.432127	1.0889	2.46466	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.00100805	0.0857622	10.4731	0.264	0.0151058	0.806862	84.2609
1	0.00107236	0.136929	7.41561	0.308	0.0125078	0.336175	23.5554
2	0.00127223	0.012136	0.136124	0	0.139347	5.774	99.5301
3	0.00134409	0.125183	4.31995	0.238	0.0113592	1.75735	81.8446
4	0.0169071	6.26808	94.0747	0.845	0.0221238	0.911929	65.2541
5	0.00102271	0.0140358	0.905808	0.046	1.10992	26.0289	97.5412
6	0.00201053	0.103921	6.99402	0.177	0.577631	13.0279	99.936
7	6.46333	22.901	91.7266	1	0.0167217	0.415607	2.93747

Table A1 (cont'd)

8	0.00203692	0.222539	12.7029	0.298	0.0190949	0.308357	3.04025
9	4.60632	23.9739	98.31	1	0.0110383	0.111142	1.11865
10	0.0124886	0.75269	22.6114	0.504	0.0105062	0.434587	4.50805
11	0.974465	9.49394	56.856	0.955	0.0140266	0.159128	1.80763
12	0.00251723	0.128412	5.38936	0.166	0.0100142	0.0715999	2.09764
13	0.00738275	0.888606	36.0791	0.558	0.0101339	0.136391	2.27413
14	0.00104266	0.00806876	0.104342	0	0.0220137	0.265005	4.91631
15	0.001011	0.058906	13.4761	0.172	0.0119127	0.169409	2.78653
16	0.00103682	0.140186	34.0713	0.317	0.0102633	0.112323	2.07546
17	0.00102408	0.246142	8.31547	0.302	0.0110528	0.181136	3.0646
18	5.8862	22.3522	86.5292	1	0.0113332	0.0763629	0.979815
19	0.0202449	5.13409	80.531	0.827	0.0104334	0.124861	2.92623
20	0.00106424	0.0374568	17.023	0.145	0.0103114	0.236619	4.99336
21	0.00166427	0.0262197	0.440641	0	0.0107198	0.156916	8.27462
22	0.00106171	0.013039	0.459314	0.006	0.0192495	0.660505	72.6126
23	0.00102806	0.0166207	0.474678	0.019	0.0103161	0.563617	34.6957
24	0.0081278	0.47946	6.67771	0.382	0.012705	0.133035	1.31818
25	0.0018383	0.279314	40.9331	0.442	0.0104411	0.209221	3.31499
26	0.0010082	0.00459117	0.0983988	0	0.0100892	0.0926541	2.82092
27	0.114008	3.68118	40.7935	0.81	0.0100584	0.227619	19.638
28	0.00116637	0.0817729	9.74336	0.195	0.0116686	0.30767	18.0194
29	0.0451132	0.751749	19.9641	0.457	0.010241	0.183993	30.3601
30	0.0012448	0.0336775	0.499716	0.011	0.0100328	0.290234	48.4662
31	0.00121394	0.020722	1.07173	0.051	0.0116849	0.337442	11.5465
32	0.0471611	0.895937	13.8144	0.433	0.0107262	0.17796	2.36135
33	0.00197481	0.0572335	2.25826	0.098	0.0147483	0.261433	2.41278
34	0.00341316	0.255335	18.7031	0.328	0.010967	0.12952	3.00241
35	0.100541	2.27699	35.2296	0.705	0.014744	0.649837	49.1359

Table A1 (cont'd)

36	0.00101973	0.0886678	6.75099	0.165	0.0113208	0.367909	14.7132
37	0.00114159	0.0308259	6.73178	0.19	0.0103186	0.312366	7.04704
38	1.64132	15.605	74.2089	0.994	0.0105285	0.102798	0.95692
39	0.00103162	0.0414146	4.30549	0.221	0.0104386	0.0955652	1.50399
40	6.78231	26.5558	86.7327	1	0.0103525	0.0854404	1.28056
41	0.130436	7.2986	96.2257	0.922	0.015688	0.581137	9.21139
42	0.00102235	0.0454548	3.0615	0.169	0.0166389	0.525057	10.4017
43	0.00139809	0.150778	3.19609	0.244	0.0160192	0.596622	7.81465
44	1.89242	13.6894	94.4713	0.964	0.0106311	0.168664	1.97439
45	0.00293653	0.633376	22.1847	0.544	0.0101577	0.135806	9.37646
46	0.00124449	0.3004	20.0301	0.477	0.0160102	0.171855	1.56937
47	0.00117653	0.0632237	2.44524	0.177	0.0115946	0.318531	7.18109
48	4.15893	25.6668	99.7738	1	0.0121767	0.192822	2.39316
49	0.769139	8.17733	74.9757	0.942	0.0105853	0.189293	2.35566
50	0.012155	0.705362	19.3618	0.503	0.0100314	0.156768	2.08814
51	11.9689	35.1157	94.4833	1	0.0103247	0.0812831	0.963407
52	1.25429	25.3998	99.039	0.953	0.0119925	0.624848	13.5241
53	0.00104508	0.0823909	9.82985	0.315	0.0101356	0.178296	23.7369
54	0.00100911	0.214823	9.39912	0.365	0.0379873	1.79127	26.1662
55	0.514402	3.83512	94.9079	0.861	0.0102049	0.0899015	6.25684
56	0.0611217	1.52605	21.4895	0.653	0.0124567	0.274312	6.82569
57	0.00101954	0.0843765	12.5778	0.252	0.010068	0.333249	10.6992
58	0.00101072	0.0576394	4.24941	0.214	0.0102055	0.402	7.84453
59	4.63219	19.4792	98.6422	1	0.0339482	4.25798	85.441
60	0.00121466	0.0987079	5.57484	0.203	0.0108684	0.71191	22.5964
61	0.00186716	0.102505	11.803	0.169	0.0173771	1.38057	98.439
62	0.0011218	0.0203618	0.734701	0.042	0.0160729	0.70398	48.4084
63	0.00233229	0.373303	25.6601	0.448	0.011124	0.640607	66.8124

Table A1 (cont'd)

64	0.00102216	0.266972	15.3828	0.472	0.0798023	3.56306	79.6967
65	0.00139407	0.0279186	7.19014	0.144			

Inv_equal_start2_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.00446666	0.00790886	0.015797	0.428035	1.07606	2.5433	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.00100907	0.125705	10.6839	0.334	0.0233585	0.874047	96.8933
1	0.00105974	0.143719	7.60071	0.249	0.0107422	0.804107	28.1658
2	0.00109097	0.0166498	0.436244	0.003	1.87133	19.4072	99.4495
3	0.00100747	0.0497398	2.5462	0.152	0.0102984	1.79422	71.0352
4	0.00297705	4.52358	67.5759	0.861	0.0106737	0.327697	34.3645
5	0.0010567	0.00989304	0.420187	0.007	1.22901	26.4878	99.1037
6	0.00106287	0.0452119	1.99331	0.092	0.172158	11.1461	97.3605
7	5.7357	24.2633	79.7177	1	0.0265742	0.35858	2.90058
8	0.00636034	0.320268	16.0661	0.37	0.0312295	0.28523	4.04812
9	6.27669	26.5318	82.4724	1	0.010854	0.139634	1.69558
10	0.00856725	0.576461	17.0669	0.381	0.0101059	0.463156	3.53432
11	0.552453	7.0638	91.7967	0.907	0.0120705	0.146583	2.41673

Table A1 (cont'd)

12	0.00291394	0.136416	4.91551	0.227	0.0102752	0.102745	2.41156
13	0.0082914	0.587562	30.2009	0.455	0.0109787	0.261871	3.75798
14	0.00100516	0.00742694	0.130895	0.002	0.0105256	0.115117	1.76734
15	0.00333167	0.163771	13.8725	0.274	0.0101499	0.123247	1.93005
16	0.00109584	0.189935	11.9487	0.314	0.0117946	0.119962	3.91788
17	0.00103836	0.192703	8.22607	0.348	0.0100811	0.137762	2.29638
18	4.88944	22.621	66.0523	1	0.0110016	0.0910147	0.951924
19	0.0769002	6.54535	90.7232	0.805	0.0108679	0.211231	10.7691
20	0.00102541	0.0154989	8.99211	0.065	0.0113002	0.405228	8.70325
21	0.00115925	0.0211499	0.552478	0.016	0.0101962	0.156121	12.0688
22	0.00101247	0.0194171	1.50941	0.067	0.0144659	1.60614	86.217
23	0.00104176	0.012079	0.163172	0	0.0149491	0.776992	27.8123
24	0.0279397	0.635591	15.9447	0.429	0.0101286	0.0736256	0.540789
25	0.00126944	0.118856	15.0762	0.307	0.0103736	0.23243	17.6919
26	0.00101132	0.00656779	0.0648818	0.004	0.0102961	0.0927211	4.63671
27	0.0343594	3.60573	39.1888	0.858	0.0144832	0.18236	15.1113
28	0.00100872	0.0544919	9.284	0.242	0.0101508	0.683723	49.8109
29	0.0365511	0.57164	12.7308	0.381	0.0108383	0.153251	14.4767
30	0.0203689	0.18591	3.66457	0.137	0.0135973	0.813953	85.2584
31	0.00126639	0.055986	1.19884	0.051	0.014666	0.709112	47.1843
32	0.0752835	0.662514	12.805	0.338	0.0102758	0.366903	8.44987
33	0.0012342	0.075278	4.96168	0.211	0.0105763	0.141415	1.88864
34	0.00240661	0.109282	19.6805	0.172	0.0107303	0.175881	29.3052
35	0.182189	1.71019	16.1353	0.678	0.0195489	1.19394	29.1392
36	0.00101974	0.0342376	1.23536	0.06	0.0133949	0.516874	37.7584
37	0.00100426	0.0301719	3.38118	0.187	0.0100976	0.578183	22.7065
38	4.36946	16.9294	62.2322	0.996	0.0106223	0.121818	1.13412
39	0.00122927	0.134233	9.17751	0.303	0.0102872	0.136067	1.40963

Table A1 (cont'd)

40	9.22647	30.0001	95.712	1	0.0125936	0.108597	1.48709
41	0.0893444	7.11942	80.7742	0.897	0.0146991	0.232295	9.35024
42	0.00101695	0.0213696	1.93871	0.07	0.0393825	0.954351	10.8365
43	0.00504926	0.225091	3.37832	0.233	0.0545706	1.11893	11.6424
44	2.26711	17.0458	91.746	0.991	0.0109884	0.136876	1.35371
45	0.00460743	0.416469	14.5506	0.487	0.0100404	0.243257	6.98706
46	0.00107801	0.193324	11.1338	0.294	0.0119189	0.243489	4.51287
47	0.00114902	0.181235	4.26222	0.299	0.0110971	0.256588	3.31582
48	4.06824	22.636	94.5934	1	0.0185031	0.368901	2.6534
49	0.712861	7.82222	76.8925	0.901	0.0105077	0.244551	2.82357
50	0.0139081	0.372348	7.28064	0.318	0.0163065	0.164507	2.73705
51	9.04328	35.992	92.6138	1	0.0106722	0.0716053	0.523532
52	0.62941	25.4923	98.1791	0.949	0.0338421	1.13365	50.7094
53	0.00103803	0.0964759	16.5583	0.236	0.0104358	0.158167	10.3263
54	0.00105147	0.197723	15.105	0.35	0.0108458	0.673085	20.4039
55	0.632017	4.39749	41.293	0.899	0.0103556	0.109509	8.8533
56	0.0129979	1.10365	22.2463	0.576	0.0116454	0.303441	5.48885
57	0.00551723	0.281551	18.2699	0.356	0.010461	0.2704	6.49247
58	0.00108526	0.0758303	7.60688	0.267	0.022836	0.663951	18.4919
59	4.7871	19.6101	69.6912	1	0.164319	7.67691	97.4882
60	0.00319379	0.184443	10.136	0.263	0.205494	3.18297	96.762
61	0.001675	0.149024	14.8588	0.235	0.0213934	3.18778	94.1924
62	0.00100935	0.0116053	0.466163	0.021	0.0109945	1.00321	56.5473
63	0.00443774	0.271236	25.6601	0.297	0.0101869	0.527549	60.9016
64	0.00109055	0.262767	25.0906	0.469	0.317582	5.78024	99.9909
65	0.00243804	0.165812	9.16107	0.31			

Table A1 (cont'd)

Inv_equal_start2_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.00318061	0.00675866	0.0160844	0.438561	1.06067	2.41172	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.00502413	0.275894	18.1013	0.3	0.0143192	1.41292	65.3637
1	0.0474332	2.17049	45.3412	0.68	0.410013	6.84451	99.8212
2	0.00102832	0.19294	16.8002	0.378	0.0100746	0.111329	4.25654
3	0.00458857	0.346685	6.58853	0.407	0.213468	15.1023	99.2657
4	1.86294	13.5125	94.7403	0.975	0.75908	21.4501	98.6561
5	0.00700134	0.304912	7.9759	0.327	0.0212627	0.744526	19.1871
6	0.0013601	0.261695	26.1536	0.463	0.133582	7.16849	97.4963
7	2.88853	20.0847	82.6775	0.981	0.0101104	0.0783194	1.33528
8	0.0290039	0.630397	9.46137	0.429	0.0208605	0.303382	3.36942
9	2.91789	28.7467	95.2546	0.978	0.0102839	0.102794	1.21645
10	0.00156492	0.112514	12.5948	0.185	0.0117909	0.383753	6.35577
11	0.935209	7.03224	43.7697	0.962	0.0100609	0.130368	2.14591
12	0.00890517	0.291862	46.2875	0.35	0.031789	0.583909	9.39267
13	0.00105665	0.132445	43.6615	0.27	0.017856	0.294552	2.91591
14	0.00256102	0.207932	8.69643	0.285	0.0181421	0.189499	2.58483
15	0.00101132	0.126156	20.6455	0.383	0.0108713	0.760869	10.3061
16	0.0119164	0.786219	32.9788	0.461	0.0120135	0.112504	0.932407

Table A1 (cont'd)

17	0.0010271	0.0241452	0.743541	0.042	0.0112094	0.0810111	0.921585
18	0.086908	12.4754	88.0088	0.916	0.0128459	0.112415	1.87788
19	0.177693	20.7888	99.5097	0.942	0.0106606	0.150458	1.69894
20	0.00135616	0.0838976	8.66682	0.272	0.0101002	0.164449	12.81
21	0.00204494	0.568019	18.3001	0.457	0.0100081	0.144975	23.5449
22	0.0013945	0.0848974	7.27401	0.247	0.0142137	0.186391	2.83818
23	0.00301625	0.232187	63.5821	0.364	0.0104427	0.551117	11.4954
24	0.00133484	0.127603	9.75935	0.306	0.0130609	0.399793	13.3466
25	0.00107714	0.346064	27.789	0.501	0.0356138	0.552334	6.26802
26	0.00236789	0.258386	10.2732	0.272	0.0147729	0.157786	3.50662
27	0.471131	5.9353	35.7387	0.943	0.0100103	0.190617	19.1439
28	0.00830152	0.4297	8.5731	0.424	0.0106082	0.140126	1.1798
29	0.00101525	0.0679147	20.1911	0.266	0.0112169	0.320862	42.3648
30	0.0012756	0.179332	7.78336	0.316	0.0149742	0.224787	5.32374
31	0.00144528	0.0988441	8.73694	0.201	0.0102755	0.168685	2.79389
32	0.00348071	0.0768184	12.9501	0.179	0.0102053	0.119157	0.941314
33	0.00110958	0.298511	13.3218	0.401	0.0101201	0.0900268	0.770271
34	0.00962275	1.47125	41.5002	0.677	0.010085	0.076181	0.83009
35	0.0110131	0.389347	22.6464	0.336	0.0128238	0.33692	5.26116
36	0.00100094	0.0138125	0.60511	0.04	0.0100732	0.071141	2.87217
37	0.00830569	0.294926	13.9284	0.322	0.0122705	0.0849288	1.47811
38	0.419885	8.44863	95.0678	0.912	0.0105016	0.0958048	0.93141
39	0.00101985	0.0313944	6.26309	0.183	0.0102249	0.216943	4.5763
40	0.0371113	11.269	99.8639	0.868	0.0108009	0.157727	2.10734
41	2.31819	14.7858	72.9467	1	0.124573	2.34339	13.2128
42	0.0010136	0.0105951	0.401469	0.032	0.011382	0.298483	3.75386
43	0.0325377	1.10058	16.4159	0.568	0.0108563	0.14642	3.56819
44	0.0358509	13.0913	98.882	0.901	0.0101136	0.23957	3.23502

Table A1 (cont'd)

45	0.00100106	0.0104729	0.381137	0.028	0.0111784	0.186445	9.19574
46	0.0147657	0.294552	9.12053	0.28	0.0101363	0.117973	1.25305
47	0.00114628	0.146867	18.5523	0.355	0.0128479	0.178052	4.00755
48	4.22747	27.4162	98.5715	1	0.0103158	0.197874	3.9395
49	0.148125	5.20371	59.0153	0.794	0.0116927	0.234271	3.39294
50	0.00104956	0.0368843	7.39984	0.122	0.0122334	0.10155	3.44961
51	13.5698	40.4231	96.1037	1	0.010617	0.0658333	0.503109
52	9.3036	37.0937	98.8099	1	0.0117185	0.335546	6.64304
53	0.00100426	0.120222	19.5526	0.269	0.0505469	2.18019	57.2668
54	0.00792383	0.237193	10.7536	0.197	0.0160651	1.03479	40.9518
55	0.151571	4.92145	68.0339	0.856	0.0183902	0.761737	15.6552
56	0.00102746	0.0563684	7.93836	0.149	0.0106865	0.0932771	1.52285
57	0.00123229	0.0525095	7.19722	0.12	0.0109731	0.148259	2.39799
58	0.00103644	0.13476	26.3938	0.376	0.0364812	0.238501	2.9092
59	7.67227	31.8465	99.6906	1	0.0105898	1.41014	31.262
60	0.00119279	0.0618987	6.12199	0.132	0.0248456	2.80661	88.07
61	0.0232324	0.446653	23.9877	0.375	0.0356153	4.34533	99.6851
62	0.00150229	0.0484313	0.933154	0.026	0.0848174	3.40899	72.7468
63	0.00365614	0.365755	6.39932	0.325	0.0660389	1.58403	93.2484
64	0.00121012	0.166467	4.63249	0.294	0.0215872	1.66289	99.6715
65	0.0013941	0.199878	10.3072	0.256			

Table A2. Key for terms for summaries for all omegaMap analyses for spotted (*Crocuta crocuta*) and striped (*Hyaena hyaena*) hyena MHC *DQB* sequences.

Prior distributions used	
impinv =	improper inverse
inv =	inverse
Codon frequency	
cat =	Codon frequencies from domestic cat (*Felis catus*)
equal =	Equal codon frequencies
Start1 or Start2 = 2 different sets of starting values of each following parameter:	
Mu	
Kappa	
Indel	
Omega	
Rho	
Rep1 or Rep2 = repetition of each set of starting values	

Table A2 (cont'd)

Impinv_cat_start1_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000612	0.020013	0.125141	0.710084	1.36911	2.67672	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	5.97E-05	0.031268	121.454	0.239	0.852488	133.524	112897
1	0.000527	0.321515	299.34	0.34	8.49E-13	1.06E-08	3.30E-05
2	0.000462	0.250333	1855.29	0.355	3.25456	4235.34	1.34E+06
3	3.35E-08	3.90E-05	0.010498	0	2.68E-06	0.000736	0.150123
4	0.022463	2.18183	76.9571	0.724	1.64E-10	3.76E-07	0.011319
5	7.29E-11	4.72E-09	8.69E-06	0	0.00044	0.291036	134.78
6	1.70E-10	4.70E-07	0.012204	0	5.43E-06	0.048918	92.7884
7	1.8718	22.8657	2842.51	0.987	7.23E-11	5.36E-08	7.59E-06
8	0.443453	5.42233	514.35	0.83	9.48E-12	4.78E-05	1.887
9	3.34814	35.675	1889.63	1	0.009325	0.287909	9.92995
10	2.50E-12	8.53E-10	1.32E-05	0	4.72E-07	8.78E-05	0.006605
11	8.99279	87.5885	2684.42	1	5.86E-07	0.084158	144904
12	0.116065	3.80096	322.03	0.707	0.000816	0.753156	557822
13	0.000193	0.080496	5.54552	0.172	0.019602	23.8671	45633.3
14	1.94E-08	2.58E-05	0.001586	0	15.7891	3.77E+06	4.79E+10
15	0.156757	5.71058	1279.35	0.826	1.19E-06	0.001504	1.03451
16	5.91E-06	0.002242	2.35675	0.027	5.71E-11	2.29E-06	0.016041

Table A2 (cont'd)

17	4.16E-06	0.000675	0.331657	0	3.93E-09	1.30E-06	0.00041
18	2.76102	43.5098	1263.44	1	2.02E-06	0.000305	0.113805
19	0.709946	8.44403	192.962	0.92	0.001958	0.077957	29.0707
20	0.000373	0.070353	7.1772	0.192	1.40E-09	5.60E-06	0.128708
21	1.85E-05	0.001866	0.396638	0	54613.2	3.79E+07	3.85E+09
22	0.000111	0.012426	0.456685	0.003	0.000541	1.17358	15339.8
23	4.62E-11	9.52E-09	4.10E-07	0	88.0354	4570	288128
24	2.45E-05	0.045498	12.638	0.388	35344.1	1.55E+09	1.14E+15
25	3.58E-06	0.002153	277.749	0.126	9.28E-07	0.015293	0.891649
26	1.98E-11	1.08E-05	21.6245	0.146	5.65E-09	1.41E-07	9.78E-06
27	0.06944	4.15913	7415.41	0.683	0.006675	0.206259	5.81414
28	0.430781	7.31962	274.675	0.91	0.003317	0.096724	2.11393
29	0.464983	5.31193	226.285	0.862	0.010499	0.695378	116.746
30	0.703382	9.1462	498.889	0.912	0.00023	0.009839	1.47263
31	5.39E-10	1.60E-07	0.000251	0	0.001612	22.9585	103668
32	2.44E-05	0.027881	19.9087	0.186	5.72E-07	0.001895	0.302467
33	0.492568	7.58116	188.533	0.937	0.000291	0.019791	1.17038
34	2.18E-11	2.79E-08	4.22E-05	0	8.09E-06	0.00482	0.196626
35	3.52E-08	0.035711	623.682	0.469	6.36E-06	0.00032	0.242324
36	0.000726	2.23779	44.676	0.791	0.001787	0.081341	3.7414
37	1.52E-06	5.31E-05	0.017223	0	0.000221	0.220756	7.88952
38	2.39023	27.2066	1162.56	0.994	2.13E-08	8.76E-06	0.005247
39	8.40E-07	0.002873	34.026	0.292	0.023083	20.7565	117289
40	2.82E-08	0.009319	1190.36	0.201	0.089838	7.58053	3259.67
41	0.09014	1.63903	25.2947	0.669	1.84E-07	0.06295	100.891
42	1.27E-09	2.12E-06	0.000794	0	1.57E-07	0.002854	1740.95
43	0.000347	0.200154	8.80417	0.344	2.92E-10	0.000222	24.2346

Table A2 (cont'd)

44	1.97439	32.6804	2297.29	0.971	0.01484	3.62135	4299.44
45	0.000647	0.481968	1047.36	0.433	9.70E-18	9.42E-13	5.16E-08
46	4.55E-06	0.001053	0.369674	0.003	25.9575	25631.5	1.53E+07
47	0.000777	0.063284	12.883	0.197	1.19E-08	1.27E-05	0.007067
48	2.98763	27.4516	489.002	0.992	0.018544	1.32628	85.8887
49	2.49E-05	1.87975	75.597	0.783	2.29E-05	0.052469	5.33812
50	2.40E-05	0.067224	12.5713	0.226	0.000355	0.596888	1254.52
51	3.55847	47.547	1479.02	1	0.072027	6488.57	4.27E+07
52	0.350819	27.2601	1100.02	0.944	13.5268	6273.88	1.57E+06
53	0.001078	0.871542	6623.46	0.396	183.047	1.72E+09	6.59E+13
54	1.75E-07	0.003539	187.962	0.209	3.37E-08	0.025704	23.0367
55	0.617308	12.0884	775.367	0.972	0.00018	0.202828	36.0185
56	9.58E-06	0.003793	0.44675	0.02	0.111308	12.178	10200.2
57	1.85E-05	0.002625	0.565761	0	7.71E-05	0.003554	5.08546
58	0.298084	14.8439	56946.6	0.898	0.000443	0.062853	174.325
59	0.964495	11.9907	744.091	0.962	0.000491	1.10088	4577.22
60	9.88E-13	1.76E-08	1.24E-05	0	1.27E-08	1.26E-06	8.18E-05
61	0.000511	0.228713	13222.8	0.185	4.28E-06	0.002161	4.99063
62	1.29E-07	0.054195	136.04	0.379			

Impinv_cat_start1_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000138	0.010137	0.103827	0.583295	1.17816	2.31723	0	0	0

Table A2 (cont'd)

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	7.82E-05	0.057206	16.1655	0.281	0.184917	6430.75	1.00E+06
1	0.000682	0.603792	72.428	0.486	6.97E-09	1.89E-06	0.00024
2	0.010142	0.601841	19.2041	0.491	19.6496	1326.26	47608.6
3	7.19E-06	0.00109	0.560504	0.006	2.67193	193.678	4526.19
4	0.25751	5.01372	196.706	0.817	0.542749	502.349	27373.9
5	1.55E-08	0.004225	8299.73	0.356	3.12E-09	5.81E-05	8.23333
6	0.000141	0.546665	4832.63	0.383	0.000805	0.145247	8.46827
7	0.576184	61.4706	13827.2	0.969	4.63E-08	0.000651	2.38945
8	4.01E-06	0.010734	5.79204	0.269	0.221839	3.42729	98.7106
9	2.45756	55.0957	5165.66	1	7.05E-13	3.82E-09	0.000439
10	0.001257	0.506328	868.913	0.353	6.49E-10	2.30E-06	0.399005
11	11.3259	102.687	12118.2	0.97	1.18E-05	0.002266	0.686746
12	0.195516	5.55289	765.618	0.699	0.001059	0.090844	2.81656
13	8.87E-05	0.027611	3.18963	0.153	464814	7.25E+07	1.73E+10
14	8.60E-06	0.002717	0.251752	0	0.000381	7.8773	15801.1
15	0.148275	13.4252	10654.8	0.745	4.20E-07	0.000263	0.163996
16	0.000284	0.107033	12.9471	0.272	0.106788	152.199	13444.2
17	0.00017	0.015293	2.53076	0.095	0.030269	11.1326	21221.8
18	4.42632	59.4858	8183.06	1	1.97E-11	2.32E-06	1.4312
19	1.17371	21.8877	994.832	1	7.86E-09	4.63E-05	0.226724
20	5.40E-06	0.039683	1815.37	0.277	7.62E-11	0.00044	3.19024
21	0.002338	1.09945	161.65	0.503	7.01E-06	0.030613	4.91873
22	0.003106	2.33227	125158	0.535	0.001911	0.105984	5.35073
23	0.014068	1.32248	390167	0.356	0.001342	0.088037	3.30321

Table A2 (cont'd)

24	0.004198	1.11628	29.9833	0.661	4.87E-08	0.000391	0.915953
25	5.72E-05	0.036882	251.269	0.273	6.51E-07	5.44E-05	0.047913
26	0.000455	0.04469	5.26646	0.106	7.09E-06	0.000984	0.131863
27	0.067825	6.57802	317.061	0.763	2.90E-06	0.000297	0.575177
28	0.279301	8.28099	313.175	0.906	0.000453	0.041023	1515.85
29	0.346772	10.9266	645.071	0.799	7.19E-06	0.01147	6.54162
30	0.382382	10.3206	1568.01	0.892	4.36E-07	0.000185	0.729609
31	0.000339	0.737932	1926.94	0.396	9.44E-07	0.001171	2.41481
32	0.000661	0.020745	1.64803	0.081	0.203482	135.346	26355.2
33	1.05124	13.2548	930.052	0.98	1.03E-05	0.016154	21.1122
34	2.46E-12	2.24E-07	0.022043	0	6.73E-11	1.27E-07	0.0013
35	0.000942	0.07768	62.402	0.13	0.000288	0.02108	1.80599
36	0.005608	0.674854	13.9294	0.583	0.080907	10023.2	6.88E+06
37	2.71E-06	0.043523	29539.9	0.29	7.25E-06	4.9053	1.03E+06
38	3.22996	64.9124	8351.22	1	0.003756	0.161753	9.66355
39	0.000967	0.663476	3489.43	0.375	0.000904	0.439393	194.566
40	6.21E-07	0.1149	144.135	0.502	7.00E-05	1.55913	18255.1
41	0.053763	14.027	5010.07	0.835	2.22E-09	2.82E-07	5.24E-05
42	4.42E-06	0.00867	13.4497	0.211	5.35E-10	4.21E-06	0.164253
43	0.058386	4.23006	1494.68	0.573	0.11558	4.91314	212.993
44	2.41994	35.3683	2138.16	0.995	0.032295	17.6623	57964.4
45	9.19E-06	0.001531	0.106445	0	9.59E-05	0.168259	138.575
46	1.52E-07	3.74E-06	0.000141	0	8.38E-08	9.17E-05	0.041385
47	3.90E-11	3.40E-07	0.297816	0.001	1.19E-06	0.000119	0.0323
48	0.432284	18.6019	3437.55	0.926	2.02E-06	0.003549	5.21173
49	0.192839	25.6443	11039.4	0.866	7.15E-05	0.001034	0.161819

Table A2 (cont'd)

50	4.80E-06	0.005669	428.562	0.176	0.003439	0.490517	32.5975
51	6.98185	82.514	4891.06	1	2.17E-10	0.003165	2.56E+06
52	4.31113	70.2999	5025.3	0.996	0.013448	5.90671	8845.64
53	0.000281	1.08895	7289.95	0.588	0.002294	1.02354	35.9976
54	0.000103	0.11359	288.152	0.27	0.000847	94.9867	451224
55	0.002909	3.64047	1004.65	0.801	0.000396	0.312021	108.177
56	5.66E-06	0.004612	778.128	0.228	65.3673	5891.76	2.50E+06
57	2.65E-07	0.00019	7.9176	0.081	0.13215	243.346	31273.4
58	0.086329	18.0377	6012.85	0.837	1.34E-06	0.005857	1.00999
59	0.92259	19.3784	1255.49	0.974	7.51247	2007.45	1.30E+06
60	0.079465	1.03789	11.1616	0.534	3.59E-10	6.88E-08	0.001201
61	4.57E-11	2.42E-08	3.51E-05	0	0.000778	29.9266	103987
62	4.60E-05	0.017551	28.321	0.25			

Impinv_cat_start2_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
6.50E-05	0.004455	0.054965	0.672072	1.34753	2.83883	0	0	0

Table A2 (cont'd)

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	1.36E-05	0.000243	0.005048	0	3.80E-07	3.21E-05	0.002412
1	2.09E-05	0.247815	12.5001	0.514423	395.236	11016.1	1.06E+06
2	0.000341	3.78772	119148	0.567308	4.85E-05	0.008222	1.20891
3	0.008309	4.07848	18242.1	0.548077	1.14699	14.1247	1308.48
4	5.99E-07	7.15E-05	0.728175	0.004808	98894.6	1.93E+07	2.13E+09
5	0.000446	0.015745	1.32527	0.040865	3.47E-10	5.30E-07	0.0006
6	3.33E-06	0.000349	0.11179	0	0.016318	0.410539	5.48102
7	8.90222	108.822	4025.43	0.992788	3.41E-08	1.67E-05	0.06749
8	0.535696	22.1937	14934.4	0.882212	1.59E-07	0.000334	0.037172
9	21.2829	538.22	61661.5	1	17.5711	3985.46	3.00E+06
10	2.78E-05	0.001003	0.32016	0	0.125273	3.14617	290.542
11	41.8368	313.402	6648.97	1	7.64E-07	3.39E-05	0.004699
12	0.00266	0.514288	18.9619	0.504808	0.001021	0.038053	0.420856
13	0.012819	0.411348	27.2569	0.360577	3.28E-05	0.001688	0.12053
14	0.000182	0.087172	2.14218	0.15625	0.332102	2.59285	74.1854
15	3.222	25.1876	437.768	1	0.006789	2.33148	80.9274
16	0.157237	5.77289	3291.81	0.574519	2.02E-07	1.56E-06	9.65E-06
17	0.100308	2.68746	618.711	0.504808	0.100752	2.0338	12.364
18	7.63942	148.596	50624.5	1	1.64E-09	1.38E-07	1.78E-05
19	4.14542	67.6101	7102.22	1	6.66E-06	0.006818	0.991289
20	0.015603	1.34081	2418.55	0.480769	1.39E-07	8.85E-07	6.47E-06
21	0.010997	6.08019	10296.8	0.673077	0.002216	0.080986	5.24648
22	8.31E-09	1.53E-06	0.000523	0	0.000754	0.123034	3.83428

Table A2 (cont'd)

23	7.08E-06	0.000116	0.002079	0	2.23E-09	5.16E-08	4.89E-06
24	1.32047	29.7151	7693.93	0.975962	4.55388	597.89	19817.1
25	6.84E-07	2.03E-05	0.000728	0	2.93E-09	3.84E-07	6.87E-05
26	8.26E-11	2.30E-08	8.69E-07	0	175.055	8272.07	325182
27	0.320784	51.8376	226276	0.867788	1.69728	63877.9	2.42E+07
28	0.000138	0.571108	59.4927	0.685096	363.884	7349.87	143933
29	1.13917	29.9062	3104.87	0.987981	5.73E-09	1.24E-06	0.000124
30	0.026511	2.75916	40.072	0.721154	0.008492	0.140294	8.0325
31	1.15E-06	0.000231	0.029598	0	2.36E-05	0.001985	0.054206
32	0.003171	0.168345	37.349	0.235577	0.045546	2.14823	135.291
33	2.55853	19.5898	426.335	0.990385	2.53E-14	6.76E-11	9.91E-06
34	4.65E-05	0.001011	0.041835	0	0.056661	223.683	64994.5
35	0.001184	0.018112	0.409395	0.004808	120.651	2894.12	72404.5
36	0.974558	11.7428	101.931	0.995192	0.004032	0.194639	18.8498
37	0.330863	25.0419	22245.4	0.850962	0.002126	0.044612	3.16187
38	0.000169	1.12454	90.7441	0.622596	0.040432	1.65743	17.6145
39	0.000231	0.150827	31.4807	0.502404	72343.6	2.03E+09	6.76E+11
40	0.005507	4.4806	42064.2	0.600962	2.69E-10	6.90E-08	1.51E-05
41	0.000593	0.074807	13.5006	0.353365	0.042219	3.22578	147.425
42	0.008957	12.536	43731.6	0.625	64.4327	686.092	8965.83
43	0.008147	1.19638	123.119	0.514423	20.9468	864.087	180298
44	15.0103	211.537	13265.4	1	2014.45	2.43E+08	2.79E+11
45	1.91E-08	1.23E-05	0.001087	0	6.40E-06	8.56E-05	0.001164

Table A2 (cont'd)

46	0.020102	0.61704	1573.02	0.302885	44.8423	1133.68	49025.4
47	0.007111	1.41597	613.834	0.512019	0.002575	3.22467	325877
48	8.87E-05	2.19389	92.3716	0.730769	8.82E-10	7.06E-07	0.000262
49	0.00526	3.21921	185.947	0.699519	8001.82	9.81E+06	1.84E+09
50	0.008516	2.05697	152.422	0.629808	2.54E-05	0.084724	10.3422
51	0.002387	8.31348	193.012	0.819712	0.000926	0.06902	3.16888
52	6.14E-05	0.547222	156.097	0.569712	0.000564	0.056447	2.59255
53	0.250095	6.0343	74.4497	0.911058	1.99E-05	0.000272	0.012159
54	3.94E-07	0.000169	0.034507	0	0.963978	19.1005	1608.12
55	2.67549	72.4816	2951.05	1	8.36111	6067.94	348085
56	0.000251	0.003975	0.233407	0	6.61E-07	0.000208	0.192836
57	1.86E-06	2.69E-05	0.000785	0	2.23E-05	0.004957	0.424925
58	1.77641	78.9923	6738.14	0.992788	0.000293	0.266236	40.1468
59	4.7661	36.7194	1002.91	1	0.098101	165.777	225386
60	0.106823	8.43672	2364.18	0.675481	6.04E-09	1.02E-06	0.001729
61	0.001592	0.47713	425.136	0.394231	17.7048	1192.43	31788.5
62	0.044756	2.49885	11347.9	0.524038			

Impinv_cat_start2_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000113	0.005877	0.067674	0.728813	1.45615	3.30693	0	0	0

Table A2 (cont'd)

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.349157	6.2811	407.906	0.707921	5.10E-10	7.15E-07	0.000146
1	7.97E-05	0.431379	20.3643	0.497525	3.14E-07	1.22E-05	0.001238
2	0.009708	2.28475	6989.47	0.480198	360.595	151967	4.64E+07
3	0.011755	0.509661	140.417	0.435644	0.120443	4.5561	56.1056
4	0.000791	1.01574	58.0973	0.608911	8.60E-12	2.59E-08	0.000237
5	2.31E-05	0.008179	1.03931	0.017327	396.191	13459.3	607466
6	4.05E-05	0.006379	0.471705	0.002475	6.91E-05	0.001951	0.074199
7	5.58E-05	0.366609	68.9146	0.542079	0.110059	16.8168	4445.49
8	0.641749	29.1827	1870.85	0.938119	2497.38	531003	1.93E+07
9	9.88E-05	0.274446	46.4787	0.537129	0.002107	0.067232	4.87017
10	0.00042	0.351206	213.354	0.475248	807.729	33819.7	1.41E+06
11	29.0421	388.144	26212.1	1	0.000213	0.010009	0.201315
12	0.669642	36.0875	713817	0.856436	0.009095	0.103849	1.0936
13	0.007976	2.01146	30.4385	0.769802	0.013551	0.214479	0.889797
14	0.448453	4.43311	71.4667	0.767327	1.34E-05	0.000503	0.072126
15	0.001526	0.663096	12.2976	0.678218	0.157285	1.47609	16.0951
16	0.075618	2.81754	66.9996	0.668317	1.31E-07	2.22E-05	0.023666
17	6.05E-06	0.182349	1941.82	0.465347	0.000254	0.04164	3.80989
18	4.01615	81.6777	3921.19	1	0.006776	0.295631	7.27762
19	0.637689	22.15	851.016	0.90099	3.72E-07	0.001631	4.29547
20	4.23E-06	0.00014	0.00196	0	4.55E-06	0.00092	2.18527
21	0.001283	0.878542	514.433	0.519802	229547	2.26E+08	9.32E+10
22	7.10E-08	1.42E-05	0.001088	0	0.000124	0.014235	0.220993

Table A2 (cont'd)

23	0.002295	2.05294	837.245	0.544554	24.8432	16916.3	1.63E+06
24	0.000113	0.402915	15.6449	0.636139	0.292324	7.42851	278.841
25	0.115564	1.83071	84.5752	0.579208	5.27571	873.515	64891.9
26	0.000509	0.024559	69.272	0.14604	0.796674	318.574	29296.9
27	9.95E-06	0.028826	5.98392	0.170792	8.30E-05	0.002914	0.167813
28	0.003865	1.60852	138.619	0.638614	3.85E-07	1.45E-05	0.000858
29	1.19E-05	0.005177	8.50334	0.123762	2.79E-06	7.35E-05	0.003538
30	0.033986	3.83888	258.58	0.772277	0.00196	0.15733	3.26967
31	0.010312	1.73572	12874.1	0.492574	2.47E-05	0.00807	2.05261
32	0.447027	26.2231	10032.2	0.839109	1.67E-08	4.21E-07	6.77E-06
33	1.01097	10.489	332.747	0.95297	0.004204	0.205999	5.27723
34	0.044649	17.0949	39210.2	0.467822	263987	2.96E+06	3.79E+07
35	2.99E-05	0.000254	0.004061	0	8.51E-08	1.58E-05	0.006284
36	1.03374	51.4116	7566.65	1	0.010363	0.459681	4.89106
37	1.49E-08	5.21E-05	0.047348	0	0.001363	0.067414	3.22235
38	3.24063	78.9167	3220.71	1	0.01027	0.241938	32.3722
39	0.013686	2.82742	1900.11	0.564356	0.064077	1.30658	70.031
40	4.47E-07	8.15E-06	7.44E-05	0	346755	5.59E+07	9.01E+08
41	0.943931	5.54799	53.9584	0.955446	3.82E-08	1.09E-05	0.001684
42	3.61E-07	4.28E-05	0.003519	0	0.589523	117.818	40318.9
43	9.65E-06	0.005571	2.13046	0.10396	1858.79	21574.6	298597
44	5.98479	83.1972	2903.27	1	135.701	5049.8	122773
45	0.002662	0.021718	0.22815	0	0.008054	1.13064	374.736
46	0.000933	0.195421	449.617	0.35396	753.479	120301	5.35E+06
47	7.56E-05	0.327013	577.705	0.410891	2.95E-05	0.000754	0.01165

Table A2 (cont'd)

48	2.58989	81.6914	4993.32	1	3.37E-08	1.77E-05	0.025162
49	4.64341	50.9738	818.844	1	1.85E-09	6.92E-08	2.45E-06
50	0.066497	10.5598	24989.5	0.554455	1.00602	554.099	162971
51	3.14537	50.4503	1395.25	0.987624	141.254	6445.97	245185
52	11.4856	561.987	37874.1	1	0.000146	0.013063	0.205862
53	0.329558	4.45566	259.673	0.74505	1.09E-05	0.000235	0.022444
54	1.76E-06	2.26E-05	0.000292	0	0.040941	2.65869	135.076
55	0.045697	2.9811	58.5666	0.772277	7.27E-05	0.006984	0.794257
56	0.001196	0.028223	0.698244	0.004951	0.013556	1.87719	64.1147
57	2.50E-06	0.026247	73.5556	0.200495	0.001636	3.26682	1590.92
58	1.58742	138.879	174174	1	0.009494	0.231983	3.66419
59	6.96E-06	0.014708	12.5777	0.319307	0.285131	3.51666	198.763
60	0.448176	1.92663	21.1453	0.685644	3.10E-06	0.000241	0.005138
61	1.80E-07	9.29E-06	0.000523	0	1.30E-05	0.002059	0.143562
62	6.49E-05	0.002084	0.040007	0			

impinv_equal_start1_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.001837	0.029257	0.108641	0.611787	1.25584	2.53889	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.002301	0.067325	3.75159	0.157	0.000932	0.070233	56.3575

Table A2 (cont'd)

1	7.33E-07	0.00889	3.37365	0.083	1.05752	104.978	1272.99
2	0.000872	0.070988	7.78016	0.177	1.77E-05	0.145236	1281.55
3	6.21E-07	8.94E-05	0.006394	0	0.001792	0.283349	29.2222
4	0.052859	2.26228	35.2107	0.764	2.55E-05	0.00205	0.193658
5	0.003068	0.144092	17.09	0.26	0.009677	324.612	2.23E+07
6	1.57E-09	0.002114	154.616	0.318	2.31E-05	0.060652	37.1238
7	0.002272	4.14048	78.4566	0.865	0.092404	586.756	6.77E+06
8	0.102615	3.45082	34.8736	0.82	7.14E-08	2.40E-05	0.009859
9	0.00233	5.73534	108.778	0.861	2.28E-17	1.95E-12	1.43E-06
10	0.000754	0.120814	110.358	0.247	0.000162	0.058017	3.44556
11	6.01577	44.0555	347.215	1	8.20E-05	0.009131	2.20153
12	0.197415	1.81262	12.966	0.79	2.13E-11	4.50E-09	3.41E-05
13	2.03E-10	7.82E-08	7.74E-06	0	2.09E-05	0.138593	472.15
14	0.016107	0.762335	25.5126	0.402	1.83E-06	0.000144	0.014609
15	0.212132	3.13035	25.288	0.827	0.636352	37.8858	5647.6
16	1.40E-07	6.97E-05	0.154081	0.019	1.99E-09	6.24E-06	0.001077
17	8.78E-08	0.013691	6.11862	0.226	2.65E+06	2.30E+10	2.65E+14
18	2.09361	21.6945	963.897	0.997	0.000533	0.050834	3.72006
19	1.87153	12.4103	75.7672	0.988	2.14E-13	1.15E-08	0.046779
20	7.96E-08	8.70E-05	0.032057	0	62878	3.65E+10	1.63E+14
21	8.87E-06	0.005998	0.622212	0.009	2.50E-08	1.32E-05	0.01163
22	5.38E-06	0.038899	647.466	0.281	2.46E-07	0.000192	0.047903
23	2.58E-07	0.000774	2.5988	0.023	6.54336	179.723	21747
24	0.080319	3.00513	385.023	0.728	2.97E-05	0.082503	57.8193
25	8.19E-05	0.034082	5.22667	0.128	2.76E-11	1.20E-07	5.98E-05
26	2.03E-07	0.01139	79.2438	0.396	1.47E-05	0.009475	145.92

Table A2 (cont'd)

27	1.27E-05	0.326229	11.752	0.517	5.99E-06	0.000507	0.030254
28	0.959295	12.978	3298.18	0.985	2.72E-05	16.0599	402122
29	0.446597	5.91408	435.828	0.95	3.59E+06	3.52E+09	5.14E+12
30	0.001111	0.655193	28.4112	0.57	2960.01	1.31E+09	1.43E+14
31	6.85E-08	2.70E-05	0.014191	0	7.14E-10	2.06E-05	0.020316
32	4.95E-07	9.48E-05	0.027251	0	2.40E-08	5.69E-05	1.40163
33	0.570531	6.29762	137.268	0.891	0.000224	0.023054	1.27991
34	3.39E-08	3.98E-06	0.001053	0	6.63E-09	6.64E-05	0.290717
35	7.16E-08	1.59E-05	0.012338	0	1.60E-14	7.47E-12	9.85E-05
36	0.296517	3.81992	32.1463	0.841	6.32E-06	0.001192	2.12217
37	0.003152	0.308617	37.0252	0.31	0.00039	0.058042	4.30675
38	3.30107	44.0892	8087.92	1	7.78E-08	0.00973	11.179
39	4.88E-08	0.003412	13.2426	0.305	4.36E-11	4.88E-08	4.46E-06
40	0.00071	1.78681	7413.16	0.442	0.000396	0.857496	4163.81
41	0.096442	3.51714	449.518	0.753	2.75E-15	5.26E-10	0.000129
42	4.72E-13	5.95E-08	0.000216	0	5.49E-12	3.06E-08	0.001088
43	0.00036	0.357428	16.2316	0.516	2975.7	1.07E+07	2.08E+10
44	0.892884	15.64	3881.39	0.966	12.0625	2828.78	1.83E+07
45	0.000144	0.015936	0.879135	0.045	2.39E-07	9.51E-05	0.01324
46	0.00169	0.26489	113.517	0.326	1.77E-05	4.21521	5616.64
47	3.20E-06	0.005249	2.48916	0.158	1.37E-07	0.0036	1921.61
48	1.85556	23.1862	1851.1	0.99	267.832	29471.3	2.58E+06
49	0.0145	6.29315	144.112	0.904	4.18E-06	0.000213	0.009114
50	8.12E-08	7.54E-05	0.018429	0	1.53588	4590.78	580782
51	3.69582	17.7579	1313.83	0.965	0.001644	1.54262	28079.4
52	0.043922	12.2181	164.444	0.9	1.18E-09	2.87E-07	0.00016
53	7.37E-11	8.40E-06	0.010524	0.007	18.0838	3949.02	863693
54	2.09E-08	4.26E-05	0.025454	0	8.19E-09	8.62E-07	0.000737

Table A2 (cont'd)

55	0.002404	2.94032	46.1456	0.79	0.000376	0.018088	0.758598
56	4.96E-07	0.005907	13.3382	0.157	0.16711	45.97	63162.4
57	6.41E-08	3.89E-06	0.000115	0	1.73E-05	0.027529	7.47059
58	0.052484	1.98864	53.499	0.612	0.015918	345.945	453502
59	0.315791	5.98414	65.9763	0.917	543.393	3.52E+06	1.09E+10
60	1.58E-05	0.021538	5904.31	0.28	0.000101	0.056496	20.3511
61	4.32E-05	0.00395	0.116893	0	0.006096	277.379	501228
62	4.67E-11	6.40E-08	2.05E-05	0			

Impinv_equal_start1_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000406	0.028861	0.130154	0.537243	1.07289	2.11866	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	4.04E-05	0.015015	4.44666	0.093	5.69159	427.776	364381
1	0.000128	0.262897	708.902	0.333	0.001713	5.06992	4552.5
2	2.74E-07	2.97E-05	0.00832	0	1.87E-07	0.000245	1.71757
3	7.20E-05	0.030857	1950.78	0.205	0.000895	0.075701	12.7664
4	0.062991	2.33339	52.0417	0.68	4.33299	19294.9	8.23E+07
5	1.32E-07	0.01119	5.83745	0.159	518748	1.23E+08	3.16E+10
6	3.26E-05	0.016363	131.172	0.13	6.78E-10	4.48E-08	1.54E-05

Table A2 (cont'd)

7	1.23E-05	0.916036	179.568	0.762	0.002345	0.228822	515.354
8	0.107233	2.94399	378.271	0.67	0.154612	6.50874	3148.19
9	2.76597	28.642	3293.12	1	1.07512	4484.32	2.01E+06
10	0.004348	0.868414	601.87	0.497	7.8931	2146.35	153831
11	5.82885	40.8707	3241.89	1	4.19E-07	0.001273	1.19232
12	0.007567	0.68447	17.8013	0.521	2.03E-09	0.002954	4.86558
13	0.000283	0.029716	3.98346	0.064	0.03253	1.96006	153.268
14	1.42E-06	0.001518	9.0567	0.108	7.05E-05	0.019628	10.726
15	0.136201	5.21782	709.181	0.798	0.001711	4.3558	23722
16	2.11E-05	0.029154	1140.49	0.246	944.674	34120.4	1.98E+06
17	0.00086	0.106486	23.5385	0.265	0.052313	186.869	283989
18	0.105896	7.26877	65.2023	0.902	0.000863	0.305115	1795.6
19	0.147082	8.10002	205.107	0.948	0.041991	1.32619	1267.65
20	4.90E-08	7.50E-06	0.001686	0	0.000396	3.37546	168221
21	0.000133	0.018543	2.57704	0.077	45.1273	1.70E+09	8.75E+14
22	3.98E-05	0.033694	63.8773	0.22	6.57E-08	0.042659	1611.26
23	6.15E-06	0.021532	0.974578	0.036	3.00E-06	0.000723	0.028297
24	0.06816	2.00234	49.4841	0.631	0.000853	0.062969	3.01403
25	9.83E-07	7.72E-05	0.005786	0	1.30E-12	3.11E-10	1.19E-06
26	0.000104	0.173419	1079.3	0.292	4.54E-05	0.002646	0.194458
27	3.06E-05	0.380881	16.8006	0.494	0.000514	0.029441	2.04485
28	0.344652	5.0649	613.272	0.892	1.03E-10	1.51E-06	0.002502
29	0.306144	6.75925	356.321	0.867	5.61E-09	0.000134	1.37792
30	0.080329	6.62395	1784.55	0.849	2.53E-06	0.012813	6.41197
31	1.50E-06	0.008843	19066.4	0.24	3.22E-11	7.78E-07	0.003836
32	1.23E-09	6.79E-06	0.576646	0	0.309488	543.124	446343
33	0.468885	7.76413	1500.66	0.887	0.069288	74020	2.15E+09
34	3.04E-12	8.28E-07	0.008936	0	0.001683	4.66273	18825.8

Table A2 (cont'd)

35	2.62E-12	1.06E-07	0.006132	0	1.96E-05	0.011509	5.06244
36	0.000148	0.364806	18.1511	0.496	7.41E-12	9.16E-08	0.000254
37	2.25E-06	0.035325	179.313	0.188	1.73E-07	0.0014	1.69963
38	0.002136	5.26989	57.2419	0.868	6.55E-14	2.25E-09	0.001516
39	1.74E-06	0.006231	2.41972	0.052	0.000168	0.101477	36.0036
40	1.13E-07	0.000903	4.45734	0.076	5.99E-07	1.09E-05	0.001919
41	0.533591	5.66208	307.371	0.87	0.000426	0.46655	77.2411
42	2.93E-05	0.030193	40.8312	0.228	354334	1.39E+08	5.82E+10
43	0.086258	3.52685	3068	0.714	11.4691	7182.56	4.17E+06
44	9.66E-05	2.79003	57.1295	0.881	2.42E-06	0.019065	15.8905
45	9.98E-06	0.012146	185.204	0.127	6.76E-08	1.45E-05	0.006916
46	2.95E-14	0.000122	4.37945	0.046	0.000926	0.507473	164.855
47	0.00144	0.603207	27618.3	0.405	0.057718	53.1877	16151.6
48	2.4214	21.1321	1550.58	1	8.23E+08	5.73E+10	4.32E+12
49	2.0188	14.057	266.015	0.992	0.060591	1.63828	14.8731
50	5.60E-07	0.000124	0.028537	0	9.21E-08	0.000141	0.028764
51	2.15302	30.0335	3288.01	0.996	1.89E-07	0.001153	4.55123
52	1.09E-06	0.183861	58.3593	0.683	41.707	4922.78	335906
53	0.00078	0.524166	125.247	0.418	4079.23	1.49E+07	1.01E+10
54	3.89E-08	0.001211	363.951	0.138	0.00017	0.0812	22.6702
55	0.669302	11.3472	239.512	0.982	150.72	1.02E+08	1.08E+12
56	4.37E-08	3.36E-05	0.027464	0	1.20E-07	3.01E-05	0.015914
57	2.72E-11	0.000912	1039.15	0.146	0.003288	65.1155	260089
58	1.19E-05	0.151866	9.7901	0.397	1.03E-06	0.000214	0.018419
59	1.10713	14.6777	3537.58	0.992	43249.9	1.00E+10	8.97E+16
60	5.93E-06	0.003309	0.402757	0.013	2.01E-05	0.56877	29075.2
61	4.90E-11	3.33E-05	0.692522	0.032	5.45E-05	0.004521	1.28342
62	2.81E-06	0.036045	30969.6	0.238			

Table A2 (cont'd)

Impinv_equal_start2_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.001812	0.04779	0.141296	0.694689	1.25348	2.50215	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	3.92E-06	0.000384	0.030573	0	0.065171	45.9934	23684.1
1	9.01E-06	0.003791	1.07207	0.006	1.42E-07	0.001752	7.04173
2	2.41E-08	5.92E-06	0.025794	0	548.761	1.30E+07	6.84E+14
3	3.55E-05	0.013151	1.16219	0.038	734501	1.16E+08	6.50E+10
4	0.012028	1.33542	18.118	0.646	2.01E-05	0.044567	72.9535
5	0.002979	0.101681	6.74053	0.171	0.025259	2.05455	424.664
6	1.23E-09	6.20E-06	0.000566	0	0.000252	0.028509	5.49469
7	0.007343	2.96973	107.885	0.863	0.006943	1.024	28.1159
8	0.10057	2.86957	3809.79	0.746	0.002794	0.561115	896.794
9	3.31487	26.4742	16432.4	1	4.65E-08	8.90E-06	0.002205
10	1.79E-07	0.008268	748.581	0.222	6424.5	7.37E+06	1.45E+09
11	1.45357	13.459	146.307	0.96	0.003657	0.192034	3.95712
12	0.097244	2.2107	3402.89	0.509	2.80E-08	0.000272	0.102571
13	2.90E-09	6.93E-05	2928.91	0.196	4.10E-06	0.000292	0.009594
14	4.30E-06	0.001153	0.450718	0.006	0.000281	0.074573	5.86328

Table A2 (cont'd)

15	0.001107	0.446301	10.5301	0.504	0.027663	1.56969	52.9493
16	8.31E-08	2.49E-05	0.004829	0	0.000295	0.04098	16.7965
17	3.47E-13	2.44E-05	47.9782	0.164	1.74E-08	0.004474	6.9396
18	1.83E-05	1.39294	28.884	0.79	1.43E-07	0.00079	0.548277
19	2.07435	18.2412	27058.2	1	820394	3.42E+11	1.09E+16
20	7.65E-05	0.204593	1038.74	0.295	138705	1.73E+08	1.04E+12
21	2.68E-07	0.000624	0.267319	0	2.37E-05	0.056965	38.03
22	0.002238	0.603892	12854.1	0.289	2.50E-15	2.93E-10	0.003488
23	2.24E-06	0.000203	0.032018	0	0.007329	10784.3	8.25E+08
24	0.024413	1.20134	338.1	0.423	19319.4	2.68E+08	2.67E+10
25	0.000109	0.025751	986.146	0.149	533.795	3.13E+07	2.05E+12
26	1.28E-06	0.00709	287.644	0.262	2.44E-05	0.017352	31.895
27	0.027968	1.5326	146.773	0.623	124.643	19537.1	9.06E+06
28	0.207429	2.12126	46.5601	0.754	28204.6	1.53E+08	2.56E+13
29	0.01055	0.959839	10.7421	0.587	1.31E-06	0.000368	1.28405
30	0.061314	4.64211	3306.68	0.801	100518	3.40E+09	6.86E+14
31	6.32E-12	9.45E-07	0.153945	0	4.78E-06	0.037059	1260.83
32	2.87E-11	1.96E-05	3909.55	0.149	4.30E-07	0.000102	0.098285
33	0.00087	0.926405	15.4115	0.724	1.44862	18982.9	834657
34	0.000392	0.2284	568.369	0.207	1.55E-07	0.00018	0.011801
35	1.55E-07	0.000223	435.749	0.123	0.014401	6.62877	2557.79
36	0.280556	3.63711	7668.23	0.738	0.000797	32.3034	46212
37	5.84E-06	0.002547	6.03403	0.069	0.006023	0.393796	7.12754
38	0.000164	1.85907	27.9847	0.785	1.57E-07	0.000724	2.50455
39	1.51E-11	0.000114	14.1994	0.178	6.09E-07	0.000151	1.80719
40	4.76E-07	3.47E-05	0.003135	0	1.44E-10	1.57E-07	2.92E-05
41	0.031808	1.33689	10.3849	0.614	0.001005	0.099925	1.68811

Table A2 (cont'd)

42	0.000806	0.170415	22691	0.184	8.20E-09	0.027336	235775
43	0.104184	2.79606	318.737	0.708	0.008436	0.819092	82.296
44	0.352342	4.83424	34.9766	0.894	1.88E-07	0.000134	0.027581
45	0.000584	0.35412	8801.22	0.329	4182.12	152479	3.28E+06
46	0.000359	0.122649	146.109	0.12	6.48E-05	0.315899	4895.58
47	1.51E-08	3.89E-06	0.844181	0.004	0.405533	556.223	2.09E+07
48	2.73961	16.0865	1176.7	1	6.14E-06	0.00634	2.88322
49	1.23E-05	0.393478	21.2203	0.606	3.16E-07	0.002621	130.487
50	2.40E-06	0.004443	1.92282	0.048	3.89E-06	0.000403	0.464184
51	1.56454	11.8668	172.439	0.974	0.024507	95.8673	22420.7
52	7.27E-05	0.815058	63.6945	0.711	1.37E-05	0.172779	23.0176
53	0.001285	0.038334	2.1662	0.09	1.97E-09	1.07E-05	0.010099
54	4.53E-08	4.72E-06	0.000583	0	0.000259	0.167966	24.8916
55	0.712579	12.3936	9154.17	0.963	1050.51	3.46E+07	2.10E+11
56	9.90E-07	0.00376	7.57124	0.104	9.42E-05	0.034872	11.2551
57	2.35E-13	4.38E-07	2.07529	0.044	1.17E-08	9.29E-07	0.000383
58	0.124282	2.16977	471.418	0.604	2.98E-07	2.37E-05	0.002482
59	0.276752	3.32751	29.1288	0.853	5.70E-08	0.000149	0.047537
60	6.79E-06	0.002417	0.617844	0.031	74.8234	8366.79	1.13E+06
61	1.86E-08	2.62E-06	0.000437	0	1617.12	419254	2.08E+08
62	0.000309	0.088043	26.7545	0.177			

Impinv_equal_start2_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000247	0.040838	0.187554	0.544065	1.17959	2.42844	0	0	0

Table A2 (cont'd)

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	3.92E-06	0.000384	0.030573	0	0.065171	45.9934	23684.1
1	9.01E-06	0.003791	1.07207	0.006	1.42E-07	0.001752	7.04173
2	2.41E-08	5.92E-06	0.025794	0	548.761	1.30E+07	6.84E+14
3	3.55E-05	0.013151	1.16219	0.038	734501	1.16E+08	6.50E+10
4	0.012028	1.33542	18.118	0.646	2.01E-05	0.044567	72.9535
5	0.002979	0.101681	6.74053	0.171	0.025259	2.05455	424.664
6	1.23E-09	6.20E-06	0.000566	0	0.000252	0.028509	5.49469
7	0.007343	2.96973	107.885	0.863	0.006943	1.024	28.1159
8	0.10057	2.86957	3809.79	0.746	0.002794	0.561115	896.794
9	3.31487	26.4742	16432.4	1	4.65E-08	8.90E-06	0.002205
10	1.79E-07	0.008268	748.581	0.222	6424.5	7.37E+06	1.45E+09
11	1.45357	13.459	146.307	0.96	0.003657	0.192034	3.95712
12	0.097244	2.2107	3402.89	0.509	2.80E-08	0.000272	0.102571
13	2.90E-09	6.93E-05	2928.91	0.196	4.10E-06	0.000292	0.009594
14	4.30E-06	0.001153	0.450718	0.006	0.000281	0.074573	5.86328
15	0.001107	0.446301	10.5301	0.504	0.027663	1.56969	52.9493
16	8.31E-08	2.49E-05	0.004829	0	0.000295	0.04098	16.7965
17	3.47E-13	2.44E-05	47.9782	0.164	1.74E-08	0.004474	6.9396
18	1.83E-05	1.39294	28.884	0.79	1.43E-07	0.00079	0.548277
19	2.07435	18.2412	27058.2	1	820394	3.42E+11	1.09E+16
20	7.65E-05	0.204593	1038.74	0.295	138705	1.73E+08	1.04E+12
21	2.68E-07	0.000624	0.267319	0	2.37E-05	0.056965	38.03
22	0.002238	0.603892	12854.1	0.289	2.50E-15	2.93E-10	0.003488
23	2.24E-06	0.000203	0.032018	0	0.007329	10784.3	8.25E+08

Table A2 (cont'd)

24	0.024413	1.20134	338.1	0.423	19319.4	2.68E+08	2.67E+10
25	0.000109	0.025751	986.146	0.149	533.795	3.13E+07	2.05E+12
26	1.28E-06	0.00709	287.644	0.262	2.44E-05	0.017352	31.895
27	0.027968	1.5326	146.773	0.623	124.643	19537.1	9.06E+06
28	0.207429	2.12126	46.5601	0.754	28204.6	1.53E+08	2.56E+13
29	0.01055	0.959839	10.7421	0.587	1.31E-06	0.000368	1.28405
30	0.061314	4.64211	3306.68	0.801	100518	3.40E+09	6.86E+14
31	6.32E-12	9.45E-07	0.153945	0	4.78E-06	0.037059	1260.83
32	2.87E-11	1.96E-05	3909.55	0.149	4.30E-07	0.000102	0.098285
33	0.00087	0.926405	15.4115	0.724	1.44862	18982.9	834657
34	0.000392	0.2284	568.369	0.207	1.55E-07	0.00018	0.011801
35	1.55E-07	0.000223	435.749	0.123	0.014401	6.62877	2557.79
36	0.280556	3.63711	7668.23	0.738	0.000797	32.3034	46212
37	5.84E-06	0.002547	6.03403	0.069	0.006023	0.393796	7.12754
38	0.000164	1.85907	27.9847	0.785	1.57E-07	0.000724	2.50455
39	1.51E-11	0.000114	14.1994	0.178	6.09E-07	0.000151	1.80719
40	4.76E-07	3.47E-05	0.003135	0	1.44E-10	1.57E-07	2.92E-05
41	0.031808	1.33689	10.3849	0.614	0.001005	0.099925	1.68811
42	0.000806	0.170415	22691	0.184	8.20E-09	0.027336	235775
43	0.104184	2.79606	318.737	0.708	0.008436	0.819092	82.296
44	0.352342	4.83424	34.9766	0.894	1.88E-07	0.000134	0.027581
45	0.000584	0.35412	8801.22	0.329	4182.12	152479	3.28E+06
46	0.000359	0.122649	146.109	0.12	6.48E-05	0.315899	4895.58
47	1.51E-08	3.89E-06	0.844181	0.004	0.405533	556.223	2.09E+07
48	2.73961	16.0865	1176.7	1	6.14E-06	0.00634	2.88322
49	1.23E-05	0.393478	21.2203	0.606	3.16E-07	0.002621	130.487
50	2.40E-06	0.004443	1.92282	0.048	3.89E-06	0.000403	0.464184
51	1.56454	11.8668	172.439	0.974	0.024507	95.8673	22420.7

Table A2 (cont'd)

52	7.27E-05	0.815058	63.6945	0.711	1.37E-05	0.172779	23.0176
53	0.001285	0.038334	2.1662	0.09	1.97E-09	1.07E-05	0.010099
54	4.53E-08	4.72E-06	0.000583	0	0.000259	0.167966	24.8916
55	0.712579	12.3936	9154.17	0.963	1050.51	3.46E+07	2.10E+11
56	9.90E-07	0.00376	7.57124	0.104	9.42E-05	0.034872	11.2551
57	2.35E-13	4.38E-07	2.07529	0.044	1.17E-08	9.29E-07	0.000383
58	0.124282	2.16977	471.418	0.604	2.98E-07	2.37E-05	0.002482
59	0.276752	3.32751	29.1288	0.853	5.70E-08	0.000149	0.047537
60	6.79E-06	0.002417	0.617844	0.031	74.8234	8366.79	1.13E+06
61	1.86E-08	2.62E-06	0.000437	0	1617.12	419254	2.08E+08
62	0.000309	0.088043	26.7545	0.177			

Inv_cat_star1_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
9.34E-05	0.012443	0.07212	0.643737	1.25848	2.58367	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.003662	0.487194	184.891	0.259	16.5797	385.94	20891.9
1	3.04E-05	0.010068	2.59851	0.135	6.73E-06	0.000295	0.017833
2	5.93E-10	1.28E-06	0.00124	0	963.7	272898	1.61E+08
3	4.53E-07	0.003407	6.58076	0.2	1.14E-05	0.038239	102.308
4	0.073106	13.0059	16286.8	0.877	1.56E-09	4.45E-07	0.000679
5	0.001006	0.456461	151.764	0.341	1.07E-05	0.013569	367.29

Table A2 (cont'd)

6	0.007941	1.79719	100.888	0.674	1.28E-05	0.053316	118.688
7	1.84997	21.414	269.118	0.996	1.01154	210.566	23561.5
8	1.53E-07	0.074876	7.99237	0.451	1.73E-06	0.000144	0.017086
9	7.65E-05	4.02721	200.991	0.847	3.27E-06	0.000178	0.028368
10	5.28E-10	1.99E-06	0.014611	0	1.23E-05	0.224107	80819.8
11	18.163	168.224	39596.1	1	8.18E-08	7.80E-06	0.002858
12	0.064266	5.4281	12175.1	0.758	6.79E-05	0.029882	408.826
13	0.070208	3.38478	10450.5	0.627	3.73E-09	3.46E-05	18.6345
14	1.95E-05	0.06037	95.4268	0.237	2.02E-06	0.021267	716.144
15	0.281377	10.4336	546.929	0.942	28.3631	45712.6	1.98E+09
16	0.000702	0.328116	2853.12	0.315	283.074	1.26E+07	2.44E+10
17	0.014429	0.60821	2946.05	0.3	1.78E-05	0.002184	0.652633
18	9.25E-05	2.57459	96.1566	0.825	9.53E-08	0.049427	11.7379
19	0.000518	3.1014	155.547	0.868	7.18E-06	0.014327	4.89434
20	2.99E-06	0.003867	0.762736	0.016	0.003317	0.173909	6.99008
21	1.57E-05	0.003526	0.409747	0.006	0.028634	2.04782	4694.01
22	0.000754	0.295093	903.36	0.271	0.000265	0.267977	3324.16
23	0.000602	0.76764	508.053	0.463	1.89E-07	4.17E-05	0.010407
24	0.000154	0.521833	33.5785	0.491	5.41E-08	1.13E-05	0.000863
25	8.79E-05	0.012265	0.276149	0.005	1.17709	4240.33	2.06E+06
26	0.007886	1.43856	392141	0.482	55537.5	9.35E+09	2.77E+14
27	0.518223	6.53316	1368.19	0.836	0.000342	14.7942	808620
28	0.003459	1.54	38.9178	0.793	6.29E-10	3.37E-07	0.022424
29	8.11E-05	0.160519	22.8306	0.53	3.62E-05	0.035058	3.16977
30	0.152259	12.7915	1543.91	0.861	5.26E-07	0.005454	8.20324
31	0.005123	0.453285	30.017	0.42	0.001419	0.490008	35568.3
32	0.003248	1.1445	33308.7	0.423	9.76E-05	0.053889	4.69182

Table A2 (cont'd)

42	0.000196	0.589459	964.861	0.38	4.17E-08	1.99E-06	3.66E-05
43	0.248646	2.92296	166.593	0.771	4.26E-11	4.33E-08	1.46E-05
44	4.07696	79.8376	24466.7	1	0.002528	11.0796	18113.7
45	0.000197	0.561857	430.555	0.512	5.88E-05	30.5352	9.24E+07
46	0.001311	0.072049	1.57221	0.073	1.07E-07	0.000128	0.013212
47	2.08E-05	0.035125	63.4103	0.211	0.016476	3.09248	4748.51
48	2.63282	23.3892	1729.31	0.971	0.526988	12307.8	2.34E+07
49	0.83378	15.9202	696.104	0.981	0.001115	0.112729	24.2539
50	0.000205	0.290367	2112.72	0.317	3.50E-08	4.39E-05	0.016994
51	4.3055	48.5566	1412.24	1	5.79E-05	0.010179	3.46297
52	5.44763	98.8527	26143.7	1	6.14E-09	9.02E-06	3.29863
53	0.003924	1.0726	1602.14	0.49	447.351	1.50E+07	6.70E+09
54	0.000262	0.306254	60.7961	0.339	6.19E-05	0.008685	2.62218
55	1.16712	27.3933	1516.3	0.996	1169.05	4.49E+07	1.22E+11
56	1.85E-06	0.000424	0.921758	0.032	2.15E-15	2.75E-10	3.82E-05
57	2.95E-08	2.96E-05	0.02222	0	0.0002	0.058494	34.7402
58	0.001186	2.92529	91.0192	0.826	6.17E-11	3.25E-08	0.000998
59	1.23384	19.5534	2754.67	0.997	5410.12	1.17E+06	2.38E+08
60	2.11E-05	0.079705	28.7408	0.384	4.69E-07	0.006761	1.57252
61	0.000578	0.028568	6.12043	0.095	3.92E-22	1.72E-13	0.000194
62	0.005899	0.164375	2.52127	0.147			

Table A2 (cont'd)

Inv_cat_start1_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.001392	0.040505	0.129146	0.575522	1.20678	2.42781	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	2.51E-08	3.56E-05	0.06461	0	3.37E-13	5.41E-09	8.04E-05
1	6.07E-06	0.004359	125.985	0.224	703.126	1.36E+06	1.81E+08
2	0.001402	0.271235	12.7263	0.409	3.69E-10	1.83E-07	0.000165
3	3.18E-16	3.50E-12	1.13E-05	0	0.011413	11.3765	2296.64
4	0.162551	5.35025	1517.74	0.832	26.6635	3740.3	316694
5	0.005748	0.229868	61.7225	0.28	9.78E-07	0.006321	1.58748
6	1.79E-06	0.000351	0.082267	0	1.06E-05	0.394526	43975.7
7	0.001593	2.5858	40.655	0.824	2.39E-12	4.78E-08	0.00018
8	0.033839	1.50945	552.824	0.554	8.14E-06	0.005201	10.9246
9	3.17453	22.4289	547.864	1	2.38E-10	1.11E-06	0.024031
10	5.84E-10	4.32E-07	0.000165	0	0.000516	0.155735	491.782
11	5.05E-06	3.94299	122.842	0.841	0.006107	1.63772	6314.36

Table A2 (cont'd)

12	2.76E-05	0.080592	4.39208	0.236	0.000816	0.043871	2.09326
13	2.81E-06	0.004632	2.32398	0.076	3.88E-05	0.173245	233.703
14	1.38E-06	0.013045	69.508	0.233	180.327	25968.9	1.36E+06
15	0.000149	0.228875	9.1941	0.394	0.205193	15.108	1431.88
16	2.73E-06	0.008643	240.576	0.158	0.002225	0.379222	31.0444
17	8.98E-11	3.60E-07	0.000524	0	16.1416	398676	4.03E+09
18	1.25388	9.98389	82.1394	0.979	6.47E-09	1.89E-05	100.44
19	1.62321	9.51548	183.987	1	1.67E-08	0.00469	346.852
20	0.000138	0.018744	3.23447	0.082	5.05E-08	4.44E-05	0.242702
21	0.018952	0.521179	621.441	0.234	1.68E-09	0.000866	10350.3
22	3.16E-05	0.007548	0.512139	0.004	2.29E-05	0.232233	147075
23	9.46E-09	0.000651	35.3204	0.162	3.80E-06	4.41E-05	0.000769
24	0.171577	1.71731	105.567	0.536	1.31E-16	3.92E-09	0.000647
25	0.000257	0.022187	3.54306	0.172	2.91E-06	0.005354	6.94597
26	4.62E-07	0.007154	4.5516	0.161	3.56E-07	3.01E-05	0.016677
27	0.034395	1.24156	185.188	0.478	4.42E-07	0.000589	1.09371
28	0.046048	1.53093	15.3562	0.72	0.001653	0.04578	1.49999
29	0.080164	2.24124	86.119	0.73	7476.51	326698	7.67E+07
30	0.029549	3.32624	2887.9	0.735	6.75E-06	10.2254	1.25E+07
31	4.60E-08	4.92E-06	0.001772	0	5.29317	1371.76	237832
32	6.57E-05	0.028263	2.80077	0.093	8.74E-07	0.043793	42.6275
33	0.235967	1.90404	63.5801	0.674	1.08E-10	0.000136	28.9057
34	2.94E-06	0.000122	0.023363	0	9.06E-05	0.003023	0.144801
35	1.28E-05	0.006499	1.81546	0.098	0.000253	0.687921	14518.2
36	0.33515	5.82689	357.955	0.846	0.003366	0.12325	1.80966
37	7.72E-09	2.42E-06	0.020534	0	1483.2	3.91E+06	1.95E+09
38	7.10E-05	3.56614	111.19	0.881	5.00E-09	9.51E-06	10.7983
39	3.27E-06	0.020349	9.69754	0.205	1.26E-09	1.26E-06	0.000948

Table A2 (cont'd)

40	7.53E-07	0.01354	421.74	0.296	1.66E-05	0.002978	0.265278
41	0.052423	2.18629	124.937	0.727	0.008318	1.45801	319.364
42	1.45E-06	9.48E-05	0.012494	0	18.1751	1570.82	2.59E+07
43	0.015979	0.833792	92.5944	0.441	2.98706	1669.87	2.33E+06
44	3.7111	19.4429	1170.42	1	2.90E-09	1.25E-07	1.13E-05
45	0.006756	0.169058	2.72594	0.17	5.82769	36011.8	1.93E+08
46	0.000107	0.015054	2.54124	0.09	6.90E-06	0.005961	1.57966
47	5.31E-08	0.000188	69.0834	0.151	1.34E-06	0.002026	0.577764
48	1.62623	13.0864	458.334	1	8.72E-09	9.05E-05	7.25357
49	0.133277	3.85892	53.5534	0.798	6.22E-13	2.54E-10	2.29E-06
50	1.24E-07	0.001001	2.1701	0.088	7.52E-07	0.008505	4.82967
51	3.20013	20.2025	165.397	1	5.27E-06	0.00154	0.934661
52	3.15E-06	1.70416	35.3926	0.808	1.06E-08	0.001251	9.64561
53	3.87E-10	3.20E-06	0.080102	0	2.47E-08	8.66E-07	0.000282
54	9.86E-11	0.000114	1137.67	0.161	0.025119	3.72747	646.692
55	1.02222	4.47548	69.7323	0.964	9.49E-11	1.18E-07	0.00092
56	1.00E-06	0.000517	0.206585	0	0.000972	0.043449	1.68608
57	1.81E-08	4.42E-06	0.00036	0	0.143281	2.7582	94.6896
58	0.338261	2.65459	95.5799	0.796	1.21E-07	0.000443	0.120284
59	3.82E-05	0.367631	17.6296	0.68	0.210922	4.28221	395.02
60	0.004004	0.343139	16.5402	0.258	0.1563	25.2848	3455.43
61	2.76E-06	0.015857	1032.74	0.273	774.646	156751	1.99E+07
62	3.35E-08	7.17E-05	38.9564	0.145			

Table A2 (cont'd)

Inv_cat_start2_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000262	0.016407	0.108063	0.622198	1.29399	2.51013	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	1.31E-06	0.000589	1.13274	0.039	3.97E-06	0.00024	0.008439
1	2.39E-11	3.66E-08	0.000561	0	0.000405	0.039779	117.832
2	0.000339	0.398537	822.932	0.399	5.11861	299710	2.30E+08
3	1.01E-08	1.44E-06	0.019279	0	2.86E-06	0.035098	119
4	0.007683	1.80603	31.1321	0.74	0.000118	0.020165	3.90826
5	0.001125	0.669329	620.321	0.406	62.2952	9036.07	9.41E+07
6	7.56E-06	0.115072	69.7579	0.39	8260.17	1.45E+06	7.07E+07
7	1.11066	24.3271	785.203	0.969	0.000523	33.2378	121278
8	0.011323	0.567577	23.3652	0.425	1931.52	343540	9.19E+07
9	3.91443	33.6205	837.419	1	4.24E-07	0.000463	4.10193
10	0.0012	0.161086	7.13227	0.284	0.000795	0.466546	765.75
11	4.48531	59.5464	3372.77	0.969	0.002798	0.060091	1.76171
12	0.058436	2.87284	92.6357	0.731	5.11E-05	0.037612	2.88545
13	0.000668	0.508934	1906.04	0.372	1.70E-05	1.09851	9554.17
14	0.00014	0.066473	9.31278	0.313	1057.04	452466	1.20E+08
15	0.363594	12.349	3934.63	0.936	1.12E-08	5.92E-05	0.70261
16	6.02E-11	0.00077	4221.99	0.429	7.98E-08	0.00011	0.011958

Table A2 (cont'd)

17	0.001692	0.813621	6497.63	0.331	706.95	52166.9	1.84E+06
18	1.58893	26.1522	636.495	1	7.60E-13	6.75E-08	0.000588
19	0.792201	12.3984	1076.85	0.962	56.3664	8.65E+08	8.16E+12
20	8.27E-06	0.20303	18209.6	0.466	1.02E-12	8.61E-09	0.000177
21	8.82E-07	0.021315	265.624	0.345	0.018594	1.90051	639.226
22	0.000107	0.010124	0.566187	0.013	769.881	6.68E+07	4.56E+10
23	8.01E-09	0.000389	9.68541	0.239	87.2422	498725	1.27E+09
24	3.01E-06	0.207113	7.82972	0.425	7.74E-12	4.38E-07	1.04862
25	4.76E-08	0.002278	376.628	0.2	6.16E-06	0.428575	41808.4
26	0.000137	0.020964	1.73628	0.046	1.48E-06	0.004454	29.5153
27	0.088362	3.07516	400.618	0.617	0.000904	0.054795	3.18623
28	0.069566	1.2304	22.2846	0.593	1.95E-13	8.73E-08	0.010139
29	0.022324	0.952577	28.6498	0.568	1.02E-05	0.025497	1345.83
30	1.65E-05	0.325575	13.0309	0.452	832.243	1.81E+06	1.14E+10
31	1.30E-06	7.77E-05	0.007095	0	41323.5	2.73E+06	1.81E+08
32	6.36E-05	0.024222	8.83798	0.222	5.80E-07	0.00046	0.069292
33	0.605695	8.79095	883.417	0.93	0.0193	0.872043	98.3773
34	7.53E-05	0.024797	6.83993	0.249	0.000302	0.036098	5.25982
35	9.09E-07	0.002459	1.39253	0.041	7.02E-07	0.025546	19196.7
36	0.000204	0.408319	21.5758	0.538	1.98E-05	0.022911	2.57732
37	4.39E-10	0.003487	50381.4	0.326	9.08E-09	6.62E-06	0.011477
38	2.31698	32.7799	1845.12	1	2.95E-07	0.002857	12.025
39	6.86E-06	0.032548	23.0686	0.338	3.02E-09	1.37E-05	0.031089
40	5.43E-06	0.006371	9.46889	0.145	264.75	397379	5.99E+08
41	0.487192	11.3687	2743.43	0.931	191.953	204040	6.40E+08
42	0.004642	0.799697	11635.1	0.336	1398.23	171272	1.57E+07
43	0.088631	5.89176	9158.49	0.778	15.4968	2414.58	1.19E+06

Table A2 (cont'd)

44	2.90422	45.1394	7148.66	1	0.00104	2.26307	5815.19
45	0.007414	0.524112	139.7	0.322	3.45E-07	6.72E-06	0.000366
46	1.63E-07	5.15E-05	0.014859	0	5920.87	2.20E+06	9.93E+09
47	0.000336	0.08902	856.86	0.251	1.22E-12	1.01E-09	0.0001
48	2.13066	27.0554	3025.56	1	1.17E-05	0.011178	2.39387
49	0.559176	30.0364	4932.84	0.955	1.36E-09	5.61E-06	0.015767
50	0.005552	0.507826	129.772	0.372	128.856	288827	1.11E+08
51	3.38967	60.741	5737.69	1	3.07E-06	0.00021	0.955821
52	2.76403	44.8983	3020.62	1	0.00083	10.3656	13109.8
53	0.000245	0.011254	0.825226	0.015	8.82791	2025.9	1.34E+06
54	4.55E-08	5.78E-06	0.001135	0	0.185655	307.7	97093.7
55	0.932046	7.44975	132.975	0.937	2.92349	342.577	20027.1
56	1.88E-07	0.000219	0.022008	0	0.005714	22.0612	11475.7
57	6.09E-05	0.003312	0.304422	0.003	0.000265	6.93883	1.22E+06
58	0.52061	7.60195	540.058	0.858	4.73E-05	0.003841	0.337097
59	4.16E-10	1.20E-07	0.000211	0	0.006779	22.1257	655980
60	0.003947	0.285311	137.637	0.247	32.1328	6208.22	202912
61	3.93E-06	0.000805	0.121496	0	2.09E-06	0.021662	29.9229
62	9.44E-09	4.36E-05	0.226527	0.003			

Inv_cat_start2_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000135	0.009511	0.117123	0.679032	1.37725	2.99815	0	0	0

Table A2 (cont'd)

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	2.43E-06	6.77E-05	0.001578	0	2.92E-07	1.07E-05	0.000643
1	6.76E-09	3.92E-05	1.02049	0.026	11.1386	3240.26	1.31E+07
2	4.15E-10	4.63E-07	0.000884	0	0.038122	2.42609	141.836
3	1.06E-05	0.100569	6.50627	0.249	721.986	181489	5.91E+07
4	0.132341	6.29667	110.902	0.78	5.00E-06	0.979274	2151.86
5	5.57E-06	0.212117	218.328	0.298	0.109676	93.9308	145343
6	0.005937	0.153977	7.33175	0.273	0.006203	6.13924	783720
7	4.59572	106.029	17797.1	1	4.53E-05	0.036534	6.22163
8	0.073712	4.67918	367.138	0.763	1.82E-05	0.021486	25.9549
9	3.77958	108.822	3873.25	1	1.07E-10	2.69E-05	1.55437
10	6.82E-07	0.002387	24818.5	0.199	5.49E-08	2.35E-05	0.352417
11	7.03978	161.52	18403.5	1	125212	4.78E+07	6.32E+09
12	0.680921	8.20384	131.139	0.899	17334.9	6.54E+06	2.59E+10
13	2.89E-05	0.005393	33.0515	0.124	83585	7.19E+06	6.76E+08
14	1.49E-07	0.000114	0.015816	0	4.90E-10	4.09E-07	0.00028
15	0.255888	26.0711	26304.5	0.928	1.68602	85.4796	8759.98
16	0.002912	0.816726	5115.3	0.451	2.62E-06	0.00016	0.113149
17	1.49E-07	0.01844	4.5162	0.163	3.64E-07	3.92286	566352
18	3.64914	56.5812	819.498	1	2.10E-08	0.003094	8.51241
19	0.262588	7.143	181.517	0.905	0.006636	40360.7	5.42E+08
20	4.14E-07	0.000649	126.816	0.136	0.290301	46.7789	111330
21	1.41E-07	5.02E-05	0.126791	0	47292.9	3.16E+09	5.90E+13
22	0.005039	0.383184	42.8903	0.276	3.20E-05	0.001221	0.139482
23	5.76E-11	4.00E-07	0.000293	0	1.18E-06	0.003781	33.044

Table A2 (cont'd)

24	0.121109	6.07333	1425.74	0.769	0.000124	0.349849	7998.91
25	2.71E-05	0.151003	1216.87	0.319	6.60E-06	0.002106	5.60237
26	3.18E-07	0.178997	589.428	0.595	1.25E-06	0.674362	28511.4
27	9.87E-06	0.07184	13.7177	0.333	3.58E-07	0.000958	4.6605
28	3.65E-06	0.435296	83.265	0.545	0.000124	0.037698	2.34339
29	0.137277	4.2751	98.5552	0.818	3.50E-09	5.08E-06	0.153338
30	0.026169	2.172	48.2027	0.7	7.21E-05	0.053908	8.76426
31	0.000377	0.375094	8114.85	0.357	6.63E-06	0.000459	0.205922
32	8.03E-10	0.002306	4464.05	0.305	73.9257	14405.3	1.42E+06
33	0.650569	17.2435	713.168	0.955	1.27E-09	2.42E-06	0.502102
34	0.000337	0.090067	11.8238	0.326	1089.97	151152	1.10E+07
35	0.006621	1.73858	3002.43	0.49	3.30E-05	0.002412	0.441106
36	0.326333	14.698	1607.61	0.893	0.000376	0.024209	1.05909
37	2.41E-05	0.198746	18.7757	0.585	3908.1	2.65E+07	1.49E+10
38	3.76621	34.77	2866.63	1	0.117156	1.84362	17.5618
39	5.21E-10	6.86E-06	0.040458	0	32.6633	34130.3	7.11E+07
40	2.52E-08	2.26E-05	0.038708	0	2.91E-11	2.78E-05	5.30094
41	0.541021	9.03043	252.798	0.91	1.44E-08	0.000227	8.21873
42	0.010447	2.15931	602.055	0.599	142.324	14178.6	1.79E+06
43	0.099935	4.32488	196.276	0.715	0.374257	831.228	413506
44	0.000654	7.37296	387.697	0.833	9.13358	1503.76	497826
45	4.73E-06	0.009723	8.13881	0.249	1963.79	1.16E+06	2.48E+09
46	0.007058	0.252629	8.23255	0.323	445.528	61496.3	1.33E+07
47	0.077272	1.27984	68.9744	0.538	1.88E-05	0.017249	2.41086
48	2.49957	58.6283	5645.16	0.994	7.67E-09	4.28E-06	0.001614
49	4.08424	66.4254	6417.89	1	50793	2.79E+10	5.04E+14
50	5.56E-11	6.22E-08	0.000266	0	0.969541	2166.23	623004
51	2.92261	116.314	6062.21	1	6.12E-05	0.011028	3.254

Table A2 (cont'd)

52	2.98957	89.012	25720.5	1	1.29046	1852.02	939270
53	5.74E-06	0.007308	2.64639	0.122	6.22E-07	0.00238	7.95937
54	2.46E-05	0.004471	1.14431	0.035	235.713	51799	7.44E+06
55	0.832264	17.5151	582.894	0.934	0.001605	0.088718	3.38795
56	3.55E-06	0.070231	96.3117	0.408	0.456085	4.33353	153.522
57	0.000292	0.02961	4.68018	0.134	4.17E-11	5.27E-08	0.000598
58	1.30042	47.6451	6535.12	1	0.00086	0.049191	2.86784
59	1.19E-07	8.59E-05	6.43756	0.157	0.123079	175.635	23878.7
60	0.000113	0.087976	43.8672	0.382	141.96	146070	2.77E+07
61	6.06E-08	1.57E-06	6.84E-05	0	63.904	22439.6	2.65E+06
62	2.65E-11	0.001003	6956.95	0.396			

Inv_equal_start1_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.001503	0.027429	0.098048	0.575923	1.13176	2.1534	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	3.39E-11	3.82E-07	0.001697	0	21.9464	1894.54	645190
1	2.11E-05	0.001857	1.41358	0.069	0.000762	0.038624	3.54716
2	3.55E-08	0.001023	1.66613	0.046	0.001315	3.75724	16566
3	0.000414	0.048594	2.49129	0.126	0.010879	48.112	23372.1

Table A2 (cont'd)

4	0.081252	6.50708	1316.81	0.837	2.21297	1966.21	64909.5
5	1.44E-09	0.000876	940.541	0.246	0.004308	99.8706	3.93E+07
6	0.01073	0.443176	7.70687	0.379	1042.76	5.54E+06	2.43E+09
7	0.384439	17.2	13482.2	0.954	6.45E-10	3.13E-07	0.000424
8	0.161691	4.2453	79.9533	0.816	0.000537	0.19026	72.6798
9	3.61731	20.2986	155.46	0.968	2627.74	134534	1.35E+07
10	0.010924	0.215661	2.37809	0.171	3.09E-15	2.47E-11	2.29E-06
11	0.002284	9.15035	288.741	0.879	1.20E-11	5.92E-07	9.50996
12	0.000723	0.529168	20.9291	0.638	6.95E-05	0.014721	1.91284
13	4.39E-07	0.003381	18.5043	0.17	4.07E-05	0.211694	8254.33
14	3.54E-06	0.017985	1.89934	0.1	1.16E-08	1.25E-05	0.011246
15	0.301461	3.45589	67.0817	0.857	3.9925	3884.64	1.41E+06
16	4.52E-11	1.78E-05	3.94957	0.065	3.38E-07	3.27E-05	0.005858
17	0.004292	0.081591	5.70683	0.141	38.9549	77669.8	9.80E+08
18	0.005599	6.74255	103.389	0.872	1.00E-05	0.024966	2.81735
19	2.4378	17.161	469.453	1	0.000139	0.022975	3.49852
20	1.52E-15	6.34E-11	6.15E-06	0	1.14E-09	8.76E-08	0.000119
21	0.000215	0.162935	81.0178	0.334	2.59E-07	0.000341	0.431422
22	7.63E-07	0.000379	223.881	0.124	198.911	127288	6.58E+07
23	1.39E-10	1.86E-05	0.689146	0.013	6.74E-05	0.005651	0.216333
24	0.00206	0.183461	7.78412	0.248	0.001916	0.049572	4.3725
25	5.35E-09	1.27E-06	0.000512	0	6.75E-08	3.82E-06	0.000222
26	0.001724	0.207156	6.84044	0.236	3.10E-05	0.03406	2.08965
27	0.206913	4.06679	117.088	0.772	0.000577	0.116612	4.51349
28	0.581259	9.86926	130.666	0.961	1.14E-08	8.80E-06	0.002073
29	2.61E-05	0.46569	62.4525	0.601	7.00E-05	0.030839	5.84047

Table A2 (cont'd)

30	0.861997	9.41577	155.072	0.975	1.99E-07	0.000177	0.692527
31	1.27E-06	0.004571	17.6976	0.237	3.52E-08	1.38E-05	0.000544
32	2.62E-07	0.001659	9.97726	0.191	1.55E-05	0.002286	1.9987
33	0.969304	9.18361	332.587	0.99	0.000958	0.071442	44.4642
34	0.000163	0.325655	533.322	0.352	127.811	379948	5.05E+08
35	3.45E-05	0.012287	175.608	0.22	0.002417	2.46021	40474.8
36	0.400937	7.90475	110.25	0.929	7.49412	616507	7.05E+08
37	1.56E-06	0.001993	22.8217	0.152	0.002582	0.261609	6.45687
38	4.59262	36.2844	509.216	1	5.46E-08	0.000346	2.6941
39	8.36E-08	0.002089	57.567	0.235	0.004518	0.664266	4685.94
40	6.34E-06	0.019278	4.46846	0.147	1.23314	531.576	576627
41	0.275248	2.82126	19.2867	0.826	670.155	3.30E+07	3.57E+13
42	1.24E-08	5.07E-06	0.000839	0	8.72E-07	0.002258	0.543963
43	0.048975	1.46598	10.2981	0.667	1.10E-05	0.0021	12.5612
44	1.81821	21.3006	939.059	1	1.47E-12	2.05E-09	1.71E-06
45	0.000455	0.219983	155.273	0.276	7.78E-12	4.66E-09	9.72E-05
46	0.000133	0.0234	4.62546	0.112	4.07E-09	2.40E-07	0.000215
47	6.28E-05	0.016611	1.91346	0.033	0.326505	29.6165	3043.85
48	0.059031	9.32123	157.018	0.918	0.503778	5777.05	7.48E+08
49	0.668473	11.2353	62.8425	0.916	9.07E-09	7.41E-06	0.002497
50	0.000293	0.016665	78.8883	0.216	0.579011	14508.4	1.73E+08
51	0.000869	6.00362	188.604	0.839	3.70E-05	0.001368	0.272128
52	6.72415	44.4518	1731.14	1	1.81E-08	3.47E-05	0.501511
53	1.86E-08	8.28E-05	2.16541	0.037	0.891505	397.234	71012.8
54	2.19E-06	0.048277	4.75196	0.252	0.002958	0.052692	2.45166
55	1.98821	16.8457	649.755	0.997	0.923441	958.082	881426
56	1.73E-05	0.003778	2.16719	0.036	0.000408	0.123546	39.0399

Table A2 (cont'd)

57	0.000259	0.131627	20.1671	0.24	26.6183	5132.97	6.84E+06
58	0.006568	1.07811	312.158	0.558	0.000205	0.026011	0.529364
59	2.57E-10	1.77E-07	5.15E-05	0	8433.39	2.14E+06	1.25E+08
60	2.05E-05	0.021416	17.3928	0.184	6.78E-06	0.001325	1.0028
61	7.52E-07	0.000187	0.356831	0.018	0.000455	7.89896	3.44E+08
62	0.006999	0.133838	7.15976	0.186			

Inv_equal_start1_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000697	0.041836	0.163419	0.594356	1.09879	2.13061	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	2.90E-17	1.11E-10	0.000171	0	16.1971	7676.83	2.13E+07
1	1.00E-08	2.57E-05	0.696831	0.005	52.9827	32398.3	2.46E+07
2	2.12E-12	6.88E-07	1.46201	0.054	1.86E-05	0.004108	0.225781
3	2.61E-06	0.033279	118.038	0.163	2.37E-08	0.002387	2.12842
4	0.050788	1.51883	15.4753	0.62	10.218	100117	7.48E+07
5	2.88E-13	2.46E-08	0.000355	0	0.000263	0.301787	791.8
6	2.11E-14	2.46E-10	0.00136	0	0.000174	0.705884	148.54
7	0.242824	5.62598	98.2921	0.837	330575	3.62E+09	1.16E+15
8	0.025952	0.834489	11.8996	0.524	1.84E-08	7.40E-07	7.21E-05
9	0.024418	5.77342	189.329	0.905	1.80269	1670.54	463972
10	4.97E-08	5.37E-05	0.388912	0	0.413318	2203.67	995371

Table A2 (cont'd)

11	4.3243	36.6342	1921.13	1	1022.1	51787.8	3.62E+06
12	0.08937	1.1348	27.2095	0.505	1.25E-06	0.000724	0.267811
13	3.69E-06	0.000102	0.017649	0	20236.9	9.88E+06	8.39E+10
14	6.85E-10	0.000133	62.5711	0.147	6.27E-12	2.82E-06	0.038904
15	0.168658	2.13576	53.193	0.695	1.75E-08	6.67E-06	0.003704
16	3.68E-09	2.66E-06	0.000424	0	3.86028	35604.3	1.12E+07
17	2.16E-07	5.18E-05	0.013024	0	2.65E-07	0.00065	4.00911
18	2.40733	19.4117	366.263	0.995	1.10E-08	0.014705	11.3493
19	0.004403	4.02503	36.1966	0.889	0.017156	10.01	10571
20	9.55E-05	0.070439	622.314	0.232	44296.2	1.28E+08	6.76E+10
21	0.009594	0.200669	11.9871	0.22	2.23E-06	0.000249	0.018225
22	3.46E-05	0.020597	25.352	0.125	8.13E-08	1.42E-05	0.056855
23	1.35E-07	1.03E-05	0.000669	0	1.46E-05	0.050592	49.2946
24	0.006945	0.27092	6.02491	0.317	6.43E-11	3.49E-08	3.12575
25	0.001149	0.231705	4373.37	0.235	2.17E-12	6.54E-09	0.000176
26	0.019743	0.733189	25.1461	0.361	2.41E-11	2.01E-06	0.001335
27	0.00567	0.950042	205.83	0.448	2.45E-07	0.000112	0.109944
28	0.005532	1.26152	14.7681	0.773	0.000597	0.058438	2.12801
29	0.000533	0.294641	8.03019	0.496	102.875	38077.6	1.84E+07
30	3.55E-05	0.238282	9.25656	0.53	0.004385	9359.38	5.76E+07
31	0.000457	0.217203	1746.9	0.306	0.001891	3.97546	4871.34
32	2.04E-10	4.21E-05	12.3961	0.097	5.43E-07	1.50E-05	0.002827
33	0.753216	7.22537	711.665	0.916	1.87E-13	2.38E-09	7.96E-05
34	1.10E-05	0.000167	0.003704	0	5.11E-05	0.033138	15.7497
35	0.000182	0.063679	26.9859	0.219	0.270832	22227.9	6.59E+08
36	0.253865	5.71817	14376.5	0.773	308.447	5871.41	157769
37	1.71E-11	0.000149	2384.81	0.176	0.000189	0.022422	0.485063
38	0.386587	17.0164	2349.54	0.963	0.092978	1.1535	9.97445

Table A2 (cont'd)

39	1.65E-07	1.01E-05	0.000758	0	9.13E-05	0.13223	16.5352
40	0.003696	0.16106	5.12582	0.239	1.39E-13	5.90E-11	4.98E-08
41	8.59E-06	0.044134	5.82958	0.218	7.31E-13	2.14E-09	1.37E-05
42	7.15E-09	9.45E-06	0.123898	0	0.004977	0.279446	32.7645
43	0.055077	1.5522	144.361	0.487	2.99E-06	0.000134	0.009107
44	1.1913	12.3719	1819.9	0.965	0.793312	1940.72	4.51E+06
45	2.00E-10	1.47E-05	0.027936	0	1.73E-05	0.001635	0.089607
46	3.96E-06	0.002035	5.79154	0.088	8.05E-07	4.69E-05	0.012608
47	1.40E-09	0.000248	293636	0.219	1.38E-05	556.39	1.97E+07
48	1.98635	14.9681	692.372	0.997	2.10E-05	0.006423	11.7643
49	0.951372	11.2209	5219.83	0.952	0.067712	5.86523	2990.14
50	0.000344	0.038343	11.7746	0.184	0.008336	63.97	65939.6
51	1.17E-06	0.148411	74.59	0.67	7.54E-08	0.000215	3.7896
52	0.051521	9.5104	189.092	0.92	1.13715	4281.29	4.40E+08
53	0.012127	0.40512	202.563	0.193	0.062896	5072.34	1.55E+07
54	4.73E-08	0.000189	1.95043	0.073	7.29E-06	0.000375	0.04686
55	0.856814	8.68639	378.614	0.993	3.38E-10	2.85E-07	0.000516
56	0.000283	0.017507	0.939783	0.032	0.031898	1.63719	80.0657
57	0.000411	0.037278	2.60449	0.065	1.17E-06	0.000649	3.44772
58	0.079207	2.37188	845.426	0.592	0.276867	3153.85	728662
59	0.465895	5.96175	1010.35	0.9	7.96E-09	1.18E-05	0.00823
60	2.19E-10	5.83E-07	0.011821	0	8.83E-05	0.011804	2.29152
61	0.000127	0.035377	163.912	0.173	0.000344	0.408797	6616.76
62	2.20E-08	0.001524	32.0659	0.164			

Table A2 (cont'd)

Inv_equal_start2_rep1

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000128	0.026347	0.165874	0.639936	1.22307	2.23314	0	0	0

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	0.017405	1.81684	15090.1	0.516	1.09E-06	0.001145	9.02229
1	1.58E-05	0.001386	0.479002	0.004	0.000119	1.97573	13748.6
2	5.58E-08	0.004785	5339.91	0.279	63.3354	46974.7	1.09E+07
3	0.000151	0.016171	6.22342	0.099	0.051351	409.422	1.67E+06
4	0.000405	0.554914	29.9751	0.447	209142	5.84E+06	1.23E+08
5	4.74E-06	0.008068	5.29221	0.104	0.16416	218.516	119885
6	0.003666	0.189348	24.0777	0.275	4.95E-07	6.34E-05	0.008356
7	0.026552	7.81118	2987.49	0.901	0.001735	0.279713	10.9829
8	0.026496	1.29706	118.611	0.556	0.306611	33.3447	16570.7
9	0.046667	8.77542	303.426	0.933	1.76E-09	2.81E-07	0.000101
10	4.92E-07	0.03187	57.375	0.207	7.89E-05	0.034763	4.21526
11	3.90733	46.8162	23213.8	1	99.5935	1.31E+06	3.82E+11
12	0.001586	0.493561	9.15607	0.454	1.43E-07	5.14E-05	0.013507
13	9.72E-08	0.040919	27864.8	0.319	1.05E-08	1.36E-05	0.43848
14	0.000504	0.194393	162.223	0.329	0.12096	2.50337	178.513
15	0.181509	5.69361	23585.8	0.723	3.86E-09	7.39E-07	0.000803

Table A2 (cont'd)

16	4.27E-06	0.022295	24.8556	0.217	0.027817	1.31401	34.5475
17	7.90E-16	3.85E-12	1.93E-07	0	7.96E-10	3.86E-07	0.000184
18	0.459558	8.45703	1128.57	0.902	1899	8.83E+06	5.43E+10
19	0.611348	6.16517	107.485	0.926	2.08E-13	2.49E-08	0.001094
20	4.31E-07	0.064818	622.224	0.377	1349.32	227599	1.61E+09
21	5.93E-11	0.00022	6.05842	0.168	1.0126	595957	8.44E+10
22	0.000121	0.032996	53.568	0.095	0.000357	0.267417	394.538
23	0.000161	0.147125	2299.2	0.17	218.352	16193.2	1.32E+07
24	5.27E-05	0.039317	3.74721	0.236	0.83823	2697.28	2.51E+06
25	6.30E-05	0.058353	181.888	0.271	976.916	9.48E+06	5.65E+12
26	2.52E-07	0.001396	1227.19	0.24	9.69E-08	0.000152	14.9504
27	0.003842	0.931374	64.9033	0.606	9.60E-14	2.31E-08	0.000463
28	0.021997	2.16706	372.455	0.581	0.001705	0.042703	1.11158
29	0.094235	4.52114	275.839	0.732	41517.4	1.03E+07	2.62E+10
30	0.06128	5.42745	446.759	0.851	8.53E-07	0.000139	0.015853
31	3.21E-05	0.045745	1224.49	0.247	3.70E-14	5.50E-08	0.325005
32	9.28E-06	0.065465	32979.6	0.34	2.28E-06	0.009113	35.9225
33	0.215841	8.41062	2653.18	0.851	1.61E-06	0.057405	9755.57
34	0.000223	0.028124	2.91422	0.112	17.2638	1565.59	571144
35	5.47E-07	9.72E-05	0.019303	0	0.331409	10.5049	316.694
36	0.133972	4.75681	933.907	0.801	734.099	5.21E+06	4.46E+09
37	1.11E-07	0.003016	80098	0.214	7.34E-10	0.000259	5095.47
38	2.70651	17.3374	661.951	0.989	6.22E-06	0.002006	3.61284
39	3.39E-05	0.065766	131.251	0.264	19534.6	2.49E+08	2.04E+12
40	3.66E-10	4.06E-08	1.73E-06	0	8.44E-06	0.005226	5.6745
41	0.125416	5.18311	1117.31	0.772	2.39E-07	1.70E-05	0.000563
42	7.97E-08	5.60E-06	0.000292	0	4.05E-05	1.22296	6890.06
43	0.109495	3.80158	2004.78	0.639	2.24E-05	0.001822	16.5618

Table A2 (cont'd)

44	0.742877	18.638	2899.61	0.945	0.08254	618.531	81663.2
45	9.63E-06	0.004932	5584.23	0.204	0.007425	178.957	1.45E+07
46	3.29E-08	0.00019	472.02	0.097	1.71E-09	3.61E-07	0.000143
47	0.000993	0.015315	0.31379	0	0.019309	23.9543	5867.54
48	1.45E-05	1.42969	33.4021	0.804	8.65E-06	0.023574	7.83887
49	2.06669	37.9797	13177.5	1	8.94E-09	7.57E-07	0.000221
50	6.44E-05	0.294672	10007.4	0.363	2.52E-18	5.00E-12	8.05E-05
51	0.046481	7.47361	103	0.889	0.000638	0.391875	4981
52	1.23E-05	0.624162	32.4116	0.731	44.2499	3873.58	296037
53	7.27E-05	0.146366	359.086	0.254	1.91E-09	2.06E-05	0.228238
54	0.001642	0.220008	9.32099	0.233	0.001749	1.59053	430.726
55	0.406692	14.4176	7558.94	0.879	1.18972	421.367	211888
56	6.89E-07	0.00024	0.852701	0.044	1.67714	326.22	31300.8
57	2.41E-07	0.009307	15012.4	0.292	346.278	2.89E+07	1.27E+12
58	0.109459	1.68131	28.5346	0.609	448.523	446216	3.43E+08
59	0.00227	1.46866	37.4993	0.78	0.515362	32.3373	13893.5
60	1.14E-07	0.001622	472.543	0.294	1.56E-05	0.003095	0.208314
61	7.52E-08	0.000207	0.206519	0	0.000111	0.644214	6734
62	2.08E-08	1.18E-05	0.041205	0			

Inv_equal_start2_rep2

	Synonymous theta			kappa			phi	
Lower	Point	Higher	Lower	Point	Higher	Lower	Point	Higher
95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD	95% HPD	estimate	95% HPD
0.000145	0.036891	0.144943	0.550709	1.25818	2.51534	0	0	0

Table A2 (cont'd)

		omega		Posterior		rho	
Site	Lower	Point	Higher	prob of +ve	Lower	Point	Higher
	95% HPD	estimate	95% HPD	selection	95% HPD	estimate	95% HPD
0	1.35E-09	2.02E-06	0.034773	0	5.06603	118838	7.77E+09
1	0.001762	0.051941	1.27514	0.042	1.28E-12	7.52E-08	0.002651
2	1.07E-05	0.004231	0.37205	0	1.10E-06	0.001	0.193055
3	5.19E-12	3.13E-08	6.97E-05	0	45.1549	47882.4	4.91E+06
4	0.069207	2.88688	10746.3	0.633	6.31E-09	3.38E-06	0.007706
5	0.001523	0.197192	917.54	0.251	9.61E-07	0.142955	8885.61
6	0.000728	0.017285	4.58413	0.063	0.602258	4978.54	3.01E+06
7	0.01323	3.26675	48.5311	0.846	5.15E-06	6.02892	2.38E+06
8	0.215842	3.29875	2225.25	0.707	0.003732	0.880736	35.9812
9	2.15341	16.9384	4014.96	0.986	0.000154	0.003034	0.172374
10	4.27E-08	9.87E-05	506.576	0.157	0.000372	0.089997	5.21379
11	0.001358	10.2533	100.75	0.903	6.69E-05	0.032985	3.69368
12	0.066634	1.86901	1018.47	0.49	3.24E-10	2.66E-07	3.97E-05
13	4.57E-06	0.006565	597.221	0.21	3.93E-09	1.28E-06	0.000233
14	8.25E-07	0.003678	1.62118	0.026	0.057289	163.363	1.49E+06
15	0.005301	2.23621	20114.1	0.631	0.376516	400.776	5.00E+08
16	7.01E-10	2.31E-07	0.000102	0	2.61E-07	4.04782	1.50E+06
17	5.97E-05	0.068418	152.472	0.151	1003.45	1.70E+07	2.14E+10
18	2.17869	9.46865	117.735	0.973	3.95E-08	0.01165	93.9896
19	0.000251	1.26419	49.6268	0.78	1.16E-08	5.11E-06	0.003659
20	2.65E-06	0.001035	0.883072	0.003	3.38E-06	0.042889	7.47576
21	8.40E-05	0.005762	0.899954	0	2.77E-12	0.000516	1.02504
22	0.000715	0.331345	12067	0.38	0.003735	0.46643	1377.24
23	0.00076	0.318428	2682.05	0.269	3.45E-05	0.006021	2.91551
24	0.006398	0.552785	7.96736	0.376	3.16E-06	0.006259	533.063

Table A2 (cont'd)

25	2.40E-06	0.00066	1.25163	0.032	973.195	1.00E+07	1.07E+12
26	0.000807	0.253282	3634	0.285	14.9774	12127.5	1.26E+06
27	0.136414	1.82891	99.7761	0.597	0.000371	0.035233	4.08494
28	0.000656	0.263849	9.29314	0.444	4.08E-10	8.77E-07	0.517771
29	0.042568	2.15942	2293.59	0.577	162180	1.74E+08	1.20E+11
30	0.163072	2.14857	62.9978	0.734	1490.92	146661	2.66E+07
31	7.31E-05	0.015726	217.388	0.147	0.013525	11.9727	2506.45
32	3.00E-08	0.008203	3255.62	0.238	0.001758	3.5856	205474
33	0.000288	0.920237	10.741	0.712	2754.38	3.39E+08	3.34E+12
34	2.27E-06	0.012967	32411.2	0.168	1333.55	861051	2.25E+08
35	4.53E-10	0.002412	10754.8	0.14	20704.8	1.43E+07	3.84E+10
36	0.084454	1.91761	18.8954	0.639	4.17E-07	0.000636	0.892626
37	9.43E-08	0.00179	778.157	0.205	2.48E-13	5.91E-08	0.147258
38	0.02018	2.23885	34.6035	0.826	0.831952	7.16287	65.7318
39	2.30E-08	2.24E-05	0.204619	0	5.17E-08	0.066925	134.151
40	1.00E-07	1.51E-05	0.012029	0	7.30E-05	0.019558	5.36786
41	0.20286	6.04605	716.569	0.893	1.50586	181.485	18308.3
42	4.42E-05	0.22686	19926.4	0.342	2.67E-07	1.12E-05	0.002286
43	0.008713	1.31323	2580.69	0.478	1.74E-05	0.306895	970.804
44	8.99E-06	1.04201	30.3537	0.771	1.41655	662599	3.14E+11
45	4.74E-06	0.063435	282.883	0.258	0.040051	1201.01	1.80E+07
46	5.61E-05	0.011173	0.977244	0.006	1.20E-09	7.81E-07	0.085504
47	7.42E-05	0.052778	207.489	0.218	49.0906	1.37E+06	2.03E+10
48	0.000652	2.64972	48.0281	0.852	0.000345	0.039435	6.61311
49	0.784615	25.2964	27841.8	0.977	0.131	1.37235	7.6901
50	7.44E-08	1.91E-05	0.015501	0	1.82E-07	9.31E-05	0.004532
51	0.418373	8.52749	151.399	0.936	7.21E-10	3.23E-07	0.000303
52	0.000549	2.61581	34.8655	0.845	9.94E-08	1.04E-05	0.001345

Table A2 (cont'd)

53	0.002105	0.073307	1.56784	0.055	8.11E-05	0.005788	0.340584
54	2.32E-08	4.62E-06	0.000692	0	1356.31	72024.4	1.54E+06
55	0.470075	6.92169	1009.39	0.912	0.000301	1.39554	42758.2
56	9.62E-08	5.64E-06	0.00203	0	1.62E-06	0.005193	3.50627
57	0.00013	0.03157	5.57117	0.155	13.8757	3.52E+08	5.26E+13
58	0.002701	0.647145	61.4766	0.562	0.00124	1.06387	28605.6
59	0.000136	0.672158	13.5883	0.744	18.5802	78307.1	1.71E+09
60	0.000105	0.126907	59177.3	0.271	7.11E-06	0.00099	1.7848
61	0.000413	0.084435	3.08715	0.162	1.10E-08	0.035715	574.798
62	6.68E-08	0.003235	30.5113	0.132			

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CHAPTER THREE

MHC DIVERSITY AND FITNESS IN SPOTTED HYENAS (*CROCUTA CROCUTA*)

INTRODUCTION

The major histocompatibility complex (MHC) is a group of genes that are the most diverse among all of the coding loci in vertebrates. The MHC complex is comprised of two "classes" of genes: class I genes, which are expressed by nearly all nucleated cells, and class II genes, which are only expressed by specialized antigen-presenting cells of the immune system, such as B cells and macrophages (Klein 1986). MHC genes are essential to the adaptive immune response, as they code for cell surface glycoproteins that recognize and bind foreign antigens, and then present them to immune system cells, initiating an adaptive immune response (e.g. Apanius et al. 1997). The high levels of polymorphism within MHC loci are thought to represent adaptations for recognizing and responding to a wide range of rapidly evolving pathogens and parasites (reviewed in: Garrigan and Hedrick 2003; Piertney and Oliver 2006; Sommer 2005). This central role of MHC molecules in the adaptive immune response has led to the view that the high levels of variation observed in MHC loci are maintained by pathogen-driven positive selection, acting via overdominance (heterozygote advantage) and/or negative frequency dependence (rare allele advantage) (Doherty and Zinkernagel 1975; Hedrick 1998; Hughes and Nei 1988, 1989; Hughes and Yeager 1998; Jeffery and Bangham 2000; Piertney and Oliver 2006; Potts and Slev 1995). Sexual selection is also thought to play a role in maintaining this diversity by favoring MHC-based disassortative mating preferences mediated by odor-based discrimination of MHC genotypes, or by selective abortion due to maternal-fetal incompatibility

(Edwards and Hedrick 1998; Fernandez et al. 1999; Milinski 2006; Ober 1992; Penn and Potts 1998b, 1999; Wedekind et al. 1995; Wedekind and Penn 2000; Yamazaki et al. 1999).

Here, we examined the relationship between MHC diversity and fitness in a natural population of a large carnivore, the spotted hyena (*Crocuta crocuta*), by investigating MHC diversity and its relationship to cub survival, longevity, measures of immune system function, parasite load, and reproductive success. In earlier work, we found that MHC loci in spotted and striped (*Hyaena hyaena*) hyenas demonstrated high allelic diversity, and we also found there is strong evidence to support the notion that this diversity is under positive selection (Califf et al. 2013). Spotted hyenas offer a unique system in which to address questions pertaining to MHC diversity and gene-based mate choice in a wildlife population, as there is unparalleled opportunity for female choice of mates in this species, and there are also potential major advantages associated with immune system vigor. Spotted hyenas are capable hunters that have recently descended from carrion feeding ancestors (Lewis and Werdelin 2000; Werdelin 1989), and they are exposed daily to a large array of pathogens, suggesting that mate choice based on genes responsible for disease resistance should be highly adaptive in this species.

Detailed data have been collected on the behavioral ecology of spotted hyenas in the Masai Mara National Reserve (MMNR) for over 25 years, elucidating many aspects of their biology including social structure, communication, and mating system (reviewed by: Holekamp and Dloniak 2010; Holekamp et al. 2012). Here, we combined our demographic and genetic data to inquire whether MHC diversity influences specific aspects of spotted hyena health and fitness.

Assuming that spotted hyena cubs resemble other young mammals studied to date, the immune systems of young hyenas are not fully formed at birth, and they acquire passive immunity after parturition in the form of antibodies transferred via their mother's milk (e.g. Brambell 1958; 1966; Mason et al. 1930). Hyena cubs are entirely dependent upon their mothers for sustenance typically until at least 9 months of age, when they become independent of the clan's communal den, and begin consuming meat, though they continue to nurse up until an average of 14 months of age (Hofer & East 1995; Holekamp et al. 1996). Despite possibly receiving maternal antibodies perinatally as do other mammals (Brambell et al. 1951), spotted hyena cub mortality during the first two years of life is high, with estimates ranging up to 61% (White 2005; Watts et al. 2009). Therefore, the time before den independence may represent a time when the protection offered by a mother's immune system may be of particular importance to the survival of her cubs, as the cubs may be unable to mount an efficient immune response to pathogens during this critical early life history stage. If maternal MHC diversity is important to early post-natal cub survival in spotted hyenas, then we would expect maternal MHC diversity to predict cub survival to the age of den independence.

Whereas all carnivores are exposed to pathogens via their prey (e.g. Reperant et al. 2008; Boone et al. 2009; Jennelle et al. 2009; Gortazar et al. 2010; Wilson and Wolkovich 2011), spotted hyenas are potentially exposed to higher pathogen concentrations, and to a greater diversity of pathogens, than are sympatric carnivores, due to their frequent consumption of carrion (Houston 1979). In addition, whereas non-Hyaenid carnivores in Africa are known to suffer high mortality from various infectious diseases (e.g. rabies: Kat et al. 1995; Maas 1993; canine distemper virus: Carpenter et al. 1998; Roelke-Parker et al. 1996; van de Bildt et al.

2002), spotted hyenas seldom exhibit symptoms of infection, and disease-induced mortality is surprisingly rare, despite evidence for infection rates comparable to those documented in sympatric carnivores (East et al. 2001; Haas et al. 1996; Murray et al. 1999; Watts and Holekamp 2009; but see Mills 1990). Low disease mortality and carrion feeding suggest that immune function in the spotted hyena might be unusually robust. If MHC diversity is important for enhanced immune function in spotted hyenas, we would expect to see positive correlations between MHC diversity and indicators of vigorous immune function. Indicators of immune function we considered here were serum levels of antibodies IgG and IgM, serum bacterial killing ability, serum titer of 6 common viruses known to infect sympatric carnivores, and parasite load. It also follows that immune system diversity, and hence ability to mount an immune response to a variety of pathogens, may contribute to spotted hyena longevity. If individual MHC diversity is important to overall long-term survival, we would expect MHC diversity to predict individual longevity, measured as known age at death.

Natural populations of spotted hyenas host a wide array of pathogens, ranging from viruses to macroparasites (East et al. 2001; Engh 2002; Engh et al. 2003; Haas et al. 1996; Harrison et al. 2004). Engh (2002) quantified the occurrence and intensity of infection by three common parasite genera in spotted hyenas. Engh found different predictor variables to be significant in predicting loads of different parasite species. For example, high-ranking hyenas were most likely to carry heavy loads of *Spirometra* sp., females were more likely to carry heavier loads of *Isospora* sp. than were males, and juvenile hyenas were far more likely than adults to carry hookworms (*Ancylostoma* sp.). As was pointed out by Engh (2002), parasite infection levels in spotted hyenas are likely influenced by many aspects of both hyena and

parasite ecologies, many of which were not accounted for in her study. If MHC diversity is one of these influences affecting parasite resistance in the spotted hyena, then we would expect that MHC diversity to correlate with individual parasite load, although whether to expect such correlations to be positive or negative is not entirely clear.

Reproduction in spotted hyenas is primarily controlled by adult females, because these females can be exceptionally choosy when selecting mates. This is due to the adult females' social dominance over all immigrant males within a clan, as well as to the female's highly masculinized genitalia, which make forced copulation impossible (East et al. 1993). Further, chemical communication via scent marking is well documented in spotted hyenas, and earlier work indicates that these scent cues convey information about relatedness, group membership, and individual identity (e.g. Burgener et al. 2009; Drea et al. 2002; Kruuk 1976; Mills 1990, Theis 2008, Theis et al. 2012). Given the significant role of chemical communication in this species, it is plausible that hyenas use MHC-mediated odor cues when making mate choice decisions. If MHC diversity in spotted hyena offspring influences the fitness of their mothers, then females should choose mates that offer an optimal MHC diversity for their offspring. As female choice is absolute in hyenas, by choosing males that possess an optimal MHC genotype, a female may be giving her offspring a selective advantage via increased offspring immunocompetence or increased ability to cope with parasites (Hamilton & Zuk 1982; Folstad & Karter 1992).

Disassortative mating based on genetic similarity, and specifically on MHC similarity, is commonly offered as a mechanism by which mate choice acts to maintain genetic diversity, and mate choice decisions based on genetic diversity or genetic compatibility are well documented in many vertebrate species (e.g. mice (Yamazaki et al. 1976, 1988; Penn and Potts 1998a,b);

humans (Ober et al. 1997; Wedekind and Furi 1997); fish (Reusch et al. 2001; Milinski et al. 2005); birds (Freeman-Gallant et al.2003; Bonneaud et al. 2006). Sexual selection theory offers three mutually non-exclusive hypotheses for the basis of genetically informed mate choice: the 'good genes' hypothesis, the 'genetic compatibility' hypothesis, and the 'inbreeding avoidance' hypothesis (reviewed by: Penn and Potts 1999; Tregenza and Wedell 2000; Neff and Pitcher 2005).

The 'good genes' hypothesis predicts that all females should choose the same mates, choosing males based on their diversity, independent of the female's diversity, that offer their offspring optimal immune system variation (e.g. Penn & Potts 1999; Ekblom et al. 2004; Eizaguirre et al. 2009). This hypothesis stems from observations that heterozygous individuals enjoy greater fitness than do homozygotes in a majority of species studied. (e.g. Amos et al. 2001; Hansson & Westerberg 2002). The second and third hypotheses differ from the 'good genes' hypothesis in that these two both predict that females should vary in their individual mate preferences, depending on their own MHC genotypes and on the MHC genotypes possessed by members of the pool of available mates. In these instances, each female should mate with a male whose MHC genotype differs from her own, thereby possibly providing her offspring with a more diverse (i.e. an optimal) MHC repertoire with which to respond to a wider array of pathogens (Apanius et al. 1997; Tregenza & Weddell 2000; Olsson et al. 2003; Schwensow et al. 2008). Further, many studies have shown that mating between MHC-similar individuals can lead to spontaneous abortion and overall increased risk of pregnancy loss (e.g. Ober et al. 1998; Mor 2006).

Female spotted hyenas are philopatric, remaining in their natal clans throughout their lives, whereas male hyenas disperse from their natal clan after reaching sexual maturity (Smale et al 1997; Boydston et al 2005). Successful immigration appears to influence mate choice in spotted hyenas, as adult natal males sire only 2-3% of all offspring born in the clan and immigrant males sire the rest (Engh et al. 2002; Van Horn et al. 2008). Long term male reproductive success (RS) is positively correlated with total time in the clan (East and Hofer 2001; Engh et al. 2002) but within any given year of male tenure, RS varies widely, which suggests there may be other factors affecting male RS aside from tenure in the clan. Further, East et al. (2003) showed that female spotted hyenas do not mate with a few "high quality" males; instead their data suggest a role for genetic compatibility in mediating hyena mate choice decisions. If mate choice decisions by female spotted hyenas are influenced by MHC diversity, we would expect to observe correlations between measures of annual reproductive success (ARS) and individual MHC diversity among immigrant male hyenas.

The extreme diversity within MHC genes has frequently proven to be an obstacle to analysis, often limiting the conclusions that can be drawn about specific alleles. In an attempt to resolve this problem, and also to be able to accurately characterize the effects of MHC diversity, here we classified MHC alleles into groups of alleles based on their similar antigen binding motifs, called 'supertypes' (Sette and Sidney 1999; Sidney et al. 1995; Lund et al. 2004). Supertype groupings are based on similarities in antigen binding sites (ABS), such that alleles of the same supertype group bind similar pathogens and are considered to contain "functionally similar" alleles (e.g. Southwood et al. 1998; Sette & Sidney 1999; Buchli et al. 2005). This practice began in biomedicine, and was soon implemented in behavioral ecology as well.

Numerous studies now offer evidence supporting the biological relevance of these supertypes to disease resistance in wild populations, and we employ this classification method here (Schwensow et al. 2007; Huchard et al. 2010; Clough et al. 2011; Sepil et al. 2013).

Our explicit goal here was to inquire whether any relationships exist in spotted hyenas between MHC diversity and fitness by determining whether MHC diversity correlates with measures of cub survival, longevity, immune system function, parasite infection, or adult male reproductive success.

METHODS

Study population and sampling

Our study population consisted of a group of wild spotted hyenas, known as the Talek clan, which has been monitored continuously since 1988 in the Masai Mara National Reserve (MMNR) in Narok District, Kenya by workers on the Mara Hyena Project (Boydston et al. 2001). Data presented here are based on samples collected from the Talek clan in the MMNR from 1996 to 2009. All hyenas in this clan were individually identified by spot patterns and other unique markings (e.g. damaged ears; Frank 1986). The sex of each animal in our study population was determined by the dimorphic glans morphology of the erect phallus (Frank et al. 1990). Mother-offspring relationships were determined based on observed nursing associations, and birth dates of natal animals were estimated to within one week based on their first appearance above ground at dens (Holekamp et al. 1996). We were able to reliably estimate ages of immigrant males (± 6 months) using a tooth wear age estimation model (Van Horn et al. 2003). Social ranks were assigned based on outcomes of dyadic agonistic

interactions, which were documented via 'all occurrence' sampling (Altmann 1974; Holekamp & Smale 1990). For all analyses presented here, the social rank value assigned to each individual was an average of its standardized lifetime rank, with one rank value assigned to each year of the individual's adult lifespan, then standardized on a scale from -1 (lowest ranking) to +1 (highest ranking). Behavioral observations were collected for approximately 6 to 7 hours each day around dawn and dusk. Morning observations were conducted from approximately 0530 hours until 0930 hours, and evening observations were conducted from approximately 1630 hours until 2000 hours.

During routine immobilizations, hyenas were anesthetized with 6.5mg/kg of Telazol administered in a plastic dart from a CO_2-powered rifle. While the animals were anesthetized, blood samples were collected and preserved for later analyses. Detailed methods used to capture animals and collect blood samples used to extract DNA are described in detail by Engh et al. (2002). All sampling procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University (AUF 07/08-099-00), complied with Kenyan law, and met guidelines approved by the American Society of Mammalogists (Sikes et al. 2011).

Endoparasite data analyzed here were based on fecal samples collected from 27 spotted hyenas in the Talek clan, for whom we also had MHC data, between June 1999 and July 2000, as previously described (Engh 2002; Engh et al. 2003). The presence or absence of microfilariae (early stage parasitic nematodes) in the blood was also determined for 10 individuals for whom we also had MHC data, using a Modified Knott's technique, as described by Yabsley et al. (2004).

Microsatellite genotyping and MHC variation

Paternity assignments in our study population were based exclusively on genotyping, as previously described (Van Horn et al. 2004; Watts et al. 2011). Genotypic pairwise relatedness (*R*) values were estimated using microsatellite data with the software program RELATEDNESS (Queller and Goodnight 1989). We focused our measure of MHC diversity on regions of functional class II MHC loci that have previously been reported to contain antigen binding sites (ABS). ABS are highly polymorphic sites known to be involved in antigen presentation (Hughes and Nei 1988, 1989; Klein 1986). We PCR-amplified loci of 198 bp and 195 bp from *DRB* exon 2 and *DQB*, respectively. In order to obtain a representative sample of MHC alleles for this population, we characterized diversity in a random sample of 26 individuals at the *DRB* locus and 20 individuals at the *DQB* locus, and sequenced individual clones to identify alleles as previously described (Califf et al. 2013). MHC supertypes were defined within both the *DRB* and the *DQB* loci of the spotted hyena by applying amino acid sequence-based cluster analysis as previously described (Doytchinova and Flower 2005; Schwensow et al. 2007; Califf et al. 2013).

The number of alleles found within an individual increased with the number of clones that were successfully sequenced for that individual (detailed by Califf et al 2013). However, a variable number of clones were sequenced per individual. In order to correct for this sampling bias, we performed random sampling with replacement to estimate an average number of alleles and supertypes per individual, using the 'sample' function in R version 2.15.2 (R Core Development Team 2012). Here we used the average number of supertypes, and the presence of specific supertypes, at both the *DRB* and the *DQB* loci as each individual's measures of MHC

diversity. Given the substantial evidence supporting the functional importance of supertype classification as it pertains to the immune system's ability to recognize pathogens, we chose to focus on supertype diversity, and used these measures in all tests presented here that assessed relationships between MHC diversity and fitness. The evolutionary significance of allelic diversity of MHC loci in spotted hyenas is discussed in detail elsewhere (Califf et al. 2013).

To test whether MHC similarity influenced mating patterns, MHC supertype similarity (D_{AB}) was measured between each potential pair of mates using a measure known as the 'band sharing coefficient.' This was calculated as $D_{AB} = 2\ F_{AB} / (F_A + F_B)$, where F_{AB} is the average number of shared supertypes between a male (A) and a female (B), and F_A and F_B are the average number of supertypes possessed by A and B, respectively (Wetton et al. 1987).

Statistical analyses

Unless otherwise specified, all tests were implemented in R version 2.15.2 (R Core Development Team 2012). All variables were tested for collinearity within models using variance inflation factor analyses, and Anderson-Darling and Shapiro-Wilk tests were used to assess normality of residuals for all variables (Bolker et al. 2009; Larivée et al. 2010; Zuur et al. 2009). Data were examined for the presence of outliers using influencePlot, outlierTest, and av.Plots in the 'car' package as well as influence.measures in the 'stats' package in in R. We used both GPOWER (Erdfelder et al. 1996) and the 'pwr' package in R to conduct post hoc power calculations for nonsignificant, univariate analyses that were based on small sample

sizes. We corrected for multiple testing using the sequential Bonferroni adjustment, and report all *P* -values in their corrected form (Holm 1979; Rice 1989).

Cub survival

To ask whether or not either a female's number of MHC supertypes or presence of specific supertypes influences the survival of her cubs to den independence, we performed generalized linear mixed models (GLMMs), with cub survival to den independence as a binomial response (i.e. the cub survived or did not). We considered a hyena cub to be independent of the den when they were found more than 200 meters from the current communal clan den in at least 4 consecutive observation sessions; this typically occurred when the cubs were approximately 9 months of age (Boydston et al. 2005). Each female in our dataset was included multiple times because all gave birth to more than one cub during the study period, so we used maternal ID as a random effect in our models. We used an ANOVA to test for significant differences between GLMMs with and without MHC diversity as a predictor. We were unable to test the influence of male (sire) MHC diversity on cub survival to den independence, as we were only able to obtain DNA for paternity determination if a cub survived to this life history milestone. Maternal social rank was included as a predictor in all of these models, as it has been found previously to influence cub survival (e.g. Watts et al. 2009).

Longevity

In order to test whether MHC diversity predicts individual longevity, general linear models (LMs) were used to ask whether individual MHC supertypic diversity at either the *DRB* or the *DQB* locus predicts age at death (in months) for hyenas with known ages at death. All

ages at death in our data were log transformed to improve normality. LMs were used to ask whether specific supertypes significantly predicted longevity. We used log-likelihood ratio tests (LRT) to determine whether LMs including MHC diversity (as indicated by the number of supertypes or the presence of specific supertypes) predicted parasite intensity significantly better than linear models that did not include MHC data as predictor variables. The log-likelihood ratio was calculated as 2[log-likelihood of model B – likelihood of model A], and was tested as a chi-squared distribution (Pinheiro and Bates 2000). Wilcoxon rank-sum tests were used to verify differences between groups, when any significant results were found using linear models, to assess the effect of specific supertypes (i.e. longevity in a group with a specific supertype versus longevity in a group without the specific supertype). Social rank, sex, and dispersal status (i.e. immigrant versus natal) were also included as predictor variables in these linear models.

Immune function

We used three measures of immune function that were quantified as described in Flies et al. (2012): *in vitro* serum bacterial killing ability (BKA), total serum immunoglobulin G (IgG), and total serum immunoglobulin M (IgM). Where individuals were measured more than once, average measures were used. All measures were log (x + 1) transformed to improve normality before analyses. General linear models (LMs) were performed to inquire whether individual average number of supertypes at *DRB* or *DQB* predicted our three measures of immune function. Social rank has previously been found to predict BKA and total serum IgM (Flies et al.

2012), so this predictor variable was included as a covariate in our models. Models were compared using log-likelihood ratio tests (LRT).

We also looked for a relationship between MHC diversity and presence in serum at sampling of six common canid and felid viruses: canine distemper virus (CDV); coronavirus (CoV); feline calcivirus (FCV); feline herpesvirus (FHV); feline immunodeficiency virus (FIV), and feline panleukopenia virus (FPV)). Sampling and serological assay details for these viruses were conducted and described by Harrison et al. (2004). Here we performed Wilcoxon rank-sum tests to inquire whether presence of specific supertypes correlated with positive viral titers. For any significant results, we also tested the relative risk of being infected by the specific virus in question depending on supertype using odds ratio tests, a common method used in epidemiological studies. The odds ratio is the ratio of the odds of an event (in this case, a positive titer) occurring in one group (here, individuals that possessed a specific supertype) versus the odds of it occurring in another group (individuals that did not possess this same particular MHC supertype) by using a 2x2 cross-classification table (Edwards 1963; Bland and Altmann 2000). The relative risk is related to the odds ratio, in that relative risk is the ratio of the probability of an event occurring in one group versus occurring in another. Relative risk and odds ratios are often reported together (Sistrom and Garvan 2004).

Parasite burden

We used a subset of parasite data that were obtained by Engh (2002) in order to test whether MHC diversity influences the intensity of parasite load in spotted hyenas. As raw measures of parasite intensity, we quantified four different measures to assess the intensity of

individual parasite load: total number of different parasite genera (NumGen) present within each fecal sample, overall total fecal egg count (FEC), and genus-specific fecal egg count (*genus*FEC) for two commonly found parasites within the population (Engh 2002; Engh et al. 2003). All egg counts were log (x + 1) transformed to improve normality. The two common parasite genera for which eggs were counted in fecal samples were: *Ancylostoma* sp. (a nematode gut parasite), and *Isospora* sp. (a coccidian protozoan). These four measures were entered into a principal component analyses (PCA), and each individual's score from the first principal component axis (PC1) was used as our measure of parasite intensity. We built LMs to ask whether individual MHC diversity predicted the intensity of parasite infections. We used log-likelihood ratio tests (LRT) to determine whether LMs including MHC diversity (as indicated by the number of supertypes or the presence of specific supertypes) predicted parasite intensity significantly better than LMs that did not include MHC data as a predictor variable.

Male reproductive success and mate choice

In order to test the 'good genes' hypothesis, general linear models were built to inquire whether a male's MHC supertype diversity at either locus (*DRB* or *DQB*) predicted his annual reproductive success (ARS). ARS was measured as the total number of offspring that survived to den independence (our life history milestone of interest) that were sired by a male, divided by the number of years during which this male was reproductively active, as indicated by his tenure in the clan. For any adult natal males included in our analyses, 'tenure' was considered to be any time spent in the clan after reaching reproductive maturity at 24 months of age. Previous research with spotted hyenas has demonstrated a strong relationship between social

rank and ARS (Hofer and East 2003; Holekamp et al. 1996; Swanson et al. 2011), so here we also included rank as a predictor of ARS in these models.

We tested a second prediction of the 'good genes' hypothesis by asking, for all litters on which we had data, whether a male's MHC diversity predicted whether or not he sired a specific litter. Throughout this paper, we refer to "sires" as males who were genetically determined to have sired cubs, and "non-sires" as males who were concurrently in the clan and reproductively active when the litter in question was conceived, but were not the sire. Hence, any individual male could be a sire for one litter, but a non-sire for a different litter. Specifically, generalized linear mixed models (GLMMs) were performed to ask whether male MHC diversity predicted whether the male was a sire or a non-sire for each litter sampled. In these models, sire versus non-sire was treated as a binomial response, and male ID was a random effect. Tenure was also included as a predictor variable. ANOVA tests were performed to compare models with and without our predictor variables of interest. Finally, in our third test of the 'good genes' hypothesis, we performed Wilcoxon rank-sum tests to inquire whether male MHC diversity at either locus differed significantly between sires and non-sires.

In order to test the 'genetic compatibility' hypothesis, we performed the same three tests explained above in testing the 'good genes' hypothesis, but here we replaced male MHC diversity with D_{AB} , our measure of pairwise male-female MHC similarity (described above in methods) as our predictor variable. First, a Spearman's rank correlation was used to inquire whether the average pairwise MHC similarity (D_{AB}) between a given male and any female who conceived a litter during that male's tenure was correlated with that male's annual

reproductive success (ARS). Second, GLMMs were performed to test whether D_{AB} between a female and each male present in the clan when each litter was conceived predicted whether or not the male sired that particular litter. As above, male ID was a random effect and male tenure was a fixed effect in the model. Lastly, Wilcoxon rank-sum tests were used to inquire whether D_{AB} between females and sires differed significantly from that between females and non-sires.

We also generated a bootstrap model in which we simulated female choice by resampling from all males available when a particular litter was conceived to estimate a distribution of the MHC supertype similarities between all males then present in the population and the mother of each litter, and then asked where the sire of the litter fell within this distribution. More specifically, males were randomly sampled with replacement 1000 times from the pool of potential sires (average number of potential sires per litter = 6.6 ± 0.58 SE) for each litter, and we estimated break points by equally dividing the lower, middle, and upper third of these data. After this was done 1000 times, the bootstrap mean of the break points between bins was estimated for each cub. We then asked where the sire for each litter fell among these three bins to ascertain whether the sire was of equivalent similarity to the non-sires (in the middle bin), or if he fell above (in the upper bin) or below (in the lower bin) the average similarity between mothers and non-sires expected from simulated random choice of non-sires. A chi-squared test was then performed to ask whether the expected three bins that resulted from random sampling were significantly different from the observed lower, middle, and upper third of our similarity data on sires. This was done to determine whether the

observed patterns we saw in similarity between mothers and sires differed from the pattern we would expect based on random choice.

RESULTS

MHC diversity

Our results detailing observed MHC diversity are summarized in Table 4, and further details on allelic diversity are available in Califf et al. (2013). Our measure of similarity (D_{AB}), the average pairwise band sharing coefficient between individuals, was 0.19 ± 0.02 SE for alleles at the *DRB* locus, and 0.47 ± 0.04 SE at *DQB*; the average pairwise band sharing coefficient between individuals for supertypes was 0.48 ± 0.02 SE at the *DRB* locus and 0.54 ± 0.04 SE at the *DQB* locus. A Spearman's rank test revealed no correlation between an individual's social rank and its average supertype diversity at either locus ($p > 0.05$ for both tests; $\rho = 0.125$ for *DRB*; $\rho = 0.436$ for *DQB*).

Cub survival

We used survival data from 128 individual cubs born to 14 different mothers in our analysis of the *DRB* locus, and 47 cubs born to 7 mothers at the *DQB* locus. The *DQB d*ata were derived from a subset of the same individuals considered at the *DRB* locus. There was no evidence that overall maternal MHC supertypic diversity at either locus predicted an individual's survival to den independence (Appendix C; adjusted $p > 0.1$ for all GLMMs; average power = 0.40). However, we found a trend suggesting that cubs were more likely to survive to den independence if their mother possessed supertype 5 at the *DRB* locus (Figure 3; Appendix

C; un-adjusted p = 0.024; adjusted p = 0.192; power = 0.335). Five out of 14 sampled mothers (35.7%) possessed supertype 5 at *DRB*, and 79.17% of the cubs born to those mothers survived to den independence, compared to only 53.75% of cubs born to females that did not possess supertype 5. More samples are needed to ascertain whether this trend is real or spurious. Possession of supertype 5 was not associated with maternal social rank in the *DRB* data set (W = 11, p = 0.343). Maternal social rank was found to be a significant predictor of cub survival in our *DQB* data set, but not within our *DRB* dataset (Appendix C; power = 0.18).

Longevity

We assessed the relationship between age at death and number of *DRB* supertypes in 26 individuals (average age at death = 104.262 ± 10.118 SE months), and number of *DQB* supertypes in 20 individuals (average age at death = 91.142 ± 11.203 SE months). Using general linear models, neither the number of supertypes nor presence of any specific supertype at either locus were significant in predicting the longevity of individual hyenas (Appendix D). However, a non-parametric Wilcoxon rank-sum test showed that, on average, individuals who possessed supertype 1 at their *DRB* locus had a significantly longer lifespan than individuals that did not possess this supertype (Figure 4; Appendix D; W = 11, p = 0.021). The average lifespan of individuals who did carry supertype 1 (n=4) was 159.12 months (± 23.65 SE) compared to 94.29 months (± 9.94 SE) for individuals who did not possess this supertype (n=22). We found no effect of specific supertype at the *DQB* locus on individual longevity. Social rank, dispersal status, and sex were all significant predictors when using the *DQB* dataset, however only

dispersal status and sex were significant predictors when considering our *DQB* data (Appendix D).

Immune function

Our measures of MHC diversity at the *DQB* locus did not significantly predict any of our measures of immune function, although we found several *DRB* supertype-specific effects on measures of immune function (Appendix E). The presence of supertype 2 at the *DRB* locus significantly correlated with lower levels of bacterial killing ability (BKA; Figure 5; $p = 0.014$) and lower concentrations of serum immunoglobulin M (IgM; Figure 6; $p = 0.040$). Additionally, presence of supertype 5 significantly correlated with higher levels of IgM (Figure 7; $p = 0.040$). Social rank was not found to be a significant predictor of BKA or IgM in our data subsets (contrasting with previous findings by Flies et al. 2012), and was not used as a predictor in subsequent models (Appendix E).

Due to lack of variation in our serology data, we were unable to examine the relationship between MHC variation and positive titers of FHV (all individuals tested negative) or FPV (all individuals tested positive). We tested data from the four remaining viruses, with an average of 23.75 ± 1.65 SE individuals at the *DRB* locus and 18.25 ± 1.18 SE individuals at the *DQB* locus. We also asked whether specific MHC supertypes influenced titers of CDV, FCV, CoV, or FPV. We found no evidence to support a relationship between the number of MHC *DRB* or *DQB* supertypes and titers for any of the tested viruses (Appendix F; $p > 0.1$ for all tests). We did, however, find a non-significant trend indicating that individuals are 5.33 times as likely to

test positive for FIV when they do not possess supertype 7 at the *DRB* locus (Figure 8; Appendix F; n= 19; $p = 0.059$; Odds Ratio= 0.071).

Parasite burden

We assessed the effect of MHC *DRB* supertype diversity on parasite intensity from 27 individuals, MHC *DQB* supertype diversity in 20 individuals. The first principal component axis (PC1) resulting from the PCA on our four parasite intensity measures accounted for 55.1% of the variance in the data, and the scores calculated from the loadings for this axis were used as our measure of parasite intensity (Table 5). All four measures of parasite intensity exhibited positive loadings with PC1, indicating that individuals with extreme values in one measure of parasite intensity are likely to have extreme values of the measures as well, and extreme in the same direction (Table 5; Number of parasite genera: 0.416, total fecal egg count (FEC): 0.623, FEC for *Ancylostoma* sp.: 0.604, FEC for *Isospora* sp.: 0.273). We tested the presence or absence of our two specific parasite genera as response variables in logistic regression models. MHC diversity at the *DRB* locus did not significantly predict values of PC1 ($p > 0.05$). We also found no evidence of an effect of the presence of specific MHC *DRB* supertypes on individual parasite infection intensity or species presence (Appendix G; $p > 0.05$ for all tests).

We found evidence supporting a relationship between the intensity of parasite infection and MHC variation at the *DQB* locus in spotted hyenas, such that the number of supertypes an individual possessed at the MHC *DQB* locus was a significant predictor of PC1, our measure of parasite intensity (Appendix G; Figure 9; $\chi^2 = 10.349$, df = 1; adjusted $p = 0.01$). These data suggest that increased MHC diversity may enable individuals to harbor relatively innocuous

parasites without a decrease in fitness (Apanius et al. 1997; Bernatchez and Landry 2003; Piertney and Oliver 2006; Spurgin and Richardson 2010). None of the variables previously found by Engh (2002) to be significant predictors of parasite intensity or presence were significant in our study, and we found no strong evidence for any supertype-specific effects on parasite load (Appendix G).

Male reproductive success and mate choice

We had MHC sequence data and corresponding complete reproductive histories for 12 males. Due to this low sample size, our statistical power for these tests was low, and no significant correlations were found between male MHC supertype diversity and ARS (Appendix H; $p > 0.1$ for all tests; average power = 0.508). Male supertype diversity did not predict whether or not the male would sire a particular litter, though tenure was a significant predictor, with sires having longer tenure than non-sires (Appendix H; $p = 0.042$).

We tested hypotheses germane to mate choice using data from 20 litters. We found no evidence that MHC similarity between males and females (measured by their band sharing coefficient, D_{AB}) predicted male lifetime reproductive success, (Appendix I; $p > 0.1$ for all models for both loci), nor did this measure predict whether or not a male sired a particular litter (Appendix I; $p > 0.1$ for all models for both loci). However, as expected from previous work, male tenure was a significant predictor in all of our models (Appendix I; $p < 0.05$ for all tests). We found no evidence that genetic similarities between females and sires differed from those between females and non-sires ($p > 0.1$ for all tests). However, when we sampled with replacement from the pool of potential sires within a population at the time a litter was

conceived to estimate expectations of female-sire similarity under random choice, we found evidence of MHC-based mate choice such that sires and dams were more dissimilar to each other than expected. To be more explicit, amino acid similarity at the *DRB* locus between females and litter sires was found to be significantly different than predicted by random choice, and this difference was confirmed with a chi-squared test (Figure 10; $\chi^2 = 7.13$, df = 2; $p < 0.05$).

DISCUSSION

Perhaps the most obvious conclusions to be drawn from our plethora of tests is that much larger sample sizes are needed to definitively detect any significant effects of MHC diversity on measures of fitness in spotted hyenas, and to perform more rigorous statistical tests. Due to our small sample sizes and low power to detect real effects, it is possible that some of our statistically significant results were spurious (e.g. Anderson et al. 2001; Zar 2010), so any results presented here must be viewed as strictly preliminary. Nevertheless, these data may be used to inform future research regarding selection acting within an ecological context in a wild population of a non-model mammalian species.

There are currently two widely accepted non-mutually exclusive hypotheses regarding which selective forces favor increased MHC diversity, heterozygote advantage and rare allele advantage, and much research exists to support both hypotheses (reviewed by: Takahata 1995; Apanius et al. 1997; Edwards and Hedrick 1998; Hughes and Yeager 1998). There can no longer be doubt that MHC heterozygosity commonly influences infection status in a variety of species (Coltman et al. 1999; Hedrick et al. 2001; Arkush et al. 2002; Penn 2002; Froeschke and Sommer

2005). MHC sequence diversity is often generated via gene duplication and copy number variation (Bernatchez & Landry 2003). Pervasive gene duplication, high rates of evolution, and positive selection have been demonstrated at two MHC loci in our spotted hyena study population (Califf et al. 2013). However, the high levels of gene duplication within this species precluded us from characterizing heterozygosity at either locus, and from directly testing either of the above hypotheses.

To reliably detect a relationship between MHC variation and a parasite, the parasite of interest must differentially affect the fitness of individuals, altering the genetic structure of the population. The endoparasite data considered here, collected by Engh (2002), were known not to have sizeable effects on reproductive success in spotted hyenas. Research of this type invariably depends on one's definition of "parasite," over which there has been much debate (e.g. Price 1973). Parasitic organisms that are extremely detrimental or even fatal to their hosts will presumably exert stronger selection pressure on related functional loci such as those found within the MHC than will organisms such as those considered here, which do not appear to greatly affect spotted hyenas. Additionally, our measure of parasite intensity (fecal egg counts) is an indirect method of quantifying individual parasite load, as many parasites are not detectable in feces. With these caveats in mind, our data revealed that individuals with higher MHC *DQB* diversity exhibited higher parasite intensities, and we observed a trend indicating that the presence of *DQB* supertype 3 might predict higher parasite egg counts.

There is ongoing debate in the literature as to whether individuals with higher MHC diversity are better able to cope with pathogens, and therefore show higher parasite infection levels, or instead that individuals with higher MHC diversity are better able to resist parasites,

and thus should show lower parasite infection intensities (e.g. Apanius et al. 1997; Bernatchez and Landry 2003; Piertney and Oliver 2006; Spurgin and Richardson 2010). There are also a multitude of studies that show a lack of relationship between MHC variation and parasite or pathogen loads (e.g. Outteridge et al. 1985; Blattman et al. 1993; Paterson et al, 1998; Schad et al, 2005; Rauch et al. 2006; Šimková et al 2006; Garamszegi and Nunn 2011).

Our data generally support the former hypothesis, that increased diversity at MHC loci enables individuals to withstand a higher parasite load, as we have observed a trend toward increasing parasite diversity (in our principal component axis PC1) with increasing MHC variation in a wild mammal that faces myriad immune threats, the spotted hyena. Our data suggest that MHC diversity in spotted hyenas may enable them to retain high levels of fitness without expending energy to remove certain innocuous parasites. We also found some support, though not statistically significant, to indicate the importance of specific supertypes in the presence of positive virus titers. Our data show a trend suggesting that individuals are more likely to test positive for a potentially fatal virus (FIV) when they do not carry a specific supertype (supertype 7) at the *DRB* locus. This supertype was common in our data, with 84.2% of individuals sequenced possessing alleles that were categorized as supertype 7, suggesting that there may be selection retaining this supertype within the population.

Our current results indicate that there may be a relationship between fitness and specific antigen binding motifs, classified by supertype, at these loci in hyenas. Exposure to many different parasites, as is likely for spotted hyenas, may select for better antigen recognition, which occurs via elevated rates of amino acid changes at antigen binding sites

(ABS) on MHC molecules (Ohta 1991; Yeager and Hughes, 1999; Garamszegi and Nunn 2011). Our previous research further supports the notion that positive selection is acting specifically on ABS, the functional sites used to assign supertypes, within spotted hyenas (Califf et al. 2013). In the present study, we found the presence of MHC *DRB* supertype 5 to be significantly associated with increased total serum IgM (Figure 7). Increased IgM does not directly indicate active infection within an individual, but it is commonly associated with early stage infections (Flies 2012). Interestingly, this supertype was rare in our dataset, and contains a unique amino acid at a site that is predicted to be under positive selection among spotted hyenas at this locus (Califf et al. 2013). Unique to supertype 5 was the substitution of the amino acid leucine at a position in *DRB* for which all other supertypes possessed either phenylalanine or tyrosine. Leucine is a linear aliphatic compound, in contrast to phenylalanine and tyrosine, which both contain benzene rings (Creighton 1993). These differing structures might alter the binding conformation of the expressed MHC molecules, and alter the pathogens to which each supertype is able to bind to initiate an immune response.

Some evidence suggests that higher parasite variation favors higher rates of evolution to maintain a diversity of alleles over time and space to combat ever-evolving parasites (Doherty and Zinkernagel 1975; Takahata and Nei 1990; Garamszegi & Nunn 2011). Lending credence to this notion, some studies have shown that the effects of MHC diversity on parasite resistance vary with environmental conditions, such as resource availability or weather patterns (e.g. Hayward et al. 2011). Taking this research even further, Wegner et al. (2006) found that the expression of MHC class II genes increased with experimental parasite infection in three-spined stickleback fish (*Gasterosteus aculeatus*). Interestingly, they also found that individuals with

lower MHC sequence diversity exhibited higher levels of gene expression during experimental infection; these data suggest that disease-specific genes may be up-regulated in some individuals in order to compensate for lower sequence variation (Wegner et al. 2006). Similarly, in 2005, Ditchkoff et al. presented evidence of a genetic trade-off, such that some individuals had elevated resistance to fewer parasites, while other individuals (who expressed a different set of MHC alleles than did members of the first group) had moderate resistance to a wider array of parasites.

It is known from previous work that spotted hyena cubs of high-ranking females survive better than do cubs of lower ranking females (e.g. Watts et al. 2009). Here we did not find any relationship between MHC diversity and social rank; this was expected, as social rank is learned and is not genetically determined. However, our data suggest the possibility that the presence of supertype 5 at the MHC *DRB* locus in females influences the survival of their offspring to den independence. As this supertype was not correlated with individual social rank, it is possible that this supertype represents a "good gene" operating within this population, increasing fitness independent of social rank.

We also found supertype-specific effects within our longevity data. MHC *DRB* supertype 1 had a significant positive effect on individual longevity, with individuals that carry this supertype living significantly longer than those that do not. It is again impossible to know the exact mechanism at work here. It is interesting to note that supertype 1 of the *DRB* locus was rare within our data set, only possessed by 4 out of 26 individuals. This would be the expected pattern if frequency dependent selection were operating on the alleles that make up this

supertype, and via an unknown mechanism, increasing longevity for individuals who carry rare alleles. Further, given the critical role MHC genes play in the vertebrate immune system, it seems reasonable to speculate that perhaps supertype 1 confers some benefit to individuals, possibly offering resistance to an infectious or otherwise longevity-reducing agent. Another observation suggesting frequency dependence is that the presence of supertype 2 at the DRB locus was significantly correlated with lower levels of bacterial killing ability (Figure 5). This would be predicted under negative frequency dependent selection, if sampling occurred at a time when selection has acted to increase the frequency of an advantageous rare allele in the population, so that it is in fact no longer rare. Under negative frequency dependent selection, the bacterium (or other pathogen) is able to adaptively evade immune cell recognition initiated by this advantageous allele, thereby shifting the forces of selection towards favoring a different allele, now at low frequency within the population, that is able to initiate an immune response against the evolved pathogen (Takahata and Nei 1990; Slade and McCallum 1992).

In addition to being affected by the arms race between parasites and their hosts (e.g. Van Valen 1973; Dawkins and Krebs 1979), variation among MHC genes has been shown to be under sexual selection via odor-based mate choice decisions (Edwards and Hedrick 1998; Penn and Potts 1998a, 1999; Wedekind et al. 1995; Wegner et al 2003, 2004; Milinski 2006; Setchell et al 2010). It has been demonstrated in some populations that preference for MHC-dissimilar mates is correlated with an increase in fitness, presumably resulting from the offspring's enhanced immunocompetence (e.g. Kalbe et al 2010). Whereas female hyenas have the opportunity to be extremely choosy, they are also highly promiscuous over the course of their lifetimes (Syzkman et al. 2007), and multiple paternity in hyena litters is common (Engh et al.

2002; East et al. 2003). This offers a non-mutually exclusive alternative hypothesis to odor-based mate choice by which hyenas might be gaining indirect benefits for their offspring, via post-copulatory sexual selection (e.g. Ober 1992). Perhaps female spotted hyenas mate with multiple males in a single estrous cycle in order to let their reproductive tract ultimately "choose" which male's sperm will sire her offspring based on MHC genes expressed in sperm.

However, it is possibly not the number of parasite species or the pathogen load, but the specific types of pathogens to which an animal is exposed that determines that population's MHC variation over time. In reality, the relationship between MHC variation and parasite resistance within populations is likely multi-layered, complex, and constantly changing. The data presented here suggest important supertype-specific, or perhaps even allele-specific, effects on individual fitness, and on an individual's ability to cope with deleterious forces. The only true way to assess the role of parasite regulation within a population is through experimental manipulation (e.g. Irvine 2006), an impossible task in any natural population, due to lack of environmental control. At the very least, more research is needed with larger sample sizes, as well as studies targeting specific immune threats important in spotted hyena populations. With many copies of genes, many alleles, and each allele potentially conferring a different benefit or responding to a different parasite, the relationship between MHC variation and parasite communities in spotted hyenas is likely very complex.

More than twenty years ago, it was stated by a leading MHC researcher that it could "no longer be disputed *what* selects MHC variants…the parasite" (Klein 1991). Klein (1991) does not define the term 'parasite' here, but does list viruses, bacteria, fungi, protozoa, and

macroparasites among the organisms considered in his discussion of this topic. Research in subsequent years has further supported the hypothesis that diversity within MHC loci is pathogen driven (reviewed by Hedrick & Kim 2000; Jeffery & Bangham 2000; Bernatchez & Landry 2003; Sommer 2005). However, the nature of this relationship is not static over time or space, and certainly not across species boundaries.

Our description here of hyena MHC variation and its relationship to fitness is still extremely limited. Because this system is not a one-allele, or even one-locus, to one-pathogen relationship, the picture of the co-evolutionary relationship between parasites and/or other pathogens and MHC diversity within hyenas could potentially be staggeringly complex. Further complicating the picture is that, in contrast to experimental studies which focus on single pathogens or parasites, natural populations are faced with many immune system threats simultaneously.

Our work, while far from clarifying the selective forces acting on MHC loci in spotted hyenas, or allowing us to make inferences about the mechanisms underlying MHC selection in this species, suggests avenues for further research that might prove fruitful. In addition to larger sample sizes, future research efforts would benefit by targeting MHC gene expression patterns when challenged with specific immune threats in spotted hyenas, including diseases that are known to infect sympatric carnivores, such as canine distemper virus (Carpenter et al. 1998; Roelke-Parker et al. 1996; van de Bildt et al. 2002). More direct sampling, investigation of other immune system genes, as well as sampling over time, specifically spanning periods of local carnivore disease-related mortality, would surely help elucidate the role of MHC diversity in pathogen resistance in the spotted hyena.

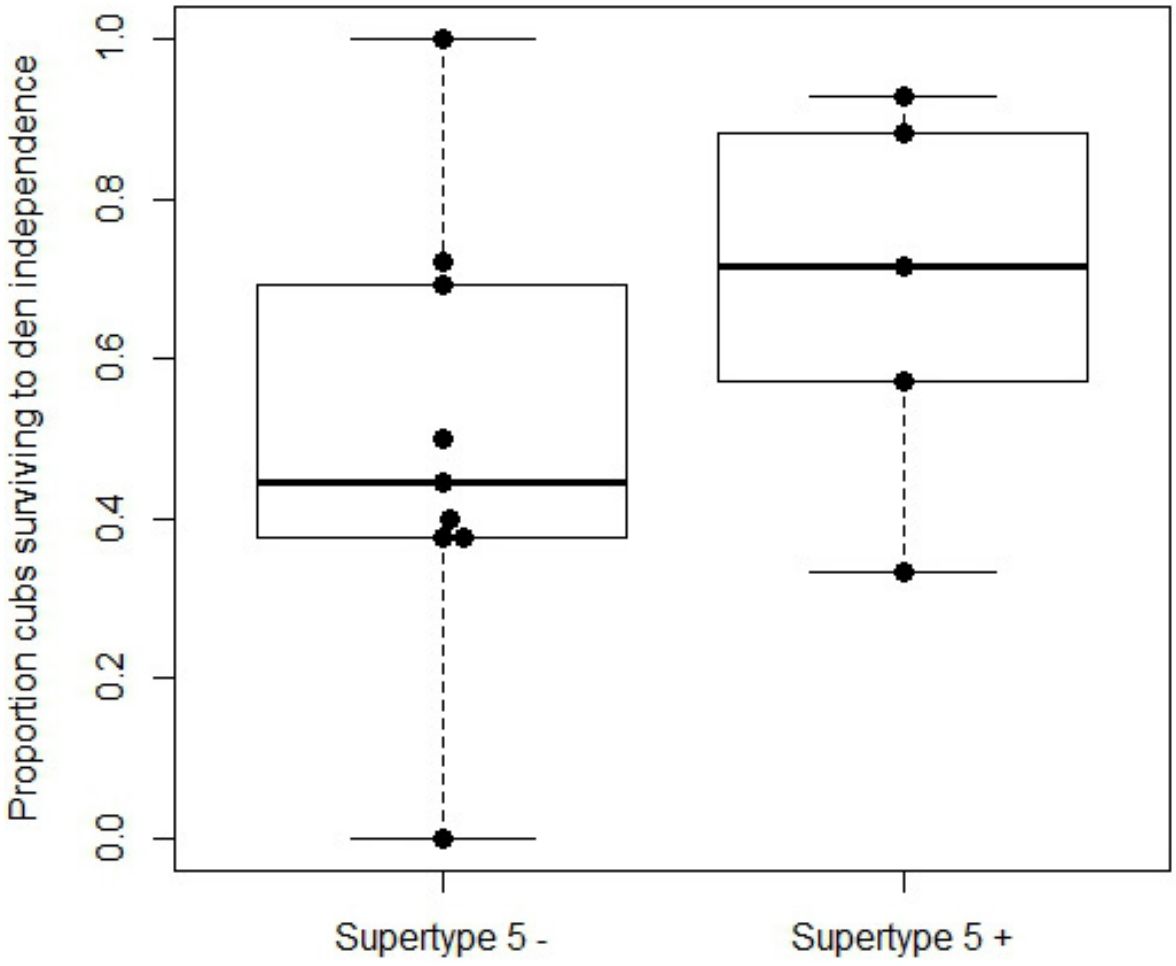

Figure 3. A higher proportion of cubs survived to den independence when mothers possessed MHC *DRB* supertype 5 (79.17 % of cubs survived to den independence; n = 5) than when their mothers did not carry this supertype (53.75 % cubs survived to den independence; n = 9). Boxes indicate interquartile range, and whiskers extend to the furthest points outside this range, but within 1.5 times the interquartile range from the median.

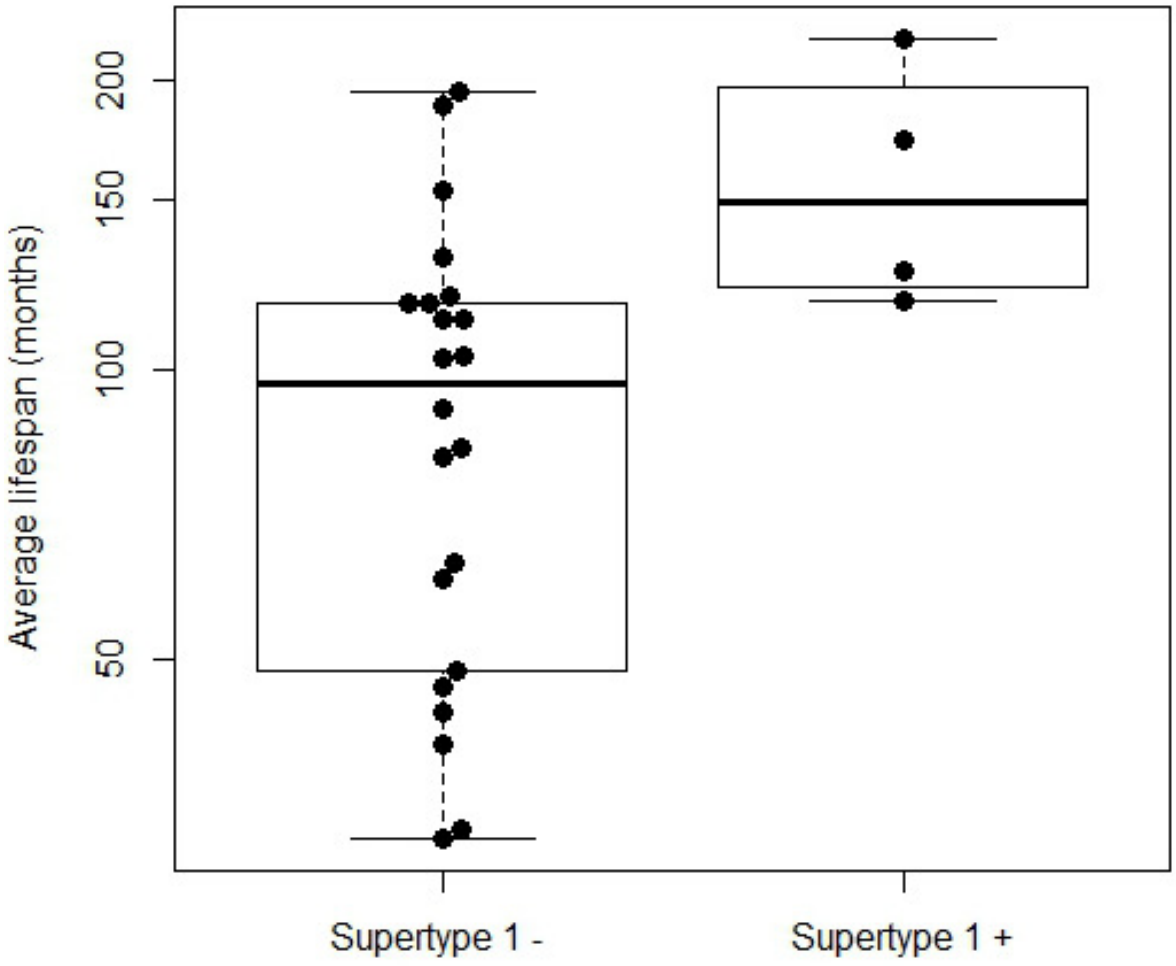

Figure 4. Average lifespan (in months) tended to be longer in individuals possessing supertype 1 (n = 4) at the MHC *DRB* locus than in individuals who do not possess this supertype (n = 22). Boxes indicate interquartile range, and whiskers extend to the furthest points outside this range, but within 1.5 times the interquartile range from the median.

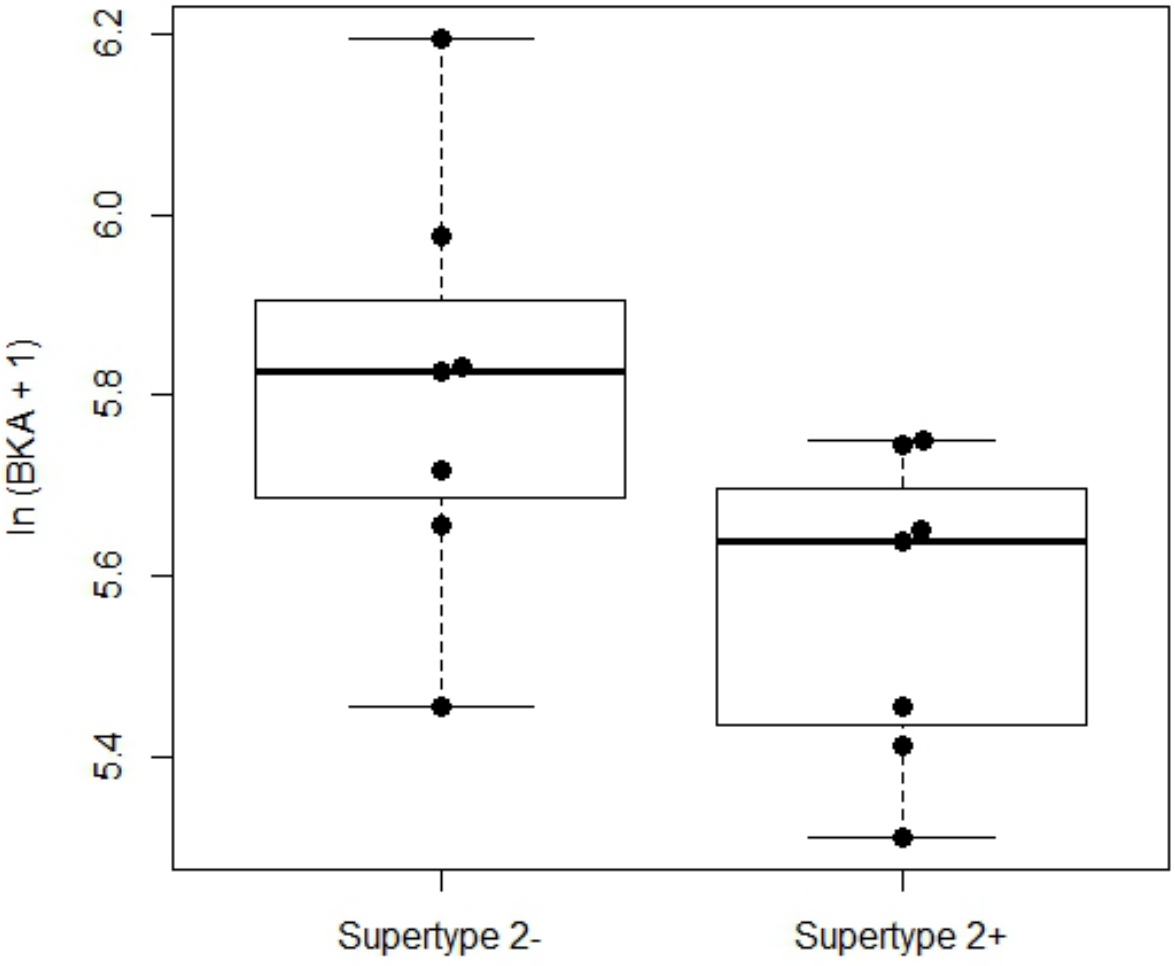

Figure 5. Measures of serum Bacterial Killing Ability (BKA) were significantly higher in individuals lacking the MHC *DRB* supertype 2 than in those that possess this supertype (n = 7 in both groups; $p = 0.014$). Boxes indicate interquartile range, and whiskers extend to the furthest points outside this range, but within 1.5 times the interquartile range from the median.

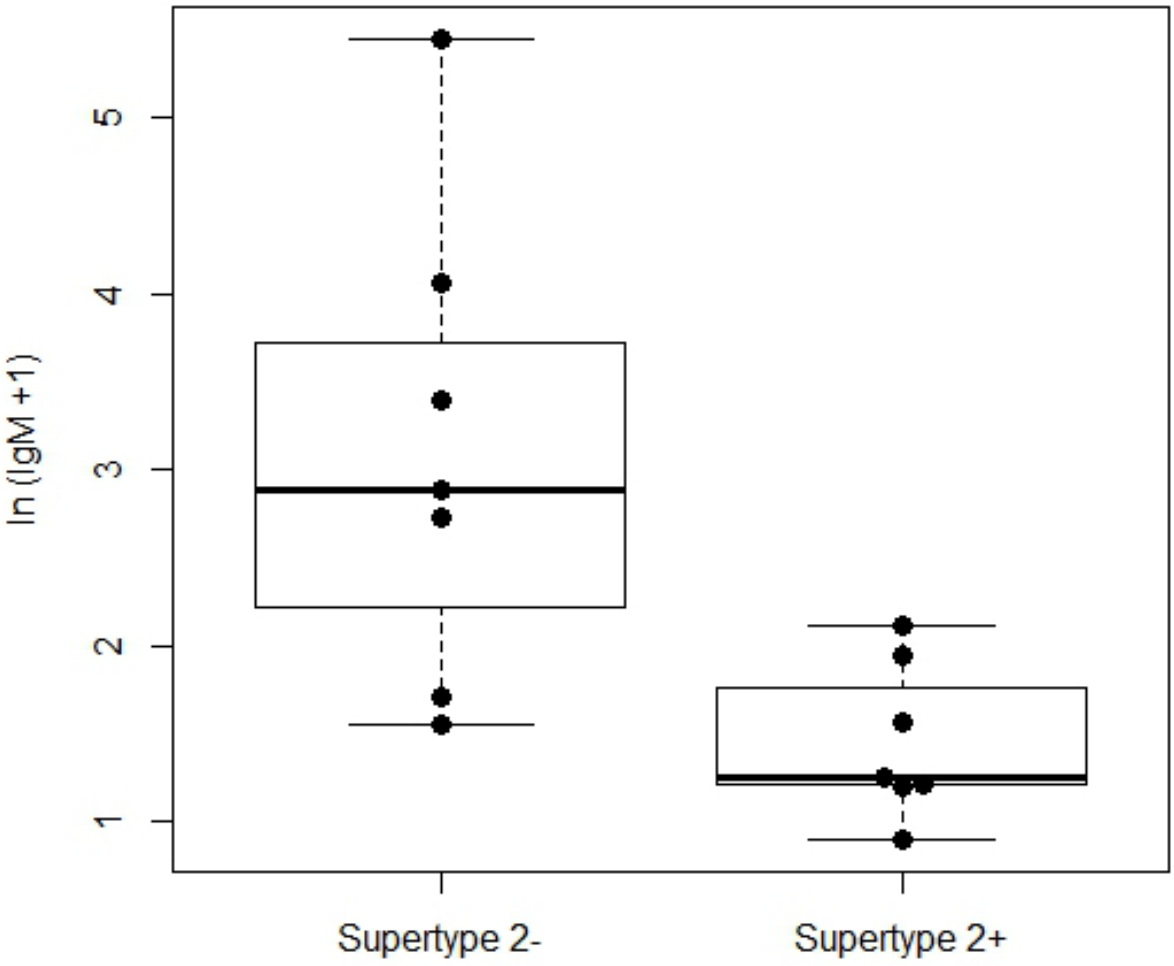

Figure 6. Measures of total serum immunoglobulin M (IgM) were significantly higher in individuals lacking the MHC *DRB* supertype 2 than those that possess this supertype (n = 7 in both groups; $p = 0.04$). Boxes indicate interquartile range, and whiskers extend to the furthest points outside this range, but within 1.5 times the interquartile range from the median.

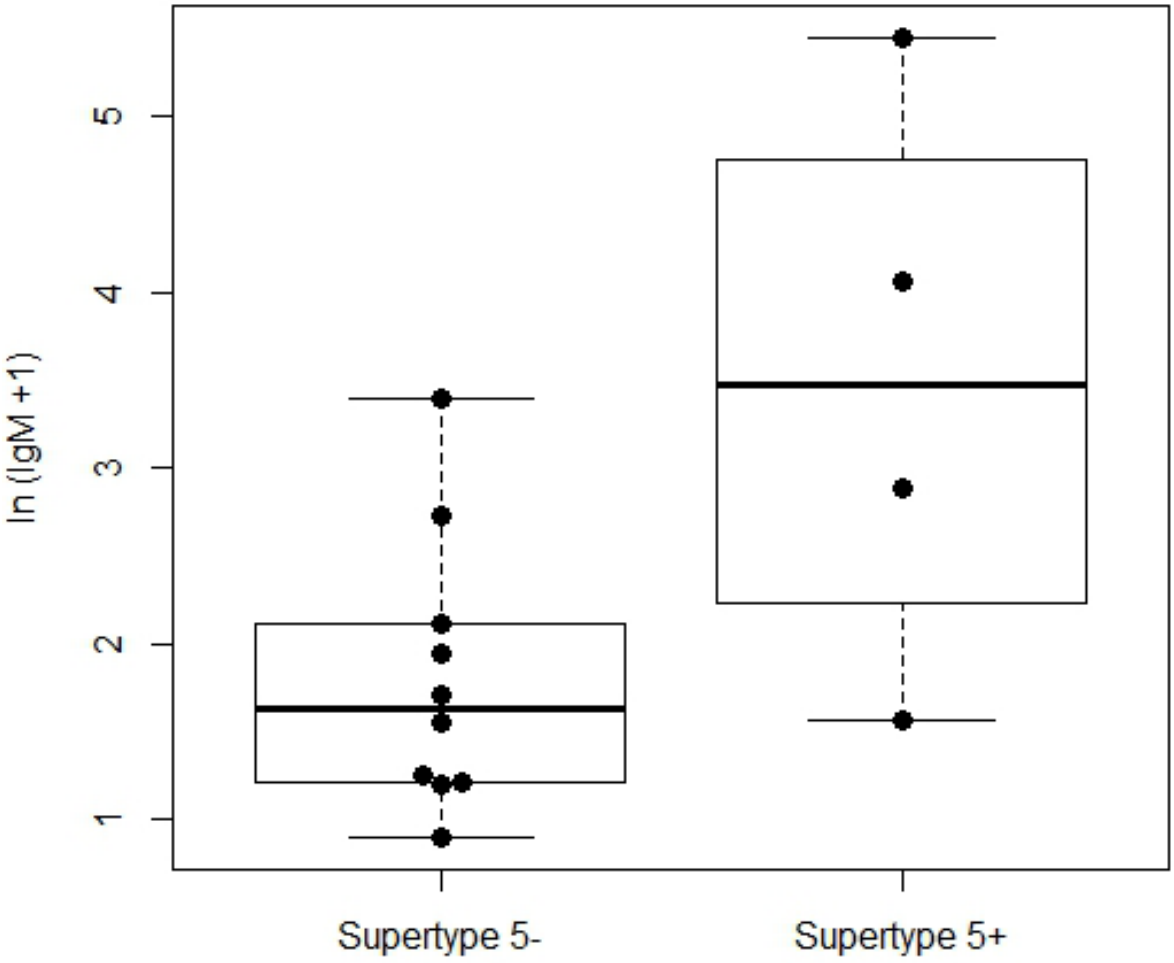

Figure 7. Measures of total serum immunoglobulin M (IgM) were significantly higher in individuals possessing the MHC *DRB* supertype 5 (n = 4) than in those lacking this supertype (n = 10; $p = 0.04$). Boxes indicate interquartile range, and whiskers extend to the furthest points outside this range, but within 1.5 times the interquartile range from the median.

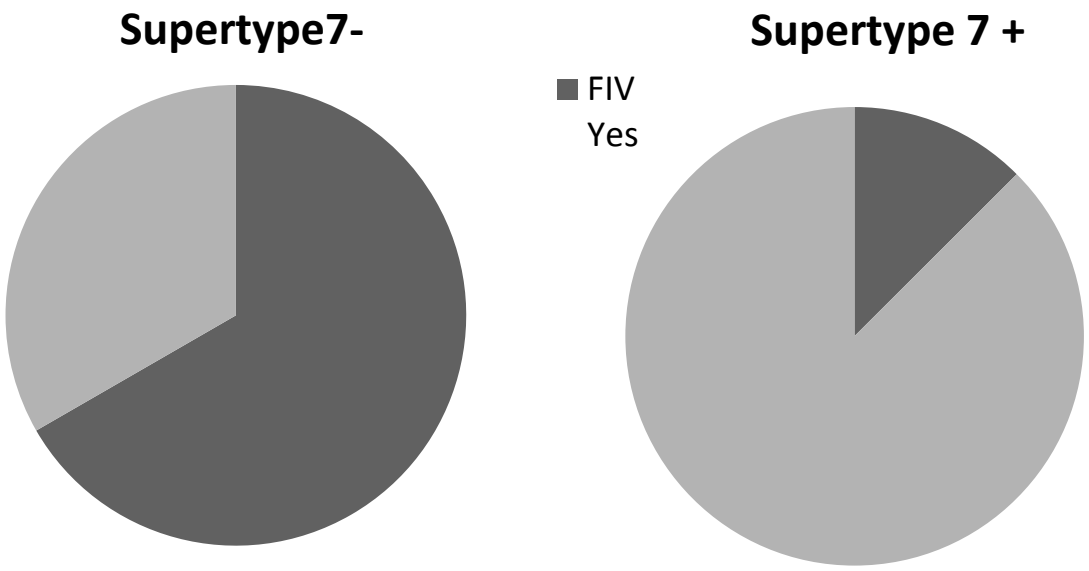

Figure 8. A higher proportion of individuals showed negative serum titers for feline immunodeficiency virus (FIV) when they possessed the MHC *DRB* supertype 7 (87.5 % negative for FIV; n = 16) than did individuals who did not carry this supertype (33.3 % negative for FIV; n = 3).

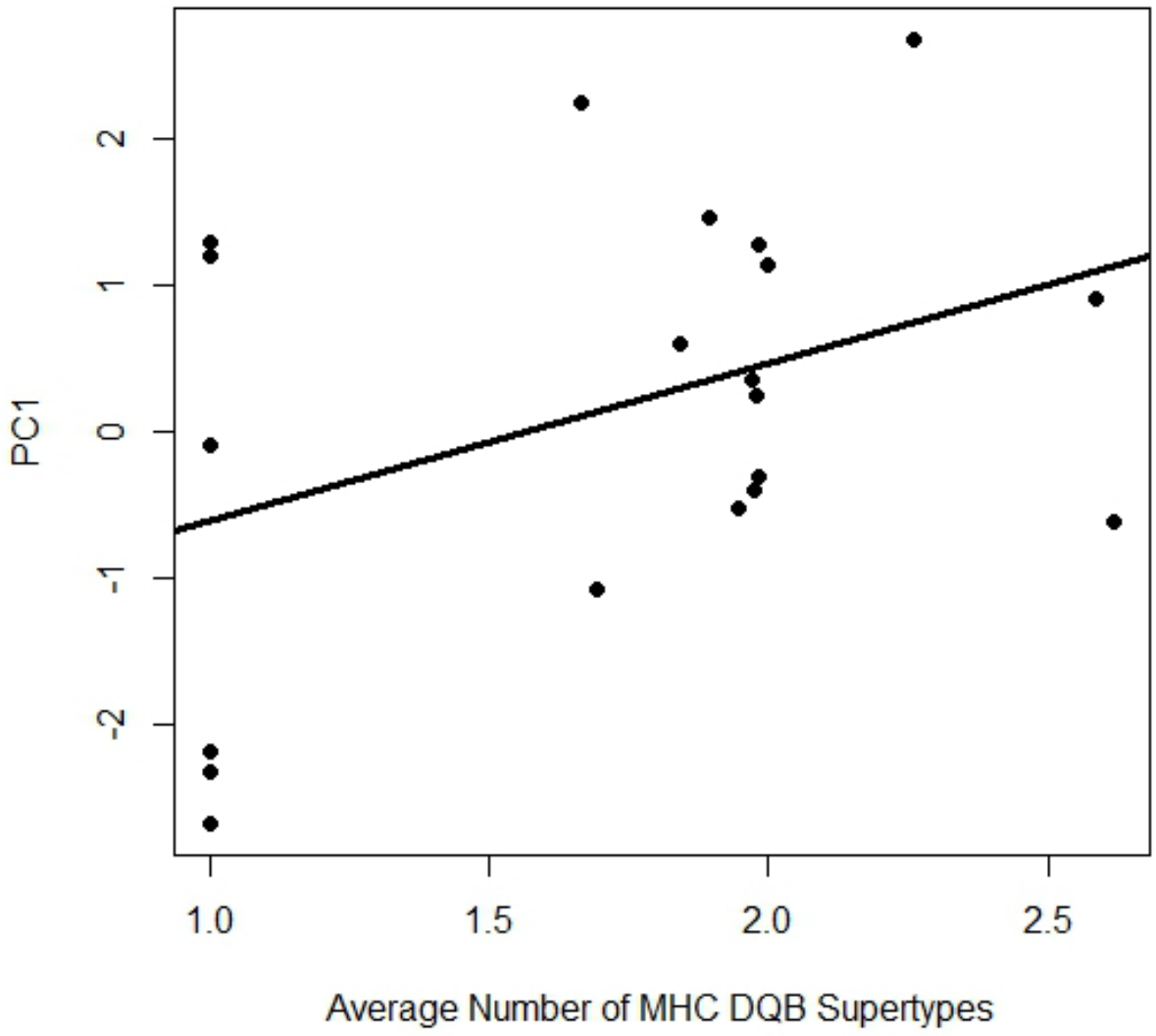

Figure 9. The average number of MHC *DQB* supertypes an individual possessed was a significant predictor of PC1, the first principal component axis, which served as our measure of parasite intensity (n = 20). PC1 included 4 measures obtained from fecal samples: number of parasite genera present, total fecal egg count, fecal egg count for *Ancylostoma* species, and fecal egg count for *Isospora* species.

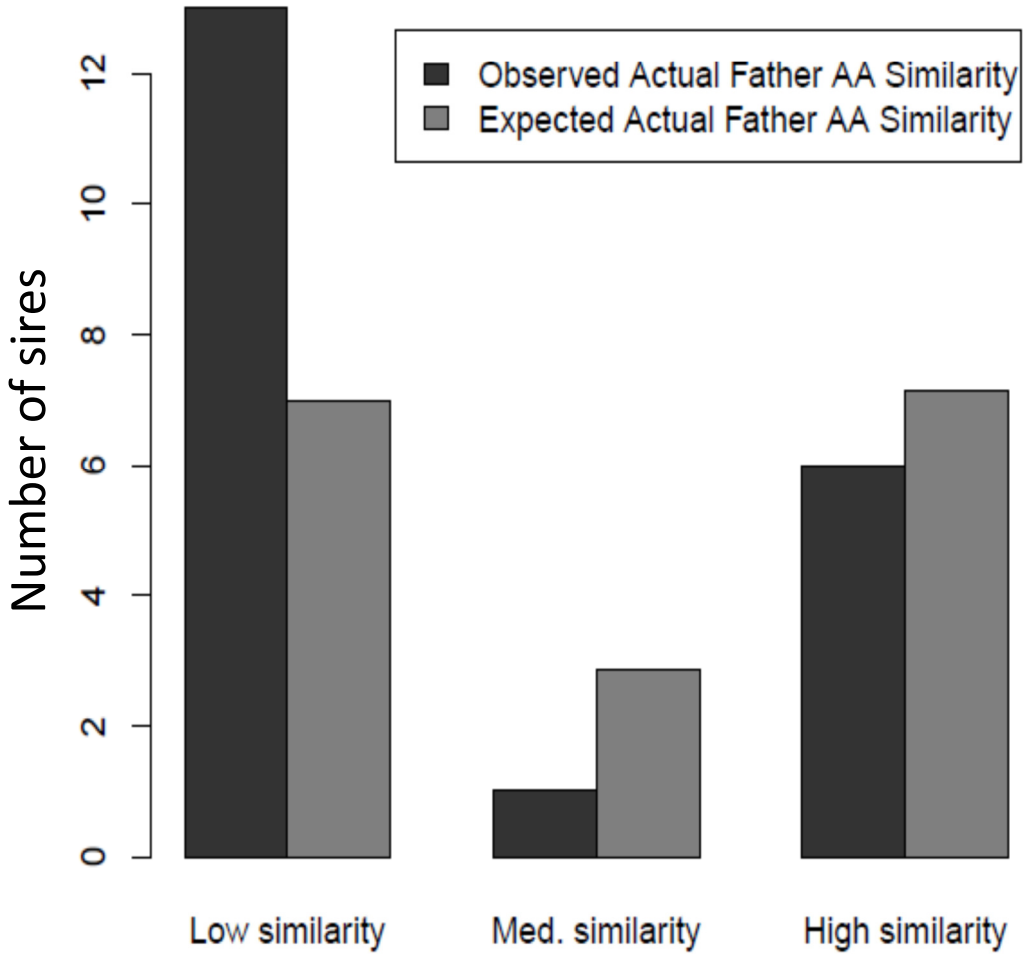

Figure 10. The observed MHC *DRB* amino acid (AA) similarity between litter dams and litter sires was lower than what was expected from randomly sampling sires with replacement from our data. The range of similarity (D_{AB}) between dam and both randomly sampled 'expected' sires and 'observed' sires, was divided into three equal proportions of similarity (low, medium and high). Expected values of similarity between dam and randomly sampled sires are plotted as the gray bars, and observed sires that fell into the low, medium and high similarity proportion of the data are plotted as black bars. The numbers of expected versus observed sires in each similarity bin compared using a chi-squared distribution.

Table 4. Sequencing results from regions of the MHC class II genes *DRB* and *DQB* in spotted hyenas. *n* = number of individuals sampled; N_A = number of alleles found per locus; N_S = number of supertypes found per locus; C_{avg} = average number of clones sequenced per individual; A_{avg} = average number of alleles per individual; S_{avg} = average number of supertypes per individual; aD_{AB} = average pairwise male-female similarities based on alleles; sD_{AB} = average pairwise male-female similarities based on supertypes. All values are given with ± standard error.

Locus	*n*	N_A	N_S	C_{avg}	A_{avg}	S_{avg}	aD_{AB}	sD_{AB}
DRB	27	26	7	10.89 ± 0.80	4.96 ± 0.29	2.52 ± 0.13	0.19 ± 0.02	0.48 ± 0.02
DQB	20	9	4	8.40 ± 0.36	2.35 ± 0.29	1.85 ± 0.15	0.47 ± 0.04	0.54 ± 0.04

Table 5. Loadings for principal component axis 1 (PC1) from principal component analyses (PCA) conducted for parasite intensity data.

Parasite intensity measure	**PC1 loading**
Number of parasite genera	0.416
Total fecal egg count (FEC)	0.623
FEC for *Ancylostoma* species	0.604
FEC for *Isospora* species	0.273

APPENDIX

TABLE A3. Cub survival results. Chi-squared (χ^2) values for ANOVA tests comparing generalized linear mixed models (GLMMs) inquiring whether MHC diversity was a significant predictor of spotted hyena cub survival. Predictors listed are number of supertypes at the given locus (# supertypes) or presence of the specific supertype listed. All *p*-values are reported in their sequential Bonferroni corrected form. Tests that were significant at a significance level of $\alpha < 0.05$ before Bonferroni adjustment are noted with an asterisk (*); tests that remained significant after the correction for multiple tests are noted with a double asterisk (**).

Locus	**Response**	**Predictor**	χ^2	**df**	**Bonferroni adjusted *p*-value**
DRB	Cub Survival	Maternal Rank	0.356	1	0.551
DRB	Cub Survival	# maternal supertypes	0.001	1	$p = 1$
DRB	Cub Survival	Supertype 1	0.997	1	$p = 1$
DRB	Cub Survival	Supertype 2	3.033	1	0.656
DRB	Cub Survival	Supertype 3	1.325	1	$p = 1$
DRB	Cub Survival	Supertype 4	0.182	1	$p = 1$
DRB	Cub Survival	Supertype 5	5.12	1	0.192*
DRB	Cub Survival	Supertype 6	0.025	1	$p = 1$
DRB	Cub Survival	Supertype 7	0.238	1	$p = 1$
DQB	Cub Survival	Maternal Rank	5.491	1	**0.019****
DQB	Cub Survival	# maternal supertypes	0.576	1	$p = 1$
DQB	Cub Survival	Supertype 1	1.901	1	0.84
DQB	Cub Survival	Supertype 2	0.17	1	$p = 1$
DQB	Cub Survival	Supertype 3	0.198	1	$p = 1$

TABLE A3 (cont'd)

Locus	**Response**	**Predictor**	χ^2	**df**	**Bonferroni adjusted *p*-value**
DQB	Cub Survival	Supertype 4	1.12	1	$p = 1$

TABLE A4. Longevity results. Chi-squared (χ^2) values for log likelihood ratio tests (LRT) comparing general linear models (LMs) inquiring whether MHC diversity was a significant predictor of spotted hyena longevity (in months). Predictors listed are number of supertypes at the given locus (# supertypes), or presence of the specific supertype listed. All *p*-values are from LRT model comparison tests and are reported in their sequential Bonferroni corrected form. Tests that were significant at a significance level of $\alpha < 0.05$ before Bonferroni adjustment are noted with an asterisk (*); tests that remained significant after the correction for multiple tests are noted with a double asterisk (**).

Locus	**Response**	**Predictor**	χ^2	**df**	**Bonferroni adjusted *p*-value**
DRB	Longevity	Social Rank	1.408	1	0.235
DRB	Longevity	Dispersal Status	10.261	1	**0.003****
DRB	Longevity	Sex	12.631	1	**0.001****
DRB	Longevity	# supertypes	0.001	1	$p = 1$
DRB	Longevity	Supertype 1	4.576	1	0.256*
DRB	Longevity	Supertype 2	0.009	1	$p = 1$
DRB	Longevity	Supertype 3	0.037	1	$p = 1$
DRB	Longevity	Supertype 4	2.161	1	$p = 1$
DRB	Longevity	Supertype 5	0.508	1	$p = 1$
DRB	Longevity	Supertype 6	1.152	1	$p = 1$
DRB	Longevity	Supertype 7	0.626	1	$p = 1$
DQB	Longevity	Social Rank	6.686	1	**0.027****
DQB	Longevity	Dispersal Status	20.207	1	***p* < 0.001****
DQB	Longevity	Sex	14.292	1	***p* < 0.001****
DQB	Longevity	# supertypes	1.508	1	$p = 1$
DQB	Longevity	Supertype 1	0.251	1	$p = 1$
DQB	Longevity	Supertype 2	0.309	1	$p = 1$

TABLE A4 (cont'd)

Locus	Response	Predictor	χ^2	df	Bonferroni adjusted *p*-value
DQB	Longevity	Supertype 3	0.47	1	$p = 1$
DQB	Longevity	Supertype 4	0.166	1	$p = 1$

TABLE A5. Immune function results. Chi-squared (χ^2) values for log likelihood ratio tests (LRT) comparing general linear models (LMs) inquiring whether MHC diversity significantly predicted three measures of immune function: serum bacterial killing ability (BKA), total serum immunoglobulin G (IgG), and total serum immunoglobulin M (IgM). Predictors listed are either number of supertypes at the given locus (# supertypes), or presence of the specific supertype listed. All *p*-values are from LRT model comparison tests and are reported in their sequential Bonferroni corrected form. Tests that were significant at a significance level of $\alpha < 0.05$ before Bonferroni adjustment are noted with an asterisk (*); tests that remained significant after the correction for multiple tests are noted with a double asterisk (**). 'NA' indicates no variation of MHC data within data subset, and the listed test was not performed.

Locus	**Response**	**Predictor**	χ^2	**df**	**Bonferroni adjusted *p*-value**
DRB	# supertypes	BKA	1.21	1	$p = 1$
DRB	Social Rank	BKA	3.098	1	0.624
DRB	Supertype 1	BKA	1.301	1	$p = 1$
DRB	Supertype 2	BKA	9.809	1	**0.014****
DRB	Supertype 3	BKA	1.977	1	$p = 1$
DRB	Supertype 4	BKA	0.571	1	$p = 1$
DRB	Supertype 5	BKA	0.635	1	$p = 1$
DRB	Supertype 6	BKA	NA	1	NA
DRB	Supertype 7	BKA	NA	1	NA
DRB	# supertypes	IgG	0.261	1	$p = 1$

TABLE A5 (cont'd)

Locus	Response	Predictor	χ^2	df	Bonferroni adjusted *p*-value
DRB	Social Rank	IgG	0.143	1	$p = 1$
DRB	Supertype 1	IgG	0.017	1	$p = 1$
DRB	Supertype 2	IgG	0.149	1	$p = 1$
DRB	Supertype 3	IgG	0.707	1	$p = 1$
DRB	Supertype 4	IgG	1.942	1	$p = 1$
DRB	Supertype 5	IgG	0.166	1	$p = 1$
DRB	Supertype 6	IgG	NA	1	NA
DRB	Supertype 7	IgG	NA	1	NA
DRB	# supertypes	IgM	0.015	1	$p = 1$
DRB	Social Rank	IgM	0.32	1	$p = 1$
DRB	Supertype 1	IgM	0.449	1	$p = 1$
DRB	Supertype 2	IgM	7.829	1	0.04**
DRB	Supertype 3	IgM	1.559	1	$p = 1$
DRB	Supertype 4	IgM	0.001	1	$p = 1$
DRB	Supertype 5	IgM	7.717	1	0.04**
DRB	Supertype 6	IgM	NA	1	NA
DRB	Supertype 7	IgM	NA	1	NA

TABLE A5 (cont'd)

Locus	Response	Predictor	χ^2	df	Bonferroni adjusted *p*-value
DQB	# supertypes	BKA	0.521	1	$p = 1$
DQB	Social Rank	BKA	4.445	1	0.28
DQB	Supertype 1	BKA	2.12	1	$p = 1$
DQB	Supertype 2	BKA	0.093	1	$p = 1$
DQB	Supertype 3	BKA	0.282	1	$p = 1$
DQB	Supertype 4	BKA	0.018	1	$p = 1$
DQB	# supertypes	IgG	0.002	1	$p = 1$
DQB	Social Rank	IgG	0.278	1	$p = 1$
DQB	Supertype 1	IgG	0.469	1	$p = 1$
DQB	Supertype 2	IgG	1.067	1	$p = 1$
DQB	Supertype 3	IgG	1.912	1	$p = 1$
DQB	Supertype 4	IgG	0.357	1	$p = 1$
DQB	# supertypes	IgM	0.252	1	$p = 1$
DQB	Social Rank	IgM	2.144	1	$p = 1$
DQB	Supertype 1	IgM	2.819	1	0.744
DQB	Supertype 2	IgM	0.383	1	$p = 1$
DQB	Supertype 3	IgM	0.186	1	$p = 1$
DQB	Supertype 4	IgM	0.146	1	$p = 1$

TABLE A6. Serology results. Wilcoxon rank-sum (W) values for tests inquiring whether MHC diversity was a significant predictor of presence in serum at sampling of four common canid and felid viruses: canine distemper virus (CDV); coronavirus (CoV); feline calcivirus (FCV); and feline immunodeficiency virus (FIV). Predictors listed are either number of supertypes at the given locus (# supertypes) or presence of the specific supertype listed. All *p*-values are reported in their sequential Bonferroni corrected form. Tests that were significant at a significance level of $\alpha < 0.05$ before Bonferroni adjustment are noted with an asterisk (*); tests that remained significant after the correction for multiple tests are noted with a double asterisk (**).

Locus	**Response**	**Predictor**	**W**	**Bonferroni adjusted *p*-value**
DRB	# supertypes	CDV +/-	40	$p = 1$
DRB	# supertypes	CoV +/-	60	$p = 1$
DRB	# supertypes	FCV +/-	95	$p = 1$
DRB	# supertypes	FIV +/-	35	$p = 1$
DRB	Supertype 1	CDV +/-	18	$p = 1$*
DRB	Supertype 2	CDV +/-	66	$p = 1$
DRB	Supertype 3	CDV +/-	75	$p = 1$
DRB	Supertype 4	CDV +/-	53	$p = 1$
DRB	Supertype 5	CDV +/-	48	$p = 1$
DRB	Supertype 6	CDV +/-	17	$p = 1$
DRB	Supertype 7	CDV +/-	15	$p = 1$

TABLE A6 (cont'd)

Locus	Response	Predictor	W	Bonferroni adjusted *p*-value
DRB	Supertype 1	CoV +/-	45	$p = 1$
DRB	Supertype 2	CoV +/-	94	$p = 1$
DRB	Supertype 3	CoV +/-	82	$p = 1$
DRB	Supertype 4	CoV +/-	44	$p = 1$
DRB	Supertype 5	CoV +/-	61	$p = 1$
DRB	Supertype 6	CoV +/-	45	$p = 1$
DRB	Supertype 7	CoV +/-	43	$p = 1$
DRB	Supertype 1	FCV +/-	54	$p = 1$
DRB	Supertype 2	FCV +/-	80	$p = 1$
DRB	Supertype 3	FCV +/-	66.5	$p = 1$
DRB	Supertype 4	FCV +/-	45.5	$p = 1$
DRB	Supertype 5	FCV +/-	79	$p = 1$
DRB	Supertype 6	FCV +/-	48.5	$p = 1$
DRB	Supertype 7	FCV +/-	60	$p = 1$
DRB	Supertype 1	FIV +/-	28.5	$p = 1$
DRB	Supertype 2	FIV +/-	27	$p = 1$
DRB	Supertype 3	FIV +/-	51	$p = 1$
DRB	Supertype 4	FIV +/-	19	$p = 1$
DRB	Supertype 5	FIV +/-	38	$p = 1$
DRB	Supertype 6	FIV +/-	11.5	$p = 1$
DRB	Supertype 7	FIV +/-	37	0.384*
DRB	Supertype 7	FIV +/-	OddsR: 0.070	**0.050***
DQB	# supertypes	CDV +/-	25	$p = 1$
DQB	# supertypes	CoV +/-	25	$p = 1$

TABLE A6 (cont'd)

Locus	Response	Predictor	W	Bonferroni adjusted *p*-value
DQB	# supertypes	FCV +/-	49	$p = 1$
DQB	# supertypes	FIV +/-	25	$p = 1$
DQB	Supertype 1	CDV +/-	47	$p = 1$
DQB	Supertype 2	CDV +/-	42.5	$p = 1$
DQB	Supertype 3	CDV +/-	14	$p = 1$
DQB	Supertype 4	CDV +/-	34	$p = 1$
DQB	Supertype 1	CoV +/-	42.5	$p = 1$
DQB	Supertype 2	CoV +/-	14	$p = 1$
DQB	Supertype 3	CoV +/-	34	$p = 1$
DQB	Supertype 4	CoV +/-	30	$p = 1$
DQB	Supertype 1	FCV +/-	50	$p = 1$
DQB	Supertype 2	FCV +/-	13	$p = 1$
DQB	Supertype 3	FCV +/-	47	$p = 1$
DQB	Supertype 4	FCV +/-	37	$p = 1$
DQB	Supertype 1	FIV +/-	32.5	$p = 1$
DQB	Supertype 2	FIV +/-	20.5	$p = 1$
DQB	Supertype 3	FIV +/-	21	$p = 1$
DQB	Supertype 4	FIV +/-	20.5	$p = 1$

TABLE A7. Parasite results. Chi-squared (χ^2) values for log likelihood ratio tests (LRT) comparing general linear models (LMs) inquiring whether MHC diversity was a significant predictor of parasite intensity or parasite presence. Predictor of parasite intensity listed is: PC1_PARA, which denotes PC1 values for the PC1 axis of principal components analyses. The 4 components of PC1 were: overall total fecal egg count, *Ancylostoma* species fecal egg count, *Isospora* species fecal egg count, and the total number of different parasite genera (NumGen) present within each fecal sample. Presence or absence of *Ancylostoma* species listed as: *Ancy* sp. y/n and presence or absence of *Isospora* species listed as: *Iso* sp. y/n. Predictors listed are either number of supertypes at the given locus (# supertypes) or presence of the specific supertype listed. All *p*-values are from LRT model comparison tests and are reported in their sequential Bonferroni corrected form. Tests that were significant at a significance level of $\alpha < 0.05$ before Bonferroni adjustment are noted with an asterisk (*); tests that remained significant after the correction for multiple tests are noted with a double asterisk (**). 'NA' indicates no variation of MHC data within data subset, and the listed test was not performed.

Locus	**Response**	**Predictor**	χ^2	**df**	**Bonferroni adjusted *p*-value**
DRB	PC1_PARA	Age	3.988	1	0.506*
DRB	PC1_PARA	Sex	1.105	1	$p = 1$
DRB	PC1_PARA	Social Rank	0.437	1	$p = 1$
DRB	PC1_PARA	# supertypes	0.018	1	$p = 1$
DRB	PC1_PARA	Supertype 1	0.06	1	$p = 1$
DRB	PC1_PARA	Supertype 2	6.027	1	0.154*
DRB	PC1_PARA	Supertype 3	1.548	1	$p = 1$

TABLE A7 (cont'd)

Locus	Response	Predictor	χ^2	df	Bonferroni adjusted *p*-value
DRB	PC1_PARA	Supertype 4	0.604	1	$p = 1$
DRB	PC1_PARA	Supertype 5	3.89	1	0.539*
DRB	PC1_PARA	Supertype 6	0.577	1	$p = 1$
DRB	PC1_PARA	Supertype 7	0.113	1	$p = 1$
DRB	Ancy y/n	# supertypes	1.396	1	$p = 1$
DRB	Ancy y/n	Supertype 1	0.794	1	$p = 1$
DRB	Ancy y/n	Supertype 2	0.413	1	$p = 1$
DRB	Ancy y/n	Supertype 3	1.299	1	$p = 1$
DRB	Ancy y/n	Supertype 4	0.761	1	$p = 1$
DRB	Ancy y/n	Supertype 5	2.608	1	0.848
DRB	Ancy y/n	Supertype 6	0.998	1	$p = 1$
DRB	Ancy y/n	Supertype 7	1.074	1	$p = 1$
DRB	Iso y/n	# supertypes	0.0007	1	$p = 1$
DRB	Iso y/n	Sex	1.791	1	$p = 1$
DRB	Iso y/n	Supertype 1	0.129	1	$p = 1$
DRB	Iso y/n	Supertype 2	3.737	1	0.424
DRB	Iso y/n	Supertype 3	1.131	1	$p = 1$
DRB	Iso y/n	Supertype 4	1.742	1	$p = 1$
DRB	Iso y/n	Supertype 5	2.128	1	$p = 1$
DRB	Iso y/n	Supertype 6	0.572	1	$p = 1$
DRB	Iso y/n	Supertype 7	0.864	1	$p = 1$
DQB	PC1_PARA	Age	9.485	1	**0.020****
DQB	PC1_PARA	Sex	0.295	1	$p = 1$
DQB	PC1_PARA	Social Rank	0.192	1	$p = 1$

TABLE A7 (cont'd)

Locus	Response	Predictor	χ^2	df	Bonferroni adjusted *p* -value
DQB	PC1_PARA	# supertypes	10.349	1	**0.010****
DQB	PC1_PARA	Supertype 1	0.091	1	$p = 1$
DQB	PC1_PARA	Supertype 2	0.086	1	$p = 1$
DQB	PC1_PARA	Supertype 3	6.705	1	0.09*
DQB	PC1_PARA	Supertype 4	3.642	1	0.56
DQB	Ancy y/n	Age	0.084	1	$p = 1$
DQB	Ancy y/n	# supertypes	1.086	1	$p = 1$
DQB	Ancy y/n	Supertype 1	1.090	1	$p = 1$
DQB	Ancy y/n	Supertype 2	0.384	1	$p = 1$
DQB	Ancy y/n	Supertype 3	0.281	1	$p = 1$
DQB	Ancy y/n	Supertype 4	1.537	1	$p = 1$
DQB	Iso y/n	Sex	0.310	1	$p = 1$
DQB	Iso y/n	Supertype 1	1.490	1	$p = 1$
DQB	Iso y/n	Supertype 2	0.224	1	$p = 1$
DQB	Iso y/n	Supertype 3	0.208	1	$p = 1$
DQB	Iso y/n	Supertype 4	1.048	1	$p = 1$

TABLE A8. Good genes and male resproductive success results. Test statistics reported comparing general linear models (LMs) inquiring whether male MHC diversity was a significant predictor of male annual reproductive success (ARS) or a significant predictor of whether the male would sire a specific litter, noted as "sire" or "non-sire." ARS is measured here as the total number of offspring produced by a male during his reproductive lifespan that survived to den independence, divided by the years this male was reproductively active, or his tenure in the clan. For any natal males included in our analyses, 'tenure' was considered to be any time spent in the clan after reproductive maturity; 24 months of age, or first sired offspring, whichever occurred first. Predictors listed are number of supertypes at the given locus (# supertypes) or specific supertype. Wilcoxon rank-sum test scores (W) are also given from tests performed to ask whether "sires" and "non-sires" differed in MHC diversity, in terms of supertype number. Unless otherwise noted, all *p*-values are from ANOVA or LRT model comparison tests and are reported in their sequential Bonferroni corrected form. Tests that were significant at a significance level of $\alpha < 0.05$ before Bonferroni adjustment are noted with an asterisk (*); tests that remained significant after the correction for multiple tests are noted with a double asterisk (**).

Locus	**Response**	**Predictor**	χ^2	**df**	**Bonferroni adjusted *p*-value**
DRB	ARS	Tenure	0.003	1	$p = 1$
DRB	ARS	# supertypes	1.822	1	$p = 1$
DRB	ARS	Supertype 1	0.262	1	$p = 1$
DRB	ARS	Supertype 2	2.631	1	0.945
DRB	ARS	Supertype 3	2.724	1	0.891

TABLE A8 (cont'd)

Locus	Response	Predictor	χ^2	df	Bonferroni adjusted *p*-value
DRB	ARS	Supertype 4	0.044	1	$p = 1$
DRB	ARS	Supertype 5	1.300	1	$p = 1$
DRB	ARS	Supertype 6	0.693	1	$p = 1$
DRB	ARS	Supertype 7	0.510	1	$p = 1$
DQB	ARS	Tenure	3.881	1	0.441*
DQB	ARS	# supertypes	1.756	1	$p = 1$
DQB	ARS	Supertype 1	0.598	1	$p = 1$
DQB	ARS	Supertype 2	0	1	$p = 1$
DQB	ARS	Supertype 3	0.764	1	$p = 1$
DQB	ARS	Supertype 4	0.836	1	$p = 1$
DRB	Sire or NonSire	Tenure	5.313	1	**0.042****
DRB	Sire or NonSire	# supertypes	3.051	1	0.162
DQB	Sire or NonSire	Tenure	3.883	1	0.098*
DQB	Sire or NonSire	# supertypes	0.273	1	1.204
DRB	Sire or NonSire	# supertypes	W = 1042		0.621
DQB	Sire or NonSire	# supertypes	W = 858		0.090*

TABLE A9. MHC similarity and male reproductive success results. Test statistic values for tests inquiring whether MHC similarity as measured by D_{AB} was a significant predictor of male annual reproductive success (ARS). D_{AB} is a measure of pairwise MHC similarity, and was calculated as $D_{AB} = 2\,F_{AB} / (F_A + F_B)$, where F_{AB} is the average number of shared supertypes between a male (A) and a female (B), and F_A and F_B are, respectively, the average number of supertypes of A and B (Wetton et al. 1987). ARS is measured here as: measured as the total number of offspring produced by a male during his reproductive lifespan that survived to den independence, divided by the years this male was reproductively active, or tenure in the clan. For any natal males included in our analyses, 'tenure' was considered to be any time spent in the clan after reproductive maturity; 24 months of age, or first sired offspring, whichever occurred first. Predictors listed are number of supertypes at the given locus (# supertypes) or specific supertype. Test statistics given as noted: Spearman's rho (ρ); ANOVA χ^2 for GLMM comparisons; or Wilcoxon's Rank Sum statistic (W); All tests given with corresponding *p*-values, which are reported in their sequential Bonferroni corrected form. Tests that were significant at a significance level of $\alpha < 0.05$ before Bonferroni adjustment are noted with an asterisk (*); tests that remained significant after the correction for multiple tests are noted with a double asterisk (**).

Locus	**Response**	**Predictor**	**Test statistic**	**df**	**Bonferroni adjusted *p*-value**
DRB	ARS	D_{AB}	ρ = 0.137		0.058*
DRB	ARS	Tenure	ρ = 0.285		***p* < 0.001****

TABLE A9 (cont'd)

Locus	Response	Predictor	Test statistic	df	Bonferroni adjusted *p*-value
DQB	ARS	D_{AB}	ρ = 0.005		0.972
DQB	ARS	Tenure	ρ = 0.517		***p* < 0.001****
DRB	Sire or Non-Sire	Tenure	$\chi^2 = 5.591$	1	**0.030****
DRB	Sire or Non-Sire	D_{AB}	$\chi^2 = 0.079$	1	*p* = 1
DQB	Sire or Non-Sire	Tenure	$\chi^2 = 1.023$	1	0.312
DQB	Sire or Non-Sire	D_{AB}	$\chi^2 = 0.029$	1	0.863
DRB	Sire or Non-Sire	Tenure	W = 1512		**0.024****
DRB	Sire or Non-Sire	D_{AB}	W = 1041		*p* = 1
DQB	Sire or Non-Sire	Tenure	W = 214		0.402
DQB	Sire or Non-Sire	D_{AB}	W = 177.5		0.958

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CHAPTER FOUR

MHC – BASED ODOR PREFERENCE IN CAPTIVE SPOTTED HYENAS (*Crocuta crocuta*)

INTRODUCTION

Genes of the major histocompatibility complex encode polymorphic glycoproteins that are involved in self / non–self recognition within the vertebrate immune system. High levels of diversity, and specifically heterozygosity, at these loci have been linked to increased pathogen resistance, as well as mate attractiveness (reviewed by: Milinski 2006; Piertney and Oliver 2006). The main predictions deriving from the hypothesis that MHC diversity influences mate choice is that individuals should be able to discriminate among potential mates based on MHC-based odors, and that they should choose mates that will provide their offspring with optimal immune function to combat parasites and pathogens via optimal MHC diversity (Hamilton and Zuk 1982; Hedrick 2002; Penn 2002). Many vertebrates tested to date, including mice, humans, and fish, can discriminate among different MHC genotypes using odor, and appear to make mating decisions based on these odors (Yamazaki et al. 1976; Wedekind and Furi 1997; Sauermann 2001; Penn 2002; Wysocki *et al.* 2004; Consuegra and de Leaniz 2008; Radwan et al. 2008). MHC–based mating preferences are also potentially adaptive in that they permit animals to avoid breeding with relatives, and hence avoid the negative consequences of inbreeding (Charlesworth and Charlesworth 1987; Brown

and Eklund 1994; Potts et al. 1994; Grob et al. 1998; Penn and Potts 1998; Keller and Waller 2002).

Spotted hyenas (*Crocuta crocuta*) demonstrate a wide variety of chemical signaling behaviors, the most common and conspicuous of which is referred to as 'pasting', in which the animals deposit anal gland secretions on grass stalks, or other objects, in their environment (Kruuk 1972; Mills 1990). Originally, pasting behavior was studied largely in the context of territorial marking (Gorman and Mills 1984). However, more recent research has shown that pasting by spotted hyenas occurs in a variety of behavioral contexts, and that paste can convey a great deal of information (Henschel and Skinner 1991; Boydston et al. 2001; Drea et al. 2002a; Theis 2008; Theis et al. 2012). Previous studies have shown that the odor of spotted hyena paste can carry information on sex, reproductive state, group membership, and individual identity (Hofer et al. 2001; Burgener et al. 2008, 2009, Theis 2008, Theis et al. 2012). In addition to depositing scent marks in their environment, spotted hyenas regularly investigate the scent glands of conspecifics and over-mark the scent deposits of others (Kruuk 1972; Mills and Gorman 1987; Theis 2008). All of these behaviors suggest the important role that odor plays in the social lives of hyenas. Here, we hypothesize that scent marks in hyenas might also convey some sort of information on genetic quality.

As well as the importance of chemical communication in this species, the ability to cope with disease is of particular importance in spotted hyenas. Hyenas are successful hunters, but also regularly consume carrion (Kruuk 1972; Holekamp *et al*. 1997; Cooper *et al*. 1999; Reperant et al 2008; Boone et al. 2009; Jennelle et al. 2009;

Gortazar et al. 2010; Getz 2011; Holekamp and Dloniak 2010; Wilson and Wolkovich 2011). Accordingly, hyenas are likely exposed to a wide variety of parasite and pathogen species from encounters with their prey, as well as with competitors at kills (East et al. 2001; Engh et al. 2003; Haas et al. 1996; Harrison et al. 2004). Further, whereas sympatric carnivores are known to suffer high mortality rates from infectious diseases (e.g. rabies: Kat et al. 1995; Maas 1993; canine distemper virus: Carpenter et al. 1998; Roelke-Parker et al. 1996; van de Bildt et al. 2002), spotted hyenas rarely die from these diseases, and rarely exhibit symptoms of infection, though they routinely test positive for these diseases at rates similar to those in other carnivores in Africa. (East et al. 2001; Haas et al. 1996; Murray et al. 1999; but see Mills 1990).

Lastly, in addition to the importance of chemical communication and disease resistance in this species, spotted hyenas also offer a unique opportunity to test hypotheses regarding mate choice. This is because female spotted hyenas are socially dominant to all adult immigrant males and have heavily masculinized genitalia, preventing forced copulation (East et al. 1993; Frank et al. 1995; Holekamp et al. 2012; Kruuk 1972). Based on their evidence that females did not mate with a few "high quality" males in Serengeti clans, East et al. (2003) suggested that genetic compatibility may play a role in spotted hyena mate choice. If female hyenas are able to discriminate males based on MHC–based odors, it may be adaptive for them to choose mates whose genes permit their offspring to develop immune systems capable of recognizing the most diverse array of pathogens possible.

To test the hypothesis that spotted hyenas base mate choice decisions on MHC gene similarity, we characterized the behavioral responses of female spotted hyenas presented with scent mark samples obtained from wild male spotted hyenas that differed in degree of genetic similarity to the female. If this hypothesis is true in this species, we predicted that females would be able to discriminate odors based on genetic similarity, and hence would vary their behavioral responses to male odors depending on their similarity to her. This study is the first step to understanding the influence of genetic relatedness, or genetic similarity, to mate choice decisions in spotted hyenas.

METHODS

To test the hypothesis that spotted hyenas discern information about genetic similarity in the scent marks (paste) of conspecifics, we examined the responses of nine captive adult female hyenas (recipients) to paste extracted from unfamiliar, wild-living adult male hyenas (donors). All donor paste samples were from adult males of the Talek clan in the Masai Mara National Reserve in Kenya, East Africa, and were collected by personnel from the Mara Hyena Project led by Dr. Kay Holekamp of Michigan State University. All animals tested were housed at the Field Station for Behavioral Research at the University of California, Berkeley (UCB) and were tested under the approved guidelines of the International Animal Care and Use Committees (IACUC) at both Michigan State University and the University of Berkeley. All odor preference testing occurred in May and June of 2011.

Prior to odor preference testing, DNA was extracted from blood samples of the nine females and we sequenced exon 2 of the *DRB* gene locus of the MHC, under conditions previously described (Califf et al. 2013). DNA sequences at this region were used to identify MHC alleles within the captive population as previously described (Califf et al. 2013). All singleton alleles were discarded from analyses. All alleles (from both wild and captive populations) were then further classified into groups of alleles, called 'supertypes', based on their antigen binding motifs (Sette and Sidney 1999; Sidney et al. 1995; Lund et al. 2004). These groups are based on similarities in antigen binding sites (ABS), such that alleles of the same supertype group are considered functionally similar and likely bind similar pathogens (e.g. Southwood et al. 1998; Sette and Sidney 1999; Buchli et al. 2005). Previous studies offer evidence supporting the functional equivalence of supertype groups, as well as the biological relevance of these supertypes to disease resistance in wild populations (Schwensow et al. 2007; Huchard et al. 2010; Clough et al. 2011; Sepil et al. 2013).

We measured MHC similarity using the 'band sharing coefficient', such that MHC supertype similarity (D_{AB}) between each potential pair of mates was calculated as $D_{AB} = 2\ F_{AB} / (F_A + F_B)$, where F_{AB} is the average number of shared supertypes between a male (A) and a female (B), and F_A and F_B are the average number of supertypes possessed by A and B, respectively (Wetton et al. 1987).

Each captive individual was genotyped at nine microsatellite loci previously genotyped in wild populations of spotted hyenas (ccr01, ccr04, ccr07, ccr11, ccr13-17;

Libants et al. 2000; Van Horn et al. 2004; Watts et al. 2011; Funk and Engh unpublished). Genotypic pairwise relatedness (*R*) values were estimated among all individuals using these microsatellite data and the software program RELATEDNESS (Queller and Goodnight 1989).

In each odor preference trial, an individual female hyena was presented with the paste of a male that exhibited a similar MHC *DRB* locus genotype and the paste of a male with a different *DRB* genotype. Each female received a total of 6 trials of 10 minutes each, and trials were modeled after those described by Drea et al. (2002a, b). Trials were conducted in covered enclosures on consecutive afternoons. Three straw piles (each $0.16m^3$ or approximately one sixth of a flake) were placed 3m apart along the wall farthest from the hyena's entryway (Figure 11). During each trial, the center straw pile was always unscented, and each outer straw pile was scented with a paste sample from a single donor (an MHC similar and an MHC dissimilar donor). On subsequent trials, the location (left side or right straw pile) of the MHC similar and dissimilar paste were randomly selected to avoid potential biases from sample location patterns among trials within test subjects.

Paste samples were collected from the scent glands of donor hyenas, snap-frozen, stored at -80^{o} C and thawed a minimum of 1 hour before testing. Using a sterile scalpel, paste (approximately 0.3 mL) was smeared on a piece of straw and placed in the center of a straw pile before each trial began. Trials began once the hyena entered the enclosure from an adjoining pen. After each trial, straw piles were removed and the room was cleaned by rinsing the concrete floor with water and squeegeeing the floor

until it was dry. All trials were videotaped and scored at a later date for behavioral responses using 'all occurrence' sampling (Altmann 1974). The ethogram of behavioral responses was taken from Drea et al. (2002a, b) and is reproduced in Table 6. Wilcoxon rank-sum and Student's *t*-tests were used to test for differences between groups (e.g. scented versus unscented straw piles). Pearson product-moment correlation tests were used to measure the correlation coefficient (*r*) between measures of genetic similarity and duration of behaviors, as well as between measures of genetic relatedness and similarity.

RESULTS

The average MHC *DRB* supertype similarity (D_{AB}) between male donor and recipient female hyenas was 0.255 ± 0.03 SEM. The average D_{AB} between recipient and 'similar' donors was 0.421 ± 0.02, and the average D_{AB} between recipient and 'dissimilar' donors was 0.089 ± 0.01. The average pairwise relatedness (*R*) among all donor and recipient hyenas was - 0.148 ± 0.02 (0.307 ± 0.04 among captive recipient hyenas only; 0.008 ± 0.02 among wild donor hyenas only).

Hyenas spent significantly more time in proximity to piles with paste applied than to piles with no paste (Figure 12, t = - 9.126, df = 141.24, p < 0.001). The average amount of time hyenas spent in close proximity to piles with paste was 45.04 seconds, when compared to 10.15 seconds spent in close proximity to piles that did not have paste. They also spent significantly more time investigating piles with paste (average

time sniffing = 8.45 seconds) than they did investigating piles without paste (average time sniffing = 2.37 seconds, t = - 7.938, df = 134.76, $p < 0.001$). Therefore, data documenting behaviors emitted in response to unscented piles were excluded from further analyses.

There was no significant difference in the amount of time a female spent in close proximity to a straw pile scented with paste from a male with similar versus dissimilar MHC *DRB* genotypes (Wilcoxon rank-sum test W = 1477.5, $p = 0.907$). Likewise, pairwise relatedness at microsatellite loci did not influence the amount of time a female spent in proximity to a particular odor ($P = 0.87$).

However, we found significant differences when we inquired whether genetic similarity influenced the amount of time a female spent investigating an odor, measured here as time spent sniffing a scented pile of straw. We found that females spent significantly more time sniffing a pile scented with paste from an MHC-dissimilar male (average time = 9.04 seconds) than if the paste was from an MHC-similar male (Figure 13, average = 5.85 seconds, W = 482, $p = 0.042$). There was also a significant correlation between the average pairwise relatedness (R) at microsatellites and time spent sniffing, such that females spent significantly longer times investigating odors from males that were more closely related to them (higher R) (Figure 14, $r = 0.371$, $p < 0.01$). However, we found no relationship between our measure of MHC similarity and R (r = - 0.008, $p >$ 0.1).

DISCUSSION

Previous authors (East *et al*. 2003; Theis 2008) have suggested that genetic compatibility may drive mate choice in the spotted hyena, but the ability of females to assess the genetic compatibility of potential mates via paste has never previously been determined. These data support the hypothesis that mate choice is odor-based in spotted hyenas, by demonstrating that spotted hyenas can indeed discriminate paste odors based on genetic similarity. These data add to existing knowledge regarding odor discrimination patterns in spotted hyenas, and offer further support that spotted hyenas glean vast information from odor cues, now including genetic similarity in addition to sex, reproductive state, group membership, and individual identity (Hofer et al. 2001; Burgener et al. 2008, 2009, Theis 2008, Theis et al. 2012).

Sexual selection theory predicts that competitors or potential mates signal their genetic quality or relatedness to conspecifics, and odor is a cue often used by carnivores to convey such information (Blaustein 1981; Andersson 1982; Gorman and Mills 1984; Halpin 1986). Data support this hypothesis in many vertebrate species, and MHC genes appear to play an integral role in the production of individual odor and volatile compounds contributing to these odors (mice: Manning et al. 1992, Penn and Potts 1998, 1999; voles: Radwan et al. 2008; humans: Wedekind et al. 1995; lemurs: Charpentier et al. 2008; fish: Forsberg et al. 2007; lizards: Olsson et al. 2003; Pearse-Pratt et al. 1999; Yamazaki et al. 1999; Kavaliers et al. 2005). The pathway from gene to odor is still not completely understood. It is known that in mice urine MHC differences

account for approximately 50 % of the individual variance in odor, and studies have shown that rodents can discriminate conspecific scents caused by single gene mutations in the MHC (Beauchamp & Yamazaki 2003; Beynon & Hurst 2004; Willse *et al.* 2006). Possible sources of odor are MHC molecules or their fragments, degradation products of their peptide ligands, or products of MHC dependent microflora (Milinski 2006). Though the exact mechanism is unknown, it is widely accepted that MHC plays a role in individual odor either through influencing microbial flora or influencing concentrations of volatile acids (e.g. Halpin 1986; Penn & Potts 1999; Tregenza & Wedell 2000). Our data contribute to this evidence, by demonstrating an additional carnivore species is able to discriminate odor based on MHC similarity.

Future studies regarding odor preference in this species should aim for larger sample sizes to tease apart the components of paste contributing to its odor. Theis et al. (2012) recently demonstrated the anal glands and paste secretions harbor bacterial communities unique to spotted hyenas. Because diet, hormones, disease status, and several other characteristics may influence individual odor, substantial opportunities exist for further research regarding the genetic basis of odor preference in these and other carnivores.

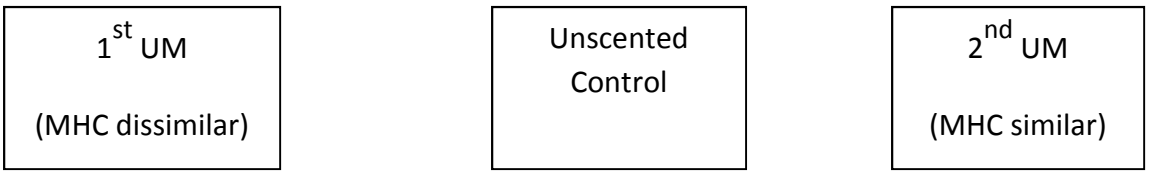

Figure 11. Example odor preference trial for an adult female. Each female entering the enclosure faced 3 straw piles. The 2 outer piles each held paste from an unfamiliar male (UM), one that was similar at his MHC *DRB* gene to hers, and one that differed. Trials were repeated six times per female, with new odors offered in every trial. On subsequent trials, the location (left side or right side) of the MHC similar and dissimilar paste were randomly placed as to have no pattern between trials within test subjects.

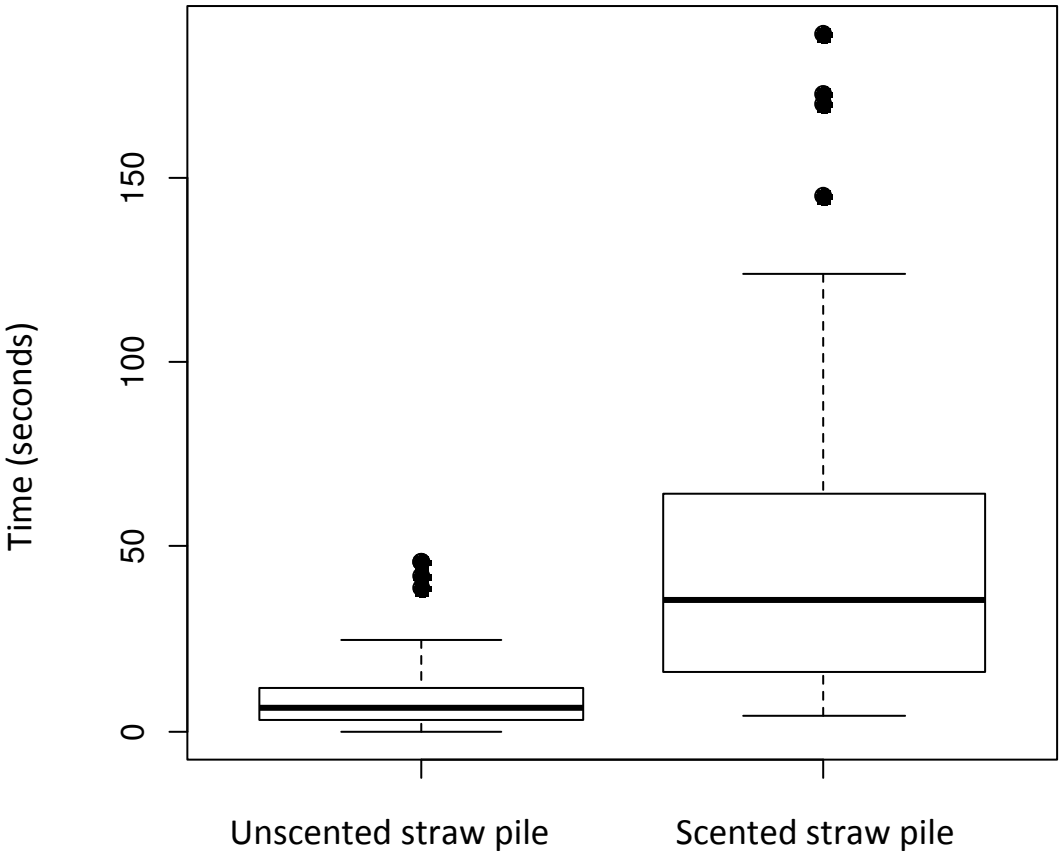

Figure 12. Captive spotted hyenas spent significantly more time (in seconds) in close proximity (less than 1 meter) to straw piles which were scented with another hyena's paste when compared to straw piles that had no paste applied to them (n = 9 hyenas, 54 trials). Each dot represents an individual trial, boxes indicate interquartile range, and whiskers extend to the furthest points outside this range, but within 1.5 times the interquartile range from the median.

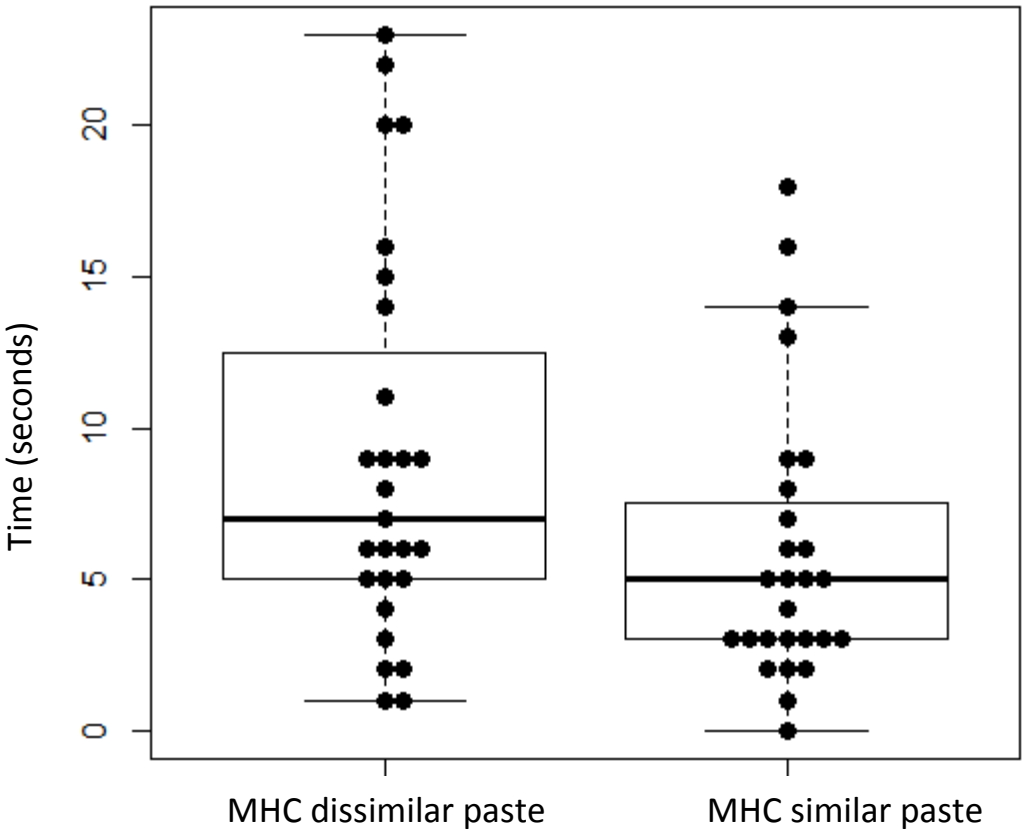

Figure 13. Captive female spotted hyenas spent significantly more time (in seconds) sniffing straw piles scented with MHC-dissimilar paste from a male than piles scented with paste from an MHC-similar (n = 9 hyenas, 27 trials). Each dot represents an individual trial, boxes indicate interquartile range, and whiskers extend to the furthest points outside this range, but within 1.5 times the interquartile range from the median.

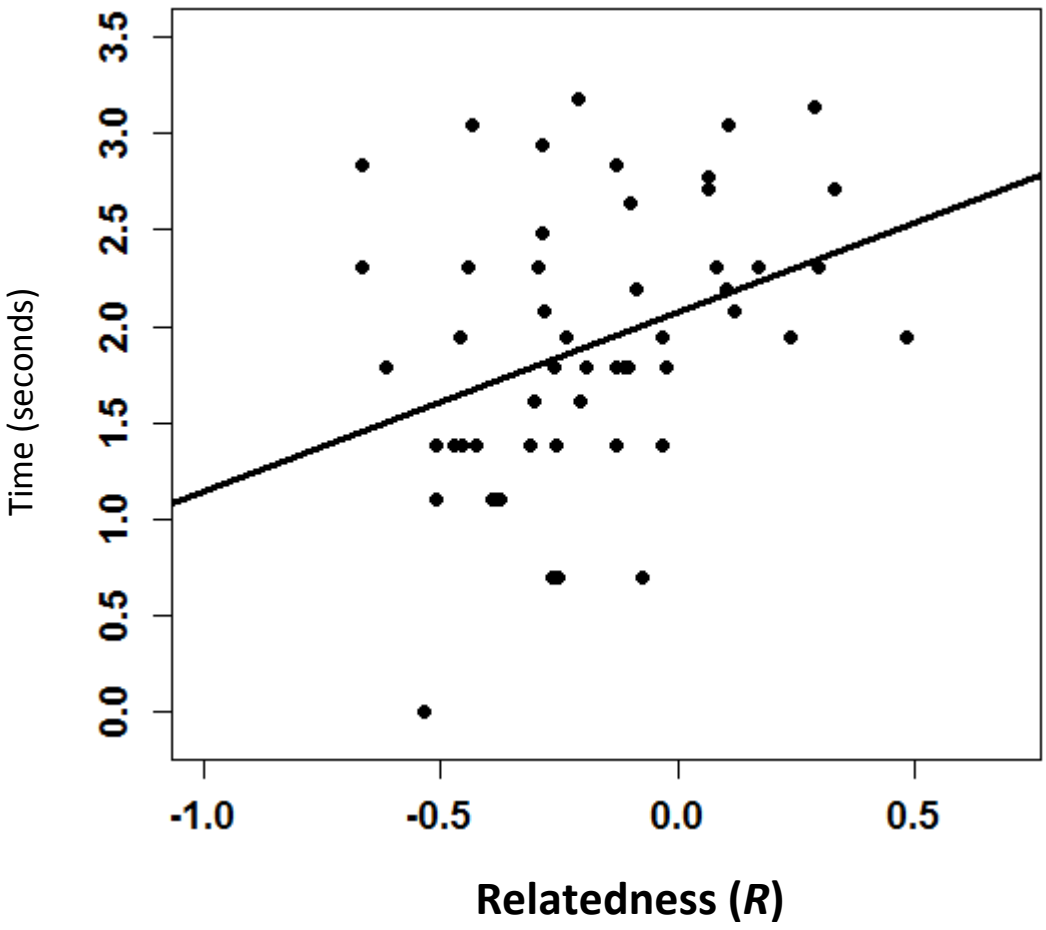

Figure 14. Adult female hyenas spent significantly more time (in seconds) sniffing the odor of male paste donors with whom they had a higher pairwise relatedness value (*R*) than when they were presented with male donor odors with whom they had lower pairwise *R* values.

Table 6. From Drea et al. (2002): Ethogram of definitions for spotted hyenas behavioral responses to odor.

Behavior	Definition
Approach	**Number of times animal comes within 1 m of target.**
Proximity	**Time animal spends within 1 m of target.**
Sniff	**Flaring of nostrils with audible sound of nasal air expulsion directed at target (i.e. nose within 10cm of target). Scored in bouts (frequency and duration), terminated by animal lifting or turning its head away.**
Lick	**Tongue in contact with target. Scored as bout frequencies, composed of several repeated licks.**
Roll	**Cheek, neck, shoulder, then back in contact with a substrate. Sometimes followed by sideways motion with feet up while in prone position. Scored in bouts terminated by the animal getting up or settling down.**
Paste	**Scent marking by depositing paste from extruded anal glands.**
Scratch	**Vigorously pawing at ground, floor or straw with front feet, presumably for depositing inter-digital scent. Usually involves repeated scraping by first one paw and then the other and is distinguished from more gentle stroking to uncover an item. Scored in bouts, terminated if activity was interrupted for 3 seconds.**

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CHAPTER FIVE

RELATEDNESS AND SPACE USE PATTERNS IN STRIPED HYENAS (*HYAENA HYAENA*)

INTRODUCTION

Mammalian carnivores tend to have large home ranges because a great deal of space often needs to be available for them to satisfy their energetic and nutritional requirements (McNab 1963, 2000; Harestad and Bunnel 1979; Gittleman and Harvey 1982). Carnivores whose diets consist largely of carrion may require particularly vast home ranges, as these food sources are often patchily distributed, rare, and unpredictable (e.g. Deygout et al. 2010). Resource dispersion is commonly cited as a predictor of space use in carnivores, though the resources required for survival and reproduction often differ between the sexes within species (Burt 1943; Carr and MacDonald 1986; Travis et al. 1999; Matthysen 2005; Poethke et al. 2011). As in other mammals, spatial distributions of female carnivores are often determined by the availability of food and other resources needed for reproduction, and female distributions in turn determine the spacing of males as they attempt to gain access to females (Emlen and Oring 1977).

When competitors for resources are closely related, or when competition is intense, selection may favor sex-biased dispersal to reduce competition among kin (Waser 1985; Perrin and Mazalov 1999; Clobert et al. 2001). Male biased dispersal is the norm among mammalian species; males typically disperse farther or more frequently than females to avoid mating with relatives; females typically incur larger costs from dispersal than do males, so instead of

dispersing, they commonly exhibit philopatry (e.g. Greenwood 1980; Shields 1982; Dobson 1982). When female dispersal does occur in mammals, it is predicted to be favored under different conditions than is male-biased dispersal, such as when high population density and/or scarce resources lead to increased competition for nutritional resources (reviewed by Clutton-Brock and Lukas 2012). Male-biased dispersal in mammals typically results in sexually dimorphic distributions of relatives, with female kin generally being found in closer proximity to one another than to male relatives (Greenwood 1980; Sherman 1981; Chepko-Sade and Halpin 1987; Waser and Elliott 1991; Smale et al. 1997; Gompper et al. 1998).

A deviation from the typical mammalian patterns of female philopatry, and female kin being found in close proximity to one another, was recently reported for a population of striped hyenas (*Hyaena hyaena*), one of the few remaining large mammalian carnivores about which little is known. Wagner et al. (2007) reported an atypical spatial grouping pattern in the Laikipia population of striped hyenas in the central highlands of Kenya, in that pairwise relatedness values between females increased with geographic distance. Further, behavioral observations and tracking of radio-collared animals revealed that no two females appeared to share home ranges, whereas males sometimes did. Taken together, these findings suggest a female bias in dispersal in the population studied by Wagner et al. (2007), and that females preferentially dispersed to ranges that were not adjacent to their natal ranges. Wagner et al. (2007) suggested that this highly unusual pattern might reflect attempts by females to avoid competing with close relatives for scarce resources in areas of range overlap.

The striped hyena is one of four extant species in the family Hyaenidae; these animals are widely distributed across northern Africa and the Middle East (Kruuk 1976; Mills and Hofer

1998; Holekamp and Kolowski 2009; Wagner 2013). Striped hyenas are nocturnal scavengers that feed mainly on bits of carrion that tend to be rare and widely scattered throughout their habitat. Though they are primarily scavengers, striped hyenas also occasionally hunt for small prey such as hares and young gazelles (Rosevear 1974; Kruuk 1976; Skinner and Ilnai 1979; Leakey et al. 1999; Kuhn 2005; Wagner 2006). Striped hyenas typically spend many hours each day traveling solitarily, presumably searching for food (Macdonald 1978; Leakey et al. 1999; Wagner 2006). When resting, they are sometimes found in pairs, or in groups of up to four individuals in some areas (Kruuk 1976; Wagner et al. 2008). They have also been observed provisioning cubs at dens, and caching food near their dens (Kruuk 1976; van Aarde et al. 1988).

Previous studies have demonstrated a high degree of plasticity in the behavior of striped hyenas, particularly in their ability to live in a variety of habitats and to forage on a wide array of food items, from fruits and invertebrates to livestock, and even human fecal matter and remains (Ilani 1975; Kruuk 1976; Macdonald 1978; Horwitz and Smith 1988; Leakey et al. 1999; Wagner 2006). Variation in home range size has also been reported across the species' range, with home ranges of males tending to be larger than those of females, and the magnitude of the sex difference varying among populations (Table 7; Kruuk 1976; Mills 1978b; Singh et al. 2010; Wagner 2008). Mills (1978a, 1982a, 1989) asserted that home range size in the striped hyena's closest extant relative and another carrion feeder, the brown hyena (*Hyaena brunnea*), is likely determined by the amount of food available in an area, and this may also be true in striped hyenas, particularly given that the diets of these two species are highly similar.

The finding of Wagner et al. (2007) of increasing female pairwise relatedness values with spatial distance raises several important questions about the factors influencing the spatial

distributions of kin in this species. For example, it is not clear whether the pattern inferred by Wagner et al. (2007) is general to striped hyenas everywhere, or simply reflective of unusual conditions specific to the Laikipia study area. The latter hypothesis predicts that, in areas with higher resource concentrations and/or lower population densities of striped hyenas, we should observe the typical mammalian pattern of more closely related females living in closer proximity to one another (i.e. decreasing relatedness with increasing geographic distance). These questions have important conservation implications regarding patterns of gene flow and population viability for this species, because striped hyenas are currently listed by IUCN as being of conservation concern, particularly due to their increasing overlap with human populations. This overlap is leading to fragmentation of the hyena's large home ranges, sometimes stranding them in marginal habitats characterized by low resource availability (Mills and Hofer 1998).

Comparative data on distinct populations in contrasting resource environments can inform our understanding of mammalian dispersal and social strategies. Here, we present data documenting genetic relatedness and space use collected from a previously unstudied wild population of striped hyenas in the southern Rift Valley near Shompole, Kenya. We used these data to explore the relationship between space-use patterns and relatedness in this species, and then compared our results to those reported from Laikipia by Wagner et al. (2007); the Laikipia striped hyena population is the only one, aside from that in Shompole, for which both genetic and spatial data are currently available. Finally, we used prey transect data to examine predictions of the hypothesis suggesting that relationships between genetic relatedness and spatial distance differ between our Shompole study population and the Laikipia population

studied by Wagner et al. (2008), due to differential food abundance or hyena population density in the two areas.

METHODS

Study populations and radio telemetry

Data presented here were collected from two populations of striped hyenas, which we will refer to as Laikipia (LK) and Shompole (SH). These two populations lie approximately 300 km apart in Kenya, East Africa, and differ with respect to many ecological variables, including elevation, rainfall, and abundance of food resources utilized by striped hyenas (Figure 15; Table 8). Data from LK have been reported previously (Wagner 2006; Wagner et al. 2007, 2008), and are used here for comparison with the SH population. Data from LK were collected from August 2000 until October 2003, and the study area of 480 km^2 was centered on the Loisaba Wilderness (284 km^2), a private livestock ranch and wilderness reserve in Laikipia District, Kenya. In the LK population, we used location data from the 18 males and 10 females in the core study area that were fitted with VHF collars (Wagner 2006, 2008). Further details of the LK striped hyena population, behavioral sampling, animal handling, and trapping parameters are given in Wagner (2006) and Wagner et al. (2007, 2008).

SH population data were collected in the Olkiramatian and Shompole Maasai Group Ranches in the southern Rift Valley of Kenya (~1,000 km^2) from February 2007 until February 2009. To maintain concordance between studies, the same data collection procedures regarding telemetry data that were followed in LK were followed as strictly as possible in Shompole as well. Briefly, animals were caught in pre-set soft-catch foot-hold traps and

anesthetized using either Telazol at a dose of 6.5 mg/kg body weight, or a combination of Ketamine HCl (dose: 3.6 mg/kg) and medetomidine HCl (dose: 0.06 mg/kg). We administered the sedative using a CO_2-powered rifle to fire a plastic dart containing the drug.

We collected blood and tissue for DNA extraction from all anesthetized hyenas in SH, as well as morphological measurements and body mass. We fit a total of nine captured striped hyenas (six females and three males) in the SH population with VHF radio-collars (Telonics, Inc., Mesa, AZ, U.S.A.; or SirTrack Ltd, Havelock North, NZ), and an additional three individuals (two males and one female) were fitted with global positioning system (GPS) collars (Savannah Tracking Ltd, Nairobi, Kenya). From these GPS collars, geographic fixes were downloaded automatically approximately every 20 minutes for 21 and 169 days on the two males, and for 26 days on the female. The GPS-collared animals were only fit with these collars, and not with VHF collars.

Behavioral sampling

Uniquely to the SH population of striped hyenas, and for the first time reported in this species, we were able to follow individuals and collect behavioral data. Striped hyenas typically move little during the day, but can travel quite far to forage at night (Kruuk 1976). In an effort to collect data during the hyena's period of peak activity within SH, we recorded behavioral data from 1830-0630 hours using focal animal sampling (FAS) and all occurrence sampling of critical incident (CI) behaviors emitted by focal individuals (Altmann 1974). An ethogram of CI behaviors collected during FAS sampling is given in Table 9. Individuals were located using their VHF radio collars and a radio antenna mounted to the roof of our vehicle. Once located, habituated individuals were followed from a minimum distance of 100 meters, so as to limit

interference with their behavior. Non-habituated individuals were often followed at distances exceeding 200m in closed habitats and at maximum sight distance in open areas.

The GPS coordinates of each focal animal were recorded every 10 minutes during each FAS in SH. Individual follows were continued until we were no longer able to follow the animal due to rough terrain or other impediments. If a FAS was abandoned, a new FAS was then begun on a new individual, if one could be located before dawn. During any FAS, other radio collar frequencies (n = 8) were constantly monitored, and we recorded the ID and location of any other collared hyenas in the same vicinity as the focal individual.

Prey density and density of striped hyenas

Densities of each prey species were calculated using DISTANCE 3.5 (Thomas et al., 1998; Buckland et al. 2001). Nine straight line transects spaced two kilometers apart were established in an east-west direction across the study area. Transect length ranged from 1-6 km and totaled 31.9 km. The variation in transect length was due to rugged terrain and our ability to navigate a straight transect line. All 9 transects were run once each month at night, using 2 spotters with handheld flashlights, and one driver who also recorded data. For every animal sighting, GPS location, as well as the group size were recorded. When possible, sex and age composition of the animal(s) sighted were also recorded. Since all data were collected after dark, we were not able to record enough age and sex specific data to conduct analyses at this level. All large birds and mammals, including livestock, alive or dead, were counted.

Spatial distances and home range size estimation

Choosing the correct sampling interval at which to select locations for use in home range (HR) size estimation for individual animals is an important consideration to which much

attention has been paid in the literature (e.g. Swihart and Slade 1985, 1997; Kernohan et al. 2001). Choosing too short of an interval can lead to autocorrelation between points, and underestimation of the animal's HR size (e.g. Dunn and Gipson 1977), yet choosing too long of an interval may involve losing much information on locations visited by the animal during travel (e.g. McNay et al. 1994). Other studies argue that, for some research questions, the time interval between points is generally unimportant (e.g. Reynolds and Laundré 1990). Because we were primarily interested in where individuals occurred and maximum range size rather than intensity of area use per se, independence of locations was not of great concern to us here (Kernohan et al. 2001). Therefore, using the 'sample' function in R version 2.15.2 (R Core Development Team 2012), two randomly chosen locations were pulled per individual per 24 hour period, one from during daylight hours (between 0631 and 1829 hours) and one during hours of darkness (between 1830 and 0630 hours), per calendar date on which data collection occurred for every individual,. Within the location data set collected for any one individual, any two points had to be recorded at least six hours apart in order to be included in the final data set for that individual. If two locations were randomly sampled that were recorded less than six hours apart, then sampling continued until this criterion was satisfied.

We calculated a HR size for every individual in both the LK and the SH populations for which we had a minimum of twenty locations, as sampled above. Home ranges for individual striped hyenas in both populations were calculated using fixed-kernel density estimation in Geospatial Modeling Environment (GME) and R (Beyer 2012; R Core Development Team 2012). We used a least-squares cross validation (LSCV) algorithm to determine the bandwidth, which controls the width of the individual kernels used, thus determining the smoothing factor

applied to the data (Kernohan et al. 2001). Although LSCV has received some recent scrutiny (Hemson et al. 2005), it is still accepted as one of the best bandwidth estimators for small sample sizes, such as we had here (Seaman and Powell 1996). We then calculated fixed kernel HR size (in km^2) for 95% isopleths for each individual. Using GME, the center of each HR was determined for each individual.

Within each population, we then calculated three spatial measurements for each possible dyad of individuals concurrently alive: 1) the pairwise distance (in km) between the centers of their calculated HRs, and the area of overlap between the two HRs, in terms of 2) total area expressed in km^2, as well as 3) a percentage of each individual's home range. Examples of these measurements are shown in Figure 16. Overlap area between HRs was calculated for each possible dyad using the intersect function in ArcGIS 10 (ESRI, Redlands, CA). Total study size area and hyena density for LK were obtained from Wagner (2006). For SH, we based total study size area and hyena density estimates on the merged composite HR areas (using Arc GIS 10) of the 8 hyenas for which we were able to obtain HR estimates (Table 7) over a continuous area.

Microsatellite genotyping and relatedness

DNA was obtained from a total 20 individuals from the SH population (11 females; 9 males). A total of ten microsatellite loci previously developed for use in the spotted hyena (*Crocuta crocuta*) were successfully amplified and genotyped in all samples from the SH population using conditions described previously (Table 10; Libants et al 2000; Wilhelm et al. 2003; Funk and Engh unpublished; Wagner et al. 2007). The tenth locus (Ccroc06) showed a lack of informative variation within the SH population (i.e. only 1 allele was recovered in any

genotyped animal), and so was not included in our analyses. As only one allele was recovered at this locus in SH, including these data did not add information to our relatedness estimates.

Wagner et al. (2007) genotyped a total of 59 striped hyenas from the LK population (25 females; 32 males). These genotypic data were obtained from the authors for the purpose of comparison with data from the SH population of striped hyenas. We chose here to exclude from our analyses the three loci among those genotyped within the LK population that showed evidence of null alleles in the work by Wagner et al. (2007). Allele frequencies at these three loci showed evidence of departure from Hardy-Weinberg equilibrium, revealing an excess of homozygous individuals, which suggests the presence of non-amplifying alleles (Guo and Thompson 1992). We calculated allele frequencies separately for both populations using the program CERVUS version 3.0 (Marshall et al. 1998), and pairwise relatedness values of (*R*) were estimated using the program RELATEDNESS (Queller and Goodnight 1989).

In seven cases where mother-cub relationships were known via a combination of behavioral observation and genotyping data, we employed a maximum likelihood-based approach (Thompson 1975; Meagher 1986) to assign paternity using the program CERVUS (Marshall et al. 1998). Our behavioral observations and radio telemetry data identified potential fathers for each cub by identifying the HRs of adult males that overlapped with those of adult females, and adult males who's HR areas were in close proximity to an adult female's HR (i.e. HR estimates for adult males were within 2 km of any edge of the adult female's HR area). We used the following parameters in CERVUS: 95% of candidate males sampled, 91% of loci typed, and a 1% error rate. A male was considered the sire of a particular cub when

CERVUS assigned him with 95% confidence, which was based on population allele frequencies at each locus (see Marshall et al. 1998 for further details).

Comparison of genetic-spatial distance relationships between populations

Due to our small sample sizes, we conducted non-parametric Spearman's rank correlations to inquire whether there were any significant correlations between estimates of genetic relatedness and spatial distance within each population. Correlations were performed on all samples available, as well as on all possible sex-specific dyads (i.e. male-male, male-female, and female-female). Significance of the correlation coefficient was tested in each case using "cor.test" in R.

RESULTS

Study populations and behavioral sampling

A total of 536.15 hours of FAS sampling were collected on 15 individuals (7 females and 8 males) within the SH population. The average FAS lasted 1.68 hours (± 0.01 SEM). During this time, hyenas spent the majority of their time traveling (69.7 %). The second most common behavior seen during our FAS data collection in SH was resting with their head on the ground (29.4 % of FAS total time; 'SO' in Table 9). Hyenas scent marked, or "pasted" 1.03 times per kilometer traveled during our follows, and this behavior did not differ between the sexes (t = -0.65, df = 8.25, p = 0.53). Hyenas of the SH population were alone during 95.6 % of the time in which we collected FAS data, independent of time spent at dens. Females were found alone a larger percentage of the time during our data collection (96.3 %) than were males (87.9 %).

Further, females were also solitary for a larger percentage (66.7 %) of focal follows during which they were observed feeding or carrying food (a total of 13.57 hours) than were males during follows when food was present (50 %). When adult females were not alone, they spent a majority of their time (58.8 %) with other adult females, followed by juveniles (26.5 % of the time), and were only found with adult males 11.8 % of the time. Adult males were found most often with other adult males (50 % of the time) when they were not alone during focal follows, and the remaining time was equally split between adult males being encountered with either adult females or juveniles.

Observations at dens

During FAS data collection, we also opportunistically recorded behavioral data at 16 dens for a total of 186.2 hours (representing part of the total FAS time given above). We saw 9 different individuals (3 adult females, 2 adult males, 3 juveniles, and 1 cub) at these dens during these observations (Tables 11 and 12). On 8 separate occasions, we saw more than one individual at a den, though we never saw more than two individuals concurrently at the same den (Tables 11 and 12). We also observed several instances (n = 11) of den provisioning by both adult males and adult females. Two different adult females (F105 and F110) were seen carrying food towards or into dens on multiple occasions. On two separate occasions, one adult male (M114) was seen carrying a scrap of food and approaching the den while, on each occurrence, a juvenile was seen exiting the den. The two juveniles (M112 and M113) seen with the adult male were known from behavioral observations of nursing from their mother (F104) to be litter-mates. This same adult male (M114) was also observed at a den with three

different adult females on different occasions (Tables 11 and 12). We present additional information regarding these individuals in our relatedness results section of this chapter.

Prey density and sample site comparison

Average prey density was significantly higher in the SH population study area (38.6 individuals/km^2) than in the Laikipia study area (11.0 individuals/km^2; t = 6.02 ; $p < 0.001$). Average hyena density was the same for LK (Wagner 2006) and SH (based on 8 individual's home range estimates) at 0.03 hyenas/km^2. Density estimates and other general ecological data from both study areas are given in Table 8.

Spatial distances and home range size estimation

Home range (HR) estimation data for both the SH and the LK populations, as well as striped hyena HR size estimates reported in previous studies, are given in Table 7. All pairwise (PW) estimates of distance between HR centers and proportion of HR overlap are presented in Table 13.

Shompole

Of the 12 hyenas fitted with collars in the SH population (9 animals with VHF collars and 3 animals with GPS collars), 8 individuals (6 females and 2 males) had a minimum of 20 locations per individual, and all independent locations (2 per 24 hour period) for each of these individuals were used to calculate home range (HR) estimates. The remaining four individuals were not encountered enough on subsequent FAS data collection dates to collect enough independent locations for these animals to estimate their HR areas. All SH hyenas were in the population concurrently throughout the period of data collection. The average HR size for

these 8 individuals was 66.89 km^2 ± SEM 13.31, and the HRs of adult males were significantly larger than those of adult females (Table 7; t = 4.14, $p < 0.01$; male average: 119.37 ± 32.03 km^2; female average: 49.4 ± 4.16 km^2). The total area occupied by these 8 individuals was 294 km^2 (Table 7). The average pairwise proportion of HR overlap for all SH individuals was 24%, and was highest for female-female dyads and lowest for male-male dyads. (Table 13). Figure 17b shows the overlap in five female HRs in the SH population with the HR of 1 adult male (M114). The five adult females in Figure 17b overlap with each other to various extents (average of 38.4 % HR overlap, ranging from 17.3 % to 98.4 % HR overlap), but nearly all are encompassed by the adult male range. The average proportion of individual female HR area overlapped by the HR area of M114 in Figure 17b is 81.2 percent.

Laikipia

A total of 19 individuals (8 females and 11 males) in the Laikipia (LK) population had a minimum of 20 locations, which were used to estimate HR size. We verified dates when each of these individuals was present in the study population to ensure that we only used data from animals that were in the LK study population concurrently. All 19 hyenas discussed here overlapped in time and space with one another for a minimum of three months, and up to 3.2 years of the study (A.P. Wagner, unpublished data). LK individuals had an average HR size of 122.24 km^2 ± 21.39, but in contrast to SH, we did not see a significant sex difference in average HR sizes in LK (Table 7; t = 0.91, $p > 0.10$; male average: 100.36 ± 13.15 km^2; female average: 152.32 ± 45.97 km^2). However, female HR size was significantly greater in LK than in SH (Table

7; t = 4.313, df = 84, $p < 0.001$). The average pairwise proportion of HR overlap for all LK individuals was 12 %, and contrary to what was seen in the SH population, was highest for male-female dyads and lowest for female-female dyads. (Table 13).

Relatedness estimates

The average pairwise relatedness (*R*) estimate for the entire SH population was - 0.07 ± 0.05 (based on 9 microsatellite loci) and **-** 0.02 ± 0.03 (based on 5 loci) for the LK population. (Table 10) The average pairwise relatedness estimate for individuals who were observed concurrently at the same den in the SH population was 0.05 ± 0.12, higher than the average population value of - 0.07, though not significantly (W = 36; $p = 0.45$). Average estimates of *R* for sex-specific dyads are presented for both populations in Table 13.

We were able to assign paternity to five cubs in the SH population, which represented 55.6 % of the genotyped offspring from this population with known mothers and putative fathers. Sires were assigned with 95% certainty by CERVUS, based on log- likelihood ratio (LOD) scores. We had one known twin litter in this population, but paternity was only assigned successfully to one of these cubs. For the remaining litters, we were unable to sample more than one individual, and thus unable to determine litter composition. A different sire was assigned to the second twin of this litter, but only at 80 % confidence, so we did not include this assignment in our results. The male whose large HR appeared to encompass those of several females (M114; Figure 17b) could be assigned as sire of one cub with 95% confidence. The mother of this cub was F104, and the proportion of her HR area that overlapped with M114 was 98.1 % (Figure 17b).

Correlations between relatedness and spatial distance

Table 13 compares the pairwise distance (in km) between centers of individual HRs to the pairwise relatedness estimate for all sex-specific dyads, divided into: female-female dyads, male-female dyads, and male-male dyads, separated by population. Figure 18a shows the same trend in female-female dyads in the LK population as that reported by Wagner et al. 2007, where average relatedness increases with increasing spatial distance, though the Spearman's rank coefficient (ρ) was not significant within our random sample of these data (ρ = 0.179; p = 0.359, n = 28 dyads). In agreement with most data on mammalian dispersal patterns, and in conflict with data from the LK population, we found that average pairwise relatedness decreased with increasing spatial distance within female- female dyads in the SH population, though this relationship was not statistically significant (Figure 18d; ρ = - 0.425, p = 0.116). We found no significant correlations in any other sex-specific dyads either (Figure 18; male-female dyads: ρ = - 0.02, p = 0.95; male- male dyads: ρ = - 0.425, p = 0.12).

The average relatedness between the five females whose HRs overlapped substantially, illustrated in Figure 17b was R = - 0.085, with the two females who overlapped the most (F104 and F105) being a mother-daughter pair (R = 0.614). In fact, F104's HR area completely encompassed that of F105 (her daughter). This was in stark contrast to the low average pairwise relatedness estimate between the three adult females with partially overlapping HRs in the LK population (R = - 0.4785; Figure 17a; here the average overlap with other females was only 3.38 % of each female's HR). The overall pairwise HR overlap in female-female dyads was in fact significantly greater in the SH population than it was in LK (Table 13; Figure 17; t = 3.477, df = 84, p < 0.001). These data further support the pattern of more highly related females being

found in closer proximity in SH, whereas more distantly related females were found closer together in the LK population.

The average pairwise relatedness within male-male dyads was similar in both populations, as was the average proportion of HR overlap between males (Table 13). However, the average proportion of HR overlap in male-female dyads was higher in SH than it was in LK, though this difference was not statistically significant (t = - 1.37, df = 26.813, p = 0.18). Figure 19 illustrates the salient differences between the two populations in regards to proportion of HR area overlap between male and female ranges. Female home ranges were significantly larger in LK than they were in SH (Table 7), and HR area estimates did not differ significantly between the sexes in LK, but male HR estimates were significantly larger than female HRs in SH (Table 13). Thus on average, one male shared space with fewer females in LK than in SH (Figure 19).

DISCUSSION

Striped hyenas have been historically understudied relative to other medium and large size carnivores (Wagner 2013). Prior to the current study, knowledge of the spatial ecology of striped hyenas was extremely limited, and derived mainly from data collected from a single population (Wagner et al. 2007, 2008). Our study is the first to offer a more comprehensive picture of the social lives of this species, and makes new data available that may be critical to informing conservation strategies. Further, our data offer the first opportunity to contrast these patterns across multiple striped hyena populations. Previous data presented a picture of

an unusual mammalian system, in which all females dispersed, preferentially moving away from their female kin, and their home ranges did not overlap those of other females, although each female home range did overlap with those of multiple males. The earlier study by Wagner et al. (2007) in Laikipia reported that, whereas striped hyenas are highly solitary, they are known to be found occasionally in groups of up to four individuals, but that these groups never include more than one adult female (Wagner et al. 2007, 2008; Wagner 2013).

In contrast, the data presented here suggest that striped hyenas in the Shompole population conform to typical mammalian patterns, and are remarkably similar, with respect to patterns of relatedness and composition of social groups, to their sister species, the brown hyena (Mills 1978a, 1982a, 1989). Here, for the first time, we report several behaviors in common between these two species, never previously observed among striped hyenas. These include incidences of multiple females overlapping in space use, and behavioral evidence of den sharing. This behavior is in stark contrast to previous reports on the striped hyena, and contributes new and useful information to the small body of existing knowledge on the behavioral ecology of this species. Studies such as ours highlight the need for population-specific data, and caution against extrapolation across multiple populations with varying ecologies. In-depth genetic analyses of these and new populations, genotyping at more loci, or at higher resolution using single nucleotide polymorphisms (SNPs), over larger spatial areas and longer time periods, would all greatly benefit the conservation of this species. These types of data are still needed to ascertain how quickly striped hyena populations are able to adapt their space use and relatedness structure in response to ecological changes, and to further investigate patterns of relatedness structuring in this species.

Males in SH maintain significantly larger home range areas than females, which presumably enables them to increase their mating opportunities by enhancing their access to females. Home ranges, specifically those of females, were significantly smaller, and prey density was significantly higher, in the SH population than in the LK population (Tables 7 and 8; Wagner 2006). These data fit predictions regarding female space use in response to resource abundance. Solitary foraging is predicted by the small, rare nature of carrion food items, on which striped hyenas rely, and solitary feeding is indeed predominant in both populations.

Our data indicate that, in comparison with the pattern seen in LK, SH females remain in their natal areas and maintain smaller home range areas. We suggest this pattern is seen in SH because there is sufficient food (see prey density data in Table 8) to support the existing density of conspecifics without intensive feeding competition, and because females may benefit from receiving help from relatives in raising their offspring, as evidenced by our den sharing and den provisioning data. However, in LK, where our own data and those obtained by Wagner et al. (2007) data provide evidence for female biased dispersal and larger female home ranges than in SH females, we hypothesize that the lower prey density in LK requires females to utilize a larger spatial area in order to acquire adequate food, and that by dispersing from their natal area, LK females also may limit kin competition for these scarce resources. We suggest that higher prey density in Shompole may relax competition over these resources such that the costs of kin competition are no longer larger than the benefits individuals might receive from cooperation with kin. Under circumstances of relatively high prey availability, such as those experienced by the SH population, it is predicted that females should remain in close proximity to close kin if the benefits of kin cooperation outweigh the costs of kin competition. Our

anecdotal behavioral data on den sharing and den provisioning further suggest that striped hyenas may receive fitness benefits from kin cooperation, even when solitary foraging remains optimal.

The pattern of increasing relatedness with increasing distance that was observed in the LK population by Wagner et al. (2007) was confirmed here as a trend for that population using more conservative genotyping methods than those utilized in the earlier study (Figures 18a and 20b). Females in the LK population appear to preferentially distribute themselves farther from close relatives than from non-relatives. One scenario that predicts this pattern is in the case of scarce resources, leading to increased competition among kin for nutritional resources (reviewed by Clutton-Brock and Lukas 2012). Under such conditions, female dispersal may be favored in mammals in order to limit resource competition among closely related animals.

Whereas female home range size and relatedness structuring differ greatly between the SH and LK populations of striped hyenas, the patterns observed within male-male dyads were remarkably similar between SH and LK. Male-male dyads in both populations average approximately 12 % overlap in the proportions of their HR areas. Further, pairwise relatedness between males of overlapping HRs is similar in both populations, and higher than other sex-specific dyads, as well as the overall population averages of relatedness (Table 13). Despite the marked differences in female HR sizes between SH and LK, male home range sizes do not differ significantly between the two populations (Table 7). Theory states that female mammals aim to optimize fitness by their access to food resources, and that males optimize their fitness by access to females (e.g. Emlen and Oring 1977; Andersson 1996; Bercovitch 1997). If this theory holds in striped hyenas, perhaps HR size for male striped hyenas remains similarly large

between the SH and LK populations because it is the largest area a single male, or possibly a coalition of two or more males, can maintain, as is suggested by consistently overlapping male-male HR areas in LK and higher than average pairwise genetic relatedness. Sexual selection theory predicts that males should behave to optimize their access to females, and home range size may be the limiting factor determining access to females among striped hyenas (e.g. Andersson 1996).

Although our estimates for home range sizes for individuals within the Laikipia population differed significantly from those reported by Wagner et al. (2008), this is a common by-product of sampling from location data (e.g. Kernohan et al. 2001). Various differences in sampling methods such as what was designated as an independent location, and differences between studies in the stringency rules for data inclusion, can influence results. In addition, any sampling from a larger dataset will always result in a range of results. The differences in these estimates calculated from the same data set illustrate some of the caveats to resampling from sets of location data to infer animal home ranges, as many home range estimators are based on randomly sampled locations from all available data (e.g. Kernohan et al. 2001).

However, regardless of whether we invoke our estimate for LK home ranges or that of Wagner et al. (2007), the following patterns hold. Male estimated home range sizes do not differ significantly between the LK and SH populations, female home range sizes are significantly smaller in SH than in LK, and the area of overlap between females in SH is significantly higher than in LK (an average of 25.6 % of the female's home range in all SH female-female dyads versus only 8.5 % in LK female-female dyads). This represents more than

a threefold difference in the amount of space shared by females in SH than in LK, and these females are significantly more closely related in SH than they are in LK (Table 13).

The Queller and Goodnight (1989) relatedness estimator, *R,* is measured on a scale from -1 to 1, where positive numbers indicate that individuals are more closely related than is expected for any two random individuals within the population (given these allele frequencies at these loci), and negative numbers indicate a lower average relatedness than expected at random (Queller and Goodnight 1989). While this measure is not suitable for making inferences about dyadic relationships, nor should it be used as the only source of information to infer pedigrees, it is a useful measure to indicate overall population wide relatedness (e.g. Van Horn et al. 2008).

The fact that we used different loci, with different values of expected heterozygosity, as well as a different total number of loci, to obtain pairwise relatedness estimates (*R*) within each population suggests that there might be population differences in the stability of our *R* measures (e.g. Altmann et al. 1996; Blouin et al. 1996). Additionally, our small sample size in the SH population likely explains why our mean *R* values of mothers and cubs (0.236 ± 0.13, n= 8), sires and cubs (0.02 ± 0.138, n = 7), full-sibling pairs (0.269 ± 0.22, n = 3) and half-sibling pairs (- 0.056 ± 0.092, n = 3) were all considerably below the coefficients of relatedness expected from known pedigrees, and exhibited large error estimates in SH. In addition to having a low sample size of individuals, the nine loci used to genotype in SH could be increased in order to strengthen the reliability of these estimates. However, these relatedness values are comparable to one another as expected. That is, *R* is highest among mother-cub dyads, full-

siblings, and sire-cub pairings, followed by half siblings, and lastly by dam-sire pairs (R = - 0.229 ± 0.076, n = 5).

Whereas using solely molecular methods to infer relatedness may often lead to incorrect conclusions regarding true pairwise relatedness or kinship within a population (e.g. Van Horn et al. 2008), when paired with behavioral data, these estimators can be very informative. In this study, we combined molecular data with behavioral and ecological data from two distinct striped hyena populations to examine the influence of ecological parameters on space use and relatedness patterns. We presented evidence that two discrete populations of the same species can exhibit markedly different space use patterns and relatedness structures when ecologies between populations differ. We contributed data from a multiyear study of a previously unstudied population of striped hyenas, and contrasted these data with those previously published by Wagner et al. (2007). These data demonstrate that the ecological circumstances experienced by a population can dictate several aspects of individual behavior and are important factors to completing a comprehensive picture of a species' biology.

Figure 15. Study areas of both the Laikipia (LK) and Shompole (SH) populations shown in Kenya. Areas within each study boundary are shaded in dark gray against the lighter gray background.

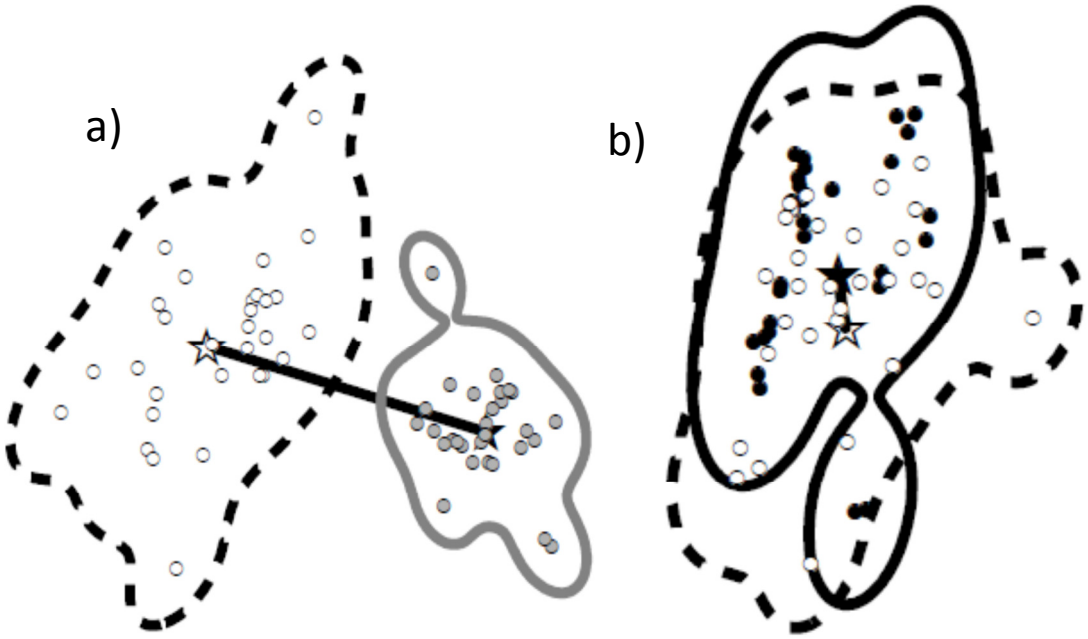


Figure 16. Examples of (a) pairwise home range (HR) center distance and (b) pairwise HR overlap. In (a), two individual HRs (one indicated by a solid line, the other by a dashed line) are estimated from the location data points of one individual each, and the distance between the centers of these HRs is depicted with a solid black line. In (b), the overlap of two individual's HR's are shown, one HR depicted by a solid line, and the other by a dashed line. In this example, the dashed line individual overlaps the solid line individual's HR by 80.7 %, while the solid line individual overlaps 74.7 % of the dashed line individual's HR, giving them a pairwise average proportion overlap of 77.7 %.

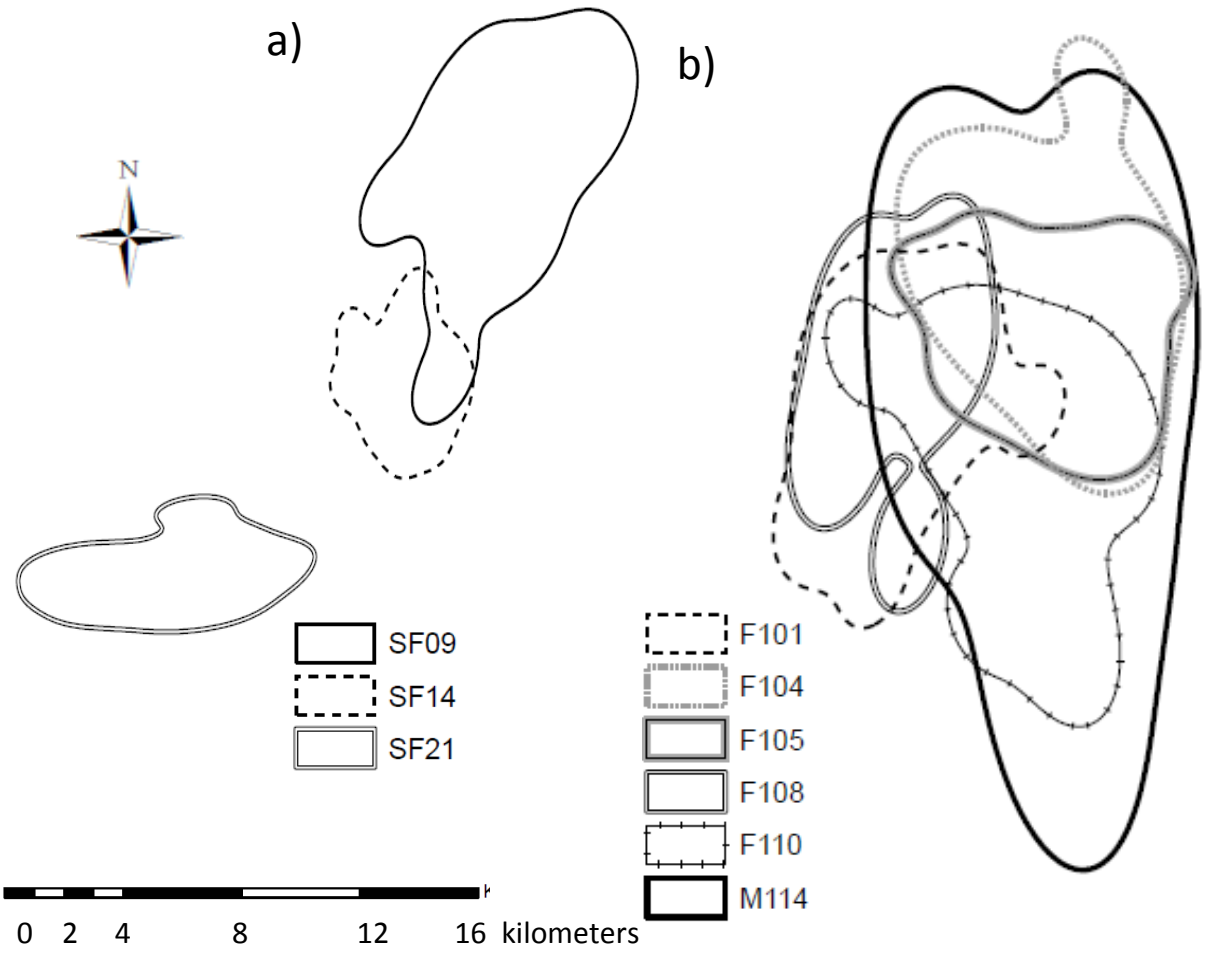


Figure 17. Home ranges (HR) shown for three adult females in the Laikipia population (a) and five adult females and one adult male (M114) in the Shompole population (b). Individual HRs are indicated by the style of line shown in each figure legend. The scale (in km) common for both figures is given in the bottom left corner.

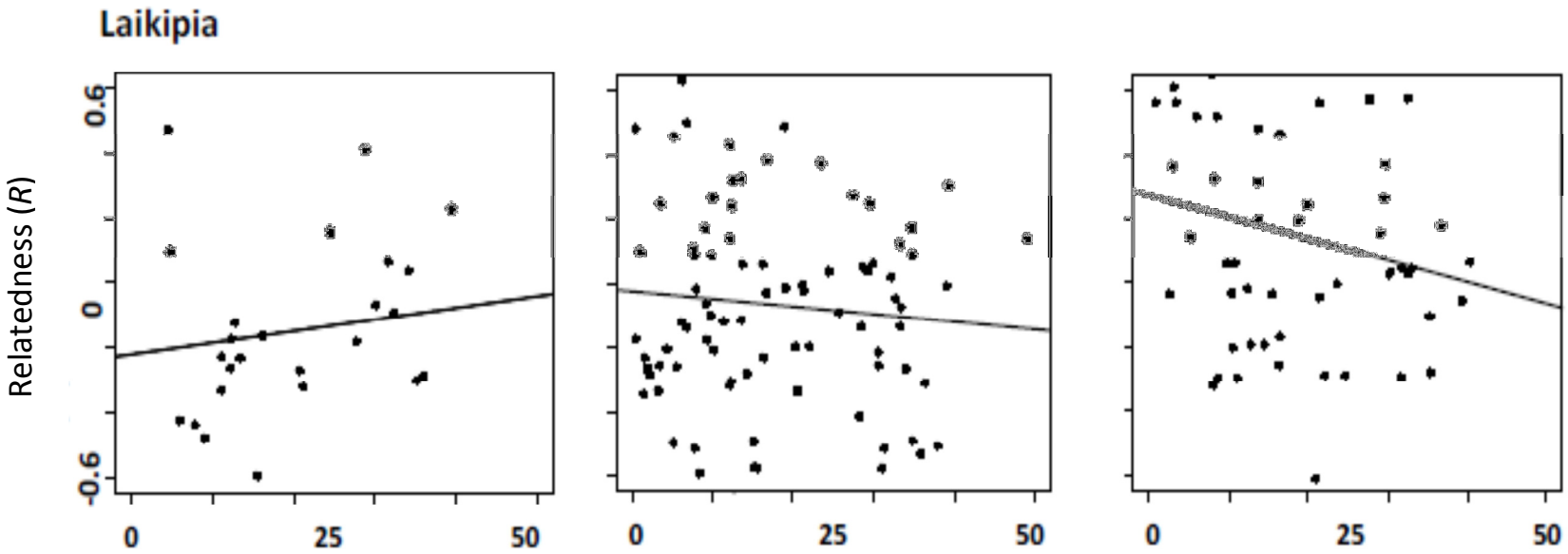
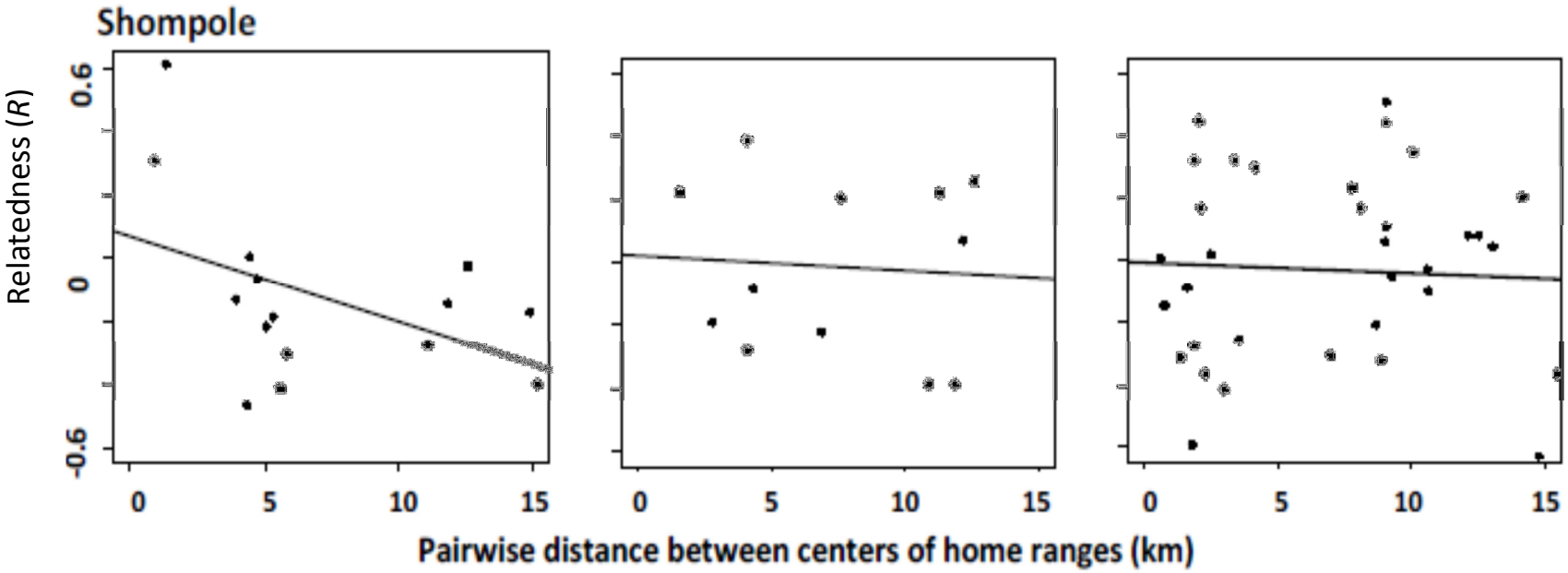


Figure 18. Pairwise genetic relatedness (*R*) as a function of spatial distances between individuals' home range centers (km) between individuals in the Laikipia population (a, b, and c; Wagner et al. 2007) and the Shompole population (d, e, and f) for female-female dyads only (left column), male-female dyads only (middle column), and male-male dyads only (right column).

Figure 18 (cont'd)



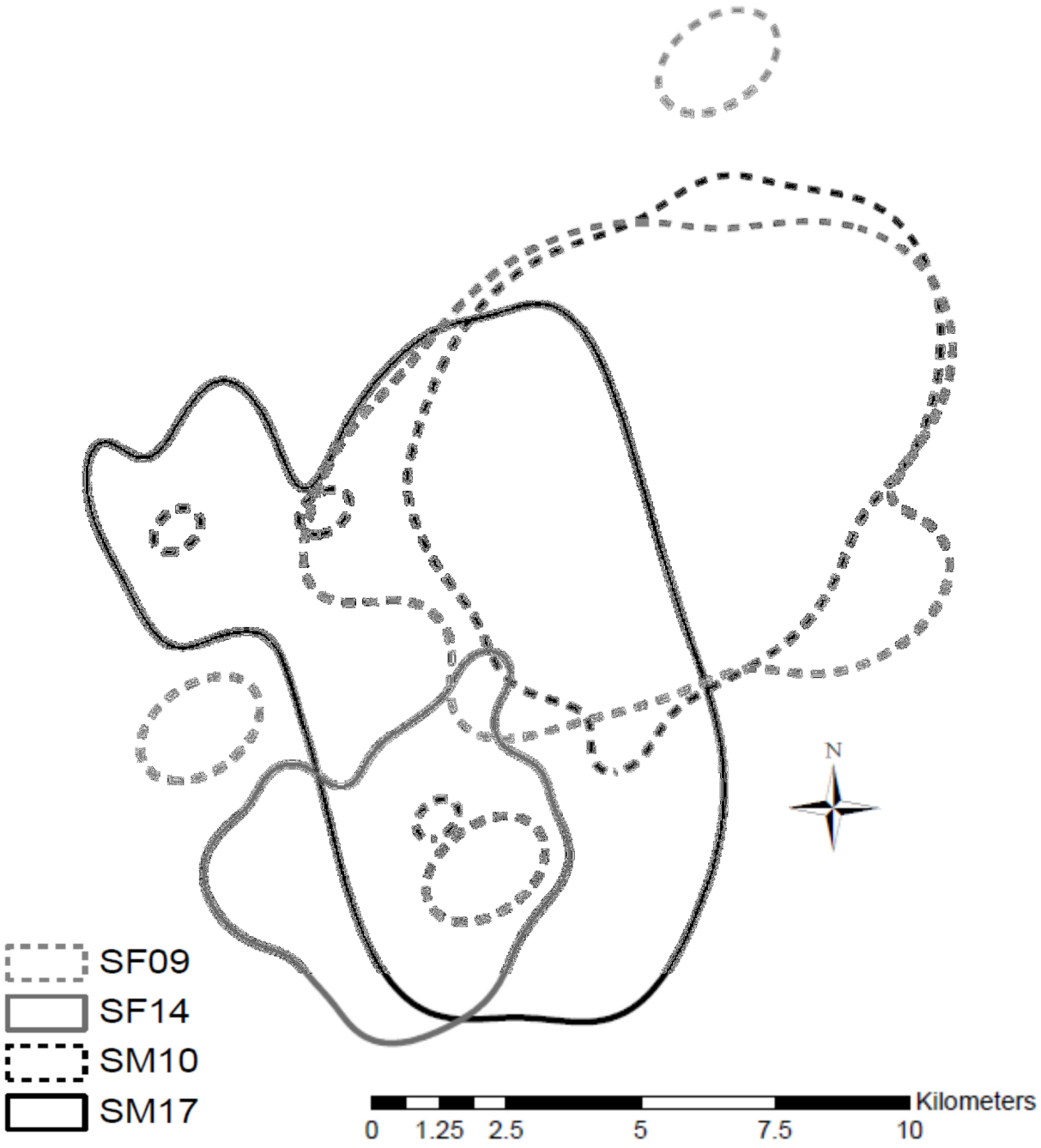


Figure 19. Home range area estimates (HR) shown for 2 adult females (SF09 and SF14) and 2 adult males (SM10 and SM17) in the Laikipia population (a) and HRs for 3 adult females (F104, F105, and F108) and 2 adult males (M114 and M115) shown for the Shompole population (b). Individual HRs are indicated by the style of line shown in each figure legend. The scale (in km) common for both maps is given in the bottom of the figure.

Figure 19 (cont'd)

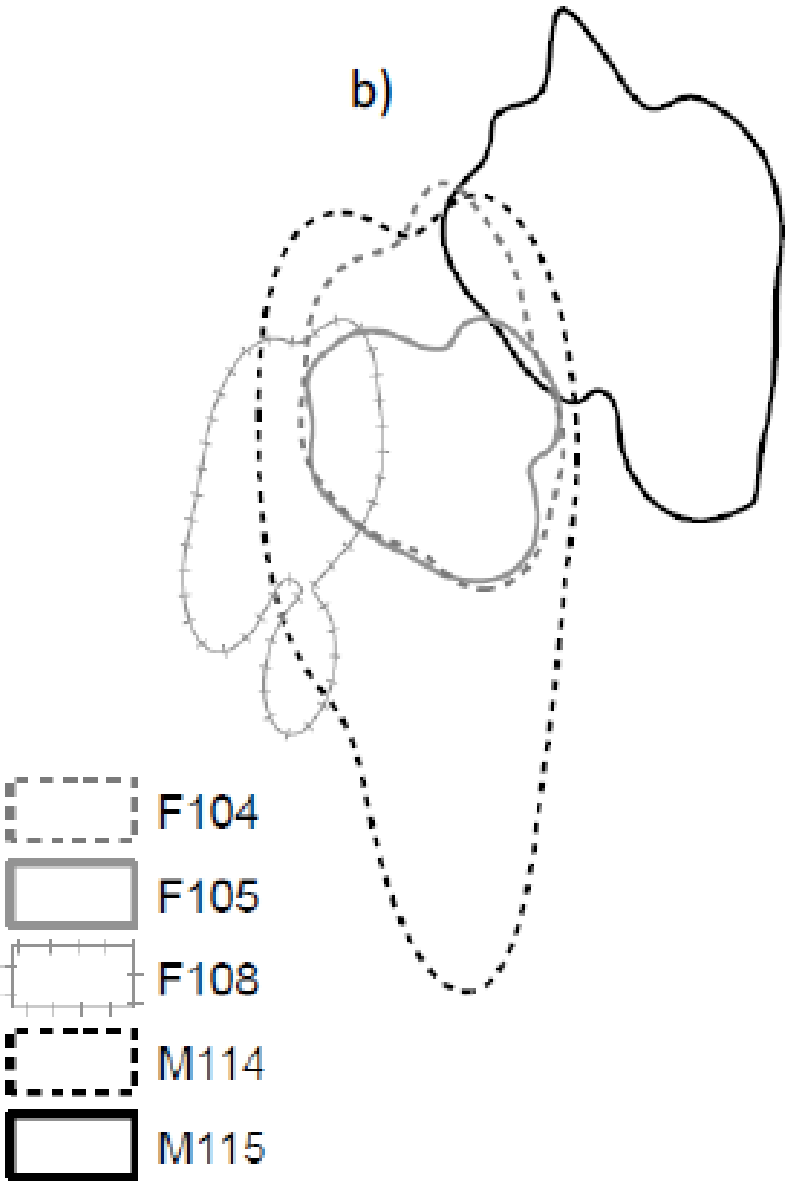


Table 7. Reported home range (HR) size estimates (in km^2) for striped hyenas (*Hyaena hyaena*) from previous studies, and those obtained using the methods of the present study. In contrast to earlier studies, only our HR estimates are based on radio telemetry data. Sex differences are shown when reported. Estimates were based on numbers of individuals given in parentheses (n). All mean values are reported ± the standard error of the mean (SEM).

Population	**Overall HR size (km^2)**	**Male**	**Female**	**Sex unknown**
Serengeti, Tanzania (Kruuk 1976)	60 (n = 2)	76 (n=1)	44 (n=1)	
Negev Desert, Israel (van Aarde et al. 1988)				60.9 (n = 1)
Laikipia, Kenya (Wagner et al. 2008)	68.9 ± 7.8 (n = 10)	76.0 ± 13.8 (n = 4)	64.2 ± 9.8 (n = 6)	
Laikipia, Kenya *(current study)*	122.24 ± 21.39 (n = 19)	100.36 ± 13.15 (n = 11)	152.32 ± 45.97 (n = 8)	
Shompole, Kenya (*current study*)	66.89 ± 13.31 (n = 8)	119.37 ± 32.03 (n = 2)	49.4 ± 4.16 (n = 6)	

Table 8. Comparison of general ecology between Shompole and Laikipia. All animal densities are given as number of individuals per km^2 ± CV (coefficient of variation). All other values are given with ± SEM (standard error on the mean). Estimates for Shompole are from the present study (see also: Schuette 2012 for similar estimates). Estimates for Laikipia are taken from Wagner (2006).

	Shompole	**Laikipia**
Location	36° East, 2° South	36° 50 East, 0° 63 North
Size of study area (km^2)	294	480
Elevation (m above sea level)	610	1780
Average Temperature (° C)		
Low	24.2 ± 0.09	16.0
High	37.7 ± 0.03	26.0
Mean annual rainfall (mm)	317.5 ± 63.5	464 ± 37
Terrain	Lava ridges and valleys Sandy Arid	Central highlands Plains Semi-arid
Ground cover	Acacia woodland/grassland Alkaline grassland flats Swamps Riverine forest	Open grassland Light acacia bush Some forest

Table 8 (cont'd)

	Shompole	Laikipia
Striped hyena density (animals/km^2)	0.03	0.03
Ungulate density (cumulative)	38.61 ± 3.38	11.0 ± 3.1
zebra (*Equus burchellii*)	6.77 ± 0.28	5.3 ± 2.4
wildebeest (*Connochaetes taurinus*)	16.33 ± 0.55	0
Grant's gazelle (*Nanger granti*)	9.97 ± 0.24	1.28 ± 0.315
impala (*Aepyceros melampus*)	4.26 ± 1.76	4.09 ± 0.464
Maasai giraffe (*Giraffa camelopardalis tippelskirchi*)	1.28 ± 0.55	0.33 ± 0.13
Livestock density (cumulative)	74.90 ± 22.68	4.3 - 5.9
Sheep/goats	59.12 ± 17.02	0
Cattle	15.78 ± 5.66	4.3 - 5.9

Table 9. Ethogram of behaviors considered critical incidents (CIs) and recorded during focal animal sampling (FAS) follows.

Code	Behavior	Description
A	active	not at rest
AD	arrive den	a hyena arriving at a den site while under observation-not applicable for short trips/patrols
AF	avoid with food…	
AL	alone	no other hyenas known to be nearby
AP	approach	
AS	air sniff	sniffing the air
AV	avoid…	avoiding
BIS	NA	back in sight
BITE	bite object/animal…	biting a thing or animal in a fight or in play (e.g. not feeding)
BM	bristle mane	mane up
BT	bristle tail	hairs erect
CA	chase after…	chasing another animal not in play or over food
CACHE	cache food	
CF	carry food	carrying a food item from one place to another
CP	chase play…	chasing in play
D	drink	drinking
DIG	digging	
E	encouters other…	crosses paths with another animal or carcass
ED	ears down	ears down flat
ENTDEN	enter den	enter den completely
ES	escort away…	
EU	ears up	as if listening
EXTDEN	exit den	emerging/coming out of the den hole
F	feeding on…	consumption usually of flesh
FL	face lick other…	licking another hyena's face
FLEE	flee from…	animal flees from something (something=event target)

Table 9 (cont'd)

Code	Behavior	Description
FOLLOW	following other…	
FS	face sniff other…	sniff another hyenas face
GO	groom other…	grooming another hyena; use E and notes to clarify
GRIN	grinning at…	ear's-back begging expression; goes with squiddling much of the time
GRM	groom	grooming self
GRS	grass sniff	sniffing a particular grass stalk
GS	ground sniff	sniffs the ground
HD	head down	posture with head down more than normal but not ground sniffing
HEARD	signal heard	signal heard, but nothing else implied
HU	head up	posture with head up more than normal but not simply looking
IA	inactive	not active (i.e. SO); can be IA without knowing LIE or SO if US
INVDEN	investigate den	investigate den hole (e.g. stick head in), but didn't enter
JA	jump away…	jumping away from something; not same as a leap or a pounce
LD	leave den	a hyena leaving a den site while under observation-not applicable for short trips/patrols
LIE	lying down	lying down, but not with head on ground (which is SO)
LL	lying and looking at…	lying on ground with HU to look around
M	moving	moving, regardless of pace (will also be walking or traveling)
MD	mane down	mane flat against back
N	nursing…	a cub nursing from a female
NM	not moving	not moving
NO	nurse other…	a female nursing her cub
OOC	out of contact	can't be heard or seen at all
P	pasting on…	marking
PEE	urinating	
PLAY	playing with…	

Table 9 (cont'd)

Code	Behavior	Description
PN	pounce on…	pouncing on something
POO	defecating	
POOC	passes out of contact	tranistions from in contact to out of contact
POOS	passes out of sight	goes from in sight to out of sight either because the animal moved or the observer moved
RCF	retrieve cached food	
ROLL	roll on/in…	rolling on ground (usually a dust bath)
ROMP	romping	running around in play
RUB	rubbing on…	rubbing on an object
RUN	running	
S	seen	the observer has the hyena in sight at that exact moment
SF	shadow food…	shadowing or chasing another animal that has food
SIT	sitting	
SL	standing and looking at…	standing still and looking in fixed direction
SNO	sniffing at…	sniffing at an object (other than the ground, air, or grass)
SO	sacked out	resting with head on ground
ST	stands up/standing	standing or standing up
STR	stretching	
T	travel	walking long distance in definitive direction
TD	tail down	between legs
TROT	trotting	also same as a fast walk
TS	travel sniff	simultaneous traveling and sniffing
TU	tail up	above spine
UNK	unknown	behavior not determined (mostly only applicable to camera photos) or doing nothing

Table 9 (cont'd)

Code	Behavior	Description
US	unseen	observer does not have the hyena in sight at that exact moment
VIG	vigilant toward…	intent watching of another animal
W	walk	walking in definitive direction but without long distance intent
WITH	with other	with another hyena
WL	walking and looking at…	simultaneous walking and looking
WN	wander	moving in small area with no definitive directional intent
WNS	wander sniff	simultaneous wandering and sniffing
WS	walking and sniffing	simultaneous walking and sniffing

Table 10. The number of alleles observed and the observed (H_O) and expected heterozygosities (H_E) of each locus used for relatedness analyses in the present study in the Shompole population of striped hyenas, and taken from Wagner et al. (2007) for the Laikipia population. The locus Ccroc06 was removed from analyses of the Shompole population due to lack of variation and is noted here with an asterisk (*). The frequency of null alleles at three loci in the Laikipia population is given by p_n. No null alleles were detected at any locus genotyped in the Shompole population.

Population	LOCUS											
	ccr01	**ccr04**	**ccr05**	**ccr06**	**ccr07**	**ccr13**	**ccr14**	**Ccroc01**	**Ccroc05**	**Ccroc06***	**ccrA3**	**ccrA5**
Shompole												
# alleles	2	6	5	3	2	6	4	3	9	1	NA	NA
H_O	0.150	0.850	0.800	0.650	0.150	0.579	0.250	0.650	0.700	0*	NA	NA
H_e	0.142	0.774	0.740	0.496	0.142	0.653	0.314	0.573	0.840	0*	NA	NA
Laikipia (Wagner et al. 2007)												
# alleles	NA	7	6	4	NA	NA	NA	3	7	2	4	5
H_O	NA	0.700	0.640	0.510	NA	NA	NA	0.610	0.830	0.150	0.340	0.640

Table 10 (cont'd)

	ccr01	ccr04	ccr05	ccr06	ccr07	ccr13	ccr14	Ccroc01	Ccroc05	Ccroc06*	ccrA3	ccrA5
H_e	NA	0.760	0.780	0.440	NA	NA	NA	0.600	0.810	0.140	0.320	0.670
p_n			0.074								0.180	0.210

Table 11. This table details the age and sex classes, as well as pairwise relatedness (*R*) estimates for any two hyenas observed concurrently at any den.

Den ID	IDs of animals seen concurrently at den	Age and sex classes of individuals	Pairwise relatedness (*R*)
F104 L2D01	F104, F105	Adult female, adult female	0.737
F104 L2D01	F104, M114	Adult female, adult male	- 0.394
F105 L1D04	F105, M112	Adult female, juvenile male	0.002
F104 L1D06	F105, M114	Adult female, adult male	- 0.336
F110 L1D02	F110, M114	Adult female, adult male	0.059
F110 L1D02	F110, F117	Adult female, juvenile female	- 0.264
F104 L1D04	M112, M114	Juvenile male, adult male	- 0.088
F104 L1D06	M113, M114	Juvenile male, adult male	0.495

Table 12. Matrix of individuals seen at dens. Juvenile individuals are noted by '(juv)' on the edge of the matrix, all other individuals listed were adults. "F" denotes a female sample number, and "M" denotes a male sample number. Numbers within the matrix indicate the pairwise relatedness (*R*) values for all pairs that were observed concurrently at a den. Relatedness values that are marked with an asterisk (*) indicate pairs where den provisioning was observed (i.e. the adult of the pair was seen carrying food at the den while the juvenile of the indicated pair was also present). Individuals that were observed alone at a den are shaded in gray along the outside edges of the matrix.

	F104	F105	F108	F110	F117 (juv)	M103	M112 (juv)	M113 (juv)	M114
F104									
F105	0.737								
F108									
F110									
F117 (juv)				- 0.264*					
M103									
M112 (juv)		0.002*							
M113 (juv)									
M114	- 0.394	- 0.336		0.059			- 0.088*	0.495*	

Table 13. Average pairwise (PW) distances (in km) are given between all possible dyads in each study population for which we estimated home range (HR) area. We present average PW distance between HR centers, average PW proportion (percent) of an individual's HR that overlapped that of the other member of the pair, and the average PW relatedness (*R*; Queller and Goodnight 1989). All measurements are given with ± SEM. Sample sizes represent number of individuals (n_i) and numbers of dyads (n_d) for all measures.

	n	Average PW pairwise distance HR centers (km)	Average % PW overlap (proportion of HR)	Average PW relatedness (*R*)
Shompole				
Total Population	$n_i = 8$ $n_d = 28$	7.37 ± 0.81	0.24 ± 0.04	- 0.07 ± 0.05
Male - Male dyads	$n_i = 2$ $n_d = 1$	9.78	0.10 ± 0.03	0.171
Male - Female dyads	$n_i = 8$ $n_d = 12$	7.50 ± 1.18	0.23 ± 0.07	0.02 ± 0.08
Female - Female dyads	$n_i = 6$ $n_d = 15$	7.11 ± 1.21	0.26 ± 0.05	0.08 ± 0.04
Laikipia				
Total Population	$n_i = 19$ $n_d = 171$	18.74 ± 0.94	0.12 ± 0.01	- 0.02 ± 0.03
Male - Male dyads	$n_i = 11$ $n_d = 55$	18.83 ± 1.66	0.12 ± 0.03	0.15 ± 0.05
Male - Female dyads	$n_i = 19$ $n_d = 88$	18.49 ± 1.36	0.14 ± 0.02	- 0.07 ± 0.03
Female - Female dyads	$n_i = 8$ $n_d = 28$	19.35 ± 2.1	0.08 ± 0.04	- 0.15 ± 0.06

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