CONSERVATION OF LEOPARDS (PANTHERA PARDUS) IN THE EASTERN AND WESTERN CAPE, SOUTH AFRICA: INVESTIGATING THE EFFECT OF LAND USE, GENE FLOW AND CONNECTIVITY

by

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Declaration

I Jeannine Stephanie McManus declare that this thesis is my own, work, and that I contributed adequately towards research findings published in the article stated below which is included in my thesis. This thesis is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

-------------------------------------- day of ---------------------------------- 2018

Disclaimer

This thesis consists of a series of chapters that have been prepared as stand-alone manuscripts, some of which were/have been submitted for publication. It is therefore possible that some unavoidable repetition may occur between chapters.

Abstract

The increasing extent of human-altered landscapes and associated human activities is projected to cause irreparable damage to biodiversity and ecosystem function by the end of the century. The conservation of species requires understanding the abilities and limitations of species persistence in modified landscapes and how this affects species population dynamics and connectivity between populations. The persistence of species in the face of altered habitat depends, in part, on the capacity of habitat patches to promote occupancy, and the ability of individuals to reach these patches and ensure fitness within them by balancing resources and threats despite the altered nature of the habitat. The distribution of animal populations across the landscape is the result of decisions individuals make in selecting and avoiding environmental characteristics over time. Carnivores interact strongly with other species and thereby have the ability to structure communities and ecosystems, often making them a focal species for conservation planning. Leopards (*Panthera pardus*) are the last free-roaming large carnivores in South Africa and, while they are considered highly adaptable to environmental changes, most leopard habitat exists outside protected areas, where they are increasingly threatened by habitat fragmentation and human-caused mortality. Their important ecological role and vulnerability to humans have raised concerns regarding the likelihood of carnivores to survive in human-altered landscapes. This thesis examines the behaviour of leopards in their environment, and how these behaviours influence leopard distribution, population structure and connectivity. This study found that conspecifics, sex-related differences and anthropogenic landscape features effect how leopards distribute themselves in the landscape, influence movement patterns, and shape their population structure. Sexes employed different strategies in selecting habitat and movement patterns, likely because of different reproductive- and conspecific avoidance strategies. Often habitat prediction modelling for solitary carnivores does not incorporate sex, and conspecifics' locations and this research found these variables are important in leopard habitat selection and movement patterns. Male and female leopards, respectively, spent 70%

and 40% of the time moving in long, straight movement patterns (inter patch behaviour). Inter patch behaviour is indicative of low resource areas, fragmented habitat, or areas with higher intraspecific avoidance and is employed to move quickly between habitat patches. Connectivity between habitat patches was reduced by human-associated features such as roads, and promoted by mountainous areas, rivers and protected areas, the latter being less affected by human-associated features. Despite the high occupancy of inter patch behaviour displayed by males, the leopard population in the region are genetically sub-structured into three subpopulations. While this population broadly conforms to a metapopulation model, gene flow between the three identified sub-populations shows low to moderate gene flow and requires management to ensure continued connectivity between these populations. These findings can contribute to improving leopard management policy at a landscape level to ensure this flagship species survives in heterogeneous environments.

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"Discovering truth by building on previous discoveries"

"nanos gigantum humeris insidentes" – "Standing on the shoulders of giants"

Bernard of Chartres 12th Century

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General Introduction

Animal ecologists study habitat use and movement because of the theory that individual fitness is strongly affected by the locations animals choose to eat, rest, mate, and travel (Boyce and McDonald, 1999; Morrison et al., 2007; Nathan et al., 2008). For example, in Norway, Eurasian lynx (*Lynx lynx*) prey, roe deer (*Capreolus capreolus*), utilise areas close to human activity to avoid predation by lynx (Bunnefeld et al., 2006). When the lynx prey on roe deer in these areas as opposed to areas further from human activity, they suffer high mortality risk due to human activities. In Russia, when female Amur tigers (*Panthera tigris altaica*) selected den sites away from roads and human activities, higher reproductive success was observed compared to females with den sites close to human activities (Kerley et al., 2002). Therefore, the locations used by individuals affect fitness because the landscapes an animal uses host different costs and benefits for that animal. Understanding these animal–habitat relationships is increasingly important, as natural landscapes rapidly alter as a result of human interventions.

Habitat selection

The persistence of an individual within a given habitat is considered to improve in high-quality habitat, which promotes reproductive success and survival, while low-quality habitat can still promote occupancy, but may reduce fitness, as these areas are associated with higher mortality risks or low reproductive success. This forms the basis of the source–sink model (Pulliam and Danielson, 1991), which is a mechanism contributing to population ecology and the landscape ecology framework (Wiens et al., 1993). Animals may select lower quality habitat, which can increase their survival, but may result in low reproductive success and survival due to the suboptimal habitat quality (Stoner et al., 2013). Alternatively, ecological traps attract individuals into areas where habitat quality is perceived to be high, but mortality risk reduces survival, usually as a result of human activity (Battin, 2004; Schlaepfer et al., 2002). When environmental factors fragment continuous habitat into a matrix of sink and source habitat patches, the fitness of individuals can be strongly affected (Battin, 2004). Conservation biologists are interested in how individuals respond to these variations, because the fitness of individuals has implications on the overall persistence of populations of species.

Sex-related differences in habitat selection

An important consideration often overlooked in habitat studies is sex-related differences. When sexes are known to interact differently within the same environment (for example, spatial requirements differ), excluding sex from models can lead to inappropriate management actions and policy. Sexual selection theory suggests that males utilise more risky habitat than females due to spatial requirements and dispersal behaviour (Trivers, 1976). In polygamous, territorial felids, males generally locate themselves according to mates, while females tend to select areas with high resources to promote successful offspring reaching adulthood, and females are often more averse to human-associated features compared to males (Colchero et al., 2011; Conde et al., 2010). If biologists use data attributed primarily to one sex the results may not represent the true situation for the species, and this may result in inappropriate management policies.

Spatial scale

Where animals locate themselves also varies at different spatial and temporal scales, and habitat selection is defined as a hierarchical process (Johnson, 1980). Scales of selection rely on each other, as large-scale distribution patterns result from individual behaviours at the fine scale, while fine-scale behaviours are constrained by large-scale distribution of the populations (Rettie and Messier, 2000). Johnson (1980) describes four spatial scales of habitat selection: the distribution of the species, the location of their home ranges, habitat used within a home range, and, at the finest scale, food items within foraging patches. Therefore, as spatial scales expand, resource selection by individuals becomes multivariate rather than a discrete process. Determining how individuals relate to the same resources across these scales contributes to understanding species ontology at different scales and is important to define in habitat selection studies. At the large scale habitat selection is considered to relate more strongly to evolutionary pressures such as reproductive success, while behaviour choices at finer scales are less affected by evolutionary pressures, such as feeding or hunting closer to dangerous environments (Rettie and Messier, 2000; Wilmers et al., 2013). For example, puma (*Puma concolor*) displayed reproductive behaviours further away from human development than other behaviours such as feeding (Wilmers et al., 2013).

The distribution of animals across the landscape and the resilience of populations to environmental change are the result of decision processes individuals undertake in selecting and avoiding environmental characteristics over time (Turchin, 1991, 1998). Untangling how animals behave in relation to specific environmental cues can give clues about the underlying causes of these behaviours, illuminating the ability of species persistence under specific conditions (Morrison, 2001).

Social implications in habitat selection

While the distribution of species can be described according to key resources, conspecifics have a strong influence on habitat selection by individuals (Elbroch et al., 2015). For example, a dominant individual can interfere with a subordinate's habitat selection, forcing it to occupy low-quality habitat (resource dispersion hypothesis) (Geffen et al., 1992), or with the result that available resources are accessible at different times (land tenure hypothesis) (Hornocker, 1969). Alternatively, conspecifics may choose to be close to others for benefits such as hunting success (Creel and Creel, 1995), to improve the survival of kin by selecting locations close to relatives (Hamilton, 1964) or, as the 'public information' hypothesis suggests, to learn from others where to find resources (Parejo et al., 2005). Although many hypotheses about the costs and benefits of associations exist, biologists agree that one must measure the patterns of relationships between conspecifics and this includes the positioning of individuals within populations and the fitness consequences of different locations within a landscape (Morris, 2003; Pulliam and Danielson, 1991).

Population dynamics and animal movement

Empirical studies have revealed how ecological processes are affected when continuous habitats are altered into isolated remnants (Terborgh, 1992; Tilman, 1994). Island biogeography theory postulates that the smaller and more distant a habitat patch is from a mainland, the fewer species it can support (Wilson and MacArthur, 1967). Similarly, in metapopulation theory, where there are disjoint habitat patches, species will rely on the patches being accessible to allow within-population dynamics (e.g. competition, predator–prey interactions) and among-population movement (e.g. dispersal, migration), which influence species survival (Hanski, 1998; Hess and Fischer, 2001; Levins, 1968). These principles thus rely strongly on animal movement within and between habitat patches.

Our understanding of movement is limited by the complexities of the internal state of the animal (Nathan et al., 2008). For example, individuals can modify their movement patterns within one habitat patch depending on time spent moving, and or the physiological state of the animal, making it difficult to isolate the mechanism causing a specific movement pattern (Morales and Ellner, 2002). Our understanding of why and how animals move has been improved by categorising animal movement patterns and linking them to the physical environment and conspecifics (Firle et al., 1998; Morales and Ellner, 2002; Nathan et al., 2008; Revilla and Wiegand, 2008). For example, convoluted path patterns are observed during exploration of a new area or in low-risk habitat where resources are easily available, which, when considered at larger scales, promote occupancy of that patch (Fahrig, 2007; Patterson et al., 2009). Fast, straight paths are effective to locate resources that are patchily distributed, or when mortality risk is higher, to improve resource search efforts and reduce time spent in high-risk habitats (Hopcraft et al., 2014; Morales and Ellner, 2002; Revilla et al., 2004). These fast-straight movements are associated with inter-patch behaviour (Hopcraft et al., 2014; Revilla and Wiegand, 2008). In fragmented or resource-poor landscapes, inter-patch behaviour usually increases, which facilitates population dynamics, thus allowing local populations to connect to one another (Fahrig, 2007; Firle et al., 1998). However, since these movements are associated with high energy costs, in low-quality habitat, as the distance between habitat patches increase, animals may be unable to cope with increased patch isolation, thereby resulting in isolation and decreased fitness (Fahrig, 2007). Inter-patch movement dynamics is therefore integral for population viability over time, and identifying where and why inter-patch behaviour patterns occur in heterogeneous landscapes has improved management policies of large carnivores in fragmented habitats (Revilla et al., 2004; Sweanor et al., 2000). Investigating the movement patterns that animals display in heterogeneous environments provides information on the way landscapes are perceived and what drives connectivity between populations (Firle et al., 1998).

If individual movement between populations is impeded, populations become isolated, which leads to inbreeding, genetic loss and high risk of extinction. Disjunct populations may not recover from such impacts, thus threatening species persistence (Epps et al., 2005; Robertson and Hutto, 2006; Sweanor et al., 2000). Sub-structuring of once continuous populations into smaller fragments and reduced gene flow have negative consequences on genetic variation. These degradations can occur rapidly, particularly in fast-changing landscapes, increased pollution and global climate change (Hoffmann and Willi, 2008). Identifying population structures and ensuring that sufficient gene flow between populations occurs, is therefore an important management effort, as this will ensure the adaptive potential of populations, particularly for species subject to changing and deteriorating environmental conditions.

If individual decisions can eventually, in some conditions, result in the persistence or extirpation of populations, then conservation biologists may wish to understand individual choices and how to intervene to prevent sinks and protect individual fitness. This is required to reduce the impact humans have on biodiversity.

Why carnivores?

The role of predation in an ecosystem is of major importance, as growing evidence points to increased carnivore absence having cascading effects on lower trophic levels, making carnivores ecosystem engineers (Estes et al., 2011; Ripple et al., 2014). Thus, top predators influence important ecological consequences, such as the regulation of prey numbers, population control of mesopredators through competition (Elmhagen and Rushton, 2007), maintaining biodiversity in a local community (Krebs et al., 1995; Logan and Sweanor, 2001; Terborgh et al., 1999), and even buffering the effects of global climate change (Sala, 2006; Wilmers and Getz, 2005). Intrinsic traits such as low population density, low fecundity, limited ability to disperse through modified landscapes, leave carnivores vulnerable to changing landscapes (Carroll et al., 2001). Additionally, high mobility and large spatial requirements increase the interface between carnivores and anthropogenic activity and landscapes, which has resulted in population declines and in some cases extinctions (Ripple et al., 2014; Treves and Bruskotter, 2014; Woodroffe and Ginsberg, 1998). The ecological importance and vulnerability carnivores face in changing landscapes makes them a focal species for conservation planning (Carroll et al., 2001; Ray et al., 2005).

Leopards (*Panthera pardus*), like other carnivore species, are considered critically endangered in some parts of their range and, conversely, pests in others (Sunquist and Sunquist, 2002). In much of their range, leopards are the last free-ranging large carnivores (Sunquist and Sunquist 2002), as is the case in South Africa. Although leopards are highly adaptable, they have been eliminated from many parts of their former range. Loss of habitat, human-caused mortality, and isolation of small populations are major concerns in the conservation of large carnivores (Clark et al., 1996; Singleton and Lehmkuhl, 2001). The impact environmental changes have on large carnivore populations has been demonstrated for leopards elsewhere: the loss of habitat and connectivity between populations, compounded by persecution of leopards by humans, and poor management of trophy hunting has caused population numbers to decline markedly within many parts of their range (Balme et al., 2009; Hunter, 1999; Rabinowitz and Winter, 2006; Swanepoel et al., 2015), resulting in increasingly fragmented and isolated leopard populations (Uphyrkina et al., 2001). These threats have resulted in a downward conservation status trend for leopards: over the last decade they have been re-categorised from least concern to near threatened in 2008 and are currently considered vulnerable on the IUCN red lists (Stein, 2016). While some wildlife species appear to be able to withstand the pressures of habitat loss and changing land use relatively well (Anderson, 1997; Nee and May, 1992; Purvis et al., 2001), large carnivores such as leopards are susceptible to such environmental changes (Belovsky, 1987; Gittleman et al., 2001; Woodroffe, 2000).

The conservation of top predators has operated primarily within the boundaries of existing protected areas. Relying solely upon this approach has several limitations, as only about 5% of the world's land area is formally protected (CIA, 2003; Gittleman et al., 2001; Mills et al., 2001), so parks and reserves are unlikely to be large enough to successfully contain viable populations of large carnivores (Marker, 2002; Woodroffe, 2001; Woodroffe et al., 1997). In Africa, an estimated 8–13% of the potential habitat is within national protected areas (Boitani et al., 1999) and in South Africa only 12% of protected areas are considered suitable for leopard (Dudley, 2008; Swanepoel et al., 2013). This makes privately owned land essential for leopard conservation. Human–carnivore conflict is a major contribution to leopard mortality, and finding effective, practical ways of reducing livestock losses is considered an important tool for leopard conservation. This suggests that privately owned land is essential for leopard conservation. Human-carnivore conflict is a major contribution to leopard mortality, and finding effective, practical ways of reducing livestock losses is considered an important tool for leopard conservation. Leopard density in the Eastern and Western Cape of South Africa area are some of the lowest reported for South Africa, with an average of 0.95

leopards / $100km^2$, ranging from 0.24 – 1.89 leopards / $100km^2$ for the Baviaanskloof and Langeberg respectively (Devens et al., 2018, Mann et al., 2014; Martins et al., 2010). These differences may be a result of modified landscapes, higher mortality risk, and depleted prey availability (Devens et al., 2017; Mann et al., 2014).

Where threats such as human–carnivore conflict are present, habitat is increasingly fragmented and altered by anthropogenic development, raising questions about the leopard's ability to survive. Understanding how animals locate themselves in the landscape, change movement patterns, and defining the population structure are therefore useful to contribute toward our understanding of leopard ecology, and to improve management of populations by predicting how they will respond to increased habitat fragmentation in the future (Cushman et al., 2013; Zeller et al., 2012).

This thesis focuses on understanding how individual decisions influence population dynamics, particularly in fragmented habitat to better understand ability and limitations of species, using leopard as the focal species. I focus on the relationship between leopards and their habitat in fragmented landscapes, and how this influences leopard population ecology at different spatial scales. My objectives were to determine how and why leopards distribute themselves and employ different movement patterns in landscapes in relation to sex, conspecifics and heterogeneous landscapes and understand how this influences connectivity over large spatial scales; and determine the genetic structure of leopards based on DNA, to establish if leopards are one continuous population or act as a metapopulation. In chapter 2, I examine how conspecifics and the physical environment relate to leopard distribution at different spatial scales. In chapter 3, I use microsatellites to determine the population structure of leopards and use the genetic variability and genetic distance to establish if leopard populations are functioning according to a metapopulation model. In chapter 4, I use changes in leopard movement behaviour to reveal relationships between individuals and inter-patch behaviour to gain insight into what drives connectivity among populations.

Study area

The study took place in the Eastern and Western Cape provinces, South Africa (33° 11'–33° 23' S and 25° 53'–18° 53' E; Figure 1.1), extending approximately 1000 km along the southern part of South Africa. Topography varies from coastal zones and low-lying valleys from sea level, to mountain peaks >1600 m along the Cape Fold Mountain range, which extends approximately 800 km between Port Elizabeth in the east and Cape Town in the west (Thamm and Johnson, 2006)..

The main geological components of the study area include quartzite, sandstones and shale of the Table Mountain, Bokkeveld and Witteberg Groups of the Cape Supergroup Thamm and Johnson, 2006). The more arid northern regions are comprised of glacial Dwyka Group tillites. These tillite deposits, form the Dwyka Group rocks of the Karroo Supergroup are overlain with Ecca and Beaufort Group rocks (Thamm and Johnson, 2006).

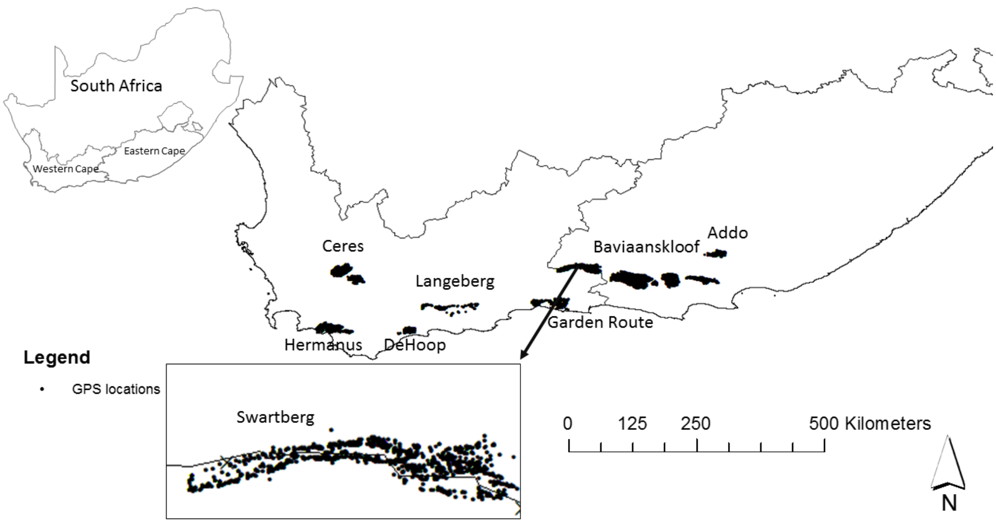

Figure 1.1. Study area of the Eastern and Western Cape, with the distribution of GPS data from sampled leopards used in this study. The magnified area provides an example of the GPS data used in this study.

Land-use zones in the area include irrigation-based agriculture (fruit, vineyards and grain crops), and pastoralists focus on livestock production (sheep, goats and cattle). Forestry and formal and informal protected areas are also present (Boshoff et al., 2000). The region was selected because there is a dearth of information regarding top predator population dynamics, and how apex predators survive in these heterogeneous and increasingly human-altered habitats is poorly understood.

Vegetation

All major biomes of South Africa, except true deserts are present in the Eastern and Western Cape. Rainfall in the region is year-round and does not exhibit a specific wet and dry seasonality. The northern reaches of the study area fall within the Succulent and Nama-Karoo biomes, characterised by low shrubs, with succulent plants present in the Succulent Karoo, and shrubs intermixed with grasses in the Nama Karoo. These biomes are characterised by high plant diversity (Mucina and Rutherford, 2006). Rainfall in these semi-arid environments averages approximately 290 mm per year. In contrast, the highest average annual rainfall in the study area occurs in the Afrotemperate forests, falling year-round, averaging approximately 950 mm per year (Mucina and Rutherford ,2006). Forests are characterised by slow-growing, tall Yellowwood species (*Afrocarpus* sp), Forest elder (*Nuxia floribunda*), and Stinkwood (*Ocotea bullata*). Other biomes present in the study area include: Thicket, which is a relatively dense, woody vegetation with an average height of about 2 to 3 meters, dominated by Spekboom (*Portulacaria afra*) and *Searsia* sp.; Sandstone fynbos, is prolific in higher altitude zones on the Cape Fold Mountain range, and commonly includes *Protea* sp. and *Erica* sp. Finally; Savanna elements are found in parts of the region, particularly in lower rainfall areas along riverine zones, which are dominated by *Vachellia karroo* trees and a herbaceous ground layer (Mucina and Rutherford, 2006).

Leopard data

Data from the sampled leopards used in this study form part of a broader leopard conservation project initiated in 2004 in the Eastern and Western Cape, South Africa. Since 2006 – 2015 27, leopards (15 males and 12 females) have been fitted with Global Positioning System (GPS) collars (Table 1.1). GPS data was set to store GPS locations between 4 and 6-hour intervals (Table 1.1). Data used for each chapter varied because of GPS storage times differing between collars and these are explained in each chapter.

Leopards were captured with walk-in, fall-door cages (2 m long, 0.7m wide, 0.8 m high) designed by the Western Cape governmental conservation body, Cape Nature (Figure 1.2). The placements of traps were based on the presence of leopard activity such as tracks, prey kill sites and scrapes. Once a leopard was captured, a veterinarian immobilised the animal, using a drug combination of Zoletol-Medetomidine at a standard dosage (1–2 mg/kg). While sedated, morphological, and DNA (tissue) samples were collected and animals were fitted with a GPS or satellite collar (Vectronic-aerospace, Berlin, Germany, or Animal Wildlife Tracking, South Africa). Data were downloaded remotely, using an ultra-high frequency receiver (UHF), or by satellite transmission. This research was in accordance with Witwatersrand University ethics authorisation (2011/05/04) and provincial research permits as issued by the Eastern Cape Department: Economic development, environmental affairs and tourism (24503), and Western Cape: Cape Nature (0035-AAA004-00797).

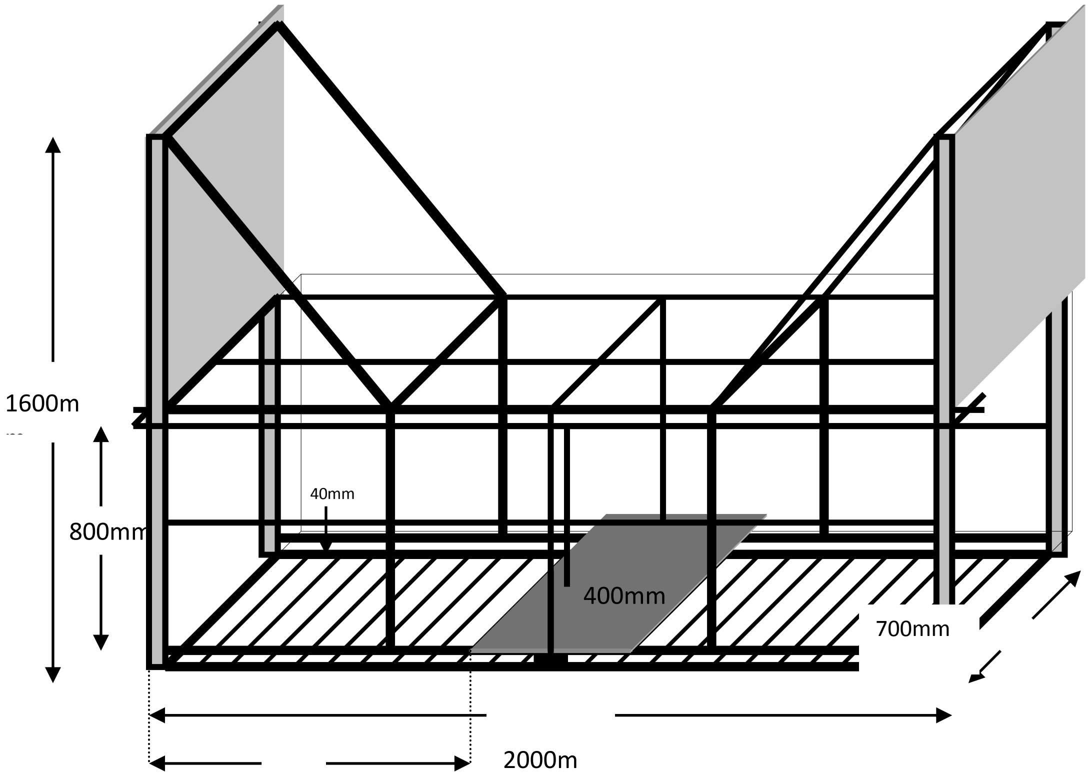

Figure 1.2. Design of the walk-in fall door traps used to capture leopards (Cape Nature, Western Cape, South Africa).

General methods

This section introduces the general methods used in each chapter. The thesis is presented as a series of chapters each as a scientific paper. Therefore, each chapter has a methods section where specific methods are discussed in detail.

In chapter two I used resource selection functions (RSF; Boyce, 2006) with generalised linear mixed modelling (GLMM) to test a) if leopard behaviour changes in anthropogenic landscapes as a result of spatial scales (i.e. large scale resource selection compared to a finer scale); b) if sex-related differences result in each sex employing different behaviours in anthropogenic landscapes at the two special scales and; c) if social interactions play a role in where individuals place themselves in the landscape. I compared the used GPS data points from male and female leopards and compared these to available random locations at two spatial scales (Boyce, 2006) to determine the relationship environmental factors had on leopard presence.

In chapter three I examined 14 microsatellites from 40 DNA samples to determine the genetic population structure of leopards in the Eastern and Western Cape to understand if the leopard population in the region is continuous, with high levels of genetic heterozygosity as has been found elsewhere in African leopard populations (Miththapala et al., 1996; Spong et al., 2000; Uphyrkina et al., 2001). I used Bayesian spatially explicit methods within a pacakge called GENELAND (Blair et al., 2012) to determine the population structure of leopards based on genetic distance, gene-flow and heterozygosity of sampled leopards in the region.

In chapter four I determine what causes leopards to use habitat as a corridor. Using a state–space statistical approach (Carlin et al., 1992), I first identified three movement patterns displayed by leopards by categorising step lengths and turning angles of leopards to describe the behavioural state of the animal (see Hopcraft et al., 2014). The behavioural states were described as 1) encamped behaviour (short steps with high turning angles), 2) search behaviour (long steps with high turning angles), and 3) inter patch behaviour (long steps with low turning angles). Each behavioural state was then modelled as a response to landscape features and other male and female leopard locations. I predicted that fast, straight movement patterns (inter patch behaviour) are associated with areas which allow animals to quickly move through but do not generally offer resources needed by the animals to remain in the area for prolonged periods. These features were therefore considered to characterise corridors based on leopard movement patterns.

All analyses were run in R 2.13.2 (R Development Core Team, 2014).

Table 1.1. Sampled leopards with Geographic positioning system (GPS) data collected from male and female leopards in the Eastern and Western Cape, South Africa. * indicates collars with 6-hour data collection intervals and the remaining collars stored data at 4-hour intervals.

Location	Province	Collar number	Period tracked	Sex	Data points
Baviaanskloof	Eastern Cape	1015*	Jan. 2015 - Feb. 2016	M	230
Addo	Eastern Cape	2996	Dec. 2006 - Dec. 2007	M	1875
Cockscomb	Eastern Cape	2997	Jun. 2007 - May 2008	M	2504
Cockscomb	Eastern Cape	2997b	Sep. 2009 - Jul. 2010	M	1554
Swartberg	Western Cape	3805*	Sep. 2012 - Oct 2014	M	1133
Baviaanskloof	Eastern Cape	3809*	Jan. 2008 - Apr. 2009	M	1828
Baviaanskloof	Eastern Cape	6776*	Apr. 2009 - Sep. 2010	M	2222
Baviaanskloof	Eastern Cape	6667*	Jan. 2009 - Jul. 2010	M	2457
Garden Route	Western Cape	6666*	Mar. 2009- Jun. 2010	M	1368
Langeberg	Western Cape	8182*	Jun. 2010 - Mar. 2011	M	1053
Ceres	Western Cape	8578*	Jun. 2011 - Aug. 2012	M	1384
Swartberg	Western Cape	8578b*	Dec. 2012 - Nov 2015	M	2777
Ceres	Western Cape	8677*	Nov. 2012 - Jun. 2014	M	376
Hermanus	Western Cape	9536*	Jun. 2011 - Sep. 2011	M	377
Hermanus	Western Cape	9648*	Feb. 2012 - Apr. 2012	M	472
Addo	Eastern Cape	1038	Nov. 2007 - Feb. 2008	F	142
Baviaanskloof	Eastern Cape	1412	Jun. 2014 - Jan. 2016	F	2439
Baviaanskloof	Eastern Cape	3704	Sep. 2007 - Jul. 2008	F	2388
Baviaanskloof	Eastern Cape	3710	Aug. 2007 - Dec. 2007	F	649
Garden Route	Western Cape	3809*	Sep. 2009 - Jun. 2010	F	997
Cockscomb	Eastern Cape	3805*	Jan. 2009 -Aug 2009	F	1126
Baviaanskloof	Eastern Cape	6775*	Apr. 2009 - Aug 2009	F	457
Cockscomb	Eastern Cape	6777*	Jul. 2009 - Sep. 2009	F	2013
Hermanus	Western Cape	8182	Sep. 2013 - Dec. 2013	F	571
Baviaanskloof	Eastern Cape	8183	Jun. 2010 - Jul. 2011	F	876
De Hoop	Western Cape	8294*	Mar. 2011 - Jan. 2012	F	1363
Rooihoek	Eastern Cape	8642*	Jan. 2011 - Mar. 2012	F	1384

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Factors affecting habitat use by leopards in human-altered landscapes

Abstract

Because species persistence depends in part on behavioural responses that determine habitat selection, understanding these responses is central to species conservation. Human activity rapidly alters landscapes, leaving remnant patches of natural habitat for wildlife. The persistence of species in the face of altered habitat depends on individual adaptability to novel habitats, and individual ability to secure reproductive opportunities and survive despite the alterations of habitats. Surviving without reproducing is as unsustainable as reproducing well then dying, so individual animals face an optimization challenge in novel habitats. Likewise, conservationists may find it challenging to promote population persistence in the face of rapidly changing habitats. Large carnivores influence ecosystem processes due to their high trophic status and are often used as focal species in conservation planning. I investigated habitat selection of the last free-roaming large carnivore (leopard, *Panthera pardus*) in the Eastern and Western Cape, South Africa to determine key correlates of habitat selection of leopards within human-altered landscapes at two spatial scales. I tested whether sex-related differences, and conspecific interactions predicted habitat selection at the different spatial scales. I predicted that large-scale selection should be more strongly associated with evolutionary pressures related to reproductive success and survival and should therefore be negatively associated with human-altered landscapes than at finer scales, where I predicted conspecific locations would predict male and female locations more strongly. Conspecific locations had an effect at both spatial scales for males, while for females, conspecifics explained fine-scale habitat selection. Distance to protected areas explained large-scale home range selection in both sexes. Human-associated landscapes correlate with habitat selection at both scales for males, and only at the large scale for females. Thus, spatial scale, sex-related differences and conspecific location play roles in habitat selection for solitary felids and should to be incorporated in habitat studies. Our model provides insight into the limitations on leopards

ranging in altered habitat. Managers might use these findings to improve habitat predictive mapping and to improve identification of corridors.

Key words

Anthropogenic threats, carnivore conservation, habitat selection, leopard (*Panthera pardus*).

Introduction

Habitat loss and fragmentation leave remnant patches of habitat for wildlife that has evolved in those habitats for millennia; those individuals that can respond to altered habitat without loss of reproductive or survival potential, may persist over generations. Understanding the causal drivers of species distribution is a central question in ecology, and particularly relevant is understanding how modified landscapes influence species distribution and persistence. The persistence of species in the face of altered habitat depends in part on the capacity of those habitats to promote occupancy, and the ability of individuals to ensure fitness by balancing resource utilisation and threats despite the altered nature of remnant patches (Hebblewhite and Merrill, 2008; Mysterud and Ims, 1998). Habitat selection by wildlife is thus the result of behavioural responses that individuals display when encountering various environmental cues, which ultimately determines the distribution of populations and species (Boyce and McDonald, 1999). Understanding the relationship between wildlife and habitat can reveal the causes of these behavioural responses, providing insight into the ability of species to persist in areas. Thus, treating environmental factors as conditions that make up the habitat of species can be considered as an investigation of an animal's niche, closing the gap in understanding the causal link between distribution and factors that influence survival (Marshal et al., 2006; Morrison, 2001). This suggests that habitat selection by animals should be based on landscape features that increase their reproductive success and reduce the risk of mortality (i.e. source habitat) (Pulliam and Danielson, 1991). These selections take place in a hierarchical process that may not be consistent over various spatial scales (Boyce, 2006; Johnson, 1980).

Evolutionary theory predicts that the selection of a specific behavioural response correlates with the fitness consequences of that response (Stephens and Krebs, 1986). Therefore, when animals select resources at large scales (such as during home range selection), these behaviours should be based strongly on behaviours that increase reproductive success, while at finer scales, should be related to behaviours that have undergone fewer pressures from evolutionary selection (e.g. hunting times driven by hunger or kill site selection) (Wilmers et al., 2013). Thus, in altered landscapes, constraints that influence habitat selection should be avoided more strongly at larger scales than those same constraints would at finer scales (Bunnefeld et al., 2006; Wilmers et al., 2013). Understanding large spatial scales thus gives insight into how animals locate themselves and may provide a more direct understanding on the effect individual selection has on population structure and dynamics.

While the distribution of species is primarily explained by the availability of key resources, social components can have a strong influence on habitat selection (Elbroch et al., 2015). Intraspecific associations, such as conspecific attraction or avoidance, influence carnivore distribution and the individuals within populations (Elbroch et al., 2015). The presence of competitors for a limited resource may result in decreased individual fitness in a specific habitat patch due to increased interference competition or, more extremely, by exploitative competition. Alternatively, increased interaction with conspecifics may have a positive influence on fitness, for example safety in numbers, which increases group vigilance against threats, or increases hunting success (Creel and Creel, 1995). Therefore, there are contradictory hypotheses relating to conspecific interactions and habitat selection. For example, the resource dispersion hypothesis predicts that dominant individuals will force subdominants to occupy less favourable habitat; the kinship hypothesis predicts that relatives should aggregate closer together within a population; the land tenure hypothesis predicts that conspecifics should avoid one another by space; and the public information hypothesis suggests that individuals use conspecifics to identify high-quality habitat effectively creating a 'copy-cat' hypothesis (Elbroch et al., 2015; Ferreras et al., 2004; Parejo et al., 2004; Seidensticker et al., 1973). The spatial partitioning of individuals may also be affected by human development, thereby influencing the severity of competition, as modified areas may act as poor habitat, increasing competition between individuals further away from these environments. Therefore, the hierarchical relationships among individuals influence species presence and are at the core of source–sink population dynamics. The location of conspecifics should therefore have a direct influence on habitat selection and are lacking in habitat selection studies, particularly in relation to anthropogenic influences on such social interactions (but see Wilmers et al., 2013).

For many large carnivores, resources and constraints cue various behaviours between sexes, often with males using altered habitat more than females (Broomhall et al., 2004; Conde et al., 2010; Gaines et al., 2005; Mace et al., 1996). Female carnivores are known to select habitat that improves raising offspring to adulthood, i.e. they usually select habitat with increased availability of key resources, while males locate themselves based on female availability (Schmidt et al., 1997). Understanding how individuals select and use habitat at different spatial scales, considering conspecific interactions and sex-related behavioural differences in habitat selection, provides insight into environmental and social implications that affect the biology of species (Boyce et al., 2002; Hirzel and Le Lay, 2008).

Carnivores interact strongly with other species such as prey, and smaller and larger competitors and thereby have the ability to structure communities and ecosystems (Carroll et al., 2001; Estes et al., 2011; Ripple et al., 2014). Leopards (*Panthera pardus*) are highly adaptable, solitary felids and are the last free-roaming, large top predator in the Eastern and Western Cape, South Africa (Skead et al., 2007). Much of their range exists outside of protected areas in the region. This makes the species vulnerable because as the interface between humans and carnivores increases, so does habitat modification and the risk of human-caused mortality (Ripple et al., 2014; Treves and Bruskotter, 2014; Woodroffe and Ginsberg, 1998).

The important ecological role of leopards and their vulnerability to humans raises questions regarding their ability to persist in human-altered landscapes making them a useful focal species for conservation planning, particularly in altered landscapes. In an effort to develop a better understanding of habitat use by leopards, my objective was to identify key factors associated with leopard distribution in the Eastern and Western Cape, South Africa, and produce a predictive habitat distribution map for this area to contribute to leopard conservation management needs. Specifically, I test 1) if leopards avoid anthropogenic landscape features more strongly at large-scale habitat selection than fine scale; 2) if sex-related differences result in each sex employing different behaviours in anthropogenic landscapes at the two special scales and; c) if social interactions play a role in where individuals place themselves in the landscape. In this regard, more specifically, I expect that female locations are not affected by male locations, but that competition may be higher between other females and therefore expect avoidance between females (land tenure hypothesis). Likewise, males should avoid males, while they should strongly select for females at both spatial scales.

Methods

Leopard data

Data collected from 24 sampled leopards (14 males and 10 females) in the Eastern and Western Cape were used in the analyses. A total of 19 052 and 15 682 GPS locations were obtained from male and female leopards respectively (Table 2.2).

Habitat covariates

I selected covariates based on environmental factors known or suspected to influence leopard behaviour (Bailey, 1993; Bothma et al., 1997; Gavashelishvili and Lukarevskiy, 2008; Swanepoel et al., 2013).

Explanatory variables used were distance to rivers, distance to roads, distance to farms, distance to formally protected areas, human population density, distance to towns, livestock density, and topographic features such as elevation and slope (Swanepoel et al., 2013). Additionally, distance to male and distance to female leopards were used to assess conspecific interaction calculated as distance from the centre of each leopard's home range. While prey distributions and abundance commonly influence carnivore distributions (Creel et al., 2001; Karanth et al., 2004), unfortunately these data are not available for the study area. Livestock can constitute some part of leopards' diets, and as livestock have been found to be a proxy for leopard habitat fragmentation (Swanepoel et al., 2013), I used livestock densities as a variable to predict leopard distributions. Protected areas in the study area do not contain livestock. Slope and elevation were derived from a digital elevation model (DEM) originally from Shuttle Radar Topography Mission. Distances to roads wider than 6 metres, distances to perennial and non-perennial rivers, and distances to conspecifics home ranges (males–males, females–females, and males–females), protected areas, farms and cities were calculated as Euclidian distances (Spatial analyst in ArcGIS 10.1, ESRI, Redlands, CA, USA). Sources of digital data are in Table 2.1.

Resource selection and scale

I estimated habitat selection at two spatial scales, first within home ranges (the third-order selection; Johnson, 1980) and second between home ranges (second-order selection; Johnson, 1980). Leopard home range sizes were estimated using minimum convex polygons (MCP) covering 100% data points for each individual leopard using Home Range Tools (Rodgers et al., 2007). For analyses between home ranges I created a 100% minimum convex polygon which included all leopards' home ranges and created random points at a sampling intensity of 10 random points to one observed point in this 'super home range' to assess available habitat for leopards (Figure 2.1). Similarly, for analyses within home ranges, I created random points within home range boundaries at a sampling intensity of 10 random points to one observed point and compared available and used locations to determine resource selection (Manly et al., 2002). This modelling framework uses a quantitative approach that estimates habitat selection of an individual by comparing used sites to a random selection of the available landscape features (Manly et al., 2002). However, presence data are only a subset of used locations because unmarked leopards might use undocumented locations and even for marked leopards, the locations between fixes remain undocumented. Therefore the 10:1 ratio also accommodates a low level of false negatives (pseudo-absence). The assumption is that most of the random locations are true absences that will far outweigh the occasional rare false absence. I used the "discrete-choice" resource selection design (see Boyce, 2006) by surrounding the used resource units with a buffer of 1 km (average distance moved by leopards over 6 hours; Figure 2.2) in order to ensure random points represented what was available to the animals (Boyce, 2006). I clipped habitat covariates to each GPS point of used and available random points, using ArcGIS 10.1 software (ESRI, Redlands, CA, USA). Pearson's correlation coefficient ($r > 0.6$) was used to identify multi-collinearity among explanatory variables (Olea et al., 2010). Finally, inclusion of the individual identity of leopards in models allowed me to examine if population-level models were more informative than those excluding individual leopard identity (Gillies et al., 2006; Koper and Manseau, 2009).

Analyses

I reserved half the dataset for model construction and half for model validation. I used generalised linear mixed models (GLMM's), using the probit link to evaluate correlations between landscape variables, sex to observed leopard locations using package lme4 (Bates et al., 2014) in R 2.13.2 (R Development Core Team, 2014). I used individual leopard identity as random effects in the models. With large sample sizes (>10,000 points), over-fitting models is a common risk in Resource Selection Function (RSF) procedures (Alexander et al., 2006; Garamszegi, 2011; Venette et al., 2010). Wald statistic and *P* values alone would lead to inclusion of spurious variables that may not be informative in describing leopard distribution. Given that the covariates used in large study areas only provide insights into correlates of the true situation on the ground, and given that leopards are known to occur in a wide range of environmental conditions, present across three continents (Sunquist and Sunquist, 2002), I wanted to include only covariates with very strong correlation to leopard distribution and high predictive strength as judged by use of the model on the reserved half of the data (Fernández et al., 2006; Treves et al., 2011). Therefore, I used three criteria to screen predictors before inclusion to multivariate models. I used the Receiver Operating Characteristic (ROC) in package pROC (Robin et al., 2011) and Bayesian Information Criterion (BIC) (Conde et al., 2010; Kaartinen et al., 2009; Treves et al., 2011) for model selection. ROC is distinct from *P* values, because it combines measures of sensitivity and specificity (false positives and false negatives respectively) to reveal how well the models discriminated between used and random points. I added a new predictor in a forward stepwise addition if the ROC value increased by 1% and if the following screening steps also indicated the utility of a new predictor. I also used BIC that relates –log likelihood scores to the number of variables, as I was seeking parsimonious but informative predictors. Any BIC change of 2 or more justified inclusion of a new predictor in the multivariate model. Finally, I examined the standard error (SE) of the slope estimator to screen out predictors whose slopes changed direction (crossed zero) when taking into account the SE. I used these strict criteria to screen predictors in initial, univariate GLMMs before combining them and their interactions in multivariate GLMMs (Alexander et al., 2006; Garamszegi, 2011; Tensen et al., 2011; Venette et al., 2010). Each method has biases, but complementary biases that justify their use in combination with screen predictors; by using them together I avoided over-fitting, and none of the biases compromise comparisons between models (Serrouya et al., 2011). Furthermore, the addition of each new predictor led me to examine its interaction with the strongest initial predictor and interaction terms had to meet the same stringent criteria above. Finally, to ensure model integrity, an individual predictor or its interaction with *a prior* predictor would only pass the final screening step if it was also informative with the reserved half of the data.

To present probable leopard occurrence visually for the Eastern and Western Cape region, I assigned the correlation coefficient values from the best RSF models for both male and female leopards to the landscape variables estimated by using the generalised linear mixed model (GLMM) coefficients best explaining the large scale habitat selection analyses (Figure 2.4) in ArcGIS 10.1. By providing the values of each variable these were layered and the total value of the pixel included all the variables used in the best models for male and female leopards (Nielsen et al., 2003).

Results

Resource Selection Functions

Model selection

Univariate analyses

I first tested all predictors individually to assess which had the strongest correlation to leopard locations in order to get closer to understanding what may drive leopard distribution in the region (Supplementary table 1). The relative probability of occurrence of both male and female leopards was strongly correlated with short distances to females (Supplementary table 1). At the large scale analyses, distances close to protected areas showed the strongest correlation to leopard presence for both sexes (Supplementary table 1). I constructed and validated the two models for each sex separately to test the effect of sex on resource selection.

Multivariate analyses

Variables did not show strong correlation to one another and could be included in models simultaneously. At the large scale analyses, the location of male leopards was positively correlated by the relationship between distance to females and protected areas, and low human population density (Table 2.3; Figure 2.3.1). The addition of distance to roads improved the

ROC and BIC indicating a positive association with short distance to roads (Table 2.3). For females, close distance to protected areas and close distances to roads appeared to best explain female leopard presence at the course scale analyses (Table 2.3; Figure 2.3.2). When testing the relationship between distance to roads and distance to protected areas for females, I found that the probability of being near a road increases as the road is closer to protected areas (Table 2.3). No other landscape features improved ROC or BIC with the requisite amounts to warrant inclusion. The best multivariate models for the fine scale analyses for male presence showed that the relationship between distance to females and cities was the most informative model (Table 2.4; Figure 2.3.3). When testing additional predictors, distance to roads and distance to rivers showed no additional explanation in the training and testing of model fit based on the ROC, but the BIC showed some improvement when distances to roads and rivers were included in the multivariate model (Table 2.4). Female presence was inferred by the relationship between distance to other females and rivers within home ranges (Table 2.4; Figure 2.3.4). The addition of distance to farms indicated that female presence had a positive association with close distances to farms, however this predictor showed little improvement in the ROC, and increased the BIC, therefore it was not selected as the best model (Table 2.4). To further compare the effect of sex in models, I used male data and fitted these to the best female models at both spatial scales, and the same was done with female data (Table 2.5).

The coefficient values calculated from the GLMM which best models fitted to male and female leopard data for the large scale analyses included distance to females, distance to protected areas, human population density and distance to roads were used to create a predictive habitat map for leopards in the Eastern and Western Cape (Figure 2.4).

Discussion

The presence of species can be explained by the availability of key resources. However, social interactions have a strong correlation to species distribution. This study suggests that leopards located themselves according to conspecifics, not resources alone. Males had a positive association with close distance to females at both scales, while females had a positive association with close distances to other females at the fine scale analyses. This suggests a more parsimonious alternative hypothesis than the resource selection hypothesis.

For solitary female felids, there have been conflicting findings on conspecific interactions, with Packer (1986) finding that females were intolerant of conspecifics, while others did not find conspecific avoidance (Seidensticker et al., 1973). The kinship hypothesis may explain conspecific attraction among related female conspecifics (Hamilton, 1964). The kinship hypothesis predicts that relatives should aggregate closer together within a population, and for solitary felids, philopatric behaviour in females is generally expected to exist and could contribute to conspecific attraction (Hamilton, 1964). Alternatively, philopatry may not occur when resources are restricted, resulting in females dispersing away from natal areas, as found in female Iberian lynx (Ferreras et al., 2004). In such cases the land tenure hypothesis and resource dispersion hypothesis may explain conspecific avoidance as was found in mountain lions (Elbroch et al., 2015). It is unlikely that all of the females in our study area were related, thus conspecific attraction may be better explained by the habitat copying hypothesis (Parejo et al., 2005), where conspecifics may use ‘public’ information from other individuals with similar ecological requirements about habitat suitability. This may increase competition between conspecifics whereby temporal avoidance may reduce competition. Elbroch et al., (2015) found that the land tenure and resource dispersion hypotheses are not mutually exclusive and that mountain lion distribution was a result of a combination of these hypotheses. The habitat copying hypothesis has been useful for individuals to identify high-quality habitat for other species, and may contribute to leopard distribution. This ‘copycat’ hypothesis may be important to investigate for future research to better understand causation of leopard distribution. For males, the location of females was important at both spatial scales. This aligns with other studies where males position themselves based on mate occurrence (Logan et al., 2009; Schmidt et al., 1997). The finding that both male and female leopards' locations are correlated to female presence at the fine scale has implications for leopard distribution. This urges for a realignment of RSFs to consider the complex and dynamic map of conspecific interactions in predicting where leopards will move, and how they will select and defend home ranges. Questioning how the constellation of leopards may respond to a female who is removed from the system is not well understood and may be important for future research to better understand how social structure influences leopard distribution and metapopulation dynamics. Further investigation into home range overlap between and among individuals would provide further insights into how leopards are influenced by conspecifics in their spatial distribution (Elbroch et al., 2015).

This study found that sexes respond differently to environmental cues at different spatial scales. The difference in resource selection between sexes is rarely incorporated into habitat models, despite known sex-related differences in behaviour among many large carnivores (Palma et al., 1999; Linkie et al., 2006; Matthew et al., 2006: Klar et al., 2008). When assigning the best model explaining female locations to predict male presence, the model indicated poor predictive power (Table 2.5). Therefore, each sex positions themselves differently to the other. These findings contribute to efforts to predict leopard distribution, as the use of sexes in models should increase the accuracy of occupancy and population abundance estimates. Studies that are based on data predominately from one sex could result in habitat predictions and management actions that consider one sex, but exclude the other (Colchero et al., 2011; Conde et al., 2010).

We expect that reproductive success is more strongly affected at the larger scale analyses (Bunnefeld et al., 2006; Wilmers et al., 2013), therefore these features could indicate a high-quality habitat, ideal for high reproductive success and survival. In the large scale analyses, areas closer to protected areas were found to be associated with both male and female occurrence. Protected areas may offer higher quality habitat, as there is less human activity, hunting is prohibited, no husbandry exists inside protected areas, and these areas are likely to contain more abundant natural prey species. As for many species, most leopard habitat exists outside of protected areas, with merely 12% of leopard habitat estimated to occur inside protected areas in South Africa (Dudley, 2008; Swanepoel et al., 2013). In the Eastern and Western Cape merely 0.08% and 0.15% of the provinces comprise protected areas respectively; of this space an estimated 71% and 79% are estimated to be suitable to leopard (Swanepoel et al., 2013). Thus, this habitat is only marginally available to leopards and, while they appear to contribute to leopard distribution at the large scale, protected areas are considered insufficient to ensure viable leopard populations (Bothma et al., 1997). Additionally, edge effects adjacent to protected areas can act as sink habitat for species (Balme et al., 2010; Woodroffe and Ginsberg, 1998). Future studies would do well to establish if these areas result in increased intraspecific competition, and how the reproductive success changes within protected areas compared to varying distances from these sites. For males the relationship between distance to females and protected areas showed a positive association with females closer to protected areas. Males also selected areas with lower human population density, probably to reduce the threats associated with people. Females at this scale also had a positive association with areas close to roads. Studies have found that animals respond differently to roads, in some cases showing ambivalent behaviour (Chruszcz et al., 2003), in other cases animals temporarily avoid high traffic volume roads using these areas when traffic volumes decrease (Martin et al., 2010; Roever et al., 2010), or in many cases, avoid roads in general (Forman and Alexander, 1998; Frair et al., 2008; Mace et al., 1996; McRae et al., 2005). Female presence close to roads may also occur when habitat further away from roads is saturated, forcing subdominant individuals to occupy areas closer to roads (Mech, 1989; Mech et al., 1995). Leopards have been found to avoid roads elsewhere in South Africa (Swanepoel et al., 2013), therefore investigating the effect of road characteristics (for example, vegetation cover adjacent to roads, road width, traffic rates) on leopard presence could further explain why females are selecting roads at this scale and thereby improve insights into what impact roads have on leopard distribution. Further investigation into the relationship between distance to protected areas and distance to roads, showed that females were closer to roads when roads were closer to protected areas, suggesting that roads may be selected more where there is less human activity (Table 2.3).

A relationship between the influence of anthropogenic factors and leopard occurrence was found for males at both spatial scales. At the fine scale males had a positive association with areas closer to towns, and at the large scale they had a positive association with areas with low human populations. These appear to be conflicting; however, males have been found to have varied responses to anthropogenic influences but these are usually reported at only within home ranges (Colchero et al., 2011; Conde et al., 2010). The selection of low human population density at the large spatial scale may provide support that male leopards are negatively associated with anthropogenic features at large scale compared to finer scales. Interestingly, at the fine spatial scale males had a negative association with areas closer to cities when closer to females. Wilmers et al., (2013) found that pumas (*Puma concolor*) occupy areas away from human developments during reproduction-related behaviours. Therefore, if males are closer to females when further from cities, this may indicate similar behaviour for leopards in this study and should be investigated.

Natural prey is known to influence predator distribution, thus a limiting factor to this research is the lack of data on natural prey availability. With these data, researchers could better understand habitat quality, and assess if and how food availability influences intraspecific interactions and the spatial distribution of individuals on the landscape.

In conclusion, understanding how individuals select and use habitat at different spatial scales provides insight into environmental requirements that affect the biology of species (Boyce et al., 2002; Hirzel and Le Lay, 2008). This study indicates that conspecifics, sex-related differences and spatial scale infer presence for solitary carnivores such as leopards. Excluding such interactions in habitat selection modelling may result in inappropriate habitat predictions and management actions, and indicates the consideration of more complex social interactions to be incorporated in RSF models.

Management considerations

Protected areas appear to be selected by leopards at the large-scale home range selection, indicating the importance of these areas when selecting home ranges. Protected areas are limited to leopards, as merely 12% of leopard habitat is estimated to occur in these areas (Swanepoel et al., 2013). The selection of such habitat could be due to low human activity, less fragmented habitat and increased natural prey availability. Shortcomings of protected areas, as a core conservation management effort, and a leopard habitat selection choice, include size and shape, isolation (lack of connectivity between other protected areas), and the areas adjacent to protected areas acting as sink habitats which threatens populations even well within protected areas (Balme et al., 2010; Soulé and Sanjayan, 1998; Woodroffe and Ginsberg, 1998). This is particularly true for wide-ranging species such as carnivores as they may use these edges (Woodroffe and Ginsberg, 1998). Connectivity between habitat patches is considered a valuable conservation tool to overcome major threats to biodiversity (Beier et al., 2011; Chetkiewicz et al., 2006). The use of RSF in predicting habitat is considered a better method than expert opinion, and studies have found that large-scale habitat selection analyses can improve the identification of functional corridors (Fattebert et al., 2015). Therefore, the second-order habitat map from this study (Figure 2.4) can be useful for managers to identify areas that are predicted promote connectivity for leopards in the Eastern and Western Cape.

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Table 2.1. Environmental variables used to model leopard resource selection function in the Eastern and Western Cape.

Variable	Description	Resolution (m)	Units	Source
Roads	Euclidean distance to roads	90	Km	a
Rivers	Euclidean distance to rivers	90	Km	a
PA	Euclidean distance to protected areas	90	Km	a
Town	Euclidean distance to town	90	Km	a
Pop	Human population density / km²	1000	Density	a
Farm	Euclidean distance to farms	90	Km	a
Slope	Digital elevation model	90	°	b
Elevation	Digital elevation model	90	m	b
Livestock	Density of small livestock / km²	5600	Density	c
Fem	Euclidean distance to female leopards home ranges	90	Km	GPS data
Male	Euclidean distance to male leopards home ranges	90	Km	GPS data

a) National Geospatial Information (NGI), South Africa, Cape Town, 2010. Department of Land Affairs, South Africa.

b) Jarvis et al. 2006. Hole-filled seamless Shuttle Radar Topography Mission (SRTM) data V3. International Centre for Tropical Agriculture (ICAT).

c) International Food Policy Research Institute, "IFPRI" (http://www.ifpri.org/)

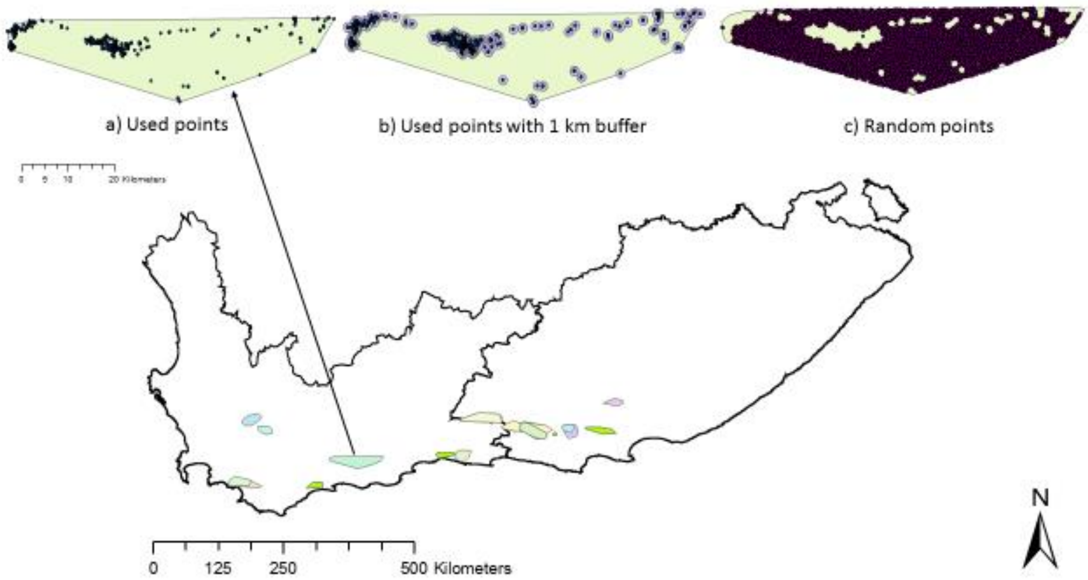

Figure 2.1. Distribution of leopard home ranges estimated from 100% minimum convex polygon (MCP). A 1 km buffer (average distance walked by male and female leopards at 6-hour intervals) was added to observed locations (used points: a) to exclude available locations (Used points with 1 km buffer: b) from observed points within home ranges (Random points: c).

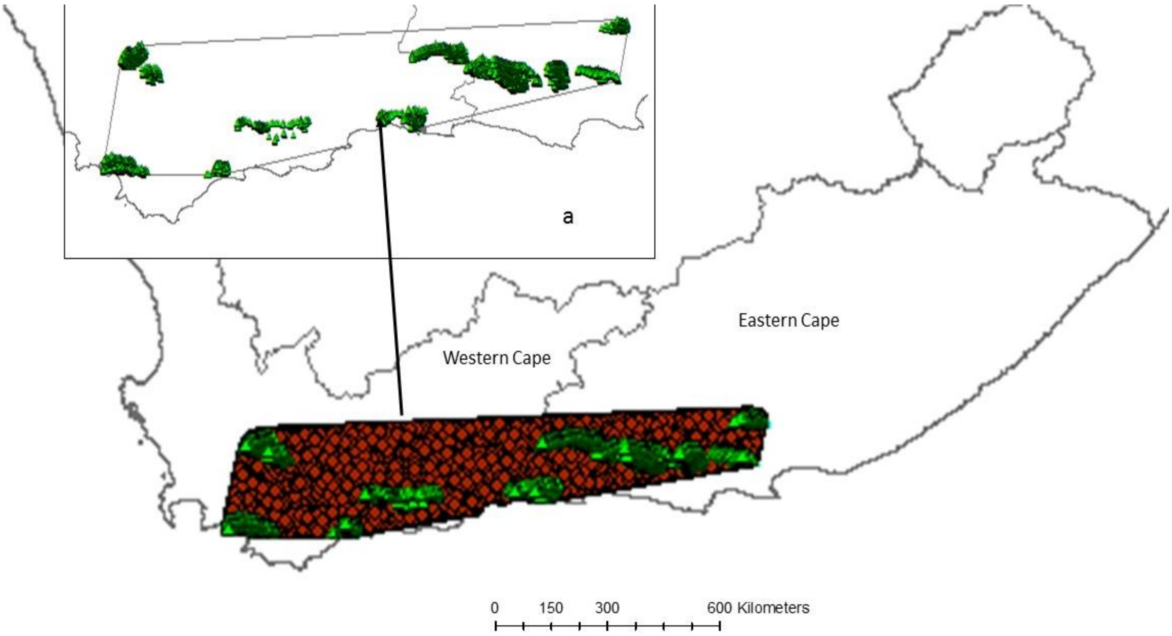

Figure 2.2. A 'super home range' was created using a minimum convex polygon (MCP) which incorporated all individual leopard home ranges to assess the large-scale resource selection analyses (a). Random points were created within this 'super home range'.

Table 2.2. Summary of the best models explaining male and female leopard occurrence at the home range selection (large spatial scale analyses), with Receiver operator curve (ROC), Bayesian Information Criterion (BIC), Standard error (SE), lower (2.5) and upper (97.5) confidence intervals. Variables included distance to females (Fem), distance to protected areas (PA), human population density (pop), distance to roads (Roads).

Home range selection (Large spatial scale analyses)

Sex	Model structure	ROC	BIC	Intercept	β	SE	2.5	97.5
Males	**Fem*PA+Pop**	0.83	48586.47	0.06		0.01	0.033359	0.08857
	Fem				-0.013	0.0002	-0.01334	-0.01255
	PA				-0.19	0.004	-0.19768	-0.18102
	pop				-0.015	0.0005	-0.01678	-0.01484
	Fems*PA				0.0005	0.00008	0.000403	0.000685
Females	**PA+Roads**	0.81	43583.61	-0.31		0.012	-0.33002	-0.28122
	PA				-0.14	0.002	-0.14324	-0.13439
	Roads				-0.15	0.003	-0.15624	-0.14619
Females	**PA*Roads**	0.72	43518.59	-0.21		0.014	-0.23269	-0.17922
	PA				-0.18	0.003	-0.19149	-0.17794
	Roads				0.016	0.0007	0.014598	0.017262

Table 2.3. Summary of the best models explaining male and female leopard occurrence within home range selection (fine spatial scale analyses), with Receiver operator curve (ROC), Bayesian Information Criterion (BIC), Standard error (SE), lower (2.5) and upper (97.5) confidence intervals. Variables included distance to females (Fem), distance to rivers (Rivers), and distance to towns (Town).

Within home range habitat selection (Fine spatial scale analyses)								
Sex	**Model structure**	**ROC**	**BIC**	**Intercept**	**β**	**SE**	**2.5**	**97.5**
Males	**Fem*Town**	0.7879	52144.64	0.039		0.021	-0.00188	0.07928
	Fem				-0.009	0.0004	-0.00917	-0.00782
	Town				-0.043	0.001	-0.04515	-0.0407
	Fem*Town				-0.00014	0.00002	-0.00019	-0.00009
Females	**Fem*Rivers**	0.6865	47296.1	-0.81		0.024	-0.84998	-0.76101
	Fem				-0.003	0.0004	-0.0041	-0.00254
	Rivers				0.02	0.014	-0.00684	0.043611
	Fem*Rivers				-0.003	0.0002	-0.00302	-0.00222

Table 2.4. Fitting the best models for male leopards to female leopard data and vice versa to assess habitat selection differences between sexes with Receiver Operator Curve (ROC), Bayesian Information Criterion (BIC). Home Range Selection (Large spatial scale analyses) and within home range selection (fine spatial scale analyses) were compared. Variables included distance to females (Fem), distance to protected areas (PA), human population density (pop), distance to roads (Roads), distance to towns (Town), and distance to rivers (Rivers). Bold indicates the best model for each sex, and the plain text indicates the opposite sex's data fitted to that model to compare if sexes respond differently to the same environmental features.

Home range habitat selection (Large spatial scale analyses)

Sex	**Model structure**	**ROC**	**BIC**
Best male model fitted to female data			
Males	**Fem*PA+Pop**	**0.83**	**48586.47**
Females	Fem*PA+Pop	0.7114	43898.8
Best female model fitted to male data			
Males	PA+Roads	0.7159	55705.24
Females	**PA+Roads**	**0.81**	**43583.61**
Best male model fitted to female data			
Males	**Fem*Towns**	**0.7879**	**52144.64**
Females	Fem*Towns	0.585	47955
Best female model fitted to male data			
Males	Fem*River	0.7588	54673.25
Females	**Fem*River**	**0.6865**	**47296.1**

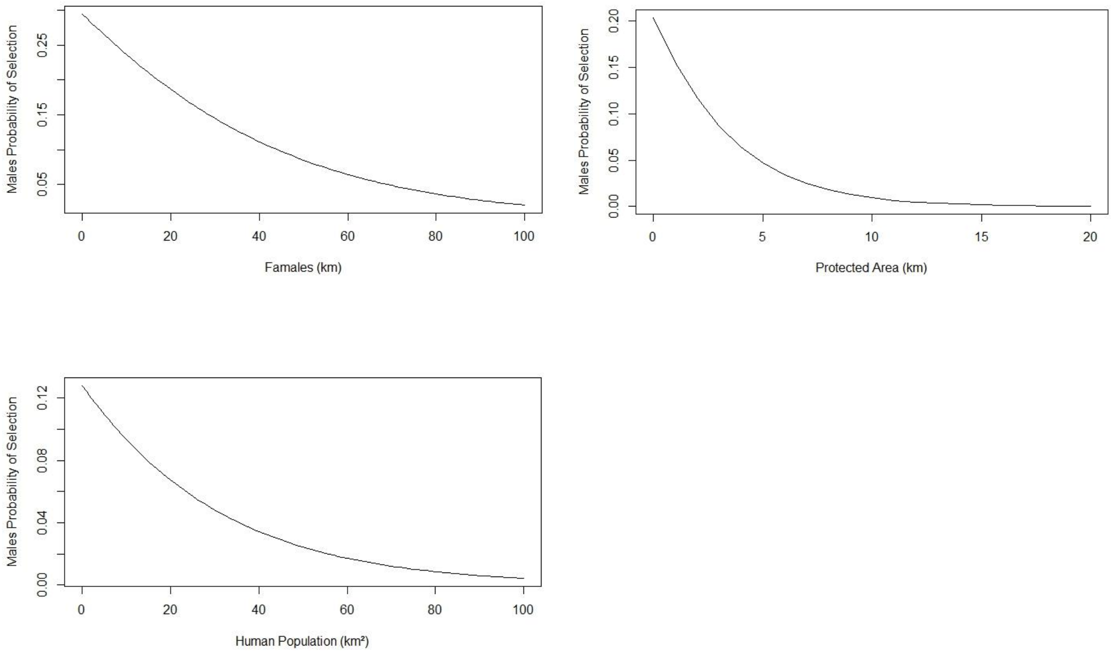

Figure 2.3.1. Covariates from the model which best explained male leopard occurrence at the home range selection (large spatial scale analyses).

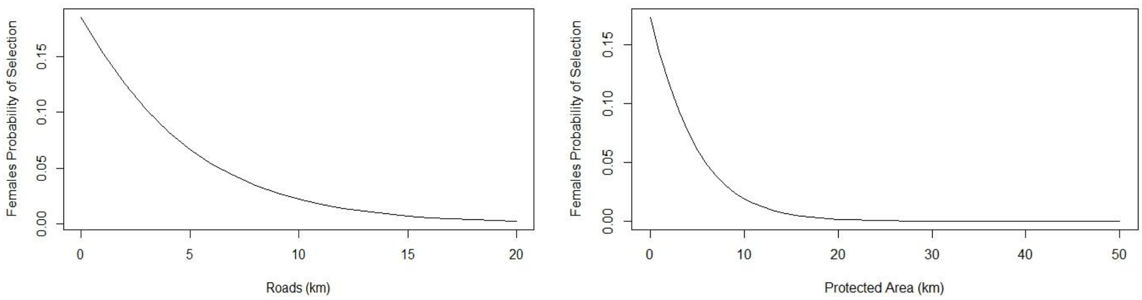

Figure 2.3.2. Covariates from the model which best explained female leopard occurrence at the home range selection (large spatial scale analyses).

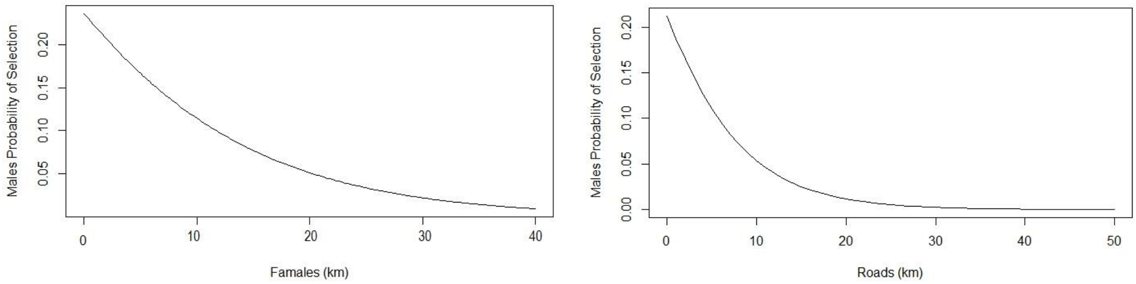

Figure 2.3.3. Covariates from the model which best explained male leopard occurrence within home range selection (fine spatial scale analyses).

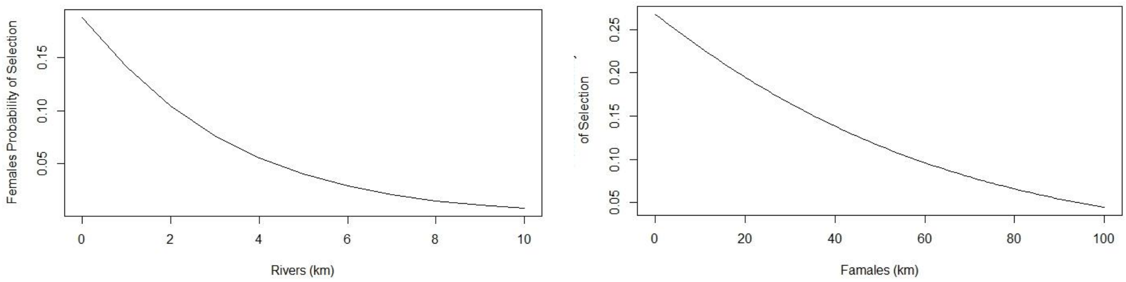

Figure 2.3.4. Covariates from the model which best explained female leopard occurrence within home range selection (fine spatial scale analyses).

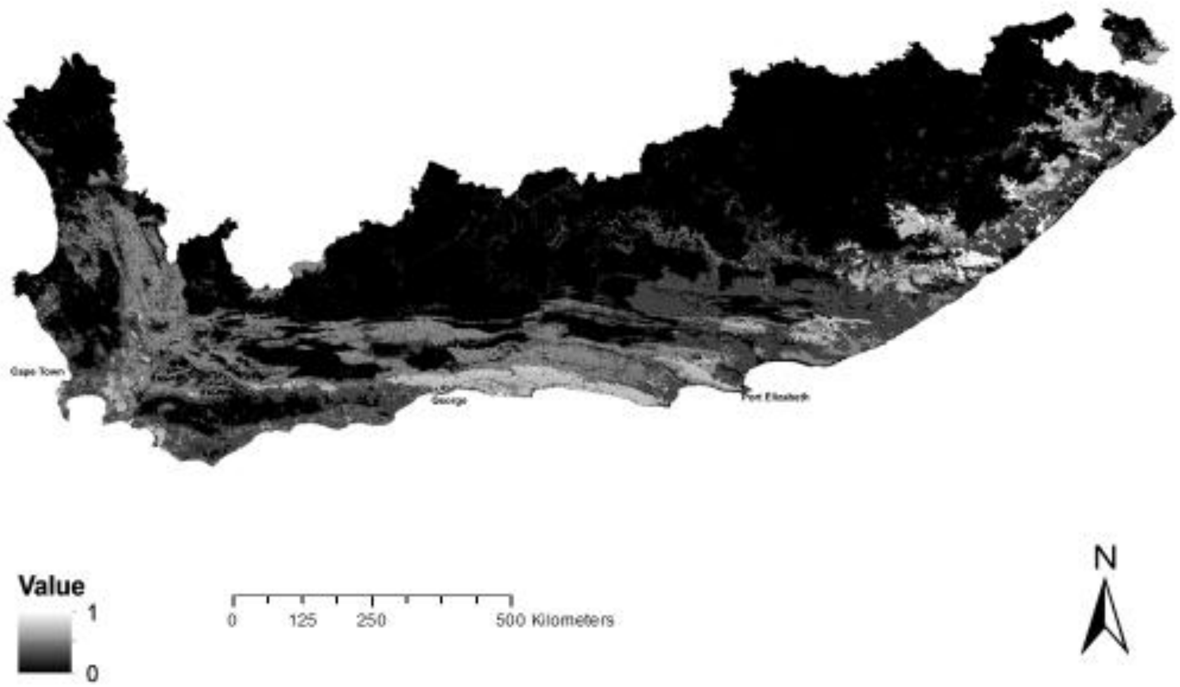

Figure 2.4. Predicted leopard habitat based on RSF coefficient values from the best models for male and female leopards at the large-scale home range analyses. Predictor variables used for modelling the presence of leopard habitat in the Eastern and Western Cape combined male and female best predictors from generalised linear mixed modelling included Distance to females, Distance to protected areas, human population, and distance to roads.

Supplementary table 2.1. Univariate analysis results for male and female leopard occurrence within home range selection (fine scale analyses), and at Home range selection (large scale analyses) with the ROC, BIC and SE.

		Within Home Range Selection (fine scale analyses)						**Home Range Selection (large scale analyses)**					
		ROC						**ROC**					
Sex	**Variable**	**Validation**	**Training**	**BIC**	**Intercept**	**Estimate**	**SE**	**Validation**	**Training**	**BIC**	**Intercept**	**Estimate**	**SE**
Males	Population	0.4335	0.4386	63226.52	-1.268	-0.0011	0.000485	0.653	0.6465	63507.03	-1.15	-0.01723	0.0005
	Cities	0.595	0.5953	62418.61	-0.9	-0.023	0.00092	0.4939	0.4944	65037.3	-1.375	0.0015	0.0007
	Protected	0.5013	0.505	63203.53	-1.3	0.016	0.003	0.7681	0.7709	55879.77	-0.850	-0.16	0.0025
	Farms	0.5119	0.5096	63160.53	-1.206	-0.056	0.0067	0.5882	0.5853	64638.85	-1.510	0.132	0.0063
	Livestock	0.5444	0.541	62957.64	-1.33	0.049	0.0029	0.5566	0.5555	64815.6	-1.385	0.0318	0.0020
	Slope	0.5448	0.5374	63080.04	-1.164	-0.0025	0.0002	0.6379	0.6397	63290.72	-0.923	-0.0086	0.0002
	Rivers	0.5538	0.5625	63069.29	-1.171	-0.055	0.0046	0.5222	0.5268	65008.16	-1.314	-0.021	0.0036
	Elev	0.7177	0.7194	57600.43	-0.356	-0.001164	0.00016	0.579	0.5766	64623.56	-0.002	0.0003	0.00002
	Roads	0.6709	0.6696	60194.66	-0.791	-0.0844	0.0015	0.5514	0.5576	65026.93	-1.380	0.00585	0.00145
	Males	0.6061	0.6041	62812.74	-1.07	-0.0023	0.00013	0.7043	0.7148	56079	-2.240	0.0162	0.0002
	Females	0.7531	0.7531	55784.94	-0.73	-0.0088	0.00011	0.7693	0.7617	58693.74	-0.707	-0.012	0.0002
Females	Population	0.5639	0.5626	49175.01	-1.32	0.0066	0.00031	0.5726	0.5827	53522.87	-1.31	-0.0013	0.000346
	Cities	0.5438	0.5464	49412.51	-1.355	0.0098	0.00069	0.6099	0.613	51789.32	-1.783	0.034	0.000856
	Protected	0.6001	0.6047	45404.44	-1.46	0.283	0.0045	0.7262	0.7286	48520.9	-0.955	-0.114	0.0021
	Farms	0.5116	0.4983	49566.19	-1.15	-0.053	0.0076	0.5617	0.5648	53471.24	-1.28	-0.036	0.00432
	Livestock	0.6029	0.6054	47448.59	-1.374	0.112	0.002	0.5736	0.5673	50897.5	-1.485	0.108	0.002

Supplementary table 2.1. continued

		Within Home Range Selection						Home Range Selection					
		ROC						ROC					
Sex	**Variable**	**Validation**	**Training**	**BIC**	**Intercept**	**Estimate**	**SE**	**Validation**	**Training**	**BIC**	**Intercept**	**Estimate**	**SE**
Females	Roads	0.5461	0.5475	49442.19	-1.103	-0.038	0.0030	0.6692	0.6673	50317.14	-0.902	-0.118	0.0023
	Slope	0.5483	0.5496	49397.26	-1.053	-0.0035	0.00024	0.5452	0.5404	53378.75	-1.213	-0.0027	0.0002
	Rivers	0.626	0.6202	48382.03	-0.91	-0.172	0.0052	0.6394	0.6395	51370.48	-1.009	-0.1667	0.0041
	Males	0.5252	0.5218	49559.88	-1.26	0.0002034	0.00003	0.6811	0.6886	51178.87	-0.88	-0.016	0.000386
	Females	0.6705	0.6756	47766.58	-0.7165	-0.0094	0.0024	0.5615	0.5661	52983.28	-1.55	0.0055	0.00023

Gene flow and population structure of a solitary top carnivore in a human-dominated landscape

Abstract

While African leopard populations are considered to be continuous as demonstrated by their high genetic variation, the southern-most leopard population exists in the Eastern and Western Cape, South Africa, where anthropogenic activities may be affecting this population's structure. Little is known about the elusive, last free-roaming top predator in the Eastern and Western Cape and this study is the first to report on leopard population structuring using nuclear DNA. By analysing 14 microsatellite markers from 40 leopard tissue samples, we predict that the leopard population in the Eastern and Western Cape prescribe to a metapopulation model. Specifically, we investigate the populations' structure, genetic distance and gene flow (*Nm*) and discuss these in relation to modified landscapes. Our results, based on spatially explicit analysis with Bayesian methods, indicate that leopards in the region exist in a fragmented population structure with lower than expected genetic diversity. Three population groups were identified, with low to moderate levels of gene flow (*Nm* 0.5 to 3.6). One subpopulation exhibited low genetic differentiation, suggesting a continuous population structure, while the remaining two appear to be more isolated, indicative of low emigration and immigration between these populations. Therefore, genetic barriers are present and, while leopards in the study region may function as a metapopulation, anthropogenic activities threaten to further reduce habitat and movement. Our results indicate that the leopard population may become isolated and suggest that management actions should aim to maintain and increase habitat connectivity and reduce human–carnivore conflict. Understanding genetic diversity and connectivity of populations has important conservation implications that can highlight management of priority populations to reverse the effects of human-caused extinctions.

Keywords: gene flow, genetic differentiation, habitat fragmentation, *Panthera pardus*, population structure.

Introduction

Ensuring the maintenance of genetic diversity and connectivity among populations facilitates the continuation of dynamic evolutionary and ecological processes. Genetic data provides insights into the population structure of a species and the rate of genetic movement between populations, which helps to determine the possibility of local adaptation and of adaptive evolution in complex landscapes (Hanski and Gilpin, 1991). Whether continuous or discrete, population structures are influenced by a variety of factors, including species-innate traits such as dispersal behaviour (Gittleman, 2013; Sork et al., 1999), climatic factors (Stenseth et al., 2004) and geographic features that may facilitate or constrain movement (Frankham, 2005; Ginsberg and Milner-Gulland, 1994; Woodroffe and Ginsberg, 1998). Metapopulations experience local extinctions and recolonizations of subpopulations through immigration and emigration (Hanski and Gilpin, 1991). Therefore, high mobility and dispersal influence the long-term survival and adaptation of species and facilitate population persistence. Large carnivores often have the ability to traverse extensive distances and can occupy a variety of environmental conditions (Sunquist and Sunquist, 2002; Sweanor et al., 2000). However, even where species are highly mobile, discontinuous habitat and anthropogenic-associated barriers, such as major roads, monoculture and human-caused mortality, constrain movements and reduce population densities (Loxterman, 2011; Sinclair et al., 2001; Walker et al., 2000; Woodroffe and Ginsberg, 1998). The proportion of declines in carnivore populations caused by human-induced mortality has been compared with declines in actively culled populations and is considered, more than any other factor, to be the main cause of extinctions for small, isolated populations (Powell et al., 1996; Wielgus and Bunnell, 1994).

If genetic transfer between populations is impeded, two major genetic threats may present themselves: firstly, alleles become randomly fixed or are lost due to genetic drift; and secondly, harmful mutations accumulate. Subpopulations may not recover from such impacts, degrading the persistence of metapopulations (Sweanor et al., 2000) and reducing the populations' ability to adapt to changing environmental conditions, diseases and other stochastic events that threaten their survival (Frankham, 2005; Keller and Waller, 2002; Walker et al., 2000). Leopards are highly mobile and are considered to be the most adaptable felid in the world, able to occupy most environments except true desert (Sunquist and Sunquist, 2002). In much of their range, leopards are the last remaining free-roaming top predator. This top predator status influences community structure in lower trophic levels, driving biodiversity (Carroll et al., 2001; Noss et al., 2002). The solitary and elusive nature of leopards has made research on the species difficult, resulting in potentially inappropriate management actions, such as lethal predator control (human–carnivore conflict; trophy hunting) when the population may already be vulnerable to extinction. Recently, however, the use of DNA has provided an opportunity to increase our knowledge of this species. Understanding the population structure and diversity within and between populations is crucial to estimate the extent of divergence among populations, recognise evolutionary significant units, preserve genetic diversity among remnant populations and highlight management of priority populations to reverse the effects of human-caused extinctions (O'Brien and Johnson, 2005).

Studies examining nuclear DNA from leopards in central Africa indicate that populations are continuous, with high levels of genetic heterozygosity (Miththapala et al., 1996; Spong et al., 2000; Uphyrkina et al., 2001). In South Africa, one study used mitochondrial DNA (mtDNA) from 29 individuals sampled across the country (Martins, 2006). These maternal phylogeographic outputs indicated high levels of genetic diversity overall, but genetic separation between the Western Cape Province and other areas of South Africa (Martins, 2006). However, the use of mtDNA for asymmetrical dispersing mammals, including leopards, where males disperse in order to mate, while females are philopatric (Bailey, 1993; Sunquist and Sunquist, 2002), may not represent the true situation (Melnick and Hoelzer, 1992; Zhang and Hewitt, 2003). An additional problem is the presence of mitochondrial pseudogenes in the nuclear genome that weaken the effectiveness of using mtDNA in population genetic studies (Zhang and Hewitt, 2003). Therefore, the genetic status and population structuring of leopards in the region is not fully understood and can be questioned. The use of microsatellites provides more insight than does mtDNA into the genetic structure, gene flow, heterozygosity and general population connectivity for closely related populations (Teske et al., 2012).

The southernmost part of South Africa (Eastern and Western Cape provinces) is characterised by a matrix of land uses, highly fragmented natural habitat and human–carnivore conflict, all of which result in carnivore mortality. Such landscape characteristics have resulted in reduced gene flow in other carnivore species around the world (McRae et al., 2005; Sinclair et al., 2001). We investigate the population structure using spatially explicit methods to determine the genetic distance, gene-flow and heterozygosity of 40 leopards by analysing 14 microsatellite markers. We present results from the Eastern and Western Cape and consider the conservation implications these have for isolated carnivore populations in a human-dominated landscape.

Materials and methods

Study area and sampling

Forty tissue samples were collected across the Eastern and Western Cape provinces (33° 11’ – 33° 23’ S and 25° 53’ – 18° 53’ E; Figure 3.1). Nine samples were from museums (oldest sample from 1976, most recent 1996), while 31 were collected from free-ranging leopards during capture and immobilization of leopards associated with a broader ecological study during 2007–2013 (Figure 3.1). All samples were accurately georeferenced. Leopards were captured in walk in, two fall-door cages 2 m x 800 mm x

800 mm. Cages were set where leopard activity was present (spoor, scat, scrapings), but no baits or lures were used in cages in order to reduce by-catch. Immobilizations were undertaken by a qualified veterinarian, using a drug combination of Zoletol-Medetomidine at a standard dosage (1–2 mg/kg). Induction times averaged seven minutes, minimizing stress to the leopards. Recovery of animals from the reversal drugs averaged eight minutes. Samples were stored in a high-salt solution (Seutin et al., 1991) that retained good quality DNA. No samples were collected from captive individuals.

DNA extraction and amplification

Genomic DNA was extracted from tissue using the QIAGEN DNeasy® Blood and Tissue Kit (GmbH, Germany), following the manufacturer's protocol. Leopard samples were analyzed using 14 microsatellite loci developed from the domestic cat (Menotti-Raymond and O'Brien, 1994). Fourteen markers were optimised: FCA024, FCA032, FCA082, FCA085, FCA096, FCA129, FCA133, FCA161, FCA191, FCA211, FCA224, FCA261, FCA275 and FCA391. Promega GoTaq® Flexi DNA Polymerase (Promega Corporation) was used for amplification in 12.5 µL reactions. The final reaction conditions were as follows: 1 x PCR buffer, 1 mM $MgCl_2$, 200 µM of each dNTP, 10 pmol of each primer (forward and reverse), 1 U *Taq* DNA polymerase and 50 ng genomic DNA template. PCR was conducted in the BOECO TC-PRO Thermal Cycler. The amplification conditions were as follows: five minutes at 95˚C, 30 cycles for 30 seconds at 95˚C, 30 seconds at 50–60˚C and 30 seconds at 72˚C, followed by extension at 72˚C for 40 minutes. The dye-labelled PCR products of the microsatellite primer sets were pooled and diluted together based on size range and fluorescent dye, so that three to six loci could be multiplexed, electrophoresed and subsequently analyzed in an ABI3130 automated sequencer. Microsatellite allele sizes were estimated by comparison with a LIZ™ 500 (ABI, Foster City, CA) internal size standard. Data was collected and analyzed using the ABI programs GENESCAN (version 1.2.2-1) and GENOTYPER (version 1.1). We conducted a minimum of two replicate PCRs per tissue sample per locus. Alleles included in the final consensus genotypes were observed twice; if observed once, additional replicates were conducted. We also included a negative and positive control in each PCR reaction as checks for contamination and to ensure standardized genotypes among experiments.

Molecular analysis

Population genetic analysis

MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) was used to detect possible genotyping errors, allele dropout and non-amplified alleles (null alleles). This software package can estimate the frequency of null alleles and adjust the dataset to correct for genotyping errors. Deviations from Hardy-Weinberg Equilibrium (HWE) proportions were calculated using GenAlEx 6.5 (Peakall and Smouse, 2006). Linkage disequilibrium between pairs of microsatellite loci was evaluated using Genepop 4.0 (Raymond and Rousset, 1995). Associated probability values were corrected for multiple comparisons using Bonferroni adjustment for a significance level of 0.05. Levels of genetic variation were inferred from the average number of alleles per locus (A), the observed heterozygosity (H_O), Nei's (1978) unbiased expected heterozygosity (H_E) and percentage/number of private alleles, all of which were calculated using the software GenAlEx 6.5 (Peakall and Smouse, 2006).

Global genetic structure of populations

Divergence of species can occur within a short time (Anderson et al., 2010), so determining if population substructure resulted on an evolutionary scale or recently was important to estimate. We used a Bayesian clustering method implemented in the software program GENELAND, version 3.1.4 (Guillot et al., 2005) to determine the genetic structure of leopard. This program was able to identify divergence between populations as recently as four generations, and found to be the best method available to determine contemporary population subdivision (e.g. Blair et al. 2012). Unlike STRUCTURE (Pritchard et al., 2000), GENELAND uses spatial location and genotypic data for all individuals to infer the number of population subdivisions and to assign individuals to each. In this way we were able to identify cryptic patterns of structure where barriers in the fragmented landscapes may not have been obvious. K was determined across 10 iterations using GENELAND. All runs were conducted using 1 000 000 Markov chain Monte Carlo (MCMC) iterations. Genetic differentiation was examined between the inferred clusters using F-statistics calculated in GENEPOP (Raymond and Rousset, 1995). Pairwise estimates of F_{ST} (Weir and Hill, 2002) were calculated in order to determine genetic distance between clusters. The rate of gene flow across the sampled units was expressed as the number of migrants per generation, *Nm*, where *N* is the effective population size and *m* is the proportion of migration per generation. *Nm* is approximated by (1/Fst−1)/4 (Wright, 1984).

Results

Population genetic analysis

All 14 microsatellite loci were polymorphic. Mean H_o values ranged from very low to very high (0.35–0.88), H_e values ranged from 0.50–0.81 and the average number of alleles per locus was 6.5 (Table 3.1). H_o values were lower than H_e in 10 of the markers, with an average observed heterozygosity level of 0.657. Pooling of the samples into one large population resulted in deviations from HWE for seven markers (Table 3.1). In addition, null alleles were observed for one marker (FCA211) and linkage disequilibrium between markers was not observed. There are many possible explanations for departures from Hardy-Weinberg proportions, including natural selection, population subdivision and null alleles. The high proportion of null alleles could be due to the markers used being developed for other species, which could result in high allele amplification failure due to mutations in primer locations. Previous studies on non-invasive samples collected from leopards that were amplified using cross-species markers identified an allelic dropout rate of 0–9% (Mondol et al., 2009). However, this estimate is probably higher than the actual rate. Thus, assuming that genotype errors were randomly distributed with respect to the population, this error rate is unlikely to bias our estimates of genetic diversity and divergence. The heterozygote deficiency was interpreted as the Wahlund effect, indicating the differentiation between leopard populations which were therefore analyzed separately.

Genetic structure of populations

This study provided important insights into the population structure and gene flow of leopards in the Eastern and Western Cape provinces of South Africa. All GENELAND runs produced a K=3 population estimate (Figure 3.2). Based on the boundaries identified by GENELAND, clusters were labelled as follows: "South population", "North population" and "Central population" (Figure 3.1). Supporting evidence was deducted from the AMOVA analysis, as 12% of the variation was shared among the different localities. The high genetic distance measure between the Central and North (F_{ST} = 0.30463; P=0.000) and South and North (F_{ST} = 0.32943; P=0.063) populations supports a strong substructuring of the diversity. Gene flow among these populations was therefore low (*Nm* 0.57 and 0.51 respectively). However, a moderate genetic distance measure was observed between the Central and South populations (F_{ST} = 0.06360; P=0.072), suggesting some gene flow between these populations (*Nm* 3.68). This may be explained by one museum sample, which formed part of the south population, collected in 1986 on the western edge of the large central population. This individual probably migrated between these two populations. Private alleles were observed in all three populations, namely 27 in the Central population, three in the South population and two in the North population.

Discussion

Although leopards in Africa are considered to have high genetic diversity and little population structuring (Miththapala et al., 1996; Spong et al., 2000; Uphyrkina et al., 2001), significant population genetic structure of leopards has been detected in the southernmost part of Africa. Three subpopulations were identified in the region with moderate to low levels of genetic differentiation. These results indicate that leopards in the Eastern and Western Cape are not panmictic, despite their high mobility and environmental plasticity. Human disturbance, leading to contemporary landscape changes, may be responsible for this low gene flow.

Predictive habitat modelling for leopard indicates that topographic features such as mountain ranges, tall vegetation cover and the close proximity of rivers promote leopard movement, while features fragmenting habitat, such as human-associated land uses and roads, are avoided (Gavashelishvili and Lukarevskiy, 2008; Swanepoel et al., 2013). Urban areas and road networks have expanded and agriculture has been intensified in the region in recent history, which has resulted in reduced gene flow and lowered genetic diversity for many species, including large mammals with longer life spans (Epps et al., 2005; Holderegger and Di Giulio, 2010; Pilot et al., 2006). Reduced levels of genetic transfer can increase genetic differentiation within a few generations (Balkenhol et al., 2009; Epps et al., 2005), so the use of appropriate analytical tools to identify these recent divergences is important (Anderson et al., 2010). Leopards live for approximately eight to 12 years in the wild and we could detect contemporary population divergences as recently as 20 generations by using Bayesian statistics and GENELAND (Blair et al. 2012).

Our findings highlight the sensitivity of leopard to landscape changes involving human occupation. The higher genetic differentiation observed in the north population (F_{ST} = 0.304 – 0.329) indicates more habitat fragmentation exists between the north and the remaining populations. While the south and central populations indicate some gene flow between them (*Nm* 3.68), one sample forming part of the south population was collected in 1986 on the western edge of the central population. Therefore, the finding of lower genetic distance between the central and south populations may be due to remnant connectivity. The high number of private alleles maintained by the central population (n = 27), compared to both south and north populations, further suggests that the latter populations have been isolated from genetic transfer of the continuous central population and have undergone a severe genetic bottleneck effect.

Our results indicate that the leopard population can broadly be described as a metapopulation (see Hanski & Gilpin, 1991) with subpopulations in habitat patches separated by human-dominated landscape, linked by some degree of genetic flow. However, low gene flow and few private alleles indicate that the south and north populations may be functioning as sink populations, while the central population may have been a source in the recent past. Furthermore, the low genetic transfer between populations may reduce potential recolonization of extinct populations (Levin, 1970). Other fundamental characteristics of metapopulations have not been demonstrated, including independent dynamics among patches, natural extirpations, and natural recolonizations of extirpated populations (Harrison and Taylor, 1997) . Therefore, we suggest that the leopard population was previously a single population with patchy, or extinction-resistant distribution (Harrison, 1991). As anthropogenic land use and human–carnivore conflict increased, this single large population became increasingly fragmented, resulting in semi-isolated to isolated populations surrounded by non-leopard habitat, as has happened to carnivores elsewhere (Beier, 1996; Cegelski et al., 2003; McRae et al., 2005). As a result, leopard populations in the region may not function to the full extent as metapopulations. If isolation trends continue and gene flow further decreases between populations, they will become isolated and separate into distinct evolutionary units and possibly experience higher probabilities of extinction (Schaffer, 1988).

Our findings are further supported by the lower than expected heterozygosity present in 10 of the 14 markers. Several factors, such as inbreeding and fragmented population structure due to the lack of genetic transfer between populations, can contribute to this. The average observed heterozygosity level (0.657; Table 3.1) was similar to those of the Indian leopard (*P.p. fusca*) (0.696) and Persian leopard (*P.p. saxilcolor*) (0.616) populations (Uphyrkina et al., 2001). Other studies have reported that the heterozygosity in Indian leopards varies between 0.57 and 0.74 (Dutta et al., 2013; Mondol et al., 2009). These differences in levels of heterozygosity may be due to different sample size and sample distribution. Because of the continuous distribution of African leopards, high levels of heterozygosity (0.77–0.80) were observed in studies undertaken in central Africa (Spong et al., 2000; Uphyrkina et al., 2001), while the lowest average heterozygosity was recorded in the endangered Far Eastern leopard (*P.p. orientalis*) (0.356) (Uphyrkina et al., 2001).

Our sample size was low and this may have reduced the potential for identifying higher rates of gene flow between populations and made it difficult to determine recolonization of historically extirpated populations. However, leopard population estimates are estimated at between 680 and 900 individuals (Devens et al., 2018) within predicted leopard habitat for the Eastern and Western Cape (Swanepoel et al. 2013; Devens et al., 2018). Our sample represents 4–6% of the leopard population, which is more than previous DNA studies in Africa (0.18%) (Spong et al., 2000). For microsatellites, sample sizes of above 25 to 30 showed minimal variability in allele frequency and expected heterozygosity, suggesting our sample size was suitable to infer genetic structure and gene flow (Hale et al., 2012).

Conservation management implications

Population subdivision may lead to decreased genetic variation within individual subpopulations owing to genetic drift (Lande and Barrowclough, 1987), thus these three subpopulations may require genetic transfer to remain as one evolutionary unit. Our estimates of gene flow, presented as relative measures of connectivity between populations, provide a useful index to assist management. The north population had low immigration and emigration (0.57–0.51 migrants / generation), with higher gene flow recorded between the central and south subpopulations (3.68 migrants / generation). The levels of gene flow are low compared with results for other carnivores (Cegelski et al., 2003; Dutta et al., 2013), which highlights the need for further research. The low gene flow estimates furthermore have important implications for human-caused mortality, particularly where human–carnivore conflict exists and harvesting is practised.

It has been proposed that only one migrant per generation is needed to prevent population differentiation (Kimura and Ohta, 1971; Vucetich and Waite, 2000), however recent evidence suggests up to 10 migrants per generation is more realistic for natural populations (Mills and Allendorf, 1996). Population viability model predictions for other large solitary felids such as cougars (*Puma concolor*) in a human-dominated landscape indicated that, even when high immigration rates were used in models, small populations became extinct within 100 years (Sweanor et al., 2000). Additionally, to withstand the threat of extinction over more than 100 years, continuous habitat had to be between 1000 to 2200 km². Considering these parameters, all three of the observed leopard subpopulations are at risk of extinction.

To ensure gene flow between populations, habitat connectivity and opportunities for genetic movement between discontinuous populations is essential (Dutta et al., 2013; Ernest et al., 2003). Finding solutions to human–carnivore conflict (see McManus et al., 2014) may reduce carnivore mortality and increase genetic transfer between populations. Active harvesting of leopards in the observed substructured populations with moderate to low gene flow will most likely increase the risk of extinction.

The detection of population divergence in leopard populations in South Africa indicates an increasingly fragmented landscape for carnivores. As the human population continues to increase rapidly, the need to maintain connectivity of natural populations is becoming greater. To ensure population persistence of carnivores, their management requires curbing human–carnivore conflict, identifying specific habitat to delineate critical habitat, increasing connectivity between populations, and ensuring that future development considers species-specific alternatives to ensure connectivity.

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Table 3.1. Genetic diversity measure across all leopard populations in South Africa. Null allele frequencies estimated with MICRO-CHECKER for 14 microsatellite loci. Hardy-Weinberg Equilibrium values as calculated by GENEPOP. NS. Non-significant; *: P<0.05; **: P=<0.01; *** P=<0.001. He values calculated to correct for uneven sample size. FIS is the inbreeding coefficient.

Locus	**No. of alleles**	**H_o**	**H_e**	**Null allele frequency**	**HWE**	**F_{IS}**
FCA391	7	0.576	0.661	0.076	0.029^{*}	0.129
FCA024	6	0.647	0.740	0.0606	0.772^{NS}	0.125
FCA129	8	0.722	0.806	0.0467	0.002^{**}	0.104
FCA032	6	0.641	0.766	0.075	0.000^{***}	0.163
FCA082	7	0.730	0.759	0.0117	0.077^{NS}	0.038
FCA275	4	0.350	0.499	0.1384	0.446^{NS}	0.299
FCA191	7	0.650	0.641	-0.0363	0.677^{NS}	-0.014
FCA133	6	0.611	0.698	0.0553	0.098^{NS}	0.125
FCA161	8	0.625	0.617	-0.0029	0.000^{***}	-0.013
FCA224	8	0.629	0.744	0.0582	0.000^{***}	0.156
FCA085	7	0.882	0.768	-0.0823	0.412^{NS}	-0.149
FCA211	6	0.577	0.793	0.131	0.001^{***}	0.272
FCA261	6	0.750	0.799	0.0251	0.010^{*}	0.061
FCA097	5	0.813	0.742	-0.0508	0.462^{NS}	-0.095
Average	6.5	0.657	0.717	---	---	0.086

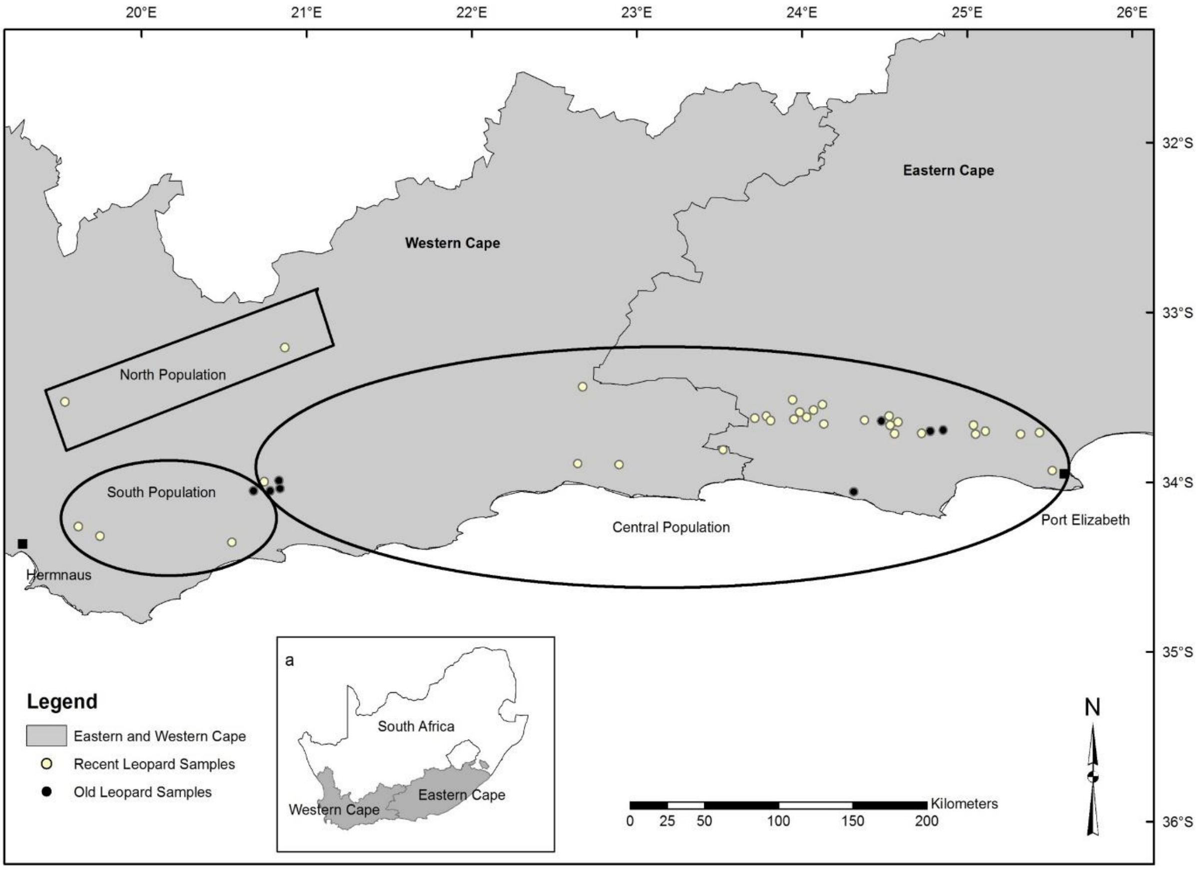


Figure 3.1. Study area within South Africa, where shading indicates the location of the Eastern and Western Cape. The locations of 40 samples (recent and museum) collected from the study area. Sample locations eveloped by black polygons denote which of the three subpopulations (K = 3) the sample was assigned to as defined by GENELAND “north popultion”, “south population”, and “central population”.

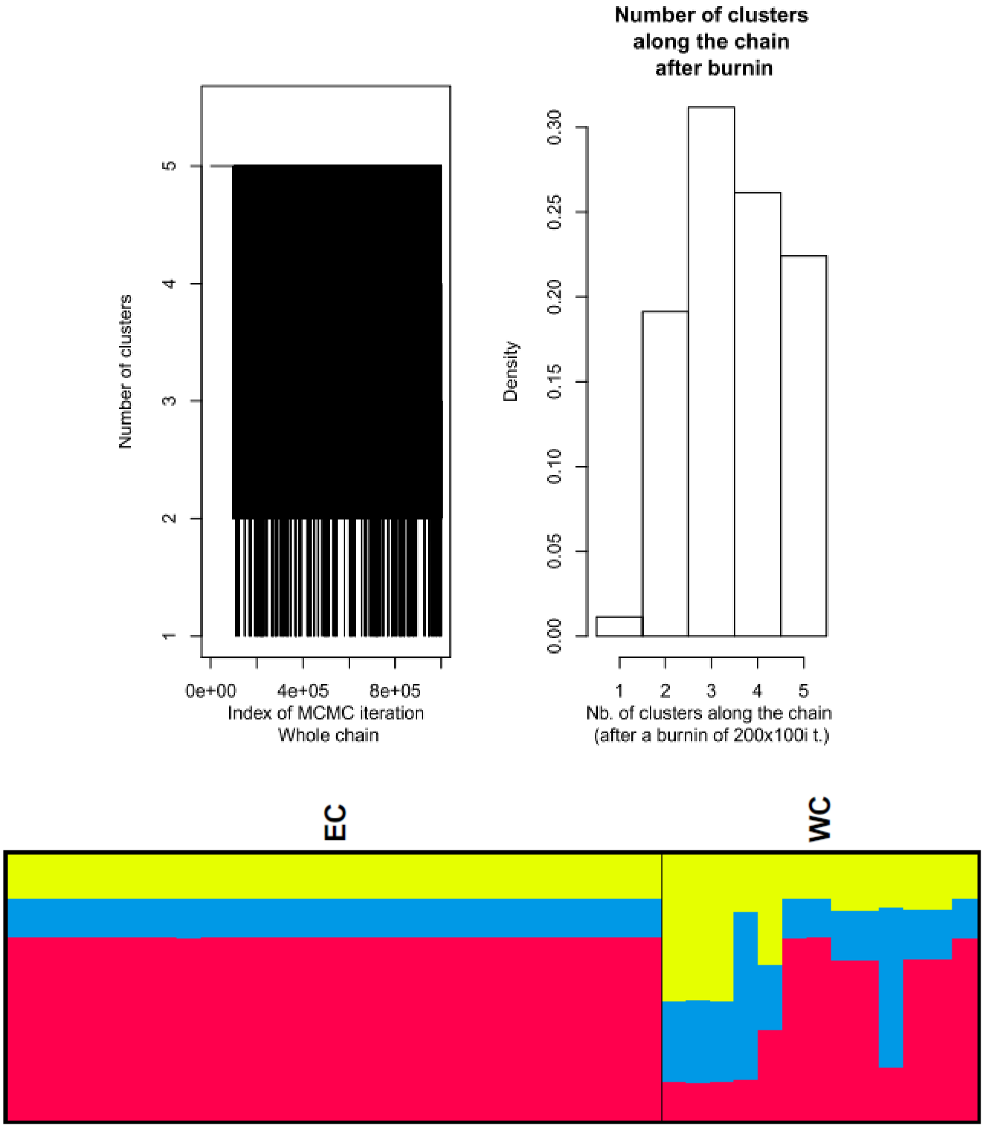
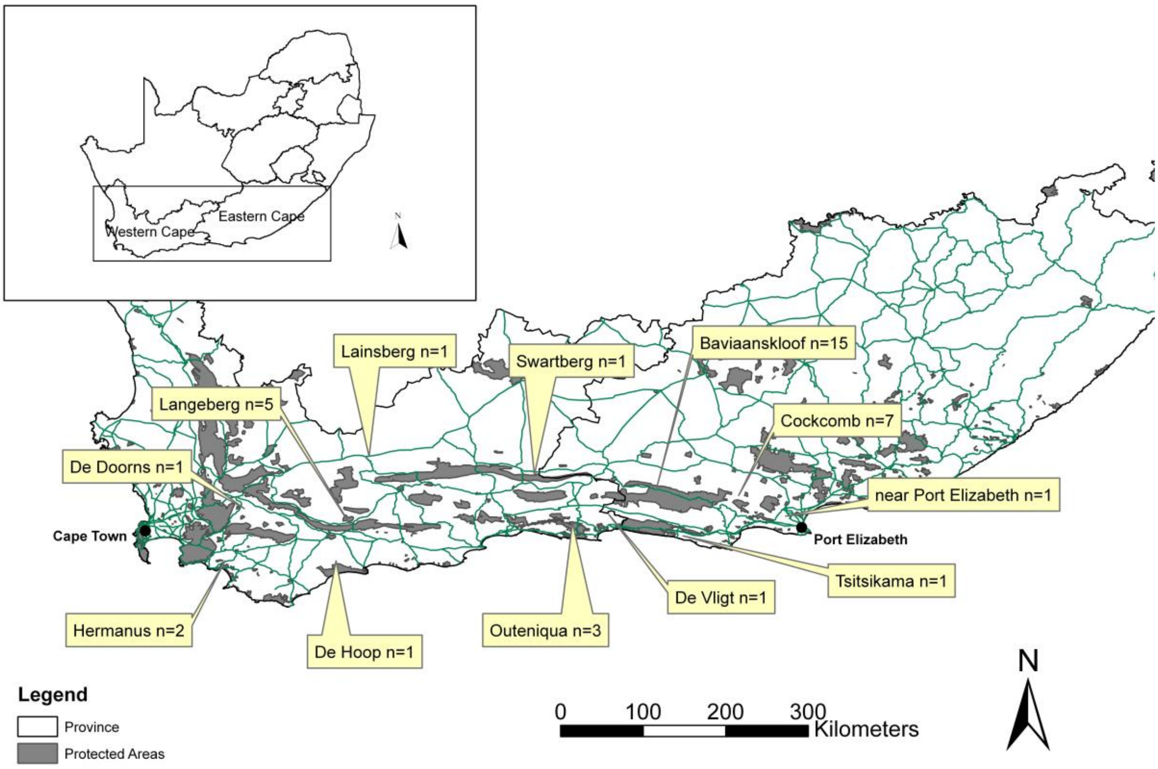


Figure 3.2. Estimated number of populations from GENELAND. (A) Posterior density distribution of the number of clusters estimated from GENELAND analysis. (B) Estimated population structure from GENELAND analyses for the model solutions K = 3. Each individual is represented by a thin horizontal line divided into K coloured segments that represent the individuals estimated membership fractions in K clusters. Black lines separate individuals from different geographic areas.



Supplementary figure 3.1 Study area with roads and number of DNA samples collected across the Eastern and Western Cape.

Using movement patterns in fragmented landscapes to understand leopard habitat connectivity

Abstract

Movement allows individuals to meet their basic requirements over time: acquire food and mates, avoid competition, and facilitates metapopulation dynamics, thereby influencing ecology and evolution. Animals display different movement patterns as a result of landscape features. For example, slow, non-directed movement patterns are associated with encamped behaviour, which is expected to occur in resource-rich habitats with low mortality risk. Conversely, fast, directed movements (inter-patch behaviour) are usually employed to move quickly between poor quality habitat patches. This movement pattern facilitates connectivity of individuals between populations which is fundamental in reducing the effect of habitat fragmentation and maintaining genetic diversity. Therefore, I predict that areas associated with inter-patch behaviour can be used to characterise corridors between habitat patches. I used a state–space statistical approach to model animal movement behaviour in response to landscape features for leopards in the Eastern and Western Cape, South Africa, with specific interest in understanding inter-patch behaviour these highly mobile animals have to the environment and the consequences these behaviours have on population dynamics. I predicted that human-associated landscape features, and the location of same-sex conspecifics would promote inter-patch behaviour, as animals should reduce time spent in these high risk, fragmented habitats and avoid competition from conspecifics. Furthermore, sexes should employ different movement strategies to various landscapes. Three movement states were displayed by leopards: 1) encamped behaviour, characterised by short step lengths and high turning angles; 2) search behaviour, with long step lengths and variable turning angles; and 3) inter-patch behaviour, with very long step lengths and low turning angles. Males spent 70% of their time displaying inter-patch behaviour, while females balanced search behaviour and inter-patch behaviour (40% respectively). Both sexes displayed encamped behaviour 20% of the time.

Search behaviour was dominant for both sexes near human-associated landscapes, indicating that these areas reduce connectivity. Sexes did respond differently to the same environmental conditions however: Males' inter-patch behaviour increased when close to human activity, while females increased inter-patch behaviour away from human activity. Males appeared to avoid male conspecifics, while females showed a variation between avoidance and attraction of female conspecifics as distance varied. Sex-related differences, socio-spatial configuration and anthropic landscapes describe movement patterns and are important to consider in management policies related to habitat connectivity.

Key words: behavioural state, connectivity, habitat, hidden Markov model, movement patterns, inter patch behaviour

Introduction

The long-term persistence of a metapopulation relies on the ability of individuals from separated populations to reach each other, thus allowing a balance between extinction and recolonisation of habitat patches (Hanski, 1998). This relies partly on the spatial structure of available habitat and on the mobility of species. Optimal foraging theory predicts that individual animals will spend more time in resource-rich, safe habitat patches. Where habitat is fragmented, there is selective pressure for movement distance to match the distance between habitat patches. Therefore, in order to maximise fitness, we might expect that movement between such patches will be direct and efficient because intervening spaces do not offer the same benefits (Alerstam et al., 2003; Fahrig, 2007 Hopcraft et al., 2014; Morales and Ellner, 2002). These movement patterns may be important to population dynamics if it increases connectivity between habitat patches (Fahrig, 2007; Graves et al., 2007; Kerk et al., 2015; Revilla et al., 2004).

Natural landscapes are generally heterogeneous as a result of resources occurring variably across the landscape (Boyce and McDonald, 1999). Increasingly, human-altered landscapes degrade habitat and intensify fragmentation of habitat into small, isolated patches, reducing species survival (Fahrig, 2007; Sala et al., 2000). Survival requires individuals to identify areas of low and high suitability and respond optimally to maximise resource access and minimise risk within these respective areas (Fahrig, 2007; Morales and Ellner, 2002; Revilla and Wiegand, 2008; Revilla et al., 2004). By studying animal–habitat relationships, ecologists can gain insight into the ability of species to persist in altered habitats (Bowler and Benton, 2005; Nathan et al., 2008).

Animal movement is governed by complex internal and external factors relating to acquiring resources, avoiding predators and outcompeting rivals. Among the resources individuals need to acquire to maximise fitness, is be near potential mates and other helpful associates (conspecific attraction) but avoid injurious conspecific rivals and competitors (conspecific avoidance) (Stephens and Krebs, 1986). Therefore, animals move based on their short-term needs as well as instinct and experience in pursuit of long-term goals. Movement patterns vary in rates and directions, as a result (Nathan et al., 2008; Patterson et al., 2009). For example, dispersing lions (*Panthera leo*) move further when solitary or when group size is small and show more directional movement than during other life stages (Elliot et al., 2014). Also, animals change movement behaviours when landscape changes occur (e.g. topographic, land use), and at different ages and reproductive status (Elliot et al., 2014; Kerk et al., 2015; Morales and Ellner, 2002). Unravelling how, when and why animals move can therefore be challenging, particularly when direct observations are not feasible.

Because animal movement patterns reflect a continuum of behaviours, ecologists quantify different movement states according to distributions of step lengths and turning angles (Berggren et al., 2002; Hopcraft et al., 2014; Morales and Ellner, 2002; Patterson et al., 2008). This approach dissects movement paths into segments that are assumed to reflect different underlying behaviours rather than being random (Morales and Ellner, 2002; Revilla and Wiegand, 2008). By relating movement patterns to different behaviours and locations, ecologists have inferred the biological processes influencing the movement behaviour of animals (Edelhoff et al., 2016; Roever et al., 2014). Despite the continuity of movement patterns, ecologists often define categorical patterns of movement qualitatively, when such categories seem useful in inferring the cause of different movement behaviours.

Three movement patterns are commonly associated with discernible behaviours. Encamped behaviour is characterised by inactive states with short step lengths associated with high turning angles (Hopcraft et al., 2014; Kerk et al., 2015). Encamped behaviour allows individuals to occupy resource-rich or low-risk environments for longer periods (Hopcraft et al., 2014; Zollner and Lima, 2005). When animals display higher turning angles, along with faster steps, ecologists infer 'search' movement patterns, which increase the likelihood of individuals finding resources (Patterson et al., 2008). Finally, fast, straight movements are associated with long-distance movement between patches of resources in separated habitat patches (Fahrig, 2007; Ferreras, 2001; Patterson et al., 2009; Revilla et al., 2004). These inter-patch behaviour might connect structured populations, and may lead to the colonisation of unoccupied areas or recolonisation of locally extinct populations, making this movement pattern fundamental to metapopulation dynamics (Bowler and Benton, 2005; Fahrig, 2007; Ferreras, 2001; Revilla et al., 2004). inter-patch behaviours are generally expected to occur in landscapes where resources are scarce and patchy or mortality risks are unpredictable or highly variable in space, and long-term occupancy of a patch is unlikely (Fahrig, 2007; Forman and Alexander, 1998; Graves et al., 2007; Revilla and Wiegand, 2008; Zollner and Lima, 2005). The importance of inter-patch connectivity has been demonstrated for long-term population persistence of Iberian lynx (*Lynx pardinus*) (Ferreras, 2001; Revilla et al., 2004) and puma (*Puma concolor*) (Sweanor 2000). Because inter-patch behaviour is an animal-landscape response (Revilla et al., 2004), understanding the relationship between specific behaviour and population persistence can improve our ability to manage and conserve populations in fragmented landscapes (Fahrig, 2007; Nathan et al., 2008; Revilla et al., 2004; Zollner and Lima, 2005). Therefore, I expect that areas associated with inter-patch behaviour can represent corridors between habitat patches.

Leopards are highly mobile, able to occupy a broad range of environments and have large spatial requirements, making them a suitable species to study the relationship between heterogeneous landscapes and movement behaviours. While leopards are considered the most adaptable of felids to environmental change (Sunquist and Sunquist, 2002), anthropogenic landscape features, such as roads, and commercial livestock farms, contribute to leopard habitat fragmentation (Swanepoel et al., 2013). The spatial structure of solitary carnivores is also related to sex-specific behaviours (chapter 2; Conde et al., 2010), and interspecific interactions (Elbroch et al., 2015).

I analyse sex-related differences, conspecific locations, human-associated models, and landscape characteristics, in relation to leopard inter-patch behaviour to identify the factors that may relate to the connectivity of leopard habitat in the Eastern and Western Cape, South Africa. I predict that sex-related differences should result in different movement strategies in threatening environments, as males and females are known to respond differently to the environment (chapter 2), and that both sexes will display inter-patch behaviour when closer to anthropogenic landscapes, as these habitats are likely fragmented and may be associated with higher mortality risk. I also predict that same-sex conspecifics should result in inter-patch behaviour to avoid competition. Understanding the relationship between inter-patch behaviour patterns and landscape features should assist in identifying areas that promote connectivity of populations and species survival.

Methods

For study site and methods for capturing leopards please see Chapter 1.

Leopard data

I used 14 591 and 9562 GPS points from 12 males and 6 females respectively (Table 1.1, indicates the collars set at six hourly collection intervals used in this chapter). I used GPS data collected at six-hour intervals to ensure standardised time-frames between data points. I pooled same sex data to represent male and female movement behaviours as responses to covariates.

Landscape variables

We measured covariates found to influence leopard movement behaviour as a result of anthropogenic features (distance to roads, distance to towns, distance to farmlands, and human population density), land use (distance to protected areas and farms), topographic features (slope and elevation) and conspecific home-range location (distance to the centre of male and female leopard home ranges: Table 2.1) (Bailey, 1993; Bothma et al., 1997; Gavashelishvili and Lukarevskiy, 2008; Swanepoel et al., 2013). While prey density and distribution influences predator movements (Carbone and Gittleman, 2002; Creel et al., 2001), these data are not available at a regional scale for the study area and, unfortunately, could not be included in the present study (Swanepoel et al., 2013). Livestock density has been found to influence leopard demographics (Swanepoel et al., 2013), so I included livestock density to assess how this might correlate with inter-patch connectivity for leopards in this study. All covariates were examined before analysis, and showed low pairwise correlations (Pearson's $|r|<0.6$). Distance to rivers, roads, farms, protected areas, towns and distance to male–male, female–male, and female–female home ranges were estimated as the Euclidean distance using Spatial Analyst (ArcGIS 10.2, ESRI, Redlands, CA, USA). Distance to rivers included perennial and non-perennial rivers, and roads were characterised as paved and unpaved roads larger than 6 m wide. Please see table 2.1 in chapter 2, for sources and scale of digital data used in these analyses.

Models

Hidden Markov models (HMMs) (Zucchini and MacDonald, 2009) provide an objective method to discern latent movement states relating to the intuitive understanding that animal movement is driven by underlying behavioural modes. This allows for the prediction of latent behavioural states, while directly accounting for the serial dependence between successive locations, and has proven to be very successful for analysing movement data (Franke et al., 2004; Kerk et al., 2015; Langrock et al., 2012; Michelot et al., 2016; Patterson et al., 2009; Towner et al., 2016).

Leopards' step lengths were drawn from a zero-inflated gamma distribution and von Mises distributions to model turning angles (Codling et al., 2008; McKellar et al., 2015; Michelot et al., 2016). Step lengths are discussed in kilometres and turning angles were calculated using radians but presented as degrees in the tables and text. Including a zero-inflation point mass in the step length distribution is necessary in the given application because leopards may remain stationary within the four-hourly data intervals. I used the underlying Markov chain to determine the behavioural states of leopards and the variation of the parameters of the turning angles and step length distributions across these states. For example, I expected that the average step length will be shorter and more directed in some states (e.g. encamped states), or longer and non-directed when moving between habitat patches (e.g. inter-patch behaviour) (Morales and Ellner, 2002; Zollner and Lima, 2005). As it was not possible to directly observe behavioural states of individuals during the study, I did not run models with more than three latent movement states to be identified by the Markov chain, in order to achieve biologically interpretable states (Kerk et al., 2015). These latent states were determined using HMMs before including covariates to these models, thereby providing null models. I used an information-theoretical approach (Anderson and Burnham, 2002), Akaike's information criterion (AIC) for multi-model comparison, and statistical inference to determine if two or three behavioural states had a greater explanation of the data (Kerk et al., 2015). (Popov et al., 2017). The difference in spatial positioning, and movement parameters between sexes for territorial carnivores is known to occur in felids (chapter 2) (Colchero et al., 2011; Sunquist and Sunquist, 2002); therefore, movement models were run separately for sexes.

Covariates

Once the latent movement patterns were identified, I used these movement parameters (the scale parameter of the gamma distribution and mean direction of turning angles) as a function of anthropogenic factors, sex, conspecific locations and other landscape variables to analyse the relationship with leopard inter-patch behaviour (Bowler and Benton, 2005; Fahrig, 2007; Patterson et al., 2009). I used AIC for multi-model comparison and statistical inference to determine which models had a greater influence on movement states (Kerk et al., 2015). Each variable was first run individually and compared to the null model to determine the explanatory value of variables in predicting leopard movement patterns (Table 4.1). Forward stepwise multivariate models were built using the variables with the lowest AIC value first. Then the second lowest variable was added to models to test global models on leopard movement. If the multivariate models' AIC reached the null models' value, these became less meaningful in explaining how leopards moved as a result of covariates. Models with the lowest AIC were selected as explaining leopard movement behaviour. Covariates in HMMs are assumed to influence the transition between states rather than the state-dependant distributions, which remains fixed for a specific variable (Patterson et al., 2009; Popov et al., 2017). The transition probability matrix (tpm) was used to examine how covariates may predict when animals switch from one state to another (Michelot et al., 2016). However, a direct interpretation of the parameters in tpm is not straight forward, as it does not consider the net effect of the covariate on the state probabilities (Popov et al., 2017). Due to complexities in interpreting Markov chain parameters, I also examined the stationary distributions for fixed levels of the covariates (Patterson et al., 2009). By holding competing variables in the multivariate models at their mean and allowing one variable to fluctuate, I could examine the behaviour of the model and the non-fixed covariate at each movement state (Patterson et al., 2009; Popov et al., 2017). This allowed me to draw firmer conclusions about the implications of the particular covariate on movement states (Patterson et al., 2009).

Finally, I used the Viterbi algorithm to predict the most likely sequence of movement states based on observed movement characteristics (Zucchini and MacDonald, 2009), and assign a likely movement mode to each observed movement segment for male and female leopards (Langrock et al., 2012; Michelot et al., 2016). Depending on the underlying behaviour (e.g.

encamped, search or inter-patch state), the proportion of time spent in each movement state could be different. Therefore, I estimated the proportion of time spent in each state. I investigated models (Table 4.2) relating to the hypothesis that leopard movements are influenced by anthropogenic landscape features, conspecifics' home-range locations, and sex.

To assess the fit of models to leopard movement data I examined Quantile-Quartile (q-q) plots for step length and turning angles (Forester et al., 2007; Michelot et al., 2016; Patterson et al., 2009; Pohle et al., 2017). I used the package 'moveHMM' (Michelot et al. 2016) in R (R Development Core Team, 2014) to discern step lengths and turning angle parameters, fitting HMM models, tpm, and for estimating state occupancy with the Viterbi algorithm. Estimating the stationary distribution required script to be developed separately, as there was no package available in R to do this.

Results

Model fit to movement behaviour

I selected the three-state movement model based on the lower AIC values compared to the two-state models (Table 4.2), because the outputs were biologically interpretable, and the residual plots for step length and turning angles indicated a good fit for three-state HMMs (Supplementary figure 1, Supplementary figure 2). Based on the state-specific parameters, the three states representing specific behaviour modes were identified as: 1) encamped mode, characterised by short step lengths and high turning angles; 2) search mode with longer step lengths and moderate turning angles; and 3) inter-patch behaviour, with long step lengths and low turning angles (Figures 4.2 and 4.3).

Movement behaviour parameters

The average step lengths in the encamped mode were shortest for both sexes (0.05 km – males (M); 0.02 km – females (F); Figure 4.4), while step lengths for males were longer than females in both search mode (5.5 km M; 0.4 km F; Figure 4.4) and inter-patch behaviour (2.2 km M; 1.4 km F; Figure 4.4). Males displayed the longest step lengths while in search mode, while females had the longest step lengths when in inter-patch behaviour (Figure 4.4). For both sexes turning angles were high in encamped states (176.1° M; 177.9° F in state 1) and in search mode (179.9° M; 137.5° F in state 2), and low turning angles in inter-patch behaviour (-6.6° M; -2.4° F in state 3) (Table 4.3).

Movement behaviour occupancy

I observed males in inter-patch behaviour 70% of the time, compared to 40% for females. Search behaviour appeared to be used more by females (40%) compared to males (10%), while both sexes spent 20% of their time in encamped mode (Figure 4.5).

Multivariate models

Male and female movement behaviours were predicted by different models (Table 4.2). The strongest model explaining movement behaviours for males was distance to females and towns, while for females, distance to roads and towns best explained movement states (Table 4.2). This supports the hypothesis that sex-related differences infer different movement patterns and that sexes employ different strategies under the same environmental conditions.

Holding one predictor constant at its mean to understand the best models, the stationary distribution for males indicated that search behaviour (state 2) increased as males became closer to females (Figure 4.6). By contrast, inter-patch behaviour (state 3) increased closer to towns (Figure 4.6). The transition from encamped mode (state 1) to inter-patch behaviour (state 3) did not show transition between states, while search mode to inter-patch behaviour (state 2 to state 3) decreased when distance to females increased (Figure 4.7). The stationary distribution for females indicates that inter-patch behaviour (state 3) increased as distance to towns and distance to roads increased (Figure 4.8). Females switched from search to inter-patch behaviour (state 2 to state 3), from 10% to approximately 40% as distance to roads increased (Figure 4.9).

inter-patch behaviour patterns were the dominant state for males in relation to livestock density, distance to protected areas, distance to rivers, distance to farms, and distance to male conspecifics (Figure 4.10). For females, inter-patch behaviour was the dominant movement pattern at higher elevation levels, steep slopes, where there was low livestock density, at variable distances to female home ranges (close and far away), further from males and further from roads and towns (Figure 4.11). While both sexes employed different inter-patch behaviour strategies in relation to anthropogenic features, search behaviour was the dominant state for both sexes, suggesting that anthropogenic environments reduce connectivity efforts of both sexes.

Discussion

Leopard movement parameters were based on both step lengths and turning angles. Three distinct movement behaviours were identified: 1) encamped, 2) search, and 3) inter-patch. During encamped mode, leopards were largely immobile (Hopcraft et al., 2014; Morales and Ellner, 2002), whereas they were mobile in both search and inter-patch behaviour, with higher turning angles when searching, versus nearly straight lines when displaying inter-patch behaviour.

Sex-related differences

This study found sex-specific differences in movement patterns. Because male leopards occupy larger home ranges than females, they need to move further to reach resource-rich patches (Bailey, 1993; Bothma et al., 1997). Furthermore, the differences in time spent in movement states between sexes can also be attributed to reproductive status. For example, female Florida panthers (*Puma concolor coryi*) with cubs occupied different movement states than females without cubs (Kerk et al., 2015). Males in most polygynous mammals can fertilise many females, so they gain a net fitness benefit from travelling further and more frequently to find females than would females that typically need only one fit male as a mate. Furthermore, females with cubs may experience a net loss of fitness if they encounter many males that have no confidence in paternity and may opt to kill the cubs (Balme and Hunter, 2013). These sex and reproductive differences might help to explain why movement modes made up different frequencies of occupation between sexes, with the most occupied state for males being inter-patch behaviour (70%) and only a short time in search mode (10%), whereas females spent equal time in inter-patch and search mode (40% each). Therefore, female leopards as in many solitary, territorial mammals, would be expected to make fewer inter-patch behaviour and more search movements to find prey in their local area. Males might be expected to optimise inter-patch behaviour to find mates and search for food and shelter once in a patch.

Conspecific interactions

If the above hypothesis about sex differences were correct, one could would expect that males increased inter-patch behaviour when far from females and then switched to search mode when near a female. This prediction assumes males can detect receptive females within a patch and that territorial males do not exclude all rivals. This was supported by the observation that search mode was dominant in response to distance to females and that the tpm indicated an increase in search mode as distance to females decreased. Without data on male hierarchies or female reproductive state, I could not refine the prediction according to the dominance status of rival males or the receptivity of females; however, the observation that males displayed dominantly inter-patch behaviour as a function of distance to male conspecifics provides support for the conspecific avoidance hypothesis.

Male spatial structure had a relationship to female movements whereby females increased inter-patch behaviour the further away males were. This is the same response females had to ‘threatening’ landscapes, such as roads and towns. This raises the question whether the average female perceives males as threats. One negative effect males have on females is the risk of infanticide. The effect infanticide has on the socio-spatial structure of leopards has been reported by Balme and Hunter (2013): high reproductive success is rewarded to the perpetrator, and male avoidance strategies are employed by females. For this hypothesis to be supported I expect that females have cubs and that females are not in oestrus. Alternatively, following the human-shield hypothesis, whereby animals select areas close to humans to reduce competition from conspecifics, and assuming that females recognise individual males, being closer to known males may shield cubs from unknown males, thereby reducing infanticide. Simpler alternatives would be that females are reproductivity receptive to males or being closer to males may provide other benefits, such as hunting success. In both of these scenarios one could expect that encamped behaviour by both sexes in response to each other increases as distances decrease. This was observed in females, but males' encamped behaviour increased further away from females. Without data on the presence of cubs, the reproductive state of females or their hunting success while near males, I could again not refine the prediction according to receptivity of females or hunting success. Nonetheless, the movement patterns of females in relation to males followed the same pattern in relation to threatening habitats. Thus, we may expect that females consider males as threats, which could have important implications in the sociospatial structure of solitary felids.

The relationship between female counterparts indicated an increase in inter-patch behaviour when both closer and further away from other females, while search mode became dominant at intermediate distances from other females. This indicates a more dynamic quadratic model in response to female–female conspecific interactions. Search behaviour at intermediate distances from other females indicates a variation between conspecific avoidance and attraction and could show support for the 'copycat hypothesis' in females (chapter 2), whereby animals identify resources based on counterpart presence, which contributes to conspecific attraction (Parejo et al., 2004). Females may search for prey when far from female rivals but then two types of female responses are seen when near rivals. Perhaps the dominant females approach rivals rapidly and subordinate females avoid their rivals rapidly, which would align with intraspecific competition avoidance. Obtaining prey availability data could allow future researchers to investigate the effect resources have on same-sex conspecific interactions. This will allow the investigation of the hypothesis that land tenure influences sociospatial structure of solitary carnivores such as cougars (*Puma concolor*; Elbroch et al., 2013). Including data on food resource availability and female hierarchical structures could help to disentangle the influence of conspecific interactions to movement behaviours, so we can better understand how social factors influence movement patterns.

Landscape features

Patch selection is predicted to reflect the richness of resources as well as the risks within different patches. Above I assumed males moved between patches in pursuit of females and females moved between patches in pursuit of prey, following the well-supported hypothesis of Crook (1970) and Sandell (1989): females distribute themselves according to food availability, whereas males distribute themselves according to female availability. The best model explained male movement patterns were related to female counterparts and distance to towns. By contrast, female movement patterns were better explained by anthropogenic factors such as distance to towns and distance to roads (Table 4.2). Anthropogenic landscapes correlated to both male and female leopard movements but did so differently. Male leopards increased inter-patch behaviour when closer to towns and roads, while females increased inter-patch behaviour further from towns and roads. I interpret the female pattern as risk averse, making riskier, longer distance movements rarely when nearer to people. The male mode might be construed as risk-taking but might also reflect a strategy of finding females they know from experience are less mobile in such landscapes.

However, both sexes also face the optimisation challenge of avoiding risks. Leopards are exposed to risk from dominant conspecifics as well as humans. Using the same study area, McManus et al., (2014) found that landowners used lethal methods (leg-hold traps, shooting and poison) to kill leopards in response to real or perceived past threats. Some of the risks perceived by leopards are associated with the physical presence of humans (e.g., shooting), whereas others are associated with secondary human aspects, such as the smell, hardware, settlements, and infrastructure of humans (e.g., traps). Moreover, the quality of a patch as a source of food may not be independent of its risk, if humans also hunt the prey of leopards or protect livestock production from predators by employing lethal or non-lethal controls, or if humans pursue leopards based on sign or other evidence. The antipredator response of large carnivores to various forms of human persecution is rarely studied. However, we know that large carnivores, such as leopards, face both opportunistic attacks by people who incidentally encounter them during other activities and deliberate search by people trying to find the large carnivore (Swanepoel et al., 2014). The two methods probably pose different risks for leopards at different times and places. Lacking such data for my study area and study period, I used landscape features as proxies for the risk posed to leopards.

In contrast to inter-patch behaviour, search mode was dominant near towns and roads for both sexes, resulting in increased time spent in these areas but less conspicuous movement patterns. Search mode may allow leopards to estimate the risk of interactions with traffic or humans by moving more carefully, but this also increases the exposure time to human encounters and exposure to human infrastructure that may include traps and poison. Towns and roads are dangerous for wildlife, as has been found for wide-ranging carnivores elsewhere (Colchero et al., 2011; Conde et al., 2010; Forman and Alexander, 1998). These landscapes reduce habitat availability, and increase edge effects and mortality due to accidental or intentional human causes, all of which contribute to major population declines for large carnivores (Mace et al., 1996; McRae et al., 2005; Woodroffe and Ginsberg, 1998).

Alternatively, large carnivores might spend time in human-altered habitat to acquire prey or other resources, or as an adaptive behaviour to avoid interference competition from counterparts, following the human-shield hypothesis (Boydston et al., 2003; Hebblewhite and Merrill, 2008; Knopff et al., 2014). For example, leopards in Maharashtra, India occupied areas with a high human density for highly available potential domestic prey, such as dogs, cats and livestock (Athreya et al., 2016). Elfstrom et al., (2014) found that young, female black and brown bears (*Ursidae* sp.), with and without cubs, occupied anthropogenic environments to reduce despotic behaviour among conspecifics. These adaptive behaviours to counter intraspecies competition can result in high risk of mortality by humans and ultimately create mortality sinks (Basille et al., 2009; Northrup et al., 2012). I did not have sufficient data on mortality risks or rates for leopards in the landscape to evaluate whether leopards were indeed suffering higher risk near towns or roads. Future research should include sex differences in mortality risk given the differentiated movement modes and patterns reported above.

For males, inter-patch behaviour was the dominant movement pattern correlated with most covariates, including distance to rivers, livestock density, distance to protected areas, distance to farms, and distance to male counterparts. Rivers and protected areas may be important 'safe' areas to promote connectivity between leopard habitat patches, as riparian areas provide vegetation cover allowing concealment from threats and protected areas exclude livestock and associated lethal controls. For females, higher elevation, steep slopes, increased distance from males, and low livestock density were associated with higher frequencies of inter-patch behaviour. Females appeared to switch between movement modes more clearly in relation to covariates than males. I infer two possible reasons for this. Either male movement patterns were largely unresponsive to major changes in habitat type or biophysical features, as befits a habitat generalist species with large ranges (Sunquist and Sunquist, 2002) or there is more uniformity among individual females' movement patterns in relation to landscape and conspecific variables, while individual males may be more variable in movement patterns for models that do not incorporate individual identity. Taking account of individual identity in models could reveal stronger associations between movement patterns and age, sex, reproductive status, competitive ability or experience (Holyoak et al., 2008; Morales and Ellner, 2002).

Sex-related differences related to reproductive strategies, large spatial requirements and conspecific interactions, and anthropogenic landscapes most likely contribute to the high display of inter-patch behaviour for males. Males appear to increase inter-patch behaviour in risky habitats. Since they spend a great amount of time in this mode, males may have increased pressure to survive, which is a conservation concern.

While it is understood that behavioural differences exist between sexes in most species, discerning the causes which underlie differences in movement patterns has been challenging. Florida panthers, for example, exhibited different movement behaviour as a result of sex, age and reproductive status (Kerk et al., 2015). Therefore, future research that considers how demographic differences other than sex alone affect landscape use could further explain what may promote or limit inter-patch behaviour patterns according to these differences. With the use of HMMs, stationary distribution and the Viterbi algorithm, I have shown that differences in movement patterns of male and female leopards appear to be a consequence of sex-specific differences in state-specific movement parameters, conspecific interactions and habitat fragmentation.

Conclusion

Connectivity between habitat patches in fragmented landscapes influences population viability (chapter 3) (Frankham, 2005; Hanski, 1998). State-space modelling can effectively describe leopard movement behaviour and be used to gain insight into the complex responses leopards have to heterogeneous landscapes, while using stationary distribution of variables helps underpin the mechanisms that promote inter-patch behaviour.

The differences in movement patterns of male and female leopards appear to be a consequence of sex-related differences in state-specific movement parameters, conspecific attraction and avoidance, and landscape features. Males displayed inter-patch behaviour in relation to most variables, suggesting a generalist species behaviour response to heterogeneous environments, while females had more specific responses. Pooling leopard data did assist in confirming that sex-related behaviours influence movement patterns in relation to conspecific locations and landscape features; however, running models for each individual should reveal population level effects of leopard movement–environment relationships, particularly for generalist species (Morales et al, 2002; Patterson et al.,2008; Hopcraft et al., 2014).

Males appear to increase inter-patch behaviour in more risky environments close to towns and roads, while females appear to employ inter-patch behaviour away from human activity. This is probably a result of different reproductive strategies. The finding that conspecific avoidance and attraction varies between sexes confirms that sociospatial dynamics relate to movement patterns and should thus influence population structure. Males appear to avoid male conspecifics indicating intraspecific competition avoidance strategies, while they increase search movements closer to females. Females appear to consider males as threats, possibly due to the high cost of reproductive strategies employed by males, such as infanticide. Females have a more dynamic interaction with female conspecifics, supporting the hypothesis that conspecifics affect movement behaviours. While future research needs to answer important questions to reach more precise results from sociospatial influences on movement patterns, this study provides a scientific-based assessment of corridor characteristics beyond theory alone. Managers should consider sex, intraspecific interactions and landscape features when identifying and implementing corridors to maintain gene flow.

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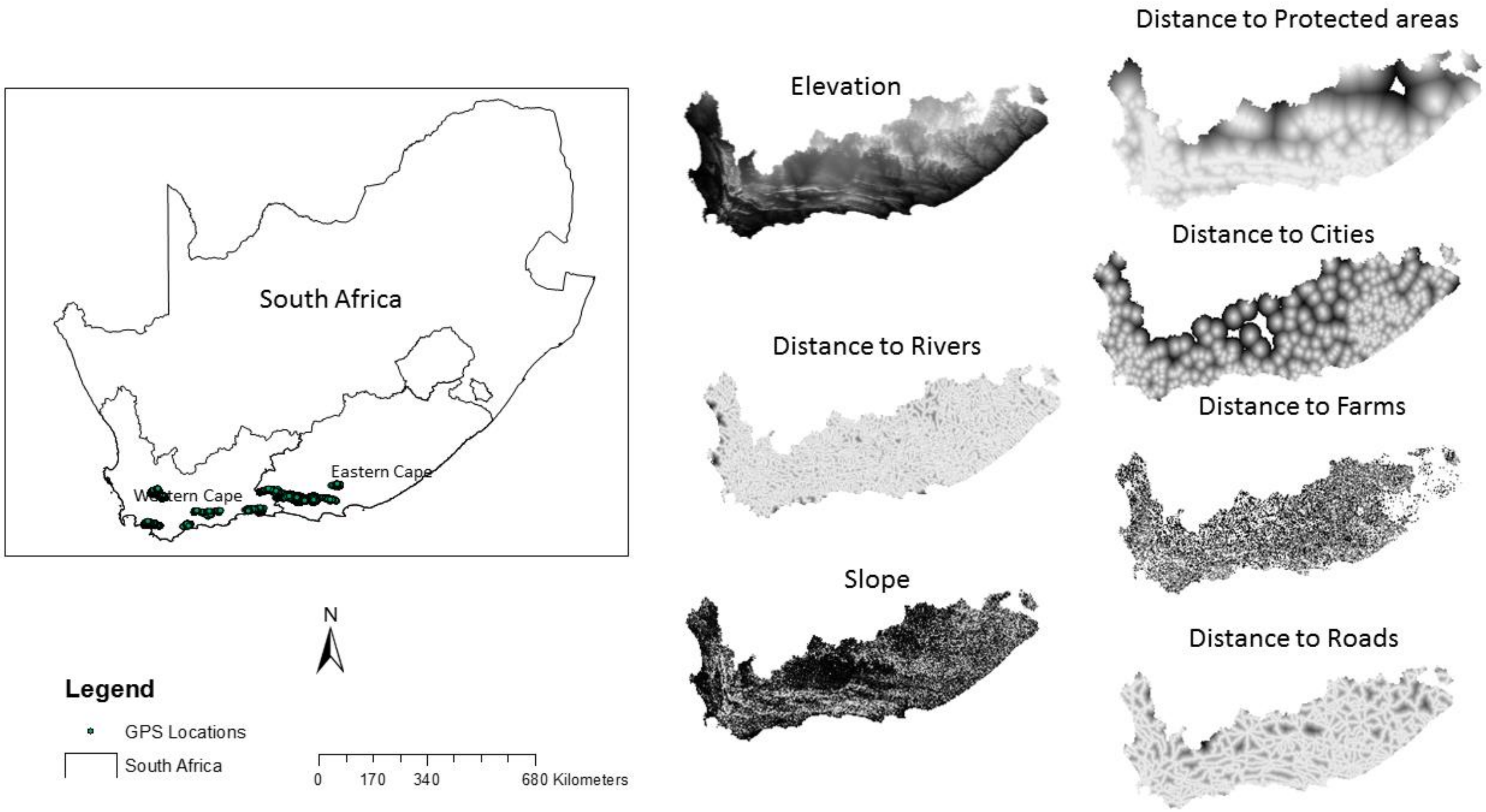


Figure 4.1. Map of the study area in the Eastern and Western Cape, South Africa, showing GPS points from sampled leopards which were used to model leopard movement patterns. Examples of the variables used to examine how movement patterns are affected by landscape features are presented alongside the study area.

Table 4.1. Univariate analyses of 2 and 3-state Hidden Markov Models (HMMs) fitted to male and female leopard GPS data. Akaiks Information Criterion (AIC) was used to inform which models best predict leopard (*Panthera pardus*) movement patterns (step length and turning angles). Bold indicates lower AIC than the null model. Population = human population density/km², Towns = distance to towns, Farms= distance to farms, PA = distance to protected areas, Livestock = livestock density/km², Slope = slope, Elevation = elevation, Rivers = distance to rivers, Males = distance to male home ranges, Females = distance to female home ranges.

	2-state model		3-state model	
Model	Males	Females	Males	Females
null	92193.05	40377.31	90768.28	39421.65
Population	92196.22	**40374.79**	**90756.34**	39427.08
Towns	**92161.54**	**40327.88**	**90741.03**	**39367.23**
Farms	92195.63	40379.3	**90763.62**	39424.69
PA	**92182.39**	40380.58	**90754.94**	39425.16
Livestock	**92176.38**	**40372.51**	**90704.77**	39422.22
Slope	92195.67	40381.04	90770.63	**39420.44**
Elevation	92192.1	**40342.82**	90775.18	**39401.78**
Rivers	**92189.47**	40379.82	**90763.76**	39427.48
Roads	92196.7	**40356.22**	90773.93	**39376.99**
Males	92247.39	**40371.99**	91447.63	39439.01
Females	92076.65	40377.44	**90657.25**	39421.57

Table 4.2. Comparison of the best multivariate models ranked in ascending order based on Akaike's Information Criterion (AIC) fitted to 3-state Hidden Markov Movement models (HMMs) for male and female leopard data. Population = human population density/km², Towns = distance to towns, Farms = distance to farms, PA = distance to protected areas, Livestock = livestock density/km², Slope = slope, Elevation = elevation, Rivers = distance to rivers, Males = distance to male home ranges, Females = distance to female home ranges.

Males		Females	
Model structure	AIC	Model structure	AIC
Females + town	90559.43	Roads + Towns	39323.31
Females	90657.25	Roads + Towns + Population	39327.27
Livestock	90704.77	Towns + farms	39366.40
Towns*Population	90713.25	Towns	39367.23
Roads + Towns + Population	90723.09	Roads	39376.99
Farms + PA (Land use)	90737.66	Elevation + Slope	39382.40
Livestock +Females	90739.42	null	39421.65
Elevation + Slope	90766.27	Livestock	39422.22
null	90768.28	Farms + PA (Land use)	39424.43
Conspecifics (Males + Females)	91992.77	Conspecifics (Males + Females)	39434.22

Table 4.3. Average turning angles (°) in each of the three movement states (State 1 = encamped, State 2 = search, State 3 = inter-patch behaviour) for male and female leopards. Bold text indicates mean (°). Included are the 95% confidence intervals for each movement state.

Turning angles

	Mean (°)		
	State 1	State 2	State 3
Males	**176.127**	**179.851**	**-6.646**
0.95	182.201	181.513	-2.979
0.05	170.054	178.304	-10.428
Females	**-177.961**	**137.510**	**-2.349**
0.95	-184.722	85.714	-6.704
0.05	-171.200	181.628	2.005

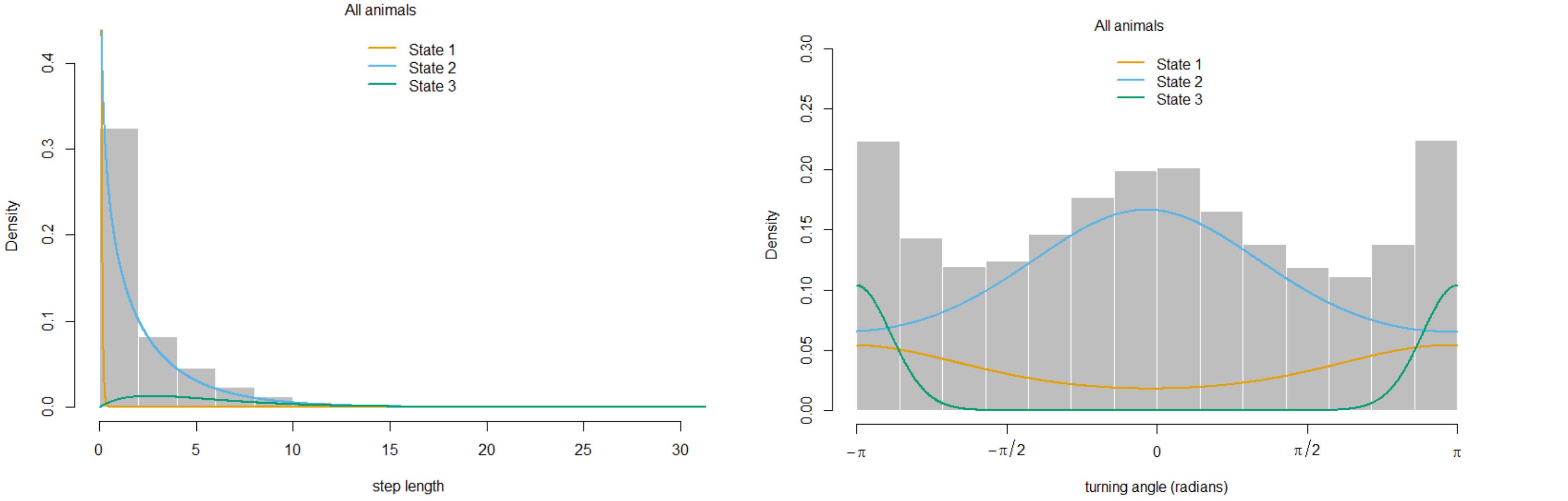


Figure 4.2. Histogram of step lengths (km) with fitted HMM distributions (left), and histogram of turning angles with fitted distributions (right) for each of the three movement states for male leopards. Turning angles were calculated using radians presented here as -π to π, which is equivalent to -180° to 180°. State 1 represents encamped behaviour (short step lengths and high turning angles), state 2 represents search behaviour (long step lengths and high turning angles), state 3 represents inter-patch behaviour (long step lengths and turning angles centered around 0).

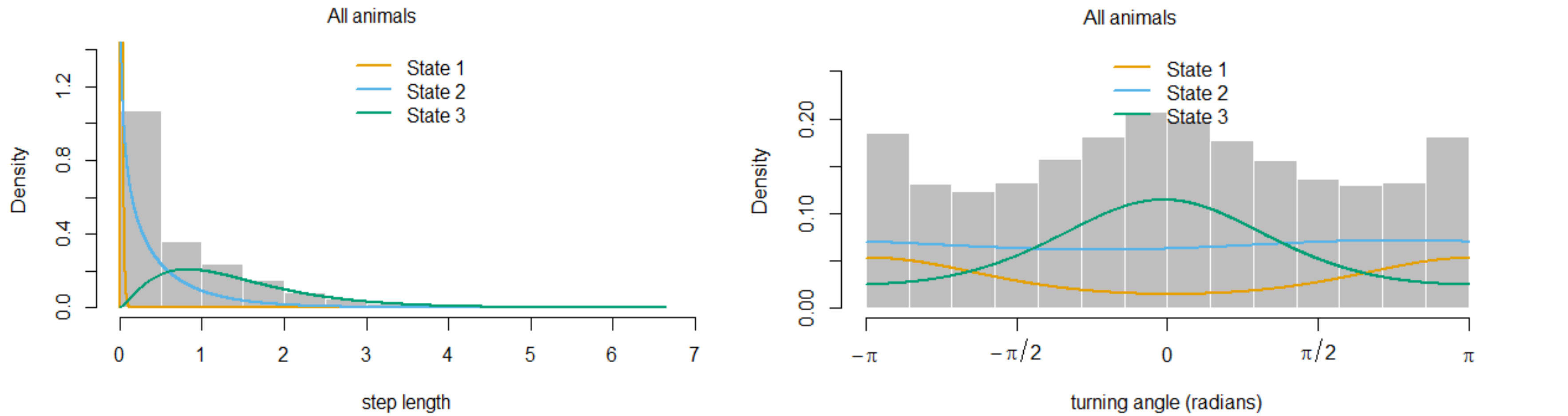


Figure 4.3. Histogram of step lengths (km) with fitted HMM distributions (left), and histogram of turning angles with fitted distributions (right) for each of the three movement states for female leopards. Turning angles were calculated using radians presented here as -π to π, which is equivalent to -180° to 180°. State 1 represents encamped behaviour (short step lengths and high turning angles), state 2 represents search behaviour (long step lengths and variable turning angles), state 3 represents inter-patch behaviour (long step lengths and turning angles centered around 0).

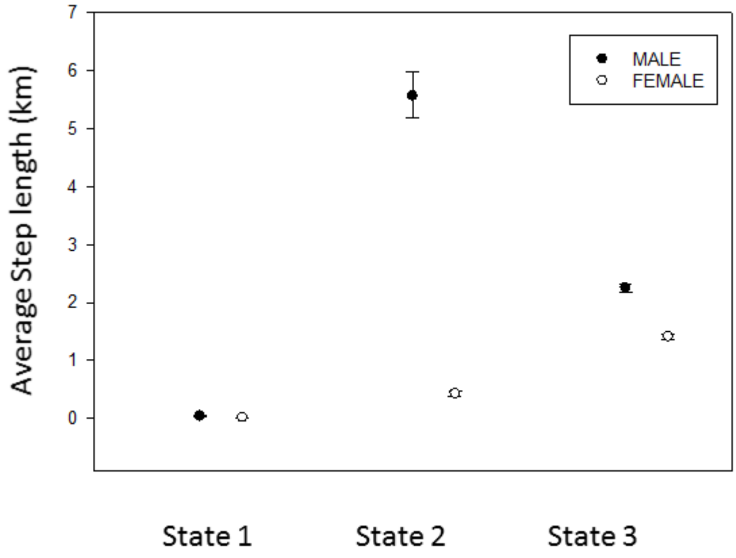


Figure 4.4. Average step lengths (km) for each of the 3 movement states (State 1 = encamped, State 2 = search, State 3 = inter-patch behaviour) for male and female leopards. Included are the 95% Confidence Intervals.

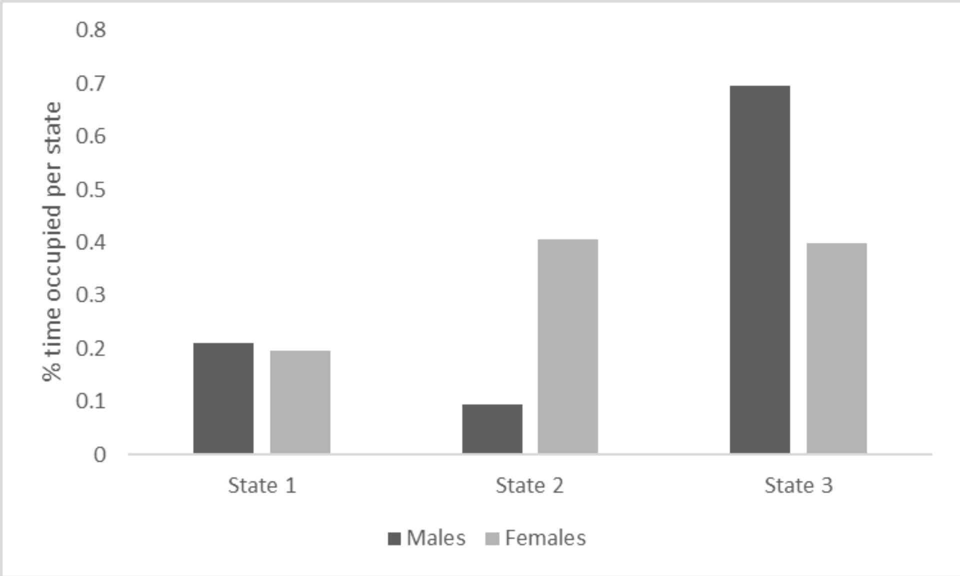


Figure 4.5. The proportion of time male and female leopards spent in respective movement states. State 1 = encamped mode, State 2 = search mode, State 3 = inter-patch behaviour.

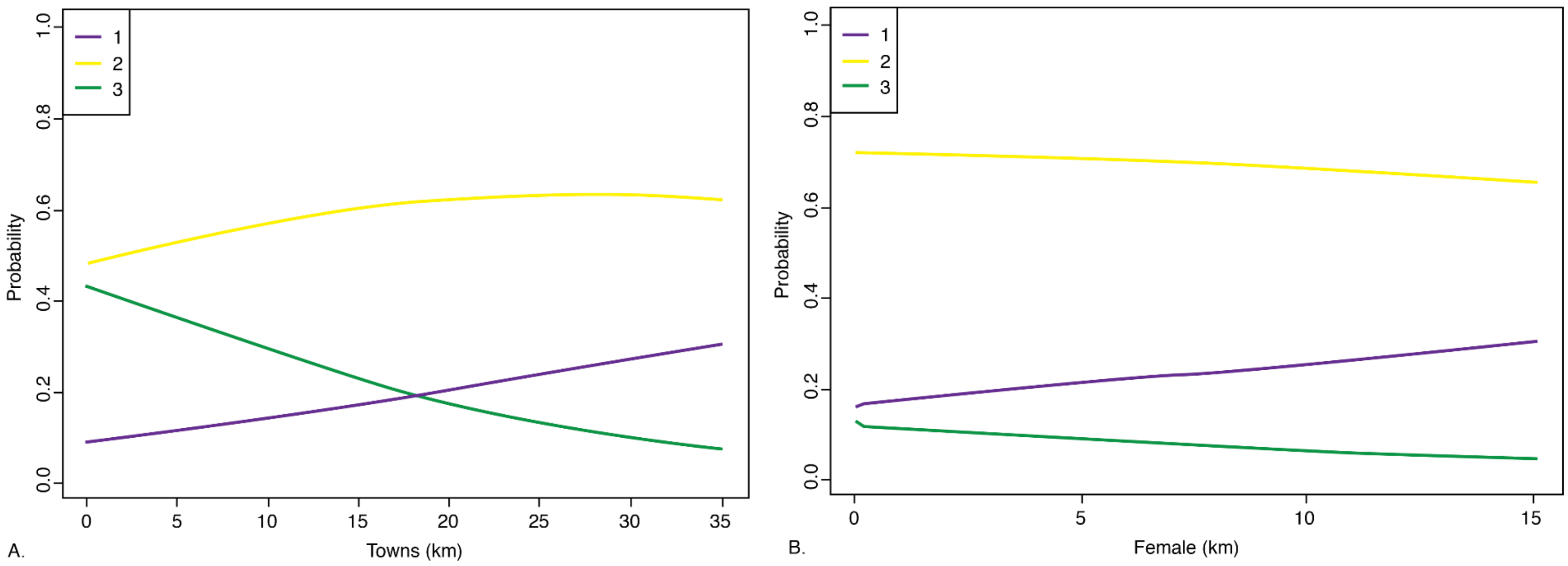
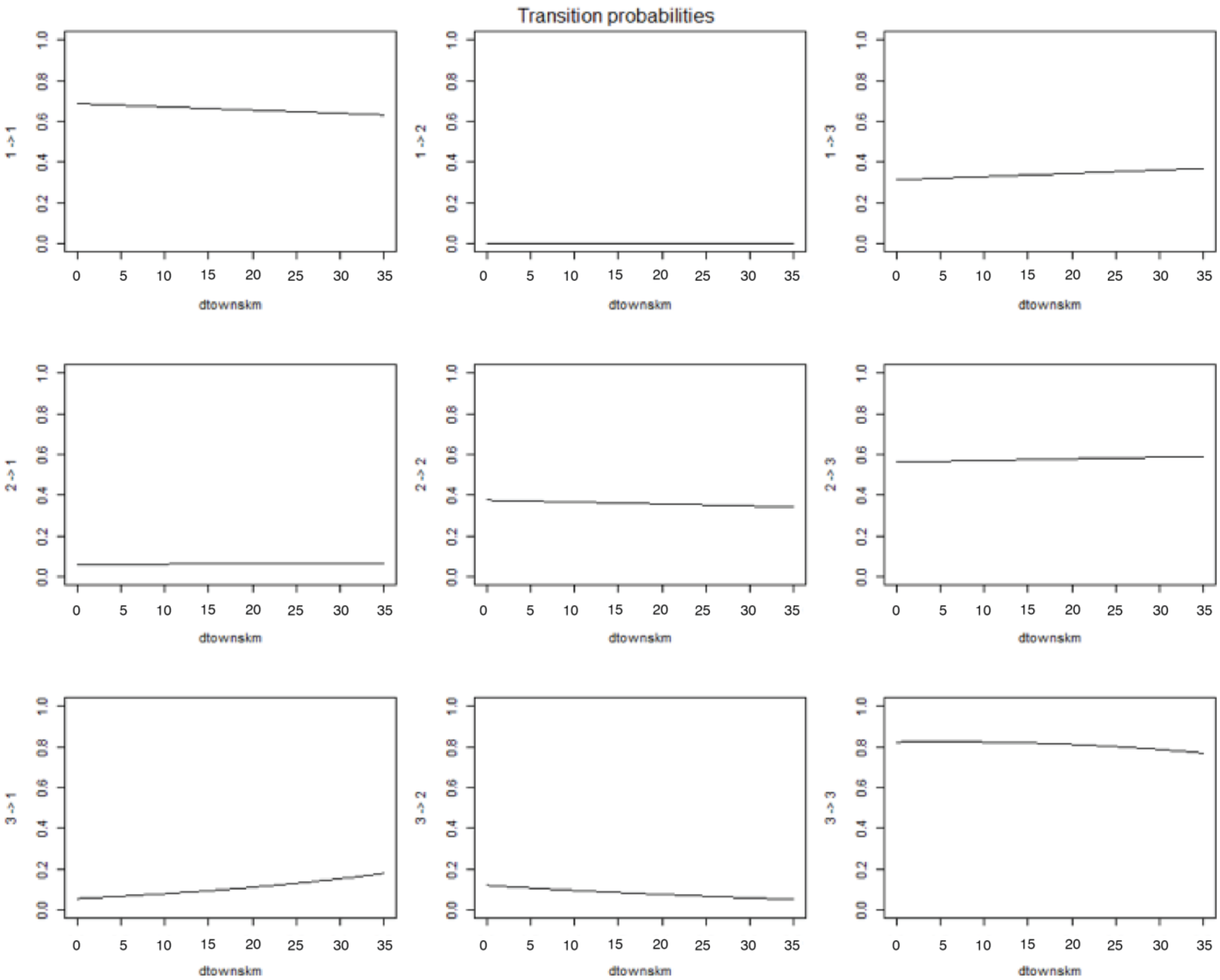
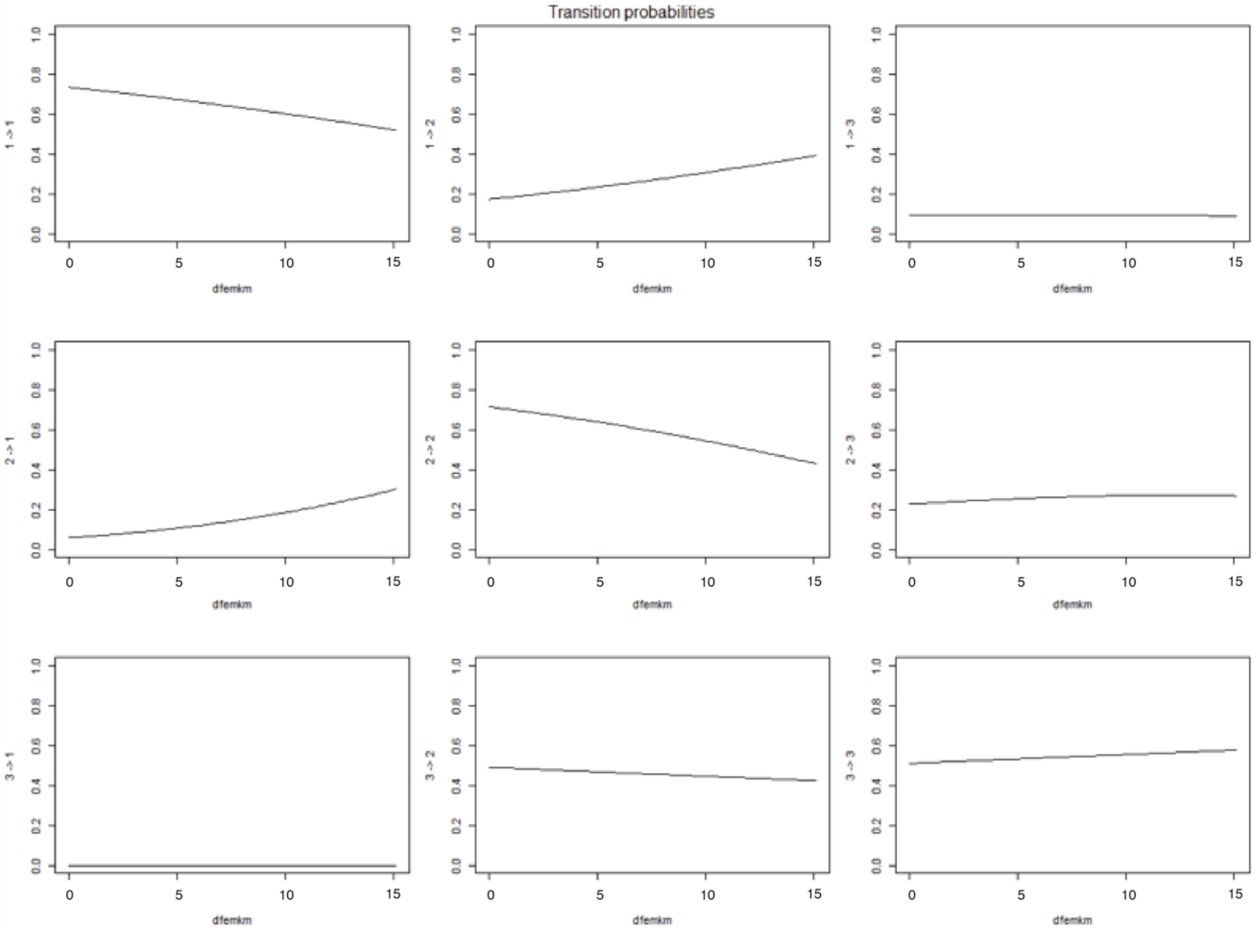


Figure 4.6. The stationary distribution of three movement states (1 = encamped, 2 = search, 3 = inter-patch behaviour) for male leopards as a function of distance to towns (km) and distance to females (km).



A



B

Figure 4.7. Transition probabilities between each movement state for male leopards as a function of A) distance to towns (km) and B) distance to female leopards (km).

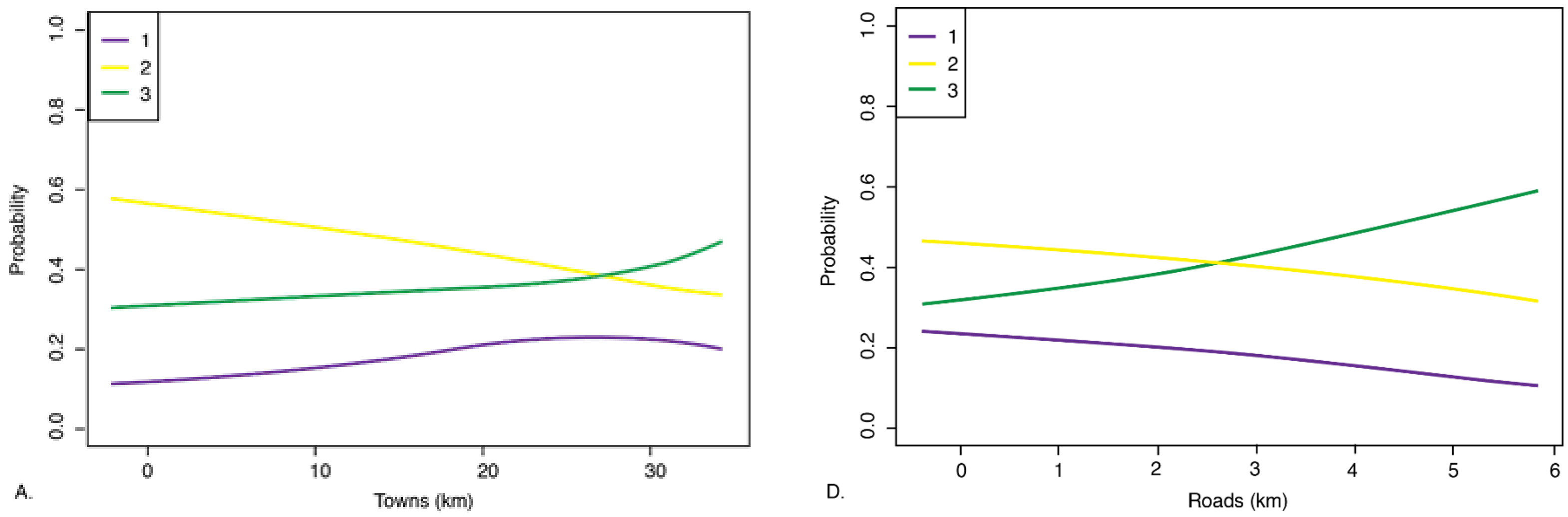
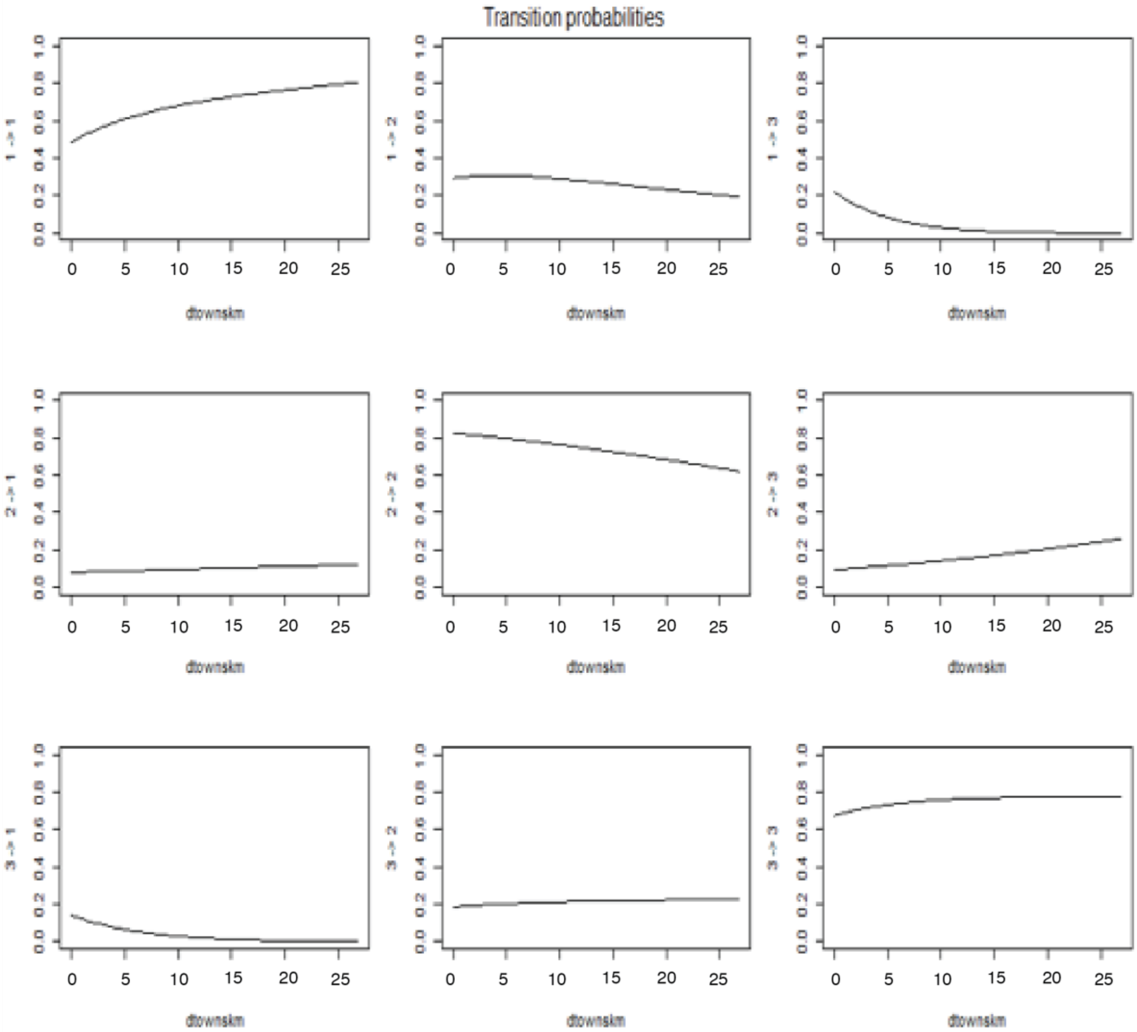
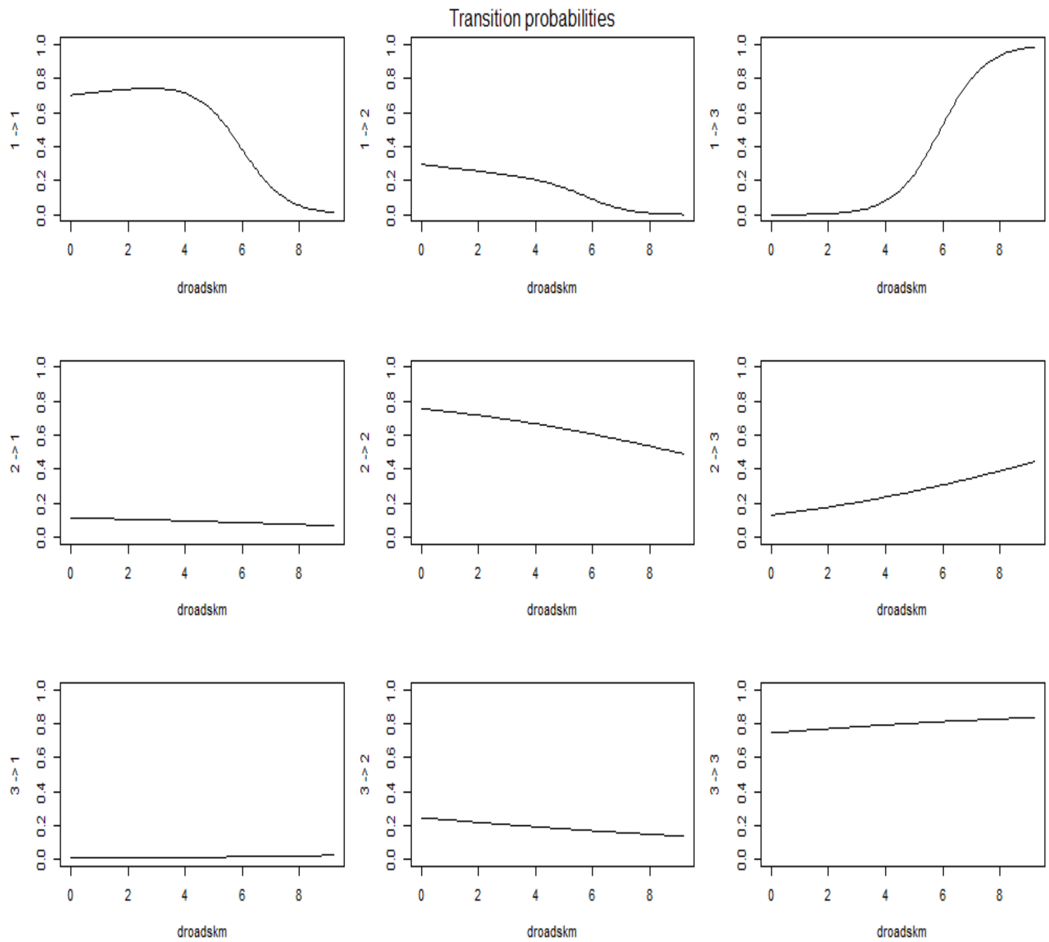


Figure 4.8. The stationary distribution of three movement states (1 = encamped, 2 = search, 3 = inter-patch behaviour) for female leopards as a function of distance to towns (km) and distance to roads (km).

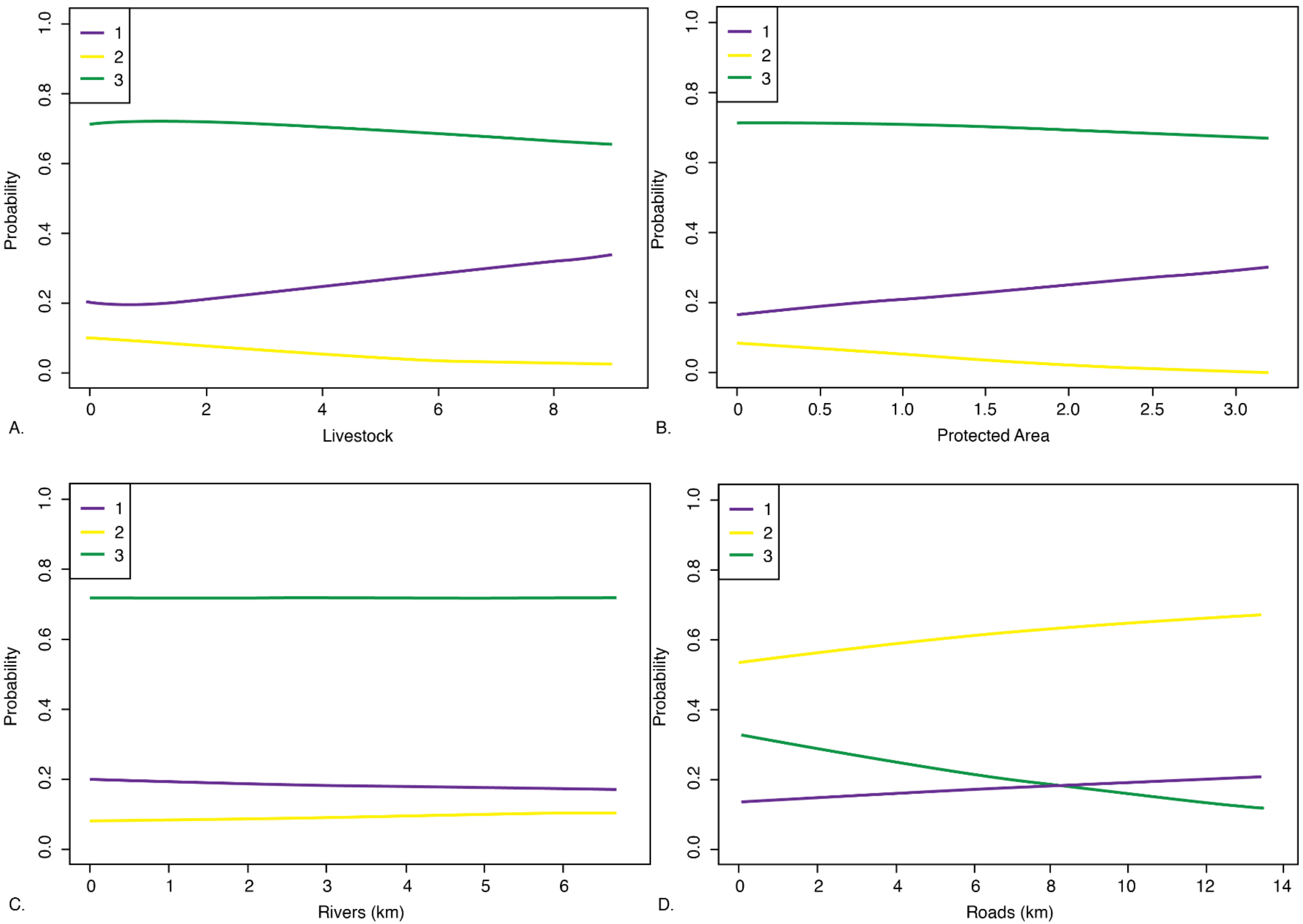


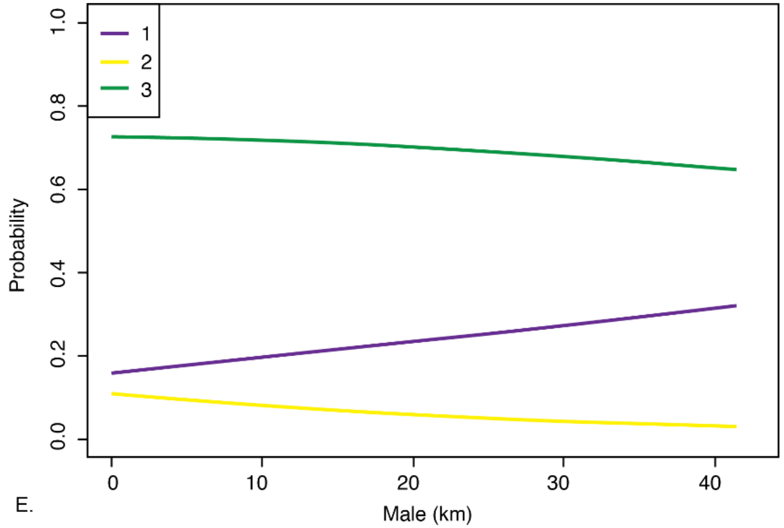
A



B

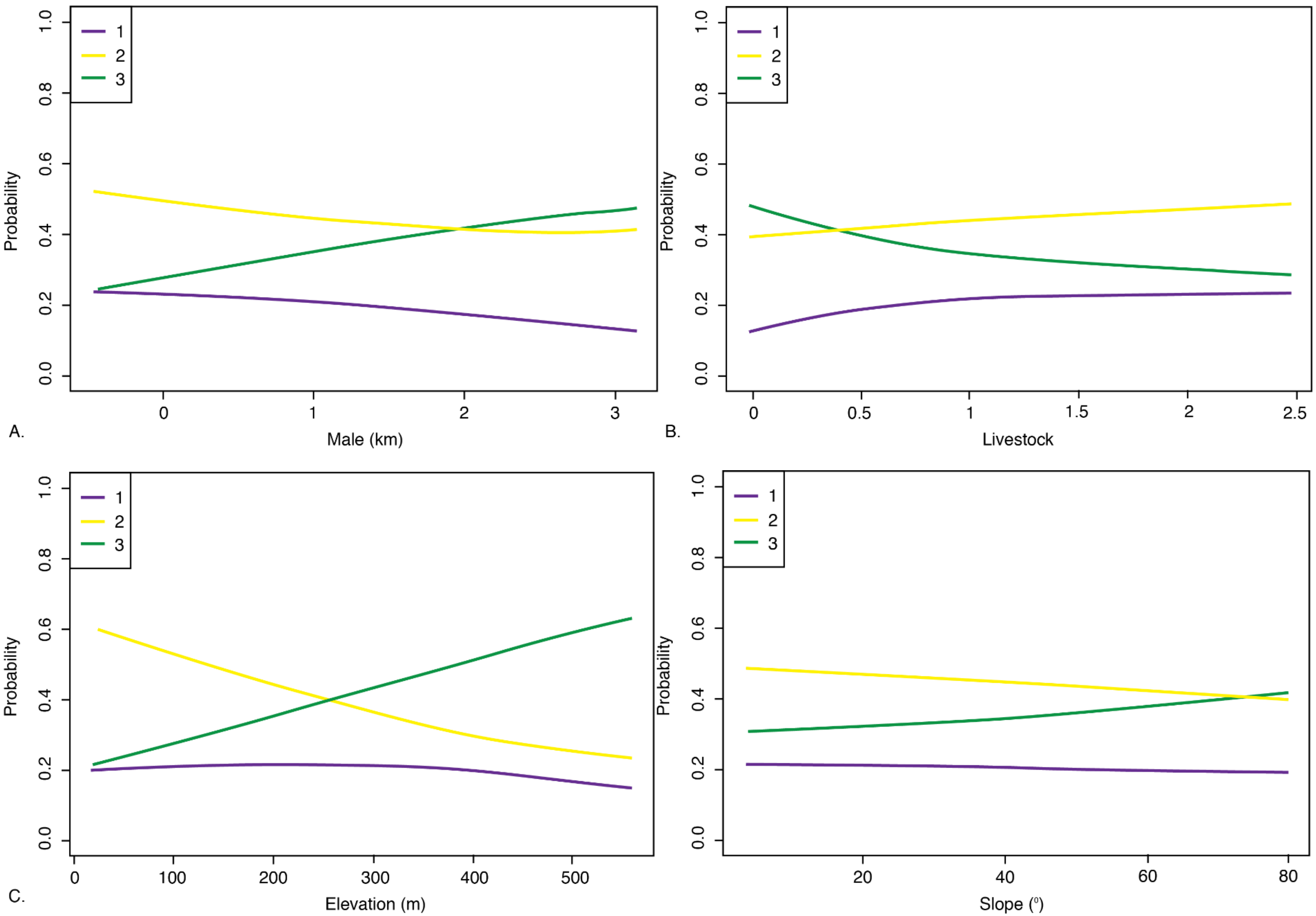
Figure 4.9. Transition probabilities between each movement state for female leopards as a function of A) distance to towns (km) and B) distance to roads (km).


A.
Livestock
Probability
B.
Protected Area
C.
Rivers (km)
D.
Roads (km)
1
2
3



E.

Figure 4.10. Stationary distribution of male leopard movement states (1 = encamped, 2 = search, 3 = inter-patch behaviour) as a function of a) livestock density b) distance to protected area, c) distance to rivers, d) distance to farms, and e) distance to male home ranges.


Probability
Male (km)
A.
Livestock
B.
Elevation (m)
C.
Slope (°)
1
2
3
0.0
0.2
0.4
0.6
0.8
1.0
0
1
2
3
0.5
1.5
2.5
100
200
300
400
500
20
40
60
80

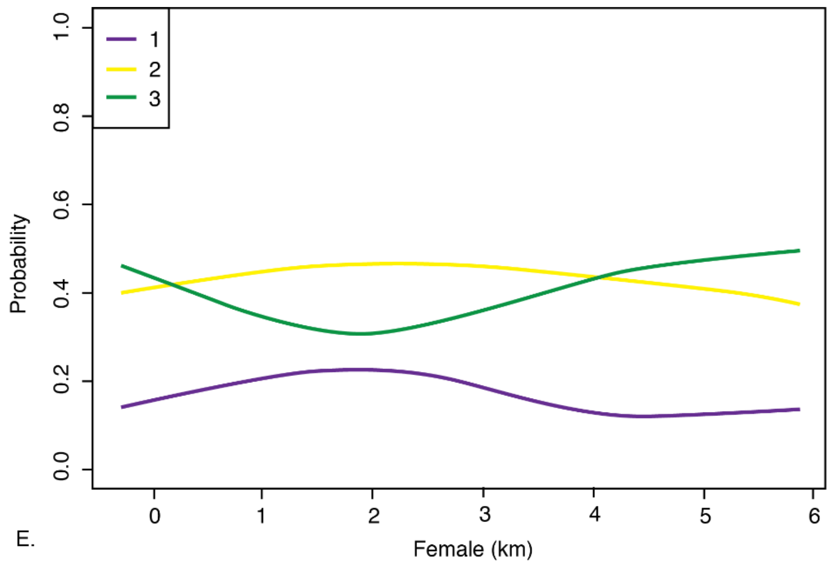
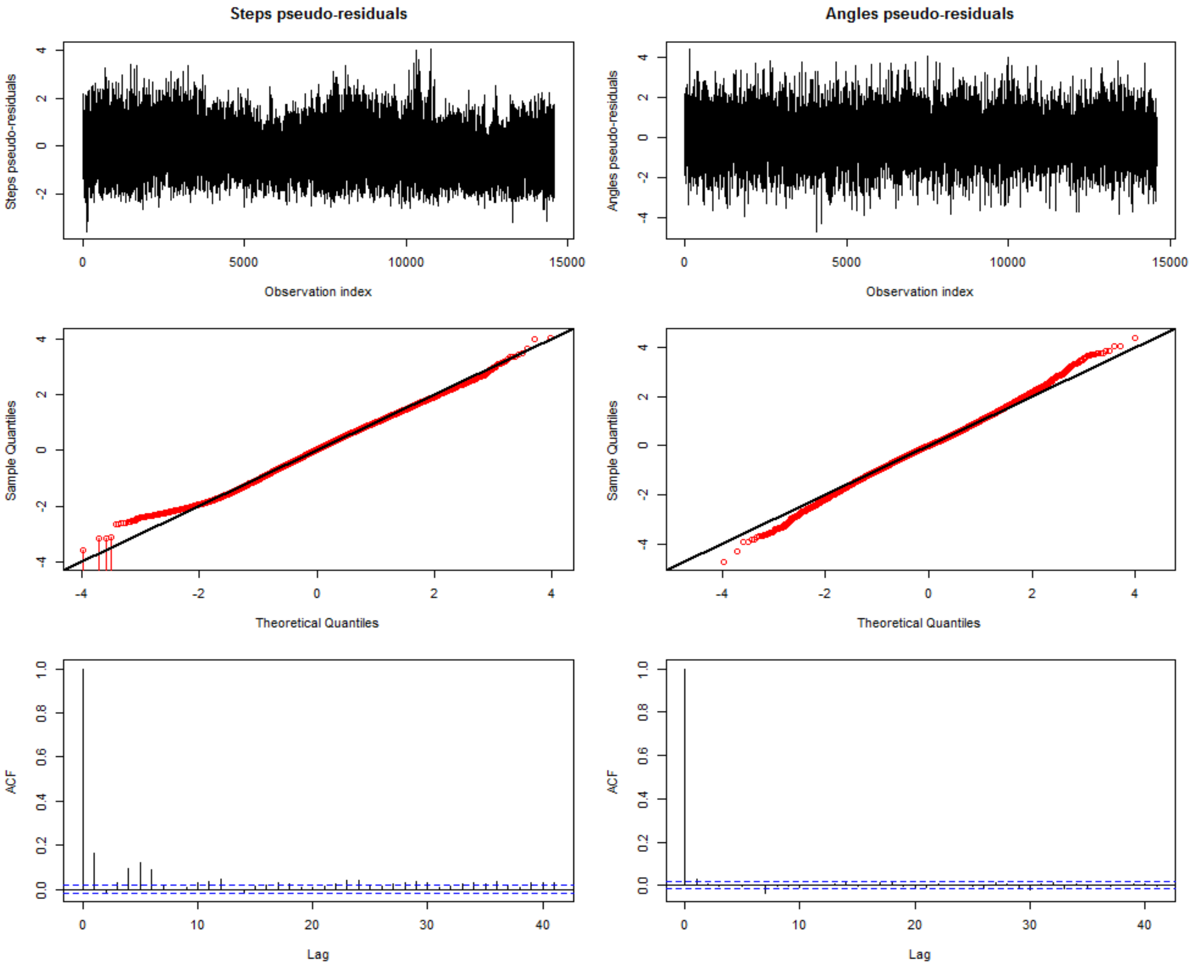
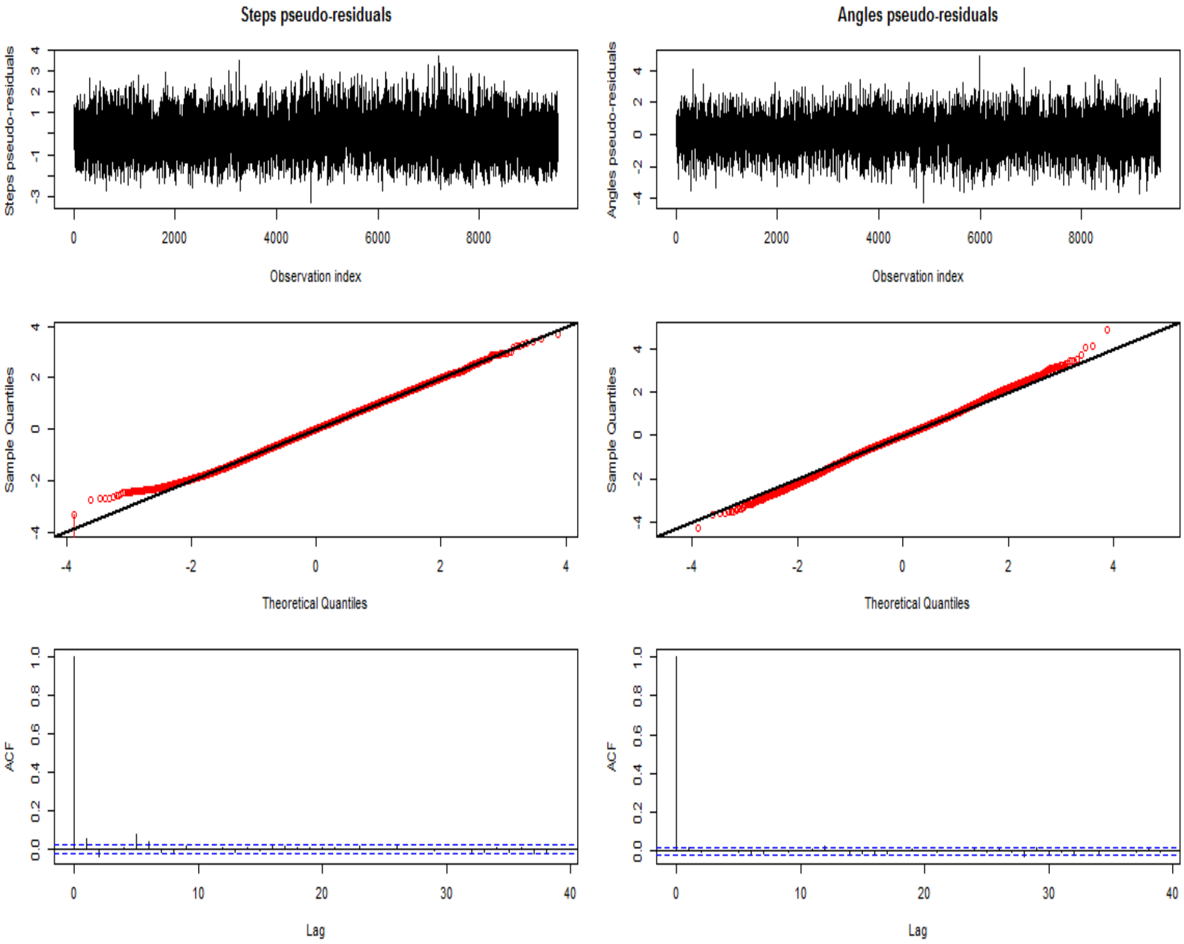


Figure 4.11. Stationary distribution of female leopard movement states (1 = encamped, 2 = search, 3 = inter-patch behaviour) as a function of a) distance to male home ranges, b) livestock density, c) elevation, d) slopes, and e) distance to female home ranges.



Supplementary figure 4.1. Diagnostic plots for three-state hidden Markov models fitted to male leopards for step length and turning angles.



Supplementary figure 4.2. Diagnostic plots for three-state hidden Markov models fitted to female leopards for step length and turning angles.

Conclusion

An individual's choice to spend more time in a particular environment dictates the fitness and survival of that organism that, at a larger spatial scale, affects population dynamics (Revilla and Wiegand, 2008). When habitats are modified or become fragmented, resource distribution and availability change, and habitat patches may become too small for individuals or viable populations to survive in or too far for individuals to reach (Fahrig, 2007; Revilla and Wiegand, 2008; Stephens and Krebs, 1986). These factors are major contributions to the loss of species globally and, for wide-ranging carnivores, these threats are further compounded by human persecution (Ripple et al., 2016).

To conserve species, identifying and protecting critical habitat in the face of rapidly expanding altered habitats is important but challenging, particularly when biological data is scarce. Understanding the relationships between individuals and the environment brings us closer to ascertaining what facilitates individuals and populations to adapt to changes and to survive, and what threatens their survival. Therefore, unravelling the ecological drivers of spatial ecology, genetic structure and movement ecology provides insight into the ecology and evolution of species which is of interest to academics and conservationists alike.

Habitat modelling provides a useful tool to predict species distribution and disentangle driving factors that relate to species presence in specific areas (Boyce, 2006). When certain resources are selected more than these resources are available, it provides insight into why animals select habitat and how their selection affects persistence (Johnson et al., 2006; Morrison, 2001). These decisions may vary as a function of spatial scale (Boyce, 2006). For example, evolution theory suggests that larger scale resource selection is related more strongly to evolutionary processes such as reproduction success during home range selection, while finer scales relate more to individual fitness with lower evolutionary pressures, such as feeding site selection (Rettie and Messier, 2000; Wilmers et al., 2013).

Therefore, understanding the ecological requirements of species involves investigating habitat selection at different spatial scales.

Chapter 2 supports the utility of hierarchical habitat selection, as leopards respond differently to resources and constraints in relation to spatial scale. Leopards selected for protected areas at the large scale. Considering the limited availability of protected areas in the region making up 8% and 15% of land surface in the Eastern and Western Cape respectively (Swanepoel et al., 2013), the associated low human threat, along with higher potential prey densities within these areas, suggests that large-scale habitat selection is related to broader evolutionary responses that are most likely associated with reproductive success.

While protected areas are important to species conservation, these areas are often too small, suffer from large edge effects, and are often areas of low productivity (Cantú-Salazar and Gaston, 2010; Morrison et al., 2007; Soulé and Sanjayan, 1998). Literature indicates that even individuals well within protected areas are negatively affected by edge effects at the borders of these areas, which act as sink habitat and have detrimental effects on populations (Woodrooffe and Ginsberg, 1998; Balme et al., 2010). Consequently, protected areas are usually islands of habitat, insufficient to maintain metapopulation processes, and ecosystem functioning over time.

At the large-scale analyses, females selected areas close to roads when these were nearer to protected areas, indicating that the use of risky landscapes appear to be used when closer to safe habitats. Responses by mammals to roads range from ambivalence to avoidance; however, avoidance strategies often relate to balancing risk, such as using roads with low traffic volume, increased vegetation cover adjacent to roads, or prey availability nearby roads (Forman and Alexander, 1998). This suggests that limiting conditions may become less limiting at different spatial scales, and that conditions may become less limiting when nearer to safe habitats. For example, Palomares et al., (2000) found that Iberian lynx (*Lynx pardinus*) avoid moving into open environments when surrounding habitat is fragmented, but use open areas when surrounding habitat is intact

(Palomares et al., 2000). Additionally, male leopards in this study avoided human-associated landscapes at the large scale more than they did at the fine scale, thus offering support for the evolutionary theory.

Nonetheless, the way animals use limiting factors appears to be related to reproductive strategies at both the fine and large spatial scale. For example, male leopards selected areas closer to towns at the fine scale, but they selected areas closer to females when females were further from towns at the same scale. Therefore, there is a balance between threats and reproductive strategies at both fine and large spatial scales. The relationship between spatial scales and reproductive strategy selection follows the evolutionary theory as leopards avoid risky landscapes more at the large scale compared to fine scale. However, animals appear to balance reproductive success in relation to threats despite spatial scales (Wilmers et al., 2013).

Where animals locate themselves is also a function of sex-related differences within species. Large carnivores display sexually distinct reproductive, social and spatial requirement behaviours. For example, males usually have larger spatial requirements than females and select habitat based on female presence, broadly following selection patterns of a generalist species. Females are known to select habitat based on key resources associated with successful raising of progeny to adulthood (Colchero et al., 2011). As a result, habitat selection drivers differ between sexes. This has been found for other carnivores, such as cheetah (*Acinonyx jubatus*), jaguar (*Panthera onca*) and Florida panther (*Puma concolor coryi*) (Conde et al., 2010). Leopards selected resources differently at both spatial scales as a function of sex, and when the best performing models of one sex were fitted to the opposite sex (chapter 2), models performed poorly. This supports the finding that avoiding sex-related differences in habitat modelling can result in biased representation of habitat if data from one sex are more prevalent than the other. This can result in inappropriate policies and conservation actions (Conde et al., 2010). It is therefore worrying that many of solitary carnivore habitat prediction studies exclude sex as an explanatory variable (e.g. Swanepoel et al., 2013; Gavashelishvili and Lukarevskiy, 2008; Linkie et al., 2006, Klar et al., 2008).

Furthermore, habitat selection appears to be influenced by social interactions as much as by key resource distribution and the associated hierarchical selection processes (Elbroch et al., 2015; Wilmers et al., 2013). At the fine scale, male leopards appeared to select areas closer to male conspecifics, while they avoided each other at the large scale. This indicates a more dynamic interaction between same-sex conspecifics at the fine scale than the widely accepted land tenure model which predicts that males forcefully exclude male counterparts from home ranges (Seidensticker et al., 1973; Ferreras et a., 2004).

However, when important resources are present, such as females, we expected that males would avoid each other when close to females as this could result in increased competition and conflict. We found that males avoided other males in relation to females at both spatial scales. Thus, the land tenure model may be employed as a function of important resource presence, supporting some combination of the land tenure and resource dispersion hypothesis for male leopard socio-spatial structure when resources are present, but appear to be more tolerant of male counterparts when resources are excluded.

Conflicting findings on socio-spatial structure also exist for female felids, as some research suggests, that females display mutual avoidance, while other females display intraspecific attraction (Packer et al., 1986; Seidensticker et al., 1973). For example, both the resource dispersion hypothesis and the land tenure hypothesis account for the behaviour of female mountain lions, suggesting that social hierarchy and resource availability are not mutually exclusive (Elbroch et al., 2015). At the fine scale, females located themselves in relation to female conspecifics, while there was a weak correlation to female conspecifics at the large-scale analyses. This, as between male counterparts, indicates an intraspecific attraction at the fine scale if resources are not limited. For females, this behaviour could be explained by the kinship hypothesis, which predicts that related animals locate themselves near to one another. However, as it is unlikely that all females in the study were related, the kinship model is an unlikely mechanism of this behaviour. We could also reject the land tenure model, as this would show avoidance of conspecifics. Thus, female socio-structure in the Eastern and Western Cape are not structured according to these models. Literature indicates that resource availability affects the socio-spatial dynamics of populations. Unfortunately, since data on prey resources were not available for my study area, I could not investigate the resource dispersion hypothesis, which predicts that conspecific attraction exists when resources are high (Elbroch et al., 2015). For example, mountain lions (*Puma concolor*) showed higher range overlap when resources were high (Elbroch et al., 2015), and female lynx were found to disperse from natal areas and establish home ranges elsewhere when resources were low, whereas philopatry was observed where resources were high (Palomares et al., 2000). Thus, prey resources could be high at the fine scale for this study, resulting in the observed female–female spatial relationship.

However, another factor known to affect socio-spatial structure in female felids is when populations face human-caused mortality, which is present in this study area. For example, persecuted carnivore populations had diminished territorial boundaries and increased female immigration, undermining kinship social structures (Beausoleil et al., 2013, Stoner et al., 2013). Alternatively, females may use a copy-cat model, whereby conspecifics perceive the presence of other females as a proxy for higher quality habitat. Testing these theories could assist in understanding the socio-spatial dynamics of solitary felid distributions and requires data on available prey. The finding that leopards select areas closer to same-sex conspecifics indicates some socio-spatial flexibility and supports the prediction that conspecifics influence population dynamics and that solitary behaviour does not contradict social behaviour (Stoner et al., 2013).

Thus, while habitat prediction studies have predominantly focused on identifying key resources for solitary felids (Gavashelishvili and

Lukarevskiy, 2008; Swanepoel et al., 2013) more recent work, including findings from this thesis, supports the need to investigate more complex relationships. These include sex, spatial scales, and conspecific interactions in heterogeneous landscapes, and the relationships these have in predicting not only species habitat but population dynamics as well.

The distribution of habitat patches defines where species should occur and thus affects population structure. As populations become fragmented by habitat deterioration, the risk of extinction increases due to reduced genetic flow and diversity (Hanski, 1998). Therefore, defining and maintaining connectivity between populations is a priority for the conservation of many species (Moilanen and Nieminen, 2002). Leopards display different movement patterns in relation to environmental factors. Inter-patch movement behaviour is characterised by fast, straight movements that allow individuals to reach and occupy habitat patches, making this behaviour a fundamental process in population dynamics (Revilla et al., 2004; Hopcraft et al., 2014).

The large spatial requirements and the polygamous reproductive strategy of male solitary felids may contribute to their predominant use of inter-patch behaviour (70% of the time) in this study. inter-patch behaviour was the dominant behaviour for males in relation to most variables: distance to male conspecifics, livestock density, distance to rivers, distance to protected areas and distance to farms. The effect these variables had on male inter-patch behaviour was unclear, suggesting that males follow a more generalist approach. By assessing individual males, we should gain a clearer picture of whether individuals undertake different choices under the same conditions. Females appeared to be more unified in movement patterns, indicating that inter-patch behaviour relate to higher elevation levels, low livestock density, variable distances from other female home ranges, being further from males and further from roads and towns.

Although both sexes were found to employ search behaviour as the dominant state near risky habitats, sexes have different movement strategies under the same conditions. Females increased inter-patch behaviour further from towns and roads, while males did the opposite. Males may be optimising finding females in these areas, while females may be undertaking less conspicuous movement patterns to reduce detection, find prey in these habitats or hide from conspecifics by means of the human-shield hypothesis (Elfström et al., 2014). Both strategies are risky, however, as interactions with humans usually results in mortality resulting in these environments being mortality sinks (Elfström et al., 2014).

Since inter-patch behaviour was the dominant behaviour in males, we could expect reasonable gene flow between populations. This assumes that males succeed in reaching neighbouring populations and in reproducing, and that barriers between populations are permeable to some degree. By using microsatellites, we identified three subpopulations in the region, with low to moderate levels of gene flow occurring between populations (chapter 3). Thus, the leopard structure in the Eastern and Western Cape can broadly be described as a metapopulation model. However, other fundamental characteristics of metapopulations have not been demonstrated by this research, including independent dynamics among patches, natural extinctions, and natural recolonisations of extinct populations (Harrison and Taylor, 1997).

The observed high genetic differentiation associated low gene flow, and few private alleles in two of these subpopulations (north and south populations; chapter 3) suggests that these populations may be functioning as sink populations, while the central population had high genetic diversity and private alleles and may be an important source population. Nonetheless, there is evidence that genetic barriers exist between populations and, although leopards are highly mobile, populations are poorly connected genetically.

Reduced genetic transfer can increase differentiation between populations over a few generations (Epps et al., 2005; Balkenhol et al., 2009); thus, ensuring connectivity between populations is important to ensure the survival of the leopard population in the Eastern and Western Cape. Leopards may be making maladaptive decisions in risky environments, which may contribute to the low success of gene flow. For example, the strategy of leopards employing search behaviour near threatening areas such as towns and roads may increase the risk of mortality, as has been found for carnivores elsewhere (Forman and Alexander, 1998). Additionally, the shortcomings of protected areas which were selected at the large spatial scale may indicate that these areas are insufficient to maintain viable leopard populations.

While this research has contributed to a better understanding of the population dynamics of solitary carnivores and their abilities to persist in altered landscapes, more questions were identified that, when answered, can further contribute to our understanding of solitary carnivore biology and conservation. For example, carnivore habitat selection and movement behaviour are strongly influenced by prey density and distribution (Carbone and Gittleman, 2002; Creel et al., 2001), but unfortunately, these data do not exist at the regional scale of this study area. Developing potential prey distribution maps and incorporating these into habitat selection and movement models would allow further understanding of socio-spatial drivers as a result of prey resources, and allow us to infer how these factors affect population dynamics. Furthermore, by incorporating other geographically extensive data sets such as leopard mortality data, one could assess ecological traps and metapopulation dynamics, as well as assist managers to identify conflict hotspots and potential barriers to corridors (Miller, 2015; Treves et al., 2011).

Incorporating multidisciplinary fields such as genetics to habitat models can also be used to generate geographically extensive estimates of abundance (Romain-Bondi et al., 2004), and could allow better understanding of the relationship between abundance and species survival capabilities to the cause of species occurrence.

As leopards have large spatial requirements, ensuring enough space for variable populations is essential. To increase the efficacy of protected areas for wide-roaming species, efforts should focus on increasing connectivity between isolated parks, and ensuring that hard edge effects at the boundaries of these areas are minimised by reducing human–carnivore conflict. Fostering gene flow between isolated populations through territorial connectivity or active management, such as translocations, may be important, because deterioration of gene flow can have detrimental effects within a few generations that would result in low population survival. Accounting for sex, social interactions and spatial scales in habitat prediction models should improve the reliability of predictions and assist in identifying where to focus our management efforts. While we still have many questions to answer, I hope that this work can be beneficial, and serve as a baseline for future studies.

Management implications

Relying on protected areas as a sole conservation strategy has major shortcomings due to small sizes, distances between protected areas, and the edge effects adjacent to protected areas which act as sink habitats. These sink habitats threaten populations living well within protected areas (Balme et al., 2010; Soulé and Sanjayan, 1998; Woodroffe and Ginsberg, 1998). This is particularly true for wide-ranging species such as carnivores which use these edges with devastating results (Woodroffe and Ginsberg, 1998). Additionally, most leopard habitat exists outside or protected areas. Connectivity between habitat patches is a valuable conservation tool to overcome major threats such as habitat modification and fragmentation (Beier et al., 2011; Chetkiewicz et al., 2006).

Males appear to increase inter-patch behaviour in more risky environments close to towns and roads, while females appear to employ inter-patch behaviour away from human activity. The finding that males spent 70% of their time moving in long, straight movements indicates a high level of risky habitat patches, which don’t offer enough for leopards to remain in the area for longer periods, but results in leopards moving through quickly. While employing inter patch behaviour can improve population connectivity, when habitats become too far for animals to reach these efforts are untenable and requires management to ensure habitat integrity and connectivity. Since leopards are not restricted by fences and come into conflict with humans, reducing human-carnivore conflict and lethal carnivore control is an important conservation strategy to ensure these areas can be used as corridors. This thesis provides a scientific-based assessment of corridor characteristics beyond theory alone. Managers should consider the socio-spatial structure of solitary carnivore populations and sex when identifying and implementing corridors to maintain gene flow.

Habitat connectivity in fragmented landscapes influences population viability (Frankham, 2005; Hanski, 1998). Our estimates of gene flow, presented as relative measures of connectivity between populations, provide a useful index to assist management. The north population had low immigration and emigration (0.57–0.51 migrants / generation), with higher gene flow recorded between the central and south subpopulations (3.68 migrants / generation). Predictions indicate that for natural populations to remain genetically viable, at least 10 migrants need to link up to adjacent populations per generation (Mills and Allendorf, 1996). The observed levels of gene flow of leopards in the region are low compared to carnivores elsewhere (Cegelski et al., 2003; Dutta et al., 2013). These findings have important implications for human-caused mortality, particularly where human–carnivore conflict exists, and harvesting is practised. Active harvesting of leopards in the observed substructured populations with moderate to low gene flow will increase the risk of extinction.

As natural habitats rapidly disappear, the need to maintain habitat connectivity becomes greater (Dutta et al., 2013; Ernest et al., 2003). To ensure population persistence of carnivores, their management requires curbing human–carnivore conflict, identifying critical habitat and increasing connectivity between populations.

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