The population dynamics of the black-backed jackal (*Canis mesomelas*) in game farm ecosystems of South Africa

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Abstract

Few studies have attempted to investigate carnivore dynamics in the privatised agricultural sector of South Africa. As such, the effect of lethal predator management on carnivore populations in private game-farms remains unclear. The black-backed jackal (*Canis mesomelas*) is one of a number of species that has historically been perceived as a threat to economic security and has frequently been targeted as a pest species. Despite efforts to reduce or remove *C. mesomelas* from livestock producing areas, recent land owner questionnaires and faecal density surveys report this species as prevalent. The mechanism by which this species persists under such circumstances is currently under debate, and remains a significant question that restricts the sustainable management of this species.

Camera-trap imagery and faecal density estimates were used to examine the influences of lethal control and food availability on the relative abundance of *C. mesomelas* in game-farm environments of the NW province, South Africa. As these factors may affect the social structure and diversity of this species, non-invasive microsatellite DNA profiles were used to quantify inbreeding, relatedness and genetic diversity. Polymorphic microsatellite loci were developed from the domestic dog (*Canis familiaris*) and characterised for cross-species amplification in *C. mesomelas*. Non-invasive DNA samples were acquired from faecal deposits and isolated via a novel technique developed for this investigation.

Camera-trapping results and faecal density estimates indicated that lethal predator control did not significantly reduce the abundance of the black-backed jackal relative to predator neutral management sites, but that predator feeding programs significantly increased the abundance of *C. mesomelas,* relative to predator neutral game farm sites. In addition, carnivore community structure was highly dissimilar between the three treatments. Predator neutral sites had the highest carnivore diversity of all treatment types and sites that practiced lethal predator control were most dissimilar from other treatment types due to the absence of large carnivore species. Reduced intra-species competition, mesopredator release, territorial instability and the perturbation effect are viable explanations for the persistence of *C. mesomelas* under lethal management practices. This study presents tentative evidence that the current localised lethal management of *C. mesomelas* is ineffective and potentially counterproductive in the control of this species.

Microsatellite fragment analysis reveals slight outbreeding at the population level, free migration between predator control and predator neutral sites and significant genetic sub-structuring at predator feeding sites. These results suggest that lethal control results in the increase in territorial turnover while high food availability effects local genetic diversity and results in a reduction in dispersal as well as a break down in territorial stability. A small degree of population structuring indicates a reduction in the dispersal of offspring from their natal range and is an attractive explanation for the high abundance of *C. mesomelas* recorded at predator feeding sites.

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Acronyms and abbreviations

bp	……	base pairs
BSA	……	Bovine Serum Albumen
CMR	……	Capture-Mark-Recapture
C_t	……	Cycle threshold
DEFRA	……	Department for Environment, Food & Rural Affairs
dsDNA	……	double stranded Deoxyribonucleic Acid
FAM	……	Fluorescein amidite
F_{IS}	……	F coefficient for Individuals relative to a Subpopulation
F_{IT}	……	F coefficient for Individuals relative to the Total population
F_{ST}	……	F coefficient for Subpopulations relative to a Total population
F primer	……	Forward primer
ha	……	Hectare
HEX	……	Hexachlorofluorescein
HSD	……	Highest Significant Difference
H_o	……	Observed heterozygosity
H_e	……	Expected heterozygosity
H_t	……	Frequency of heterozygotes in generation
IBD	……	Identical By Descent
IUCN	……	International Union for Conservation of Nature
MCMC	……	Markov Chain Monte Carlo simulation
NCBI	……	National Centre for Biotechnology Information
NMDS	……	Non-metric Multi-Dimensional Scaling
PCR	……	Polymerase Chain Reaction
PI_{ave}	……	Average probability of identity
qPCR	……	Quantitative Polymerase Chain Reaction
RDH	……	Resource Dispersion Hypothesis
RIA	……	Relative Index of Abundance
R primer	……	Reverse primer
SSR	……	Simple Sequence Repeat

Author's declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed

Dated

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Chapter I

General introduction and background information to this study

1.1 Human carnivore conflict

Human-carnivore conflicts pose a significant threat to carnivores that range outside of protected areas due to the frequent use of lethal control strategies in an attempt to mitigate the risks to safety and economic security (Woodroffe, 2000, Packer et al., 2009, Yirga et al., 2012). Woodroffe *et al* (1998) report that members of the order *Carnivora* can be highly susceptible to such active population control methods because their high trophic position and restricted population sizes make them prone to population declines in response to stochastic environmental disturbance (Woodroffe and Ginsberg, 1998). Examination of the conservation status of the largest 31 extant terrestrial carnivore species has revealed that 24 species are experiencing a significant reduction in population size as a direct consequence of human persecution (Ripple *et al*., 2014). In addition, the historic distribution of carnivores such as the leopard (*Panthera pardus*), lion (*Panthera leo*) and grey wolf (*Canis lupus*) have decreased by 45%, 83% and 33% respectively over the last century due to agricultural land use change and resulting human wildlife conflicts (Ripple *et al*., 2014).

Conflicts between humans and carnivores are common within rural and agricultural landscapes as the risk of disease transmission and livestock depredation are of

substantial concern to farming communities (Zielinski, 1997, Harcourt et al., 2001, Treves and Karanth, 2003, Graham et al., 2005, Packer et al., 2009, Roemer et al., 2009, Zimmermann et al., 2010). The use of free shooting and organised culling events to limit or remove local carnivore populations is often viewed as the quickest way to reduce livestock depredation and to prevent disease emergence (Conover, 2001, Mitchell *et al*., 2004), yet very few studies have found empirical evidence for economic remuneration through reduced livestock mortality rates using these techniques (Treves and Naughton-Treves, 2005, Berger, 2006). A lack of species selectivity and the failure to target problem individuals are the frequently highlighted disadvantages associated with many other commonly used lethal control strategies such as gin traps, poisoning and snaring (Avenant and du Plessis, 2008, Mc Manus *et al*., 2014). The inability to accurately target conflict species using such control strategies often results in unintended fatalities of non-target species prompting a debate about their use at a time of ongoing global biodiversity loss (Mc Manus *et al*., 2014).

Advances in ecological knowledge and the progression of research into animal husbandry have increased the use of many 'passive' conflict resolution strategies with the aim of producing economically viable methods of limiting the impact of carnivore species without the requirement of reducing or removing local carnivore populations (Mc Manus *et al*., 2014). The positioning of trained guardian dogs within livestock herds (Gehring *et al*., 2010) and the use of predator-proof fencing to segregate livestock from wild carnivores (Landry et al., 2005) are just some examples of passive conflict resolutions used in agricultural landscapes. For example, over 73% of farmers

in Namibia reported a significant reduction in livestock depredation from wild carnivores after associating guardian dogs with their livestock (Marker *et al*., 2005). Despite the significant financial investment and effort required to successfully undertake active population control programs (Treves and Naughton-Treves, 2005), and the substantial advances in passive conflict resolution strategies (Treves and Karanth, 2003), lethal removal of carnivores often remains a commonly considered conflict management strategy in many agriculturally intensive countries (Woodroffe, 2000).

The carnivores of South Africa have been recognised to be in decline since the mid-1970s (Ginsberg and Macdonald, 1990, Mills *et al*., 1998, Burton *et al*., 2011). During the early 20th century, carnivores ranging within South Africa were subject to a sustained level of persecution in an attempt to protect domestic livestock from depredation and disease (Bothma *et al*., 2009). During this period a vast number of carnivore species such as the leopard (*Panthera pardus*), caracal (*Felis caracal)* and black-backed jackal (*Canis mesomelas*) became locally and nationally extinct (Stuart et al., 1985, Bothma et al., 2009, Funston et al., 2013). Today, the majority of large African carnivores (>15kg) are classified by the International Union for Conservation of Nature (IUCN) as threatened or near threatened but continue to persist in areas protected by legislation (Zielinski, 1997, Chapman and Balme, 2010).

The reduction in inter-guild competition arising from the removal of large carnivores in agricultural landscapes of South Africa (Ritchie and Johnson, 2009, Balme *et al*., 2010), and the change in attitudes towards carnivores in response to the expansion of the ecotourism industry, has permitted many smaller, generalist carnivores such as the black-backed jackal and caracal to recolonize a large proportion of South Africa's rural areas, prompting conflict with many livestock owners and breeders (Cardillo *et al*., 2004, Balme *et al*., 2010). Carnivores such as the black-backed jackal and caracal, which commonly range outside of South Africa's nationally protected areas, are often targeted by active and lethal population control strategies (Infield, 1988, Castley *et al*., 2002, Ogada *et al*., 2003, Balme *et al*., 2010, Thorn *et al*., 2013). It is therefore unsurprising that snaring, poisoning and shooting are recorded as the most common causes of African carnivore mortality in privately owned agricultural land (Woodroffe and Ginsberg, 1998, Harcourt *et al*., 2001, Castley *et al*., 2002).

The black-backed jackal is one of a number of mesocarnivore species in South Africa that is perceived to be a threat to livestock and thus is frequently the target of population management as a conflict species (Thorn, 2009, Loveridge and Nel, 2004, Klare *et al*., 2010, Kamler *et al*., 2012, Thorn *et al*., 2013). Recent studies have indicated that over 17,000 black-backed jackal individuals are killed annually by humans in the North West Province of South Africa (Thorn, 2009). Despite the threat posed to livestock by the black backed jackal and the significant financial investment in lethal predator control, the consequence of active removal of individuals on the ecology, abundance and population dynamics of the black-backed jackal outside of

South Africa's nationally protected areas has received little attention (Klare *et al*., 2010, Kamler *et al*., 2012). However despite this lack of information regarding ecological impacts of large scale culling operations, it has recently been highlighted by local media that licences were granted in 2011 by the democratic alliance and Cape Nature to the Western Cape Province of South Africa to cull a combined total of 90,000 black-backed jackal and caracal individuals in response to ongoing livestock depredation and rabies transmission. This culling program has been colloquially named "the Bredell cull", after the Western Cape MEC for environmental affairs, Anton Bredell.

Efforts to reduce or remove populations of *C. mesomelas* from livestock-producing regions of South Africa are currently perceived to be ineffective by many livestock farmers as recent land owner questionnaires and faecal density surveys commonly report this species as remaining prevalent (Thorn, 2009, Thorn *et al*., 2013). Due to the abundance of *C. mesomelas* reported by farmers in livestock-producing areas of South Africa (Thorn, 2009), and the level of persecution currently recorded for this species, demographic factors such as resource availability, dispersal patterns and source-sink dynamics are potentially influential in the persistence of *C. mesomelas* in a landscape of sustained persecution. Therefore this study aims to investigate the effect of lethal control and food availability on the abundance, dispersal and genetic diversity of black-backed jackal populations in private game-breeding farms of the North West Province of South Africa.

1.2 Ecology and biology of the black-backed jackal

The silver or black-backed jackal is a medium sized carnivore (4-8 Kg) that follows a monogamous life history strategy with adult jackal pairs holding territories in which they hunt, forage, scavenge and breed (Ginsberg and Macdonald, 1990, Estes, 1992, Sillero-Zubiri, 2004). Territory size is highly variable across their range, with the average territory size in South African estimated at 18.2 km^2 (Rowe-Rowe, 1992), while territory sizes in Zimbabwe and the Rift Valley in Kenya are much smaller with a range of between 0.6 – 3.0 km^2. Density estimates of the black-backed jackal are also highly variable across their range with estimates of between one individual per 2km^2 to 22 individuals per km^2 (McKenzie, 1993). The high variability in territory size was shown to be inversely correlated with the abundance of prey and scavenged food availability (Fuller *et al*., 1989, Loveridge and Macdonald, 2001) dictating a necessity to defend territorial boundaries. Territorial boundaries of the black backed jackal are defended from transient individuals and neighbouring residence by aggressive interactions from the territory holding pair (Loveridge, 1999, Stuart and Stuart, 2000). Territorial defence is reliant on the co-operative behaviour between the resident pair (Lamprecht, 1979, Ginsberg and Macdonald, 1990, Estes, 1992, Sillero-Zubiri, 2004). Aggressive interactions over territorial disputes are often gender specific (Estes, 1992) with competing males and females failing to engage opposite genders in combat, possibly due to the slight sexual dimorphism with males being generally larger than females (Ginsberg and Macdonald, 1990, Estes, 1992, Sillero-Zubiri, 2004). The death or removal of one of the territory-holding pair often results in the loss of territory for the remaining individual (Moehlman, 1979, Estes, 1992, Jenner *et al*., 2011). Predation

success is also significantly increased by the co-operative hunting behaviour observed between mated pairs (Estes, 1992, Sillero-Zubiri, 2004).

The black-backed jackal is an opportunistic scavenger and accomplished predator of small mammals and reptiles (Ewer, 1973, Estes, 1992, Merwe *et al*., 2009). *Canis mesomelas* feeds on a wide variety of food sources including invertebrates, small mammals, reptiles, carrion, fruit, human refuse, fish and juvenile or small livestock species (<50kg) (Smithers and Abbott, 1983, Dreyer and Nel, 1990, Bernard and Stuart, 1991, Kok, 1996, Kingdon and Pagel, 1997, Merwe *et al*., 2009, Klare *et al*., 2010). Stomach and faecal content analysis indicates that the black-backed jackal has an omnivorous diet and is able to undertake diet switching to consume abundant nutritional sources (Estes, 1992, Klare *et al*., 2010). Faecal analysis of the black-backed jackal in a South African livestock farm indicated that sheep and small domesticated ungulate species (<50kg) comprise up to 48% of the total annual biomass consumed, with the seasonal peak in livestock consumption recorded as coinciding with the calving season (Kamler *et al*., 2012).

Within South Africa, the mating of territory-holding pairs takes place between the months of May and August with an average gestation period lasting 60 days (Sillero-Zubiri, 2004). Pups are born between the months of July and October, with substantial localised variation in the peak of birth dates (Stuart, 1981, Bernard and Stuart, 1991, Forbes, 2012). This large geographic variation in peak birth across the range of the

black-backed jackal is thought to be largely due to the seasonal timing of births to coincide with local peak resource availability (Ginsberg and Macdonald, 1990, Sillero-Zubiri, 2004). Late winter livestock mortality and early summer population expansions of small mammals provide the sustained level of dietary nutrition required to gestate and support a litter of pups to independence (Ginsberg and Macdonald, 1990, Estes, 1992, Sillero-Zubiri, 2004). Litter sizes can be highly variable with observations of births ranging from 1 – 6 pups per season (Moehlman, 1989, Ginsberg and Macdonald, 1990, Estes, 1992). Sexual maturity in the black-backed jackal occurs at approximately 11 months (Ferguson *et al*., 1983, Ginsberg and Macdonald, 1990, Estes, 1992). After 12 months, the offspring usually move out of their natal range and become transient individuals in response to increased aggression between offspring and parents (Smithers and Abbott, 1983, Estes, 1992). Once reaching sexual maturity, black-backed jackals have a predicted lifespan of between 3 - 12 years in the wild (Rowe-Rowe, 1992, Rhodes *et al*., 1998, Stuart and Stuart, 2000).

Similar to many other predominantly non-social canids such as the red fox, the black-backed jackal does not frequently form large social groups with a high degree of social organisation. However, alloparenting by individuals from previous generations is an observed characteristic of these species (Ginsberg and Macdonald, 1990, Baker *et al*., 2004). A small proportion of non-parent adult kin will delay dispersal and remain within their natal range to provide food to pups at the den site via regurgitation (Moehlman, 1979, Moehlman, 1983). The presence of alloparents or “helper individuals” has been shown to significantly increase pup survival rates (Moehlman,

1979, Moehlman, 1983, Estes, 1992). The tolerance and dispersal of helper individuals by territory-holding individuals is not fully understood in this species but is thought that dispersal patterns are mitigated by resource availability (Moehlman, 1979, Estes, 1992).

1.3 Dispersal and resource availability

Dispersal rates of many taxa including insects, birds and territorial mammals have been empirically shown to be highly correlated with population density (Travis *et al*., 1999, Matthysen, 2005). Density dependant dispersal is a model used to describe the movement of individuals from areas of high population densities to areas of low population densities driven by the competition for resources such as food, territory and breeding partners (Travis *et al*., 1999, Matthysen, 2005, Méndez *et al*., 2012). Source-sink dynamics are also used to describe the movement of individuals within a metapopulation, from populations with a high growth rate *per capita*, to populations with relatively low or negative growth rates *per capita*, and can be used to explain the persistence of species in heterogeneously favourable environments (Amarasekare, 2004). These theories state that in patches of favourable habitat, the birth rate will be greater than the death rate resulting in an increase in population size and subsequent emigration of excess individuals into less favourable environmental patches where the death rate is greater than the birth rate (Amarasekare, 2004). Continued migration from source populations into sink populations can therefore sustain a population in unfavourable conditions indefinitely, providing the source population remains stable (Amarasekare, 2004).

In many mammalian species active dispersal has long been regarded as paramount in maximising the chance of an individual's reproductive success (Howard, 1960, Greenwood, 1980). As a result, dispersal is thought to be a principle factor in reducing the fitness costs associated with inbreeding within a population (Wright, 1946), and plays an important role in population size and the spatial distribution of a species (Taylor and Taylor, 1977). However, in many highly social animals such as the grey wolf *(Canis lupus*) and African wild dog (*Lycaon pictus*) the mechanisms underlying dispersal are poorly understood due to the behaviour and complexities of group living and social organisation (White and Harris, 1994).

The resource dispersion hypothesis (RDH) was first described to explain the evolution of group-living in birds when resource availability is heterogeneous in space and/or time, inferring a benefit to communal behaviour, with tolerance of individuals outweighing the costs of social independence (Crook, 1964). By extenuation RDH therefore predicts that resource distribution dictates the smallest possible territory size that will support a minimum social unit size of a species alongside other occupants of the same species without the requirement for group co-operation (Carr and Macdonald, 1986, Macdonald and Carr, 1989, Bacon *et al*., 1991). Very few studies have presented empirical evidence for the action of the resource dispersion hypothesis. However, the assumptions of RDH have previously been used to explain the territorial size and distribution of many mammals such as the European badger (*Meles meles)* (Johnson et al., 2001), red fox (*Vulpes vulpes*) (Johnson *et al*., 2002) and brown hyaena (*Hyaena brunnea*) (Yarnell *et al*., 2013) where territories are thought to

be constructed in response to the predictability and availability of resources (Revilla and Palomares, 2002, Johnson *et al*., 2002).

Resources, such as food and denning sites, are highly unlikely to be spatially and temporally randomly distributed throughout an environment. Therefore RDH assumes that the optimum territorial size is dictated by the availability of resources while minimising the effort required for foraging and defending territories (Bacon *et al*., 1991, Johnson *et al*., 2002). As with a number of other Canid species such as the red fox (*Vulpes vulpes*) and coyote (*Canis latrans*), the black-backed jackal is a territorial mammal that is not known to form large and unrelated social groups with a significant degree of social structuring (Nel *et al*., 2012). However, despite their highly territorial behaviour, groups of up to 10 individual jackals have frequently been recorded scavenging from large ungulate kills and human refuse sites (Sillero-Zubiri, 2004). Under certain circumstances of high resource availability, large conglomerations of *C. mesomelas* around clumped abundant food resources have been shown to follow the predictions of the resource dispersal hypothesis (Jenner *et al*., 2011, Nel *et al*., 2012). For example the black-backed jackal populations residing amongst the seal colonies of the Namibian coast have been reported to have an average home range of between 7.1 – 24.9 km^2 (Hiscocks and Perrin, 1988, Nel *et al*., 2012, Van de Ven e*t al*., 2013), with very few antagonistic interactions reported between neighbouring individuals (Hiscocks and Perrin, 1988). Therefore the territorial nature of this species, and the ability for the black-backed jackal to utilise a wide variety of food sources, make the

availability of resources a considerable factor in the variation in dispersal and migration rates of *C. mesomelas* across its distribution (Nel *et al*., 2012).

The density and stability of a given population are regulated by the key elements of resource competition, recruitment and migration/dispersal. As jackal populations persist in areas where they are persecuted, it is possible that a high and continued rate of removal results in a destabilisation of these factors, prompting a significant change to expected dispersal and migration rates (Krebs, 2008). It is suspected that the anthropogenic removal of alpha individuals may result in an increased rate of territory turnover, providing an opportunity for sub-adult, territory seeking, transients to take up new residence. In addition, the high density of livestock in game breeding farms of South Africa may also destabilise territorial limits following the assumptions of RDH. Thus both source-sink dynamics and density-dependant dispersal may be important factors in the migration and dispersal of individuals from areas of high resource availability into areas of high persecution. Thus this study aims to use camera trapping and faecal density surveys to estimate the relative abundance of local jackal populations in areas of high and low persecution, and in areas of high and low food availability. The genetic diversity, inbreeding and structure of *C. mesomelas* populations will be analysed using short tandem repeat motifs in an attempt to investigate the effect of predator management strategies on the population dynamics of the black-backed jackal residing outside of nationally protected areas of South Africa.

1.4 Population surveying methods

Reliable and robust estimates of abundance are fundamental in both the conservation and management of free roaming carnivore species (Stander, 1998, Mills *et al.*, 2001, Sollmann *et al.*, 2013). Direct sampling techniques such as physical Capture-mark-recapture and absolute observational counts can provide highly informative data, but are often unfeasible due to the logistical constraints synonymous with sampling a population. Furthermore, many direct sampling methods attempt to estimate the abundance of cryptic, nocturnal and/or low density mammalian species, often conclude with samples sizes of insufficient power to undertake any relevant statistical analysis (Wilson and Delahay, 2001, Plumptre, 2001).

Non-invasive population sampling techniques provide a means to estimate species abundance, density and distribution without the need to capture, mark and disturb study organisms in their natural habitat (Wilson and Delahay, 2001, Plumptre, 2001). However, many limitations are apparent when attempting to derive abundance and density estimates using ecological sampling techniques in the wild. Extrapolation of absolute abundance and density estimates from direct sampling methods can be highly informative yet subject to inaccuracies due to variation in detection probability in a heterogeneous habitat (Webbon *et al*., 2004).

Methods for both direct and indirect non-captive sampling such as, camera trapping; sign surveys; faecal density; and DNA analysis, have been developed and refined with

the aim of quantifying mammalian population sizes, however each technique has limitations in explanatory and predictive power (Karanth *et al*., 2004, Thorn, 2009). The number, scope and diversity of potential survey methods described within the literature highlight the difficulty in obtaining accurate and suitable data from sampled populations through any singular method. The advantages and disadvantages of a variety of indirect survey methods and their accuracy in producing absolute and relative abundance estimates has been examined in a substantial number of studies (Doran *et al*., 2002, O'Brien *et al*., 2003, Thorn, 2009, Thorn *et al.*, 2010, Harmsen *et al*., 2011, Espartosa *et al*., 2011, Noss *et al*., 2012, Parrott *et al*., 2012, Sollmann *et al*., 2013). Many of these studies have concluded that relying on singular indirect sampling techniques may lead to huge biases in estimates of abundance in a sampled population. The automated camera trap is one distinct direct but non-invasive sampling technique that has received a considerable amount of attention in this matter due to its practicality and data productivity (Griffiths and Van Schaik, 1993, Carbone *et al.*, 2001, Jennelle *et al*., 2002, Carbone *et al*., 2002, Espartosa *et al*., 2011, Sollmann *et al.*, 2013).

1.4.1 Camera trapping

Camera traps are battery operated, remotely triggered cameras that are positioned in the environment to photograph or "capture" target species in their natural environment. Camera trapping was first used in ecological based population studies during the 1980s and were originally developed using physically triggered white flash and film-based cameras in an attempt to extrapolate basic information on nest

predation, nesting behaviour, feeding ecology, species presence and population size (Cutler and Swann, 1999, York *et al*., 2001, Carbone *et al*., 2001, Swann *et al*., 2004, Hegglin *et al*., 2004). Advancement in digital photography technology, reduction in cost and a decrease in disturbance through the use of passive infra-red illumination has made the use of camera trapping a valuable and practical tool in the monitoring of wild carnivore populations (York *et al*., 2001). Following the digitisation and an increase in the trigger reliability of modern camera traps, their use in population studies of low density mammalian species has increased exponentially in popularity over the last decade (Sollmann *et al*., 2013). Camera trap photography is now commonly used as a direct sampling technique to assess and monitor rare and cryptic mammalian populations (Griffiths and Van Schaik, 1993, Cutler and Swann, 1999, York *et al*., 2001, Swann *et al*., 2004).

The large scale, long term sampling ability and relatively low effort associated with modern camera trap technology, make camera trapping methodologies suitable for monitoring mammalian species outside of protected areas where land access, trap site visitation and camera maintenance may be limited (York *et al*., 2001, Carbone *et al*., 2001). The camera trapping sampling technique offers a non-invasive, repeatable and standardised sampling procedure, requiring low maintenance levels and minimal user interaction (Thorn, 2009). However, the advancement in camera technology and the ability to capture and store thousands of pictures per sampling session increases the total effort and time in analysis of large scale data sets (Rowcliffe and Carbone, 2008). The development of camera traps for ecological surveying has prompted the

advancement of methodology, hardware and computing software used in the analysis of large scale ecological data sets of this type (Pritchard *et al*., 2000, Carbone *et al.*, 2001, Tobler, 2007, Rousset, 2008, Thomas *et al.*, 2009) and is discussed further in chapter III.

Camera traps provide an easy and robust sampling opportunity which may passively accumulate data without disturbing a study organism. Yet experimental design, variation in detection probability and choice of statistical analysis of photographic capture rates are highly influential in the accuracy of population estimates (Sollmann *et al*., 2013). Detection probability is described as the probability of detecting an individual during a sampling session if the species is present within the sampling area. Detection probability can be highly influenced by habitat, camera position, species behaviour, home range size and experimental design (O'Brien *et al*., 2003) which can result in a reduced reliability of abundances estimates. In an attempt to account for detection, camera trap studies estimating abundance are often based around a fixed camera trap arrangement with a known camera trap density and spatial distribution (Cutler and Swann, 1999, Carbone *et al*., 2001, York *et al.*, 2001). To increase the accuracy and to standardise photographic capture rates, studies investigating cryptic carnivore populations often bait or lure camera trap sites (Thorn, 2009, Thorn *et al*., 2009, Guil *et al*., 2010, Harmsen *et al.*, 2011). A wide range of baits and lures have been used as attractants at camera trap sites. Fresh meat and blood have often been used as camera trap lures by a number of studies to attract carnivore species to camera trap sites (Thorn *et al*., 2010). Lures are often made from extracted scent

glands from foxes and coyotes, for example, and have been successfully used as species-specific attractants in both camera trap studies and live trapping protocols. The use of the male aftershave, Obsession by Calvin Klein™, containing a synthetic form of civetone, has also been trialled with varying rates of success in an attempt to lure large feline species such as leopards and cheetahs to camera trap sites (Marker and Dickman, 2003, Thorn *et al*., 2009).

Capture-mark-recapture (CMR), occupancy analysis and relative index of abundance (RIA) are three common methods for analysing camera trap data sets with the aim of inferring population characteristics such as density, presence and abundance respectively (Chapman and Balme, 2010, Thorn, 2009, O'Brien *et al.*, 2003). The presence of identifiable individuals within a population provides the opportunity to employ image-based non-invasive Capture-mark-recapture calculations in order to extrapolate estimates of absolute population density (Karanth and Nichols, 1998). However, the assumption of 100% accuracy in the identification of individuals must be maintained in order for this method to be of any practical use (Carbone *et al*., 2001, Rowcliffe and Carbone, 2008). In addition, the spatial and temporal variation in detection probability and often low density and recapture rates makes this sampling method an inappropriate choice for many cryptic mammalian studies. With the exception of a few well studied carnivores with unique morphological characteristics, the lack of visible and distinguishable morphological characteristics in many species reduces the effectiveness of capture-mark-recapture techniques dramatically (Carbone

et al., 2001) and render this method defunct for the study of the black-backed jackal as visual identification of individuals is difficult.

The ability to detect carnivores in an ecosystem and produce occupancy results has become an emerging trend among terrestrial ecological studies (Linkie *et al.*, 2007, O'Connell *et al*., 2011). Analytical techniques employing the use of photographic encounter rates and presence / absence data have been developed to estimate the population indices of non-distinguishable individuals within a study population (O'Brien *et al*., 2003, Rowcliffe *et al*., 2008, O'Connell *et al*., 2011). However, the adequate sampling effort required to undertake occupancy studies requires prior knowledge of likely occupancy and detection probability of the study organism (MacKenzie and Kendall, 2002, Thorn, 2009). As such, occupancy analysis is best suited to study low-density organisms with known attributes where capture-mark-recapture techniques are not applicable. Previous studies have concluded that the capture effort required to employ occupancy models on *C. mesomelas* is not feasible given the current technological and logistical constraints associates with its use. For example, due to the recorded prevalence of *C. mesomelas* in game farm land use of South Africa (Thorn, 2009), it is unlikely that density estimates can be resolved to a greater degree of accuracy by implementing occupancy analysis based on presence/absence data alone. The potential for large and variable population sizes makes accurate estimates of occupancy unfeasible using this technique (Merwe *et al*., 2009, Thorn, 2009).

The use of relative indices of abundance, calculated from photographic capture frequencies, is a comparative method used to quantify the abundance of one or more species at multiple locations (O'Brien *et al*., 2003). This method can be used to make both spatial and temporal comparisons between populations (Karanth and Nichols, 1998, Edwards *et al*., 2000, Thorn, 2009, Thorn *et al*., 2009) and is practically suited to the study of populations with indistinguishable individuals at both the local and landscape scale (O'Brien *et al*., 2003). Independent photographic capture events as described by O'Brien *et al* (2003) can be used to calculate an index of population abundance, and is based on the fundamental principal that areas of high population densities will produce higher photographic capture rates when compared with areas of low population densities if sampling effort and detection probability remain constant (York *et al*., 2001, Carbone *et al*., 2001, O'Brien *et al.*, 2003). The time used to define independent capture events is considered ecologically relevant to the dispersal and movement patterns of each species investigated, and is used to limit the over estimation of abundance through consecutive capture events by the same animal in a single site visit. A standard of > 30 minutes between independent events is deemed adequate for the majority of mesocarnivore species due to their mobility and often wide ranging foraging patterns (Carbone *et al*., 2001, O'Brien *et al*., 2003). It would be naïve to assume that the relative indices of abundances, calculated from camera trap frequency data, is free of biases arising from a variation in spatial and temporal detection probability (Jenner *et al*., 2011); however, in situations where capture-mark-recapture and occupancy analysis are not suitable for population monitoring, abundance estimates such as this provide a valuable tool in the quantification of relative population sizes (Carbone *et al*., 2002).

Significant limitations are inherent in the use of photographic capture rates to estimate species abundance (Carbone *et al*., 2002, O'Brien *et al*., 2003, Espartosa *et al*., 2011, Noss *et al*., 2012, Sollmann *et al*., 2013). Variation in spatial and temporal detection probability, behaviour of study organisms and home range size has been shown to dramatically affect relative abundance indices (RIA/RIAs) calculated from camera trap data (Sollmann *et al*., 2013). The correct application of relative abundance indices in ecological studies is therefore primarily suited to local scale comparative studies that do not require the estimation of absolute population data, and where detection probability remains constant (Griffiths and Van Schaik, 1993, O'Brien *et al*., 2003, O'Connell *et al*., 2011). As detection probability is rarely constant in the natural environment, recent studies have cautioned the use of relative abundance estimates from camera trap data alone (Sollmann *et al*., 2013). Authors have advised the use of additional sampling techniques, such as faecal density surveys and genetic analysis, in order to corroborate findings when calculating relative abundance index from camera trap data and discourages the use of RIAs in non-comparative studies (Sollmann *et al*., 2013).

1.4.2 Faecal density surveys

Transect-based direct counts have provided invaluable information for the management of species such as the White rhinoceros (*Ceratotherium simum)* and the Plains zebra (*Equus quagga boehmi)*, where the species range across an open and homogeneous habitat (Tainton, 1999). In addition to the difficulty associated with sampling low density cryptic mammalian species outlined above, sampling methods

that are reliant on capture, direct sighting and/or identification of individuals are often hindered by the variation in capture and detection probability. This is due to the non-random spatial distribution of individuals across a complex and heterogeneous habitat. (Davison *et al.*, 2006). Direct counts and capture-mark-recapture models additionally result in a limited explanatory power when investigating large population sizes due to the sampling effort and cost required for an analysis of sufficient statistical power (Harmsen *et al*., 2011). As such, a number of indirect population level sampling techniques have been developed with the specific aim of confirming the presence, and estimating the relative abundance, of wild terrestrial mammalian species in complex habitats where direct counts are considered non-viable (Wilson and Delahay, 2001).

Transect-based sign surveys, print and hair traps, and faecal density estimates, are commonly implemented indirect sampling techniques which attempt to detect the presence of species and quantify an estimate of abundance based on the frequency of encounters over a fixed sampling effort (Wilson and Delahay, 2001). Indirect sampling techniques used to estimate abundance indices are rooted by the assumption that the proportion of signs observed are directly correlated to the abundance of the study organism (Wilson and Delahay, 2001). Faecal counts have previously been used to quantify a relative index of abundance in a number of *Canid* species both across the globe and within South Africa (Sharp *et al*., 2001, Moehrenschlager and Moehrenschlager, 2001, Harrison *et al.*, 2002, Thorn, 2009). The persistence and longevity of faecal deposits in this environment provide advantages over other potential indirect sampling techniques such as scent stations and print counts which

deteriorate over a period of a few hours or days (Kohn *et al*., 1999, Wilson and Delahay, 2001). It has been proposed that the use of faeces as prominent territorial markers by many *Canid* species (Kleiman, 1966, Barja *et al.*, 2004, Sillero-Zubiri, 2004, Sillero-Zubiri and Macdonald, 2006) increases the suitability of faecal counts as a proxy for abundance due to the ease of observation and identification this allows (Thorn *et al*., 2010). A prerequisite of species-specific knowledge is required to define the defaecation rate of an organism in order to successfully derive accurate absolute density estimates from faecal counts (Wilson and Delahay, 2001, Davison *et al*., 2006). This information is available for a number of *Canid* species and has permitted the estimation of density in organisms such as the red fox (Webbon *et al*., 2004). However, the required species-specific information quantifying mean defecation rate is often lacking due to the absence of sufficient information gleaned from captive studies. As such, faecal density estimates are frequently implemented in a comparative nature in order to define a relative index of abundance between sites of specific interest where detection remains both temporally and spatially consistent (Wilson and Delahay, 2001, Webbon *et al*., 2004, Barea-Azcón *et al*., 2007, Thorn *et al*., 2010).

Factors such as diet, resource availability, behaviour, and identification error can be highly influential on the accuracy of any population estimators derived from faecal counts (Davison *et al*., 2006). The high rate of faecal degradation, as a result of seasonal coprophagic insect action and weathering, can be highly influential on the persistence and quantity of faecal signs observed during sampling periods (Davis, 1996, Davison *et al*., 2006). In South Africa, coprophagic action by dung beetles reaches a

seasonal peak during the summer wet season with minimal action recorded during the winter months (Davis, 1996). Seasonality and the duration between repeated sampling sessions are therefore important factors to consider when designing a sampling regime which attempts to quantify organism abundance from faecal count data. A standardised sampling regime with either multiple repeats over a short period of time or prolonged repeated sampling over one or more years can be used to minimise the inaccuracies in detection brought about by seasonality itself (Webbon *et al*., 2004). Other seasonal fluctuations in population abundance such as the juvenile recruitment during the breeding season prior to dispersal and subsequent variation in territorial stability would bring substantial biases in to abundance indices based on faecal density estimates thus multiple abundance estimates should be derived from multiple sampling techniques to ensure the most reliable measurements of population abundance possible.

The black-backed jackal is thought to use linear features such as roads and game trails to traverse a territory and define territorial boundaries (Estes, 1992). The prominent positioning of faeces along road networks and at significant features of the environment has been observed and recorded in this species within South African game farm ecosystems (Thorn, 2009, Thorn *et al*., 2010). Due to this feature of jackal ecology, a faecal transect method focusing on the anthropogenically constructed road networks of game farms has been developed for this species to quantify a relative abundance estimate based on faecal counts over a known area (Thorn et al., 2010).

1.4.3 Simple sequence repeat polymorphisms

Over the past three decades a number of molecular marker-based techniques have been developed to quantify diversity, structure and evolutionary history of a given population or species. The ability of a molecular marker to detect and resolve genetic dissimilarities between sample groups have been shown to be highly dependent on the marker type selected (Jarne and Lagoda, 1996). Therefore careful consideration should be given to marker choice with respect to the required resolution and application of a study.

The simple sequence repeat (SSR) is one such type of molecular marker that offers a high degree of resolution at the individual and population level. Simple nucleotide sequence repeat motifs, also known as microsatellites, are short fragments of nucleotide sequence comprised of repeated nucleotide motifs of between 2 – 6 base pairs, which typically repeated between 5 – 30 times. Simple sequence repeat motifs are present in both coding and non-coding genomic DNA, however microsatellites present in non-coding DNA are of particular use in estimating population parameters due to their selective neutrality. These simple sequence motifs primarily originate, accumulate and extend as a function of slipped strand misparing during replication (replication slippage) and point specific mutations. As such, the mutation rate recorded at microsatellites often range between 10^{-6} to 10^{-2} per generation and can be well above background mutational substitution event rates (Schlötterer, 2000). Due to the absence of sophisticated DNA repair mechanisms acting on non-coding Eukaryote DNA, the accumulative bias in replication error surrounding nucleotide repeat motifs

(Jarne and Lagoda, 1996), and the lack of effective selection on non-coding DNA (Jarne and Lagoda, 1996, Falconer *et al*., 1996, Metzgar *et al*., 2000) microsatellites have the potential to act as powerful and informative Mendelian molecular markers which are capable of resolving both genetic dissimilarities and similarities at the local and landscape scale (Sunnucks, 2000). Thus the identification, development and characterisation of molecular markers specific to the black-backed jackal are therefore of paramount importance for the estimation of the metapopulation parameters of this species both within and outside of nationally protected areas of South Africa.

1.5 Concepts of population genetics

The term "population" is widely used in many fields of biology to describe a group of individuals in close proximity that belong to a single reproductively isolated species (Falconer *et al*., 1996). However, when considering the genetic construction of a population, a more accurate definition is described as a group of individuals that have the potential to breed and thus are able to transfer genetic components vertically from one generation to the next (Falconer *et al*., 1996). The genetic composition of a population can be described in terms of its genotype frequency which can be expressed as the proportion of individuals within the population that contain each possible combination of alleles for a given locus or combination of loci (Falconer *et al*., 1996).

Following the seminal work by Dobzhansky (Dobzhansky, 1937) and Mayr (Mayr, 1942) on the genetic mechanisms of the evolutionary process, it is now commonly regarded by many that genetic isolation is a key prerequisite for divergence in the natural environment (Wright, 1946, Wright, 1965, Dobzhansky, 1970, Eldredge and Gould, 1972, Spurgin *et al*., 2014). Thus complete genetic mixture within a population prevents differentiation and results in genetic similarity between individuals. Systematic processes such as the creation of novel alleles through mutation, and the immigration of individuals into a population can counter differentiation by preventing the fixation of alleles due to dispersive processes such as genetic drift (Falconer *et al*., 1996). The rate of migration and mutation are therefore highly influential factors in the rate of genetic differentiation within a population (Falconer *et al*., 1996).

With a few rare exceptions, namely the golden viscacha rat (*Pipanacoctomys aureus*) and plains viscacha rat (*Tympanoctomys barrerae*), the vast majority of mammalian species are diploid with an autosomal number of 2n, where n = the number of haploid chromosomes. The remainder of this thesis will therefore be solely concerned with the assumptions associated with diploid organisms.

The Hardy-Weinberg equilibrium (Hardy, 1908) is the standard model used to describe the relationship between allele frequencies and expected genotype frequencies in an idealised randomly mating non-differentiating population of infinite size. The principle of the Hardy-Weinberg equilibrium states that under neutral selection, the allele and

genotype frequencies within an infinitely large and randomly mating population will remain consistent between generations (Falconer *et al*., 1996). Natural populations that exhibit genotype frequencies in proportion to the allele frequencies are said to be in Hardy-Weinberg equilibrium. In sexually reproductive species, the genotype frequency in subsequent generations are dictated by the allele frequency in the parental generation, thus if the allele frequencies of two alleles at a locus are *p* and *q* in the parental generation, then expected genotype frequencies in the subsequent generation can be described as p^2 2pq and q^2. For example, at a locus (A) with alleles (i and j), the Hardy-Weinberg relationship giving the expected frequencies (or probabilities, Pr) of genotypes is:

$$\Pr(A_i A_j) = p_i^2 \qquad i = j$$
$$\Pr(A_i A_j) = 2 p_i p_j \qquad i \neq j$$

Where p_i and p_j are the relative frequencies of alleles *i* and *j*, respectively. A population can therefore be tested for signs of genetic differentiation by the deviation of observed genotype frequencies from those expected under Hardy-Weinberg expectations. Deviation of the observed frequencies from the Hardy-Weinberg expected frequencies can be measured using a Chi-Squared or Fisher's Exact test (Upton, 1992, Sasieni, 1997).

The degree to which a population deviates from the expected heterozygote frequency can be described by the F statistics derived from the initial work by Sewall Wright (Wright, 1965). The inbreeding coefficient F for individuals within a subpopulation (F_{IS}), subpopulations within a total population (F_{ST}) and individuals within the total population (F_{IT}) where, generally:

$$F = \frac{(Ho - He)}{He}$$

Where H_o = observed heterozygosity and H_e = expected heterozygosity derived under the Hardy-Weinberg expectation (Wright, 1965, Cockerham and Weir, 1984, Falconer *et al*., 1996).

Populations in their natural environment rarely conform to the assumptions necessary for a population to be in Hardy-Weinberg equilibrium. For example, they are not of infinite size and geographical, anthropogenic, behavioural and physiological barriers to gene flow exist. Often, mammalian populations are of a limited size, ranging between the hundreds and thousands of breeding adult individuals rather than in the hundreds of thousands. Considering the random assortment of alleles in a population of small and finite sizes, allele frequencies can stochastically alter from one generation to the next by chance alone. Sexually mature individuals generate excessive numbers of gametes, the vast majority of which fail to fertilize. The proportions of gametes that

succeed in fertilisation are effectively random in nature. In addition, not all sexually active individuals produce the same number of offspring, leading to a bias in inherited genotypes. There are so many complex reasons for these biases in inheritance, that the outcome appears fundamentally random (Gillespie, 2010). The random selection of parental alleles that go on to form the offspring generation has important consequences for populations of finite size. The random cumulative effect of this sampling error over time is termed genetic drift. Because genetic drift is due to the random sampling of a population of finite size, the magnitude of the effect of genetic drift is reduced as the population size tends to infinity. The problem that drift causes for populations can be summarized by the notion that half of the offspring of heterozygous individuals are expected to be homozygous (two *Aa* individuals can produce *AA*; *Aa*; *aA* and *aa* offspring in equal proportions). This loss of heterozygosity in each generation is described by:

$$H_t = H_0\left(1-\frac{1}{2N}\right)^t$$

Where t = the number of generations, H_0 = the frequency of heterozygotes in the starting generation, H_t = the frequency of heterozygotes in generation t and N = the population size. This model of genetic drift states that over an infinite number of generations, and in the absence of migration or selection, drift will ultimately culminate in the fixation of a single random allele for each locus in a randomly mating population of any finite size, at a rate determined by 1/2N. Thus, as genetic drift

progresses and random alleles migrate towards fixation, p_i, for example, tends to 1; hence p_j tends to zero; homozygosity (p_i^2) tends to 1 and heterozygosity ($2p_ip_j$) tends to zero.

1.5.1 The effects of inbreeding in natural populations

Inbreeding occurs when individuals related by ancestry mate within a population. Reproduction between related individuals can be envisaged as the increased probability of the homozygous genotype being composed of alleles that are identical by descent, in addition to being identical in state. Inbreeding resulting from random mating is therefore directly related to population size as the number of prior generations required to contain ancestors of separate lineages to present individuals reduces as population size decreases (Falconer *et al.*, 1996). Thus the smaller the population size, the greater the likelihood of individuals sharing a common ancestor over fewer numbers of generations compared with larger population sizes. As population sizes decrease, there comes a point where individuals cannot avoid inbreeding as potential mates are all related. This is distinct from the effect of assortative mating or non-random inbreeding where individuals who share a phenotype and/or genotype are more likely to breed than dictated by chance. The mating of individuals related by common ancestors increases the probability of inheritance of common alleles, resulting in an increase in homozygosity and can lead to reduced fitness costs through the increased homozygosity of deleterious recessive alleles (Hartl and Clark, 1997, Gillespie, 2010). Therefore assortative mating and non-random inbreeding will result in an increase in F_{IS} values as the homozygote genotype

form will dominate but in contest to drift, the population can be comprised of many homozygote forms containing different alleles. However random inbreeding due to a reduced or limited population size can result in an increase in overall population homozygosity while no change in allele frequencies occurs within individuals compared with the sampled population / deme / line as a whole (Falconer *et al*., 1996). Inbreeding in small populations can thus be thought of as a polar opposite to the effects of migration, which increases genetic diversity within a subpopulation by the immigration of individuals containing alleles from the total population. A measure of inbreeding can be estimated by examining the inbreeding coefficient, which is the probability of two alleles at any locus being identical by descent. In summation, non-random inbreeding promotes homozygosity at a higher rate than expected under the assumptions of the Hardy-Weinberg equilibrium through the non-random assortment of alleles identical in state. However, random inbreeding in small populations ultimately results in the fixation of alleles and a change in allele frequencies between generations; it does not result in a positive F_{IS} value as the heterozygote frequency at the individual level does not change relative to the sampled population.

1.5.2 Migration and dispersal

Migration is an important systematic process which changes the allele frequencies in a subpopulation by bringing in new alleles, present in the base (total) population, into the immigrant (sub) population. The effect of migration or dispersal is therefore reliant on the successful breeding of the immigrant with an individual or individuals within the subpopulation. In the natural environment, many species exhibit forms of genetic

isolation across their distribution. Isolation by distance and physical barriers to migration are two factors that can impede gene flow and produce population substructure, i.e. subpopulations within the total population (Wright, 1965, Weir and Cockerham, 1984). The subsequent subpopulations, or demes, produced by isolation will undergo genetic drift at a level of independence determined by the magnitude of isolation. Over time, the number of demes fixed for a given allele will be proportional to the frequency of that allele in the total population by chance. As such, in a hierarchical, sub-structured population differentiating under drift, the total genetic variation can be partitioned by the ratio of genetic variation within demes relative to the genetic variation between demes (Weir and Cockerham, 1984). The progression of differentiation over time will therefore result in the shift of genetic variation from within demes to between demes leading to a conservation of genetic diversity across the population as a whole. For example, the theoretical endpoint of such a scenario will be an infinite number of demes, fixed, and hence homozygous, for different alleles. The degree of population structure due to subpopulation differentiation can be quantified by examining the proportion of genetic variation contained within subpopulations relative to the genetic variation present within the total sampled population. A measure of population structure was developed by Sewall Wright in 1969 and is known as the fixation index (F_{ST}) which ranges between 0 and 1. An F_{ST} value of 0 infers complete genetic mixture within the sampled population, and thus a lack of population structure, as the frequency of alleles are consistent across the total population. If the variation in allele frequencies is not consistent across the total population and can be partitioned within subpopulations, population structuring through subpopulation differentiation is apparent. The degree of population

structuring (F_{ST}) is proportional to the genetic variance contained within the subpopulation relative to the total population. A high degree of population structuring therefore results in high F_{ST} values which implies substantial genetic isolation of subpopulations from the total population and is indicative of population differentiation.

1.6 Quantification of inbreeding and population differentiation

When population data and pedigrees are lacking, genetic marker-based techniques are used to answer questions about population ecology, origin, differentiation, migration, isolation and inbreeding. Simple-sequence repeat polymorphisms are often used in population studies due to their selective neutrality and highly polymorphic nature (Balloux and Lugon-Moulin, 2002). F-statistics were introduced to the scientific community as a method of analysis to describe the partitioning of genetic variation within and between individuals and populations. This method is particularly suited to neutral molecular markers, such as microsatellites (Balloux and Lugon-Moulin, 2002). Many different approaches have been developed to estimate values of F_{ST}, ranging from the correlation coefficient of differentiation described by Sewall Wright in 1965 (Wright, 1965) to the analysis of variance approach described in the seminal papers by Weir and Cockerham in 1984 (Weir and Cockerham, 1984, Cockerham and Weir, 1984). However in a simplistic form when H_T = calculated heterozygosity in the total pooled population and $\overline{H_S}$ = average heterozygote frequency of the subpopulations:

$$F_{ST} = \frac{H_T - \overline{H_S}}{H_T}$$

For an infinitely large undifferentiating population, F_{ST} values will be zero as the variation in heterozygote frequency between demes is equal to the variation within demes. In a fully differentiated population with complete isolation between demes, F_{ST} would be equal to 1 as the entirety of the genetic variation is entirely between demes and none by variation within demes. The cause of this discrepancy in variation between and within demes is gene flow. With complete gene flow, demes do not vary in allele frequency and F_{ST} = 0. When there are barriers to gene flow, variation between demes arises and $F_{ST} > 0$. F_{ST} can thus be used as an indirect measure of gene flow, or migration between demes. Thus the estimate of F_{ST} has become a standard quantification of the degree of genetic differentiation within a population. F-statistics can be used to describe the genetic variation at different levels of a hierarchical structured population:

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$$

Where F_{IT} = the inbreeding coefficient of individuals relative to the total population, F_{IS} is the inbreeding coefficient of individuals relative to the subpopulation and F_{ST} is the inbreeding coefficient of the subpopulation relative to the total population. In summary, F_{IS} can be used as an indication of inbreeding within a population due to the

departure of homozygote and heterozygote frequencies expected under the Hardy-Weinberg equilibrium. F_{ST} is a measure of population sub-structuring given the difference in allele frequencies resulting from isolated populations, experiencing barriers to gene flow, diverging under the independent effects of genetic drift. Isolated subpopulations can maintain the ratios expected under the Hardy-Weinberg equilibrium given the potential for random mating in a large subpopulation, resulting in F_{IS} values of near zero. If isolated subpopulations are small and inbreeding occurs due to the lack of unrelated potential mates, both inbreeding and population structuring can result: $F_{IT} > 0$.

1.6.1 Population structuring and the Wahlund effect

When the subpopulations have not been correctly identified, due to the absence of obvious barriers to gene flow, clusters of subpopulations can be erroneously treated as a single randomly mating population. However, because this population is actually composed of subpopulations with allele frequencies that differ from subpopulation to subpopulation, an excess of homozygosity over HW expectation results (Hartl and Clark, 1997). This effect is known as the Wahlund effect and can often be confused with inbreeding: confusing F_{IS} for F_{ST} (Wahlund, 1928). For this reason, whenever a positive estimate of F_{IS} is found, the question as to whether the population studied is actually a conglomerate of several subpopulations needs to be addressed using iterative structure finding algorithms such as the program STRUCTURE.

1.7 Summary

To summarise, a greater understanding of the ecological consequences and effectiveness of unregulated predator control and food availability is needed in order to more accurately inform the management process of the black backed jackal. As such, this study aims to assess the effects of predator control and predator feeding relative to predator neutral sites on population dynamics of the black-backed jackal outside of South Africa's nationally protected areas. A multidisciplinary approach will be used combining camera trap derived abundance estimates and faecal density surveys with traditional molecular ecology to provide an insight into the population response to these anthropogenic pressures.

It is hypothesised that predator management significantly affects the population dynamics of the black-backed jackal in the game farm environments of South Africa, with predator control resulting in a lower abundance of the black-backed jackal relative to predator neutral treatment group and an increase in genetic diversity relative to the total population. In addition it is hypothesised that predator feeding leads to an increase in the abundance of the black-backed jackal relative to predator neutral treatment group and results in the formation of genetically isolated subpopulations of the black-backed jackal relative to the total population due to a reduction in dispersal rates.

Chapter II

Study Area and Experimental Design

2.1 A brief history of agriculture in South Africa

The modern day agricultural industry of South Africa has been strongly influenced by the country's complex political history. Prior to the arrival of European colonists in the mid-17th century, South Africa had been inhabited by a number of different human settlers with a variety of attitudes towards farming and agriculture. The first known people to settle in sessile communities within what is now South Africa were the San people (Khoisan) circa 44,000 BC (Thompson, 2001). The San people followed a Hunter-gatherer style existence and were once extensive throughout the southern African subcontinent; however, the San people were substantially displaced from South Africa during the southern expansion of the Bantu-tribes between 10,00BC and 500 AD (Thompson, 2001). The expansion of the Bantu tribes, and associated Iron Age technology, brought with it agricultural practices such as crop production and animal domestication (Thompson, 2001). The origins of many of the modern day tribes in South Africa, such as the Zulu, the Tswana and Basotho, can be traced to this population expansion (Schuster *et al*., 2010).

By the 16th century the Cape of Good Hope had been colonised by the Dutch as it offered a strategic and opportune stopover point on the long trading route between Europe and the East. Colonialists constructed and managed arable and domestic

livestock farms under Dutch patronage in order to resupply trade vessels passing the cape. Throughout the 17th century, the cape colony became a prosperous trading point in spices and goods; however, by the late 18th century, war between the Dutch and the Portuguese left the Cape of Good Hope open to contention between the French and the British (Thompson, 2001). After the recognition of British sovereignty of the Cape of Good Hope in 1815, many of the decedents of the original Dutch colonialists moved north away from the British occupied cape in order to establish small, self-sustaining farming communities. These people became known as the Boers, or "farmers" in Afrikaans (Thompson, 2001).

By 1909, following numerous conflicts involving the British, the Boer republic and the Zulu empire, South Africa was eventually unified as a British territory under the concessions of parliamentary home-rule with limited democratic voting rights (Thompson, 2001). After the construction of the Union parliament in 1909, the Natives' Land Act was implemented in 1913, which restricted the private ownership of land to white South African residents. This racial segregation of land ownership continued in place until the end of the apartheid era in 1994.

Under the Land Restitution Act of 1994, communities or persons who lost land and property as a result of the apartheid laws of 1913 were invited to submit claims for restitution and compensation. The Land Re-distribution for Agricultural Development Act (Act 22 Restitution of land rights 1994) was implemented to give previously

disadvantaged people the opportunity to own their own land and become effective farmers in rural communities. By 2011, approximately 6.2 million hectares of agricultural land was either redistributed or restituted to individual and tribal control, with many further claims awaiting settlement in court (Byamugisha, 2014). A large proportion of this re-distributed agricultural land is currently used as communal grazing areas for domesticated livestock such as cattle and goats; however studies into the ecology and biodiversity of wildlife in tribal farming areas of South Africa are currently limited.

Between 1913 and the 1960s, westernised agricultural practices such as domestic livestock ranching and arable farming continued to dominate the agricultural land use types of South Africa (Carruthers, 2008). Unlike the neighbouring countries of Botswana and Zimbabwe, where wildlife "game species" were primarily used for sport, the long standing farming traditions of South Africa championed the segregation of domesticated livestock and wildlife in an attempt to reduce domestic livestock loss through depredation, resource competition and disease (Carruthers, 2008, Thompson, 2001). As a result of this paradigm, large scale eradications of game and wildlife took place during the first half of the 20th century with many wild species being pushed to near extinction (Carruthers, 2008, Biggs et al., 2008, Stuart et al., 1985, Bothma et al., 2009).

In the early 1960s a revolution in wildlife management began in South Africa. In concordance with the seminal works of Raymond Dasmann and Reay Smithers throughout the 1960s, attitudes towards conservation were in flux prompting the establishment of a number of government funded nationally protected areas within the country (Carruthers, 2008). Concurrent to this, the increasing protein requirements of a developing country highlighted the enormous financial potential in the private production and hunting of game species for the meat trade. This introduction of an economic value to wildlife, and the associated legislation permitting the private ownership and sale of wildlife, brought about a huge shift in agricultural focus away from traditional cattle ranching techniques and towards the concept of game farming (Carruthers, 2008).

Over the latter part of the 20th century large areas of private rural land (up to 180 000 ha), including many former cattle farms, were used to breed and harvest naturally occurring wild herbivore species such as the Springbok (*Antidorcas marsupialis*), Impala (*Aepyceros melampus*), Kudu (*Tragelaphus strepsiceros*) and Blue Wilderbeest (*Connochaetes taurinus*) (Carruthers, 2008, Thompson, 2001). As the game meat and hunting industry began to grow, interest in the management and production of non-domesticated game species became increasingly popular. Due to the remotely managed nature of non-domesticated livestock production, much of the population management strategies employed in game farm environments focus on the manipulation of resource availability. Methods such as rotational burning of grassland land to promote growth of grazing material; the addition of watering holes across a

property and the use of modified cattle fencing in an attempt to contain closed populations of game species are used to control livestock populations and increase the local carrying capacity of the farm (Palmer, 2003). However, once a procedure for the safe chemical immobilisation, capture and transport of wild game was developed in the 1970s (Young, 1973), a large industry in the breeding, sale and stocking of live herbivore species subsequently developed. The knock-on conservation effects of breading wildlife for the game industry were paramount in the recovery of much of the previously persecuted herbivore species of South Africa. The recovery of the once endangered bontebok (*Damaliscus pygargus pygarus*) from critically low population levels is just one example of the conservation effect of private breeding programs associated with the game farming and hunting industry of South Africa (Lloyd, 2008).

Beginning in the mid-1960s, modified cattle fencing was used by newly formed game farms and pre-existing cattle farms in an attempt to restrict the movement of desirable herbivore species and confine them to within a property boundary (Carruthers, 2008). Modern game fencing was soon developed, which consisted of long fence lines of between 8 and 14 horizontal wire strands suspended on intermittent posts termed “droppers”. As a result of the high permeability of such fence lines to both desirable game species and unwanted carnivore species, efforts were made to produce “predator-proof” fencing in order to segregate stocked game species from potential predators and to better control the movement of wild herbivore species within a property. Such fences consisted of partial or full diamond-pattern expanded metal fencing set into a subterranean concrete foundation, with the addition of electrified

fencing and tripwires. Predator-proof fencing is commonly used to surround nationally protected areas, where segregating large carnivores, such as lions and wild dogs, from the wider local community and agricultural industry is paramount. However, the cost and effort associated with installing and maintaining this predator proof fencing made the adoption of such perimeter fences rare in the private game breading industry itself.

Today the breeding, transportation and auctioning of wild game species is a thriving $1.1 billion a year industry, with the auction price of wild ungulates (Impala: $110, Blue wilderbeest: $250, bontebok: $ 2,575, sable (*Martes zibellina*): $23,000, Roan antelope (*Hippotragus equinus*): $25,500), specimen stud animals (e.g. cape buffalo (*Syncerus caffer*): $ 2.6 million) and rare polymorphs of wild ungulates (e.g. White blesbok: $ 250, black impala: $1,300, golden wildebeest: $4,500, black kudu: $20,000, King wilderbeest: $210,000) increasing substantially over the last decade (Farmers Weekly 2014). Presently, private game breeding and farming remains a strong factor in South Africa's economy with many secondary industries such as veterinary science, game farm management, ecological consultancy companies and professional hunting outfitters reliant on the industry for success.

As a result of the rapid change in agricultural land-use in South Africa, the levels of wildlife are estimated to be at their highest in the last century (Carruthers, 2008). Due to this, South Africa is considered to have undergone one of the great agricultural

transitions in Africa's recent history (Bothma *et al*., 2009, Carruthers, 2008), However, as a result of the rapid transformation and shift in conservation focus and the novel and wide ranging approaches to farming wildlife, many relevant questions on the effectiveness and appropriate management strategies in game farming environments remain unanswered.

2.2 Location of the North West Province

The North West Province of South Africa (Figure 2.1) was chosen as the focal region of this study due to the reports of a high prevalence of the black-backed jackal in the private game-breeding farms in this area, and the associated effort invested in lethal control of this species (Thorn *et al*., 2009, Thorn, 2009, Thorn *et al*., 2013). Study sites were chosen within a 4,800 km^2 area of this Province in an attempt to reduce the variation in habitat heterogeneity across the study sites, while considering the constraints of the sampling effort associated with a study of this type (Thorn *et al*., 2010, Yarnell *et al*., 2013).

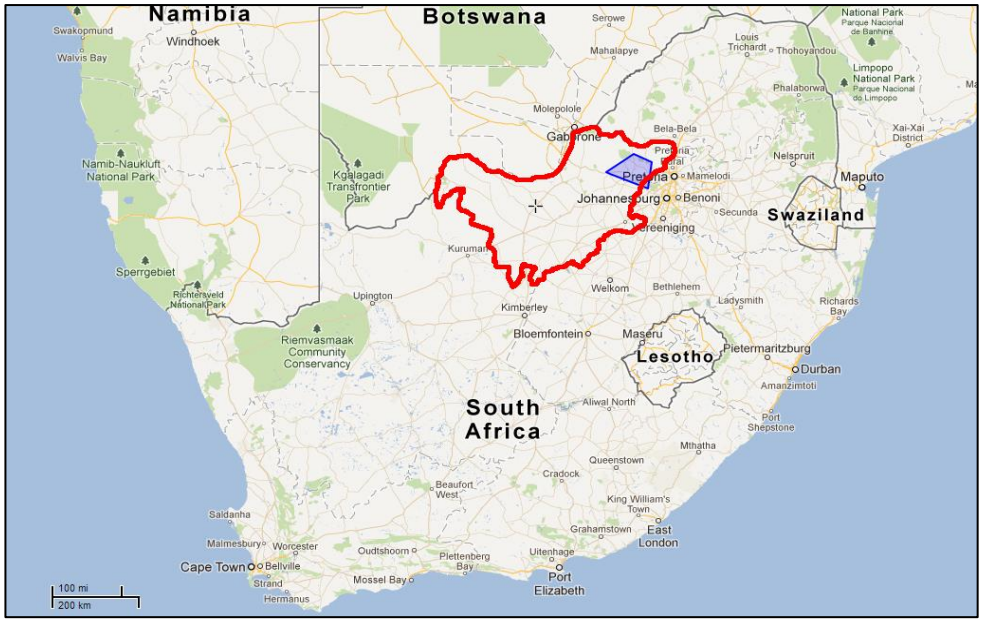


Figure 2.1. *A map showing the North West Province municipal boundaries (red: 104,882 km^2). In order to maintain habitat homogeneity, study sites were selected from an area of 4,800km^2, highlighted in blue. Map modified and compiled from Google Earth.*

2.2.1 Land use types of the North West Province of South Africa

The North West Province of South Africa has an estimated population size of between 3.1 – 3.6 million people, 65.1 % of which inhabit rural areas (Lehohla, 2003). A mosaic of land use types are present across the North West Province, with land use within the region consisting mainly of agriculture; livestock grazing; and rare mineral mining (Figure 2.2) (Tladi *et al*., 2002). Approximately 85% of the total provincial land ownership is private (Tladi *et al*., 2002), with 81% of the Province's land declared as suitable for agricultural development. Livestock grazing accounts for 46% of the total land use (Tladi *et al*., 2002) with agriculture and mining cited as the main economic contributors to the region's gross domestic product (de Villiers *et al*., 2002). Other areas of the Province's economy showing recent growth and investment include the energy and manufacturing industries (Tladi *et al*., 2002).

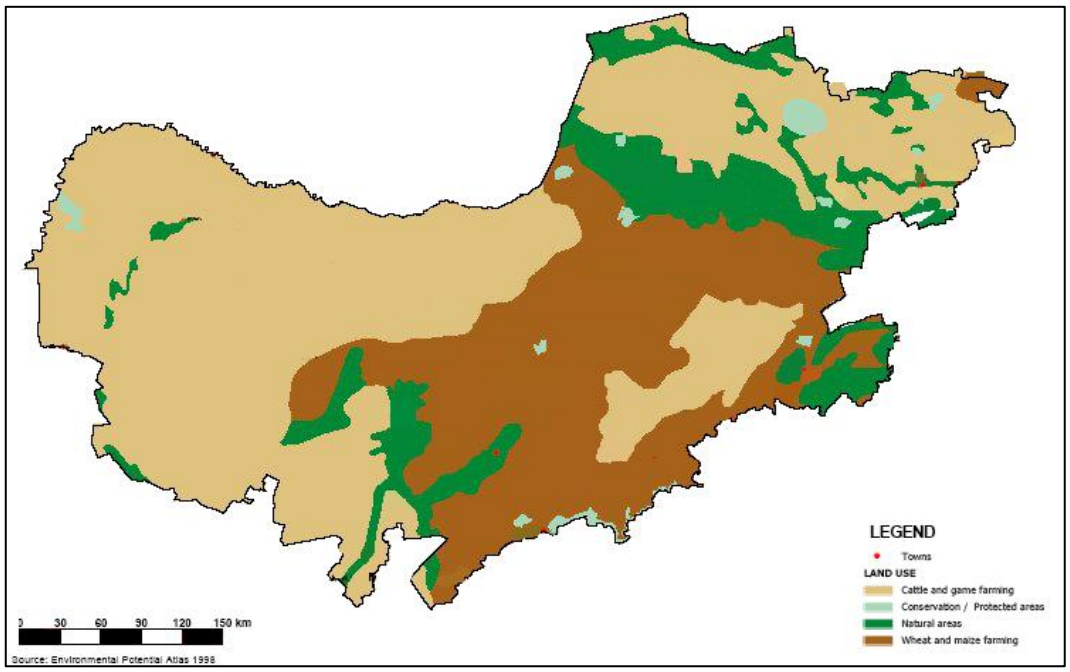


Figure 2.2. *A map depicting the major land use types of the North West Province, South Africa. (Breytenbach, 2002).*

2.2.2 Biome and habitat types of the North West Province

The majority of the North West Province is situated within the savannah biome which is prevalent over 70% of the Province (de Villiers *et al*., 2002). The remainder of the Province is situated within a mixed grassland biome that is dominant over the south east of the region (Figure 2.3) (de Villiers *et al*., 2002).

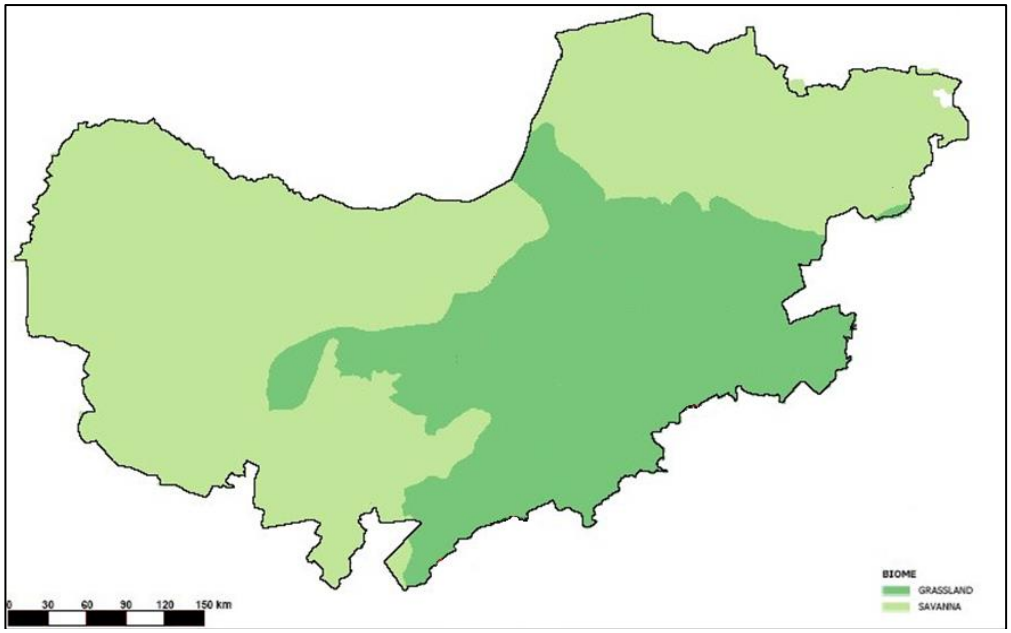


Figure 2.3. *A map depicting the major biomes of the North West Province, South Africa. (Breytenbach, 2002).*

A longitudinal gradient of vegetation biodiversity is apparent across the Province, with the highest number of vegetation types being recorded in the North East of the region (de Villiers *et al*., 2002). The most common vegetation type within the Province is bushveld, and is subcategorised into sourish mixed bushveld; sour bushveld; and mixed bushveld (Figure 2.4).

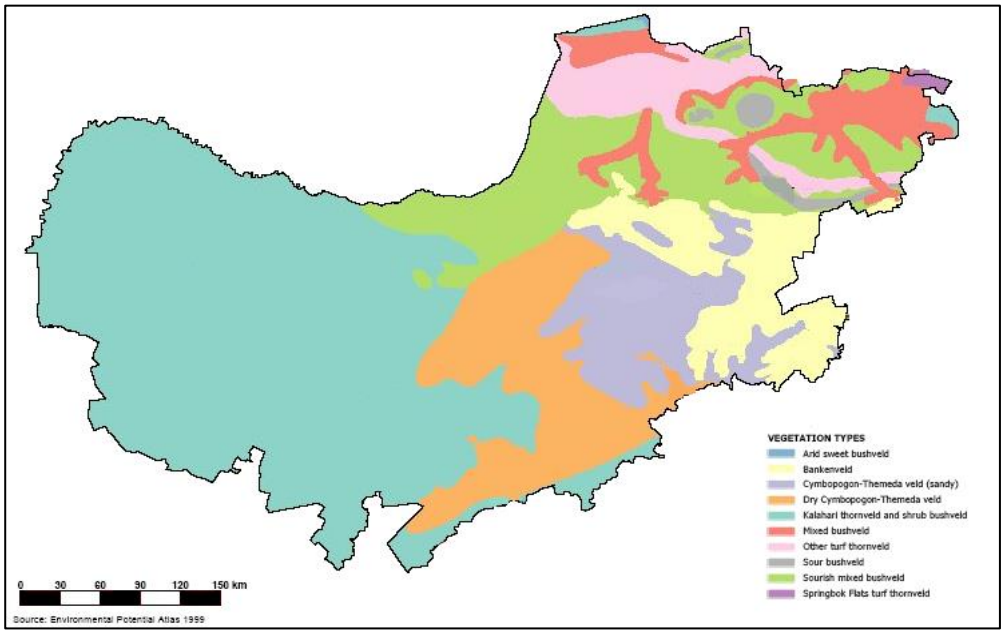


Figure 2.4. *A map depicting the most common vegetation types and coverage in the North West Province of South Africa. (Breytenbach, 2002, Hooper et al., 2012).*

2.2.3 Average precipitation and temperature of the North West Province

The annual precipitation is both temporally and spatially variable across the Province. The majority of rainfall occurs between the months of October and March (de Villiers *et al*., 2002) with an average annual rainfall of between 300 and 520 mm (de Villiers *et al*., 2002, Blignaut and Van Heerden, 2009). Measurements of over 600 mm pa have been recorded from within the study area, with peak rainfall commonly occurring in December (de Villiers *et al*., 2002). Rainfall is restricted across much of the western semi-arid domain, with rainfall concentrated to the northern and eastern temperate regions of the Province (Figure 2.5). Vegetation diversity and biomass are positively correlated with precipitation distribution within the Province.

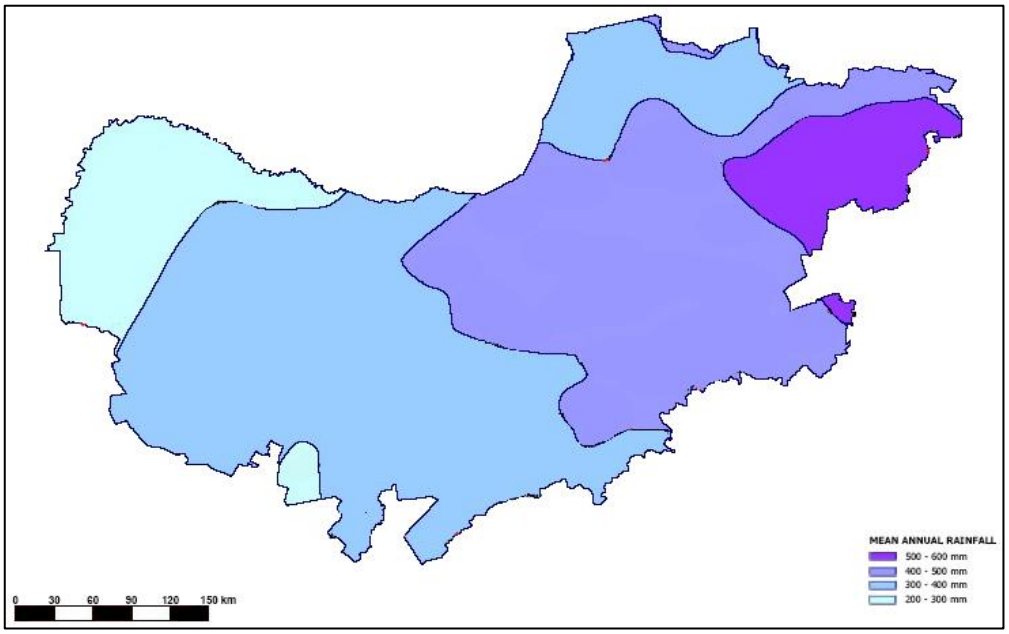


Figure 2.5. *A map showing the mean annual rainfall across the North West Province of South Africa in mm. (Breytenbach, 2002, Hooper et al., 2012).*

The study area experiences a large seasonal variation in average daily temperatures, with annual minimum and maximum temperatures of 0.9^{o}C and 32^{o}C respectively. Maximum and minimum daily temperatures occur within the months of January and July respectively (de Villiers *et al*., 2002, Blignaut and Van Heerden, 2009). Minimum night time temperatures within the study area have been recorded as low as -9^{0}C during the month of July.

2.2.4 Topography of the North West Province

With an elevation range of 826m, topographical relief is considered limited across the province as the maximum elevation within the region is 1,852 m above sea level. The

point of maximum elevation is located within the Magaliesburg mountain range. Other notable topographical and geological features within the study area include the ancient super volcano and associated igneous and ferrous constructs of Pilanesberg National Park. Topographical features are largely absent from the central and western regions of the province (de Villiers *et al*., 2002).

2.3 Experimental design and study site descriptions

In order to test the hypotheses of this investigation, the population parameters of the black-backed jackal were investigated under the effects of lethal control (predator control) and excess food availability (predator feeding), and by comparison with areas that practiced no predator management strategies (predator neutral). A spatially randomised split plot experimental design was used to reduce bias in site-specific variation in habitat coverage and local jackal abundance. Six privately owned game farms were selected from within the North West Province to represent the carnivore management strategies investigated in this study. Each treatment type was studied in two spatially independent sites (Figure 2.6).

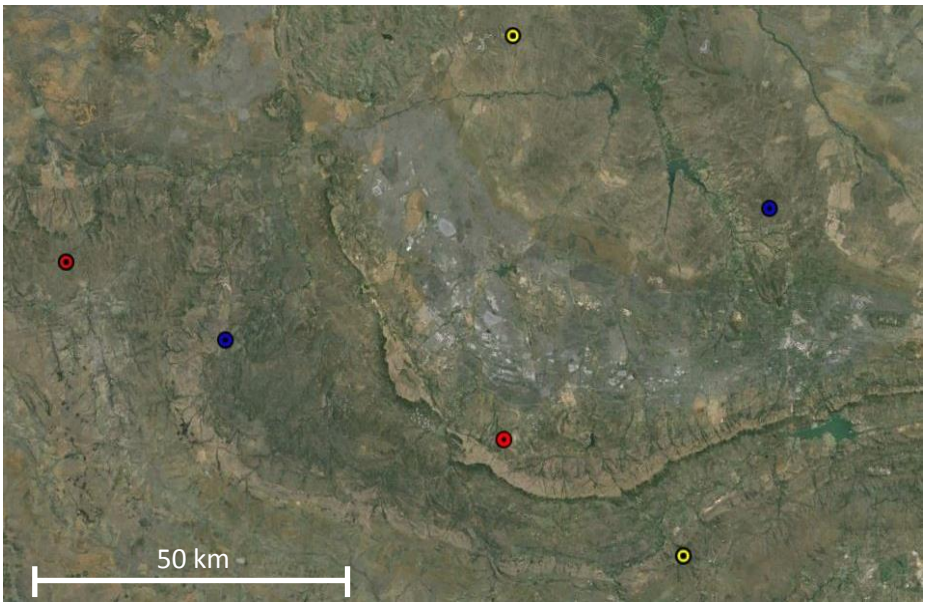


Figure 2.6. *An aerial photograph showing the position and proximity of paired study sites used in this investigation. Red = Lethal predator control sites, Yellow = Predator feeding sites, Blue = Predator neutral sites. Image sourced and modified from Google earth.*

The six study sites were selected from a pool of potential study sites within the study area in the order in which they responded to requests to undertake this investigation. The exact GPS locations for each site are not given as site anonymisation was requested in order to negate potential poaching events resulting from species occupancy described at each site. Livestock and wildlife species stocked and observed by landowners at each site is summarised in Table 2.1. All farms had standard game fencing surrounding the parameter of the property; no internal fencing was present within study sites.

Table 2.1. *A summary table showing the game species stocked at each study site as described by individual landowners PC = predator control, PF = Predator feeding, PN = Predator neutral.*

Species	PC1	PC2	PF1	PF2	PN1	PN2
Impala (*Aepyceros melampus*)	x	x	x	x	x	x
Blesbok (*Damaliscus pygargus phillipsi*)	x	x	x	x	x	x
Common reed buck (*Redunca arundinum*)	x	x	x	x		x
Mountain reedbuck (*Redunca fulvorufula*)			x	x		x
Tsessebe (*Damaliscus lunatus*)			x			
Waterbuck (*Kobus ellipsiprymnus*)			x			x
Gemsbok (*Oryx gazelle*)	x		x	x	x	
Greater kudu (*Tragelaphus strepsiceros*)	x		x	x	x	x
Blue wildebeest (*Connochaetes taurinus*)	x	x	x	x	x	x
Black wildebeest (*Connochaetes gnou*)				x		
Burchell's zebra (*Equus quagga burchellii*)	x		x	x	x	x
Common eland (*Taurotragus oryx*)	x		x	x		x
Giraffe (*Giraffa camelopardalis*)	x		x	x	x	x
White rhinoceros (*Ceratotherium simum*)			x	x	x	x
Black rhinoceros (*Diceros bicornis*)						x
Hippopotamus (*Hippopotamus amphibius*)					x	x
Nyala (*Tragelaphus angasii*).				x	x	x
Sable (*Hippotragus niger*)						x
Cape buffalo (*Syncerus caffer*)					x	x

2.3.1 Predator control sites

Predator control sites selected for this study had practised the lethal removal of the black-backed jackal for a minimum of 10 years prior to this investigation. The average number of jackals removed at each site as described by landowners ranged between 4 and 16 individuals per year. Lethal control was undertaken during the livestock breeding season using free shooting methods only. No other predator species were controlled or removed from these study sites.

Predator control site 1 (Figure 2.7) operates as a small scale commercial game farm specialising in hunting and the livestock trade. With a total area of 1,200 ha, predator control site 1 is the smallest site included in this study. A gradient of topographical relief is present across the farm with the highest point towards the northern perimeter of the study site. A mixed habitat type of open grassland, acacia woodland and forest communities are present supporting a wide variety of naturally occurring wildlife. Notable carnivores recorded on this site by landowner observations include the black-backed jackal, brown hyaena and caracal.

Predator control site 2 (Figure 2.8) is a game farm comprised of 1,250 ha of privately owned agricultural land used for livestock production and local community education. The main habitat type of this site is open grassland with interspersed areas of mixed scrub. Topographical relief is limited across the reserve, with mild undulating hills present in the north of the site boundaries. Notable carnivore species described by landowners of this site include the black-backed jackal, brown hyaena, leopard and caracal.

Figure 2.7. *An aerial photograph of predator control site 1 with overlaid land boundaries (white). Aerial imagery sourced and modified from Google Earth.*

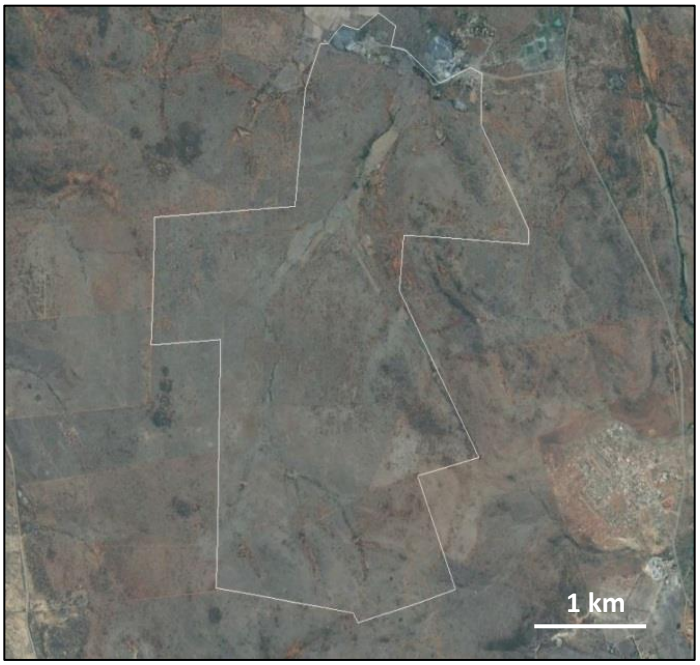


Figure 2.8. *An aerial photograph of predator control site 2 with overlaid land boundaries (white). Aerial imagery sourced and modified from Google Earth.*

2.3.2 Predator feeding sites

In order to investigate the effect of food availability on the population parameters of the black-backed jackal, two sites that had active vulture restaurants for at least four years were selected for this study. A number of private game farms and nationally protected areas of South Africa are involved with a program to provide a stable and non-contaminated carrion source for endangered species of vultures to feed from, in an attempt to conserve native vulture species. These sites provided between one and five large (>50 kg) cattle, pig and horse carcasses with a known veterinary history each week at designated sites known as vulture restaurants in order to ensure an abundant supply of food for vulture species. No attempts are made to exclude other scavenger species from the carrion areas thus clustered and abundant food resource areas were available for many other scavenger species including the black-backed jackal and brown hyaena.

Predator feeding site 1 is a 4,760 ha privately owned exempt game farm located in the North West Province of South Africa (Figure 2.9). Topographical features are limited at this site with a mildly undulating terrain interspersed with occasional rocky outcrops known as Kjoppis. Open grassland is the most common habitat type found at this site, with frequent areas of mixed bushveld and acacia woodland throughout the property. The main economic enterprises of this farm include private ecotourism, education, commercial hunting and game breeding. This site does not practise any form of predator control and supplements carnivore diet in the form of a vulture restaurant where carcasses are gathered on a regular weekly basis. Frequently observed wild

carnivores present on this site include the black-backed jackal, caracal, brown hyaena and serval (*Leptailurus serval*).

Predator feeding site 2 is a 3,120 ha exempt game farm in the Gauteng Province of South Africa (Figure 2.10). The foci of this reserve include ecotourism, ecological research, education, rare species breeding and the game meat industry. Large areas of flat open grassland surround a central mountainous section comprised of dense woodland and thicket. Topographical relief is confined to the central and northern areas of the reserve. The diet of scavenger species is regularly supplemented in the form of a vulture restaurant. No lethal control of carnivores is practised on this reserve. Common carnivore species recorded on this site include the brown hyaena and black-backed jackal.

Figure 2.9. *Aerial photograph of predator feeding site 1 and overlaid land boundaries (white). Aerial photography sourced and adapted from Google Earth.*

Figure 2.10. *Aerial photograph of predator feeding site 2 and overlaid land boundaries (white). Aerial photography sourced and adapted from Google Earth.*

2.3.3 Predator neutral sites

Predator neutral game farms were selected as sites to which black-backed jackal population indices, measured in supplementary feeding sites and lethal control sites, were to be compared. Game farms were considered predator neutral when no forms of predator control or supplementary feeding were undertaken at these sites.

Predator neutral site 1 is comprised of 4,660 ha of privately owned agricultural land (Figure.2.11). The main economic drivers of this farm include rare species breeding and exclusive luxury ecotourism. Rare species breed in fenced areas on this site and include the golden wildebeest; a polymorphism of the blue wildebeest (*Connochaetes taurinus*), sable and tuberculosis-free African buffalo. The main habitat types of this

site are classed as open grassland acacia woodland and mixed scrub. Topography is highly variable and has both flat and rocky mountainous areas. Wildly occurring carnivores frequently observed include the black-backed jackal; brown hyaena; caracal; leopard and serval.

Predator neutral site 2 is a 3,700 ha game farm in the North West Province of South Africa (Figure 2.12), specialising in the breeding of the Sable subspecies as well as luxury ecotourism. Specialised breeding camps of approximately 100 ha or less were used to segregate Sable from the wider game farm. These breeding camps were not included in this sampling area of this study. Overall topographical range is limited in this study site; however a large number of high rocky outcrops are present across the area. A mix of vegetation types is found at this site with open grassland, dense acacia woodlands and open sodic areas. Commonly observed carnivores include the leopard, black-backed jackal, caracal, brown hyaena and serval.

Figure 2.11. *Aerial photograph of predator neutral site 1 and overlaid land boundaries (white). Aerial photography was sourced and modified from Google Earth.*

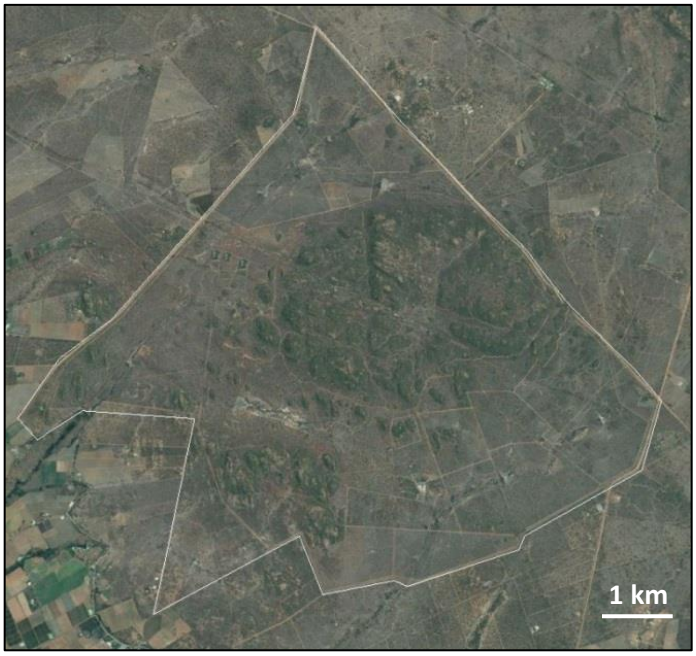


Figure 2.12. *Aerial photograph of predator neutral site 2 and overlaid land boundaries (white). Aerial photography was sourced and modified from Google Earth.*

2.4 Summary of experimental work

This section provides a brief summary of the experimental chapters within this thesis and outlines the methods used in this investigation. The specific details of the methods used to answer the questions posed in this investigation are presented in detail within each chapter.

Chapters III and IV of this thesis use camera trap capture rates and faecal density estimates to quantify the relative abundance of *C. mesomelas* at each of the six sites described above. Furthermore, relative abundance indices are calculated for black-backed jackal populations under predator control and predator feeding treatment types and compared with the average relative abundance of *C. mesomelas* populations under the constraints of predator neutral management. Chapter V is primarily concerned with the development and characterisation of cross species microsatellite markers for use in population studies of the black-backed jackal in South Africa. Chapter VI provides evidence for the successful amplification and use of these molecular markers using quantitative PCR from DNA sources acquired from non-invasively sampled faeces collected from the natural environment. Chapter VII summarises the use of these molecular markers in an attempt to highlight the effect of excess food availability and lethal removal of *C. mesomelas* on the metapopulation dynamics of the black-backed jackal by examining the overall population structure of the sampled metapopulation, and by estimating the inbreeding coefficient and relatedness within each site.

Chapter III

Camera trapping of the black-backed jackal for the photo-capture rate and relative index of abundance in game farm ecosystems of the North West Province, South Africa

3.1 Introduction and aims

The cryptic and nocturnal nature of the black-backed jackal makes quantifying local abundance difficult (Nowell and Jackson, 1996, Sillero-Zubiri, 2004, Thorn *et al*., 2011). Commonly used direct count surveying methods such as transect observations and spotlighting are prone to low sample sizes and are highly susceptible to a variation in species detection due to habitat heterogeneity (Dempsey *et al*., 2014). Camera traps are now regarded as a non-invasive tool that allows the collection of data such as relative abundance and distributions patterns of cryptic mammalian species (Carbone *et al*., 2001, Rowcliffe *et al*., 2008, Rowcliffe and Carbone, 2008). This chapter describes the use of camera trap capture frequencies to quantify the relative abundance of black-backed jackal populations in game farms of the North West Province in order to investigate the effect of predator control and predator feeding on the local scale. It is hypothesised that if predator control is an effective strategy for reducing predator populations then a significant difference in relative abundance of the black-backed jackal would be expected between sites of high offtake and sites of low offtake. Furthermore, it is hypothesised that if resource availability is a major driving force of jackal population density, dispersal and migration, then the frequency

of occurrence of jackals in areas of high food availability will be significantly greater than those in areas of low food availability.

3.2 Methods

3.2.1 Experimental design

The six spatially randomised game farms within the North West Province described in chapter II were used as study sites for this experiment. Each study site was a privately owned rural game farm on un-developed land outside of nationally protected areas. Each site was sampled twice, in a randomized order, at approximately six month intervals to account for seasonal variation in detection. The duration of each camera trap sampling sessions lasted for 16 consecutive days, were broken down into four day independent blocks. After each four day block, camera images were recovered and each camera site was baited and reset.

Camera stations were equally spaced in each site at a distance of 2km apart in all directions. Camera density was maintained at between 0.1 and 0.2 cameras / km^2 depending on the size of the study site (min =1200 Ha; max = 4700 Ha). Between three and seven cameras were used at each site to maintain a consistent camera density. A total of 10 individual, passive, Infrared Scout Guard SG550V cameras (supplied by UK Cameras) were used in this study. Each camera was powered by 8 x AA batteries and had capacity to store >1100 images at a resolution of 5 megapixels. Cameras were

numbered and camera selection at each camera site was randomized and recorded for each sampling session in order to minimise and quantify the impact of camera performance bias. Camera positions remained constant for the duration of the study period and were recorded using a Garmin GPS Map-62 device, Garmin Part Number: 010-00868-00 (+/- 3m).

The exact locations of the cameras were chosen via an assessment for suitable locations within a 50m radius of the predicted camera sites, which were dictated by the 2km spacing intervals from the origin of the first camera location set in the centre of each study site. Cameras were attached to large and sturdy trees at a height of 40 cm using 20mm steel wire and a nylon strap. Cameras were orientated in a northerly direction to reduce the chances of over exposure and sun glare. For accurate identification purposes the cameras were angled and aimed at a point 2.5m directly in front of the camera location (Swann *et al*., 2004). All cameras were set to a default medium sensitivity and programed to take a burst of three pictures upon being triggered. Cameras were triggered via the detection of heat and movement within the sensor range (120^{o}). All cameras were set to have a default delay of 30 seconds between each potential trigger to conserve battery life. Picture resolution was set to a maximum of 5 mega pixels in order to aid species identification. Accuracy of date and time stamps were checked and adjusted upon setup. Nocturnal images were illuminated with a passive infrared flash to reduce disturbance at the camera sites. All cameras were supplied with 2GB compatible SanDisk memory cards to store and transport the recorded media. Each camera site was baited with approximately 2kg of

fermenting ungulate offal, retained in place by a 30 cm dual pronged steel pin. Bait was placed at a distance of 2m, directly in front of each camera. The area in front of the camera's field of vision was cleared of residual high vegetation to reduce the probability of false triggers. Clearance was undertaken using hand tools to minimise site disturbance.

As jackals and other South African scavengers are known to use man-made dirt roads as transport routes with territorial boundaries (Sillero-Zubiri and Macdonald, 2006), a drag consisting of 2kg of fermenting ungulate offal was attached to a vehicle by wire and towed 1km along the road, away from the camera site. Drags were undertaken in a single direction from the camera site, towards the perimeter fence at each camera and study site. This was done in an attempt to increase and standardise the sampling area of the baited camera traps (Thorn, 2009). The camera sites were re-baited with approximately 2kg of fermented ungulate offal every four days throughout the 16 day sampling session (Thorn, 2009). During the re-baiting process, camera battery levels were checked and SD cards exchanged and formatted for use. Images from the collected SD cards were transferred to an external storage device for analysis.

3.2.2 Camera trap analysis

Camera Base v1.5.1 (Tobler, 2007), a customisable Microsoft access program, was used to store, collate and analyse camera trap data collected from the study area. All camera stations and temporal repeats were combined at the site level to yield a

sample size of six in order to avoid psudoreplication within each sampling site (n = 2 per treatment). The mean relative frequency of abundance (Relative Index of Abundance (RIA)) for each site, and treatment type, was calculated for the black-backed jackal. RIAs were also calculated for the leopard and brown hyaena, as they represent other competitive scavenger species within the region that have the potential to affect the density and distribution of the black-backed jackal through inter-guild competition (Roemer *et al*., 2009, Merwe *et al*., 2009). The relative frequency of abundance was calculated using the "independent events" principle outlined in the studies by O'Brien *et al* (2003). Independent events are classified as the number of photographs taken of a given species, over a set duration, interspersed by an ecologically relevant period of dispersal, in order to reduce overestimation of abundance. In this study the dispersal time given between each independent event was standardised at 30 minutes (O'Brien *et al*., 2003, Thorn, 2009). As camera density has been maintained between sites, the relative frequency of abundance for a site, or block, can be expressed as the total number of independent events for a given species, divided by the total number of camera trap hours (number of camera traps multiplied by duration) (O'Brien *et al*., 2003).

Analysis of variance and Tukey posthoc test statistics were used to test the significance of variation in RIA estimates at each site and treatment type. A Generalised Linear Model was used to investigate the explanatory power of the treatment type against the RIAs of *C. mesomelas.* Inter-site variation within and between treatment types were examined for statistically significant explanatory powers. A non-metric multi

dimensonal scaling ordination technique was used to indicate the bias in carnivore community structure in response to treatment type. A subsequent Jaccard disimillarity matrix was used to cluster carnivore community structure in response to treatment type and site. In order to implement the rank-order Jaccard dissimilarity algorithm, data was first log-transformed using the decostand standardisation function to remove negative and zero type values in the root data and to give higher weighting to presences over quantification of abundance. The "complete" hierarchical clustering algorithm was used to cluster Jaccard disimillarity indices and to produce a tree structure depicting site clusters by similar species presence and abundance. All statistical analysis was undertaken using the program R v 3.0.2 (Venables and Smith, 2005).

3.3 Results

3.3.1 Sample size, duration and species diversity

A total of 1,056 camera trap nights were surveyed between the months of May 2010 and November 2012 across the six study sites described in chapter ll. The combined area of all six study sites has been calculated as 18,260 ha. Camera trap stations were standardised at fixed distances of 2km across all study sites. A gross sum of 27,462 camera trap images containing one or more identifiable species were collected and analysed, culminating in a total of 9,154 camera trap triggers containing identifiable species. A total of 52 mammalian species and 12 avian species were recorded within the duration and conditions of the study period. The total number of species recorded

during the sampling period was 24 in the predator control sites, 43 in the predator feeding sites and 38 in the predator neutral sites. The accumulated sum of all identifiable individuals within the camera trap images, across all species, was 14,839 individuals.

3.3.2 Black-backed jackal relative index of abundance and sample size

The relative index of abundance (RIA) for black-backed jackal populations at each study site was calculated as:

$$RIA = \left(\frac{\Sigma t}{s}\right)\Big/_{i}$$

Where t = number of independent events attributed to *C. mesomelas* per sampling session, s = number of camera stations within a study site, and i = number of camera trap nights per repeat.

The accumulative sum of all black-backed jackals recorded in all trigger events at all sites study sites was 4,555. A total of 2118 camera trigger events were attributed to one or more black-backed jackal capture events. A combined total of 1,197 identifiable camera trigger events across all sites were classified as independent for the black-backed jackal.

No significant difference in *C. mesomelas* abundance indices between sites of the same treatment could be distinguished (Figure 3.1 Welch t-tests: Predator control site 1 + 2: t = 1.535 p = 0.158, Predator feeding sites 1 + 2: t = 0.66, p = 0.526, Predator neutral t = 0.6429, p =0.536). However, a significant variation in *C. mesomelas* RIA estimates could be detected between all study sites (Figure 3.1) (ANOVA: F(5,41) = 10.17 P<0.05).

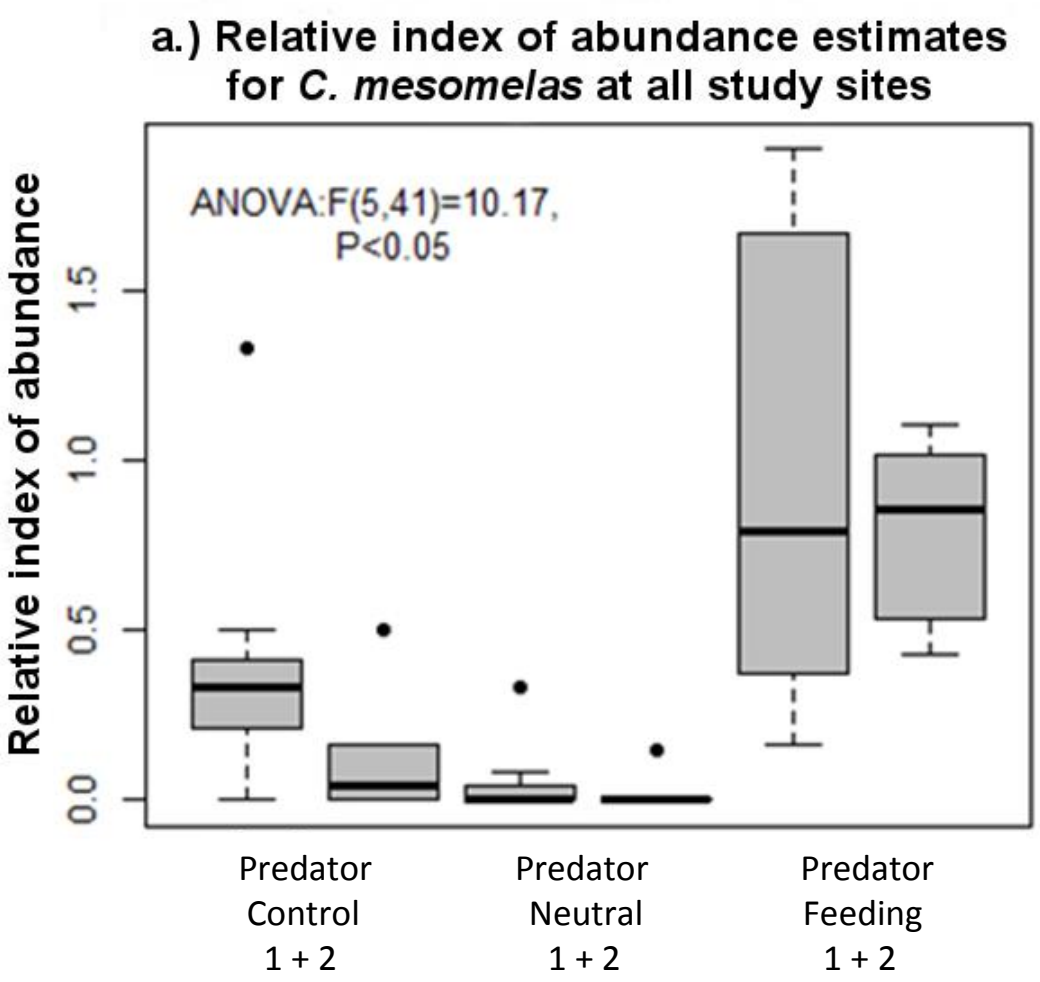


Figure.3.1. *Mean RIA of C. mesomelas at all study sites (ANOVA: F(5,41) = 10.17 P < 0.05).*

A generalised linear model was used to investigate the explanatory power of the treatment type against the relative abundance of *C. mesomelas.* Inter-site variation within and between treatment types was examined for a statistically significant explanatory power (Table 3.1). Following a step-wise model simplification procedure, site-specific residual deviation was not found to significantly influence or increase the explanatory power of the minimum adequate model.

Table 3.1 *A summary of the general linear model of C. mesomelas relative index of abundance values with treatment type and site as fixed effects. (Quassipoisson response, n = 42.) Total deviance explained: 62.2%. Residual variation within sites did not significantly increase the % deviance explained in this model (P = 0.195).*

Fixed effects	df	Parameter estimate	% deviance explained	p
Treatment	2	17.0175	57.554	$2.36x10^{-13}$
Site	5	1.3746	4.649	0.1954

As no significant variation between sites within the same treatment type can be detected, treatment-specific site data was accumulated to examine the effects at the treatment level by combining site RIA averages (Figure 3.2).

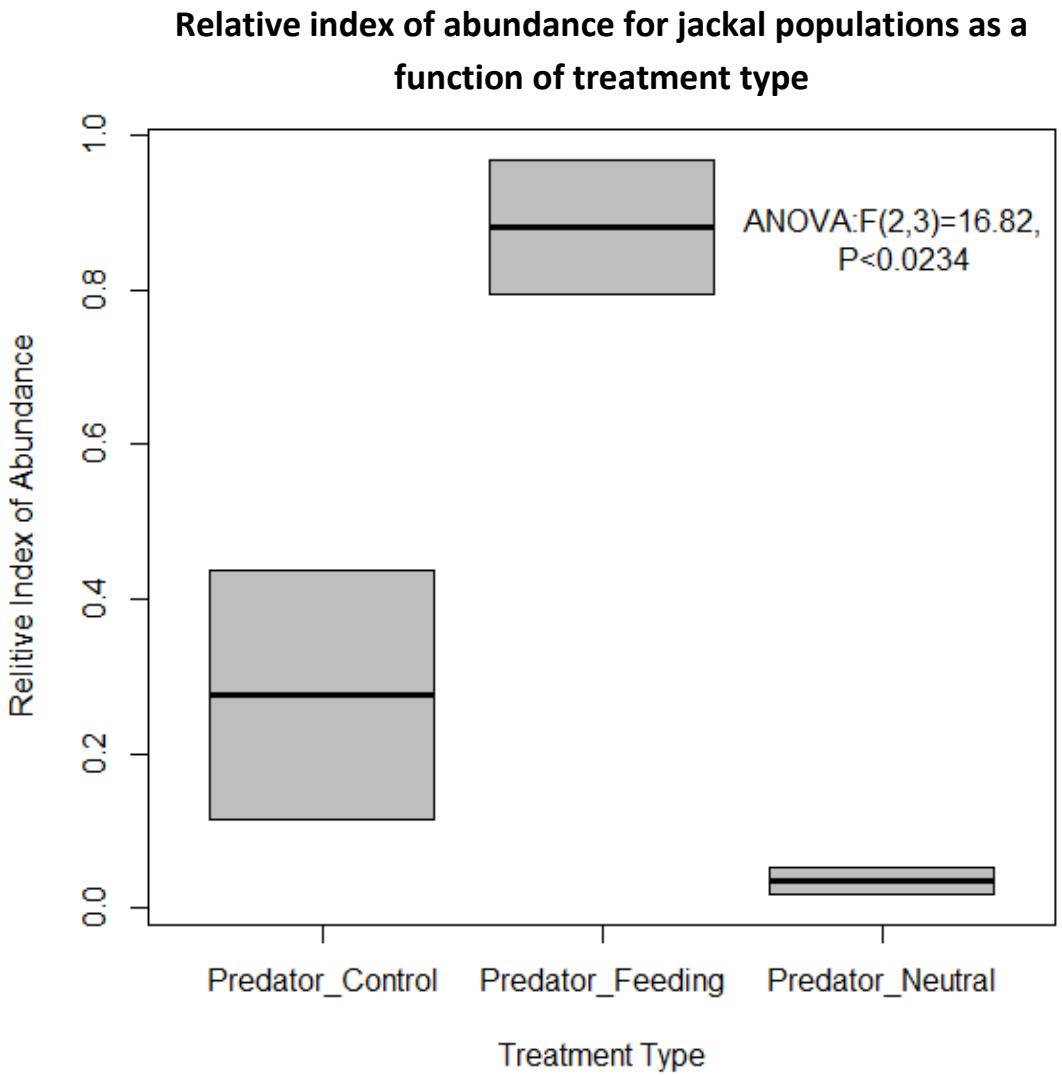


Figure 3.2. *Relative index of abundance for C. mesomelas under predator control, predator feeding and predator neutral conditions. ANOVA and post hoc Tukey Highest Significant Difference (HSD) tests reveal the relative abundance of jackals at predator feeding sites are significantly greater than both predator control or predator neutral sites, F(2,3) = 16.82, P<0.0234. TukeyHSD: Treatment type pairwise analyses of C. mesomelas relative index of abundance revealed that the predator feeding treatment of C. mesomelas had significantly higher jackal abundance relative to the predator neutral treatment type. TukeyHSD* p = 0.0225*. There was no significant difference in RIA between the predator control and the predatory neutral treatment types*: p = 0.370. For completion t*here was a significantly higher abundance of C. mesomelas under the predator feeding treatment compared with predator control treatment type*: p = 0.045*.*

Sites that supplemented the diet of black-backed jackals (predator feeding sites) had significantly higher relative abundance estimates than either predator control and predator neutral sites. The treatment groups were examined independently under the constraints of the sum of squares F-test assuming equal variance; Pairwise interactions were shown to be significant between the predator feeding and predator control treatments ($F = 10.9$ $df = 1$, $p < 0.05$) and the predator feeding and predator neutral treatments ($F = 91.08$, $df = 1$ $p < 0.05$). No significant difference in jackal RIA could be detected between predator control and predator neutral treatment types ($F = 2.205$, $df = 1$, $p = 0.276$.)

The predator control treatment had a significantly higher mean RIA value for the black-backed jackal than the predator neutral treatment type (Figure 3.2.) The predator feeding treatment had a significantly higher mean RIA value than both predator neutral and predator control treatments (Figure 3.2.) The two predator neutral sites had the lowest mean RIA values of all treatment types (Figure 3.2).

3.3.3 Carnivore abundance and community structure

A combined relative index of abundance was calculated for the black-backed jackal, brown hyaena, leopard, caracal and serval to provide a relative estimate of carnivore abundance for each treatment group and site (Figure 3.3). No significant difference in carnivore relative abundance was detected between sites of the same treatment type (Welch two sample t-test $P > 0.05$). In addition, a General Linear Model was used to

test for significant effects arising from site-specific variation within treatment types (Table 3.2). No significant increase in model explanatory power could be attributed to residual site-specific variation outside the variation explained by treatment type ($p = 0.1524$).

No significant difference in mean carnivore abundance could be determined between sampling locations of the same treatment type. Site-specific variation does not significantly affect carnivore abundance within treatment types (Welch paired samples t-test: $p > 0.05$).

Table 3.2. *Summary of the General Linear Model of carnivore relative index of abundance with treatment type and site as fixed effects. (quasi-Poisson response, n = 42.) Total deviance explained: 23.7%. Residual variation within sites did not significantly increase the % deviance explained in this model (P = 0.152), however the total explanatory power of the model is very low.*

Fixed effects	**df**	**Parameter estimate**	**% deviance explained**	**P**
Treatment	2	13.136	20.3	<0.001
Site	5	2.232	3.4	0.152

Carnivore abundance was compiled by treatment type (Figure 3.4) and examined for significant variation between treatment-group means (Figure 3.4).

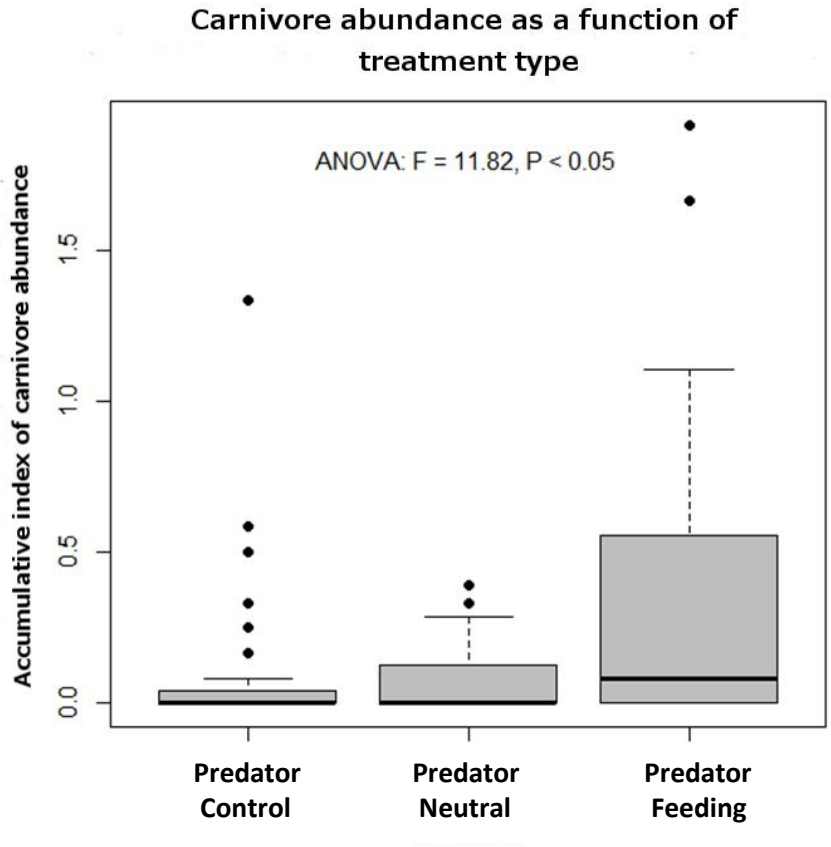


Figure 3.3. *A significant difference in total carnivore abundance was detected between treatment types. (ANOVA: F = 11.82 p < 0.05.) Predator feeding significantly increased carnivore abundance relative to the predator neutral treatment type (t = -3.9392 p < 0.05). Predator feeding had the greatest effect on carnivore abundance indices when compared with predator control (t = -3.333, p = 0.0014.)* ***c.)*** *Predator control did not significantly reduce carnivore diversity relative to the predator neutral treatment group (t = 0.66, p =0.512).*

3.3.4 Carnivore community structure

A non-metric multi-dimensional scaling (NMDS) ordination technique was used to indicate the bias in carnivore community structure in response to treatment type (Minchin, 1987, Clarke, 1993) (Figure 3.5). A subsequent Jaccard dissimilarity matrix (Pekalska *et al*., 2002) was used to cluster carnivore community structure in response

to treatment type and site (Figure 3.5). In order to implement the rank-order Jaccard dissimilarity algorithm, data was first log-transformed using the "decostand" standardisation function to remove negative and zero type values in the root data and therefore give higher weighting to presences over quantification of abundance. The "complete" hierarchical clustering algorithm was used to cluster Jaccard dissimilarity indices and produce a tree structure of similar clusters (sites) based on species presence (Figure 3.6). Sub tree clusters were organised from left to right in order of their observation sequence followed by clustering height.

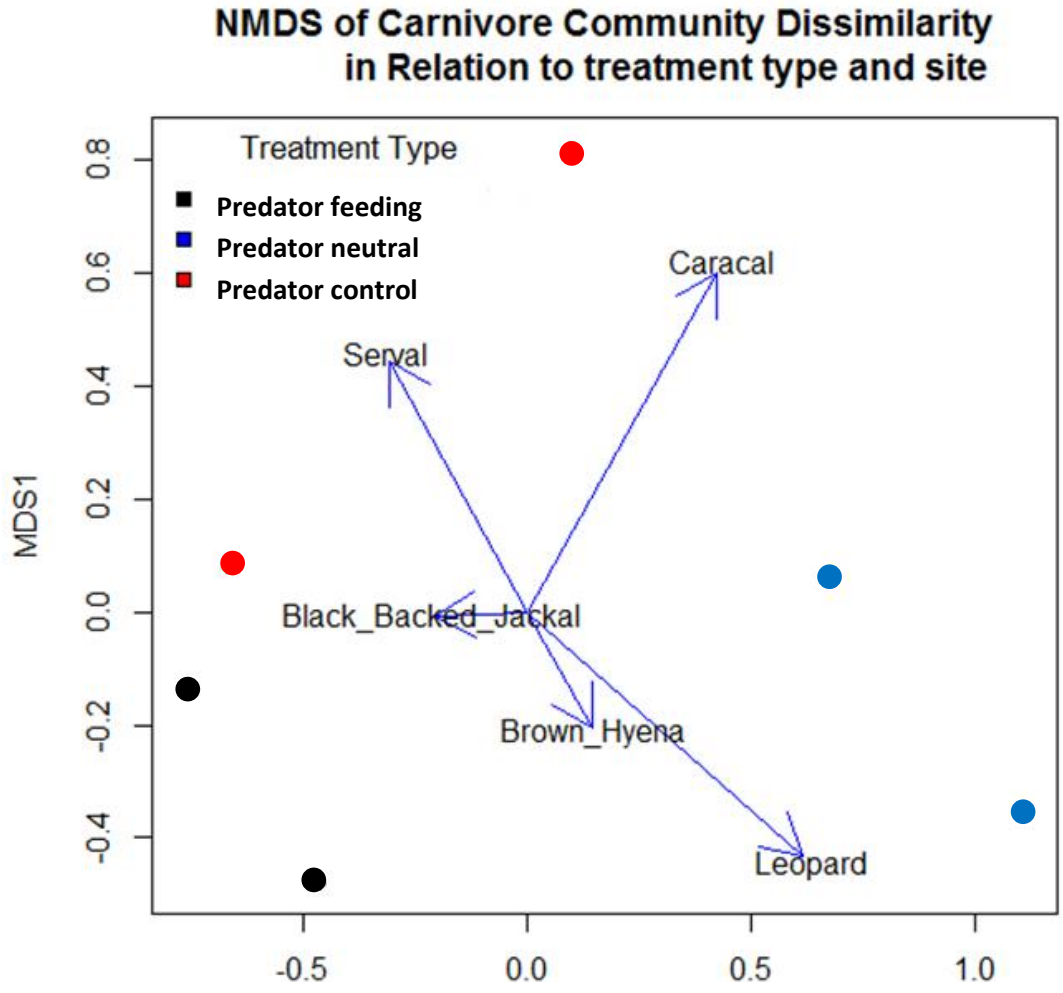


Figure 3.4. *NMDS of carnivore abundance dissimilarity in relation to treatment type. Sites are represented by coloured points and clustered by community composition dissimilarity. Predator feeding sites = Black, Predator neutral sites = Blue and Predator control sites = Red.*

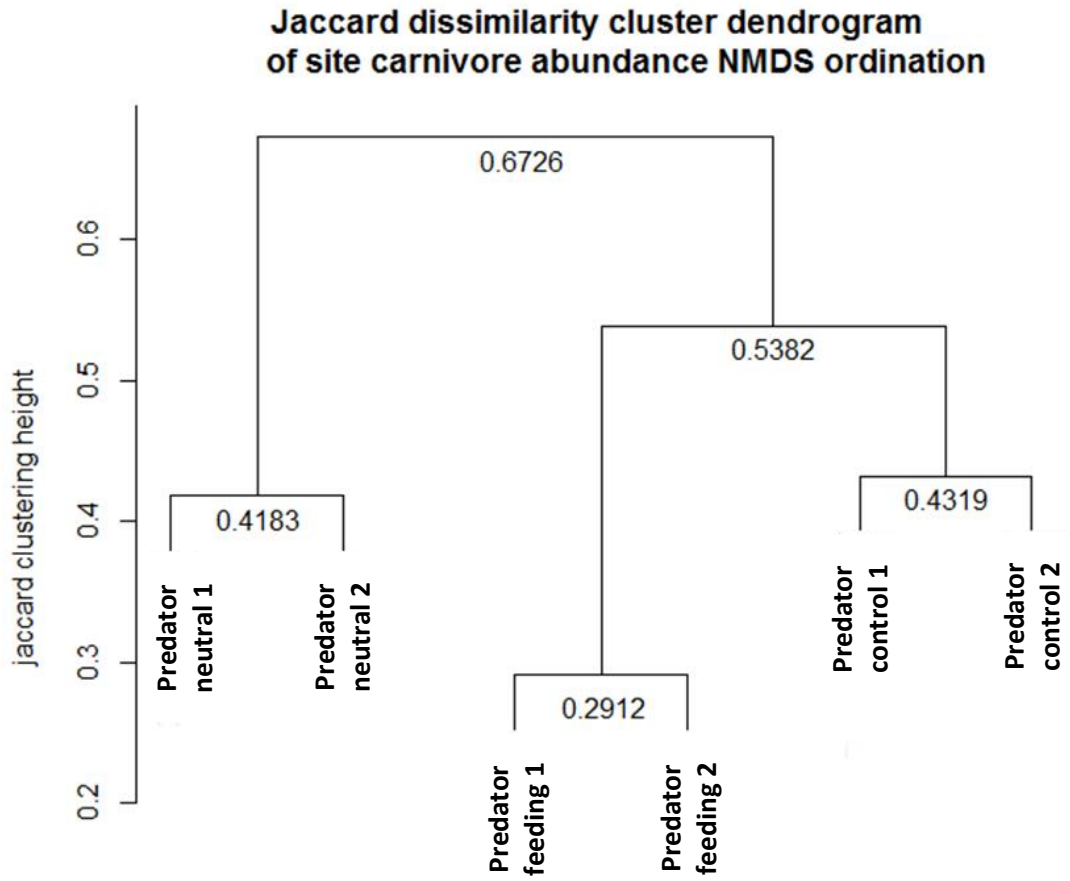


Figure 3.5. *Log-transformed Jaccard dissimilarity clustering dendrogram showing sites nested by carnivore abundance dissimilarity indices.*

The carnivore community structure in sites of lethal predatory control predominantly differed from other treatment groups by the presence and relative abundance of caracals and servals. In addition, the relatively low abundance of the black-backed jackal, brown hyaena and leopard, distinguishes this carnivore community from other sites and treatment groups. Sites that supplemented the feeding of carnivores (predator feeding) were dissimilar from other sites in the high relative abundance of the black-backed jackal. Sites that did not manage carnivore populations (predator neutral) were characterised by the combination of the relative abundance of the brown hyaena and the leopard.

When clustered via the Jaccard clustering algorithm, Log carnivore abundance dissimilarity was seen to consistently nest sites within treatment groups (Figure 3.6). The predator neutral sites were shown to have a greater dissimilarity in carnivore abundance than the lethal predatory control and predatory feeding treatment groups; in extenuation, the predator feeding and predator control treatment groups clustered tighter than the predator feeding, predator neutral treatment groups.

3.4 Discussion

3.4.1 The abundance of *C. mesomelas* in predator control sites

No significant difference in the relative abundance of the black backed jackal could be detected between sites of the same treatment type (Predator control site 1 + 2: t = 1.535 p = 0.158, Predator feeding sites 1 + 2: t = 0.66, p = 0.526, Predator neutral t = 0.6429, p =0.536 Figure 3.1). When site data was pooled by treatment type, no significant difference could be detected between the predator control group and predator neutral treatment group (Figure 3.2 AOV F test: F = 2.205, df = 1, p = 0.276, Figure 3.2.)

These abundance estimates imply no difference in the number of jackal individuals between predator control and predator neutral treatments, and indicate that the current level of removal of *C. mesomelas* implemented in the game farms studied in this investigation are ineffective as a predator management strategy. This evidence suggests that the current rate of removal of jackal individuals is not only ineffective in

reducing conflict, but potentially counter-productive in reducing black-backed jackal numbers in the private game farm environments studied. Therefore the hypothesis that predator control significantly reduces the abundance of black-backed jackals can be rejected. However, given the limitations to the camera trap sampling methodology; further investigation is required to corroborate the findings of this study.

The effects of predator control on the territorial stability and dispersal rates of *C. mesomelas* remain largely unknown and unpredictable as the metapopulation dynamics of the black-backed jackal, and many other similar conflict species, remain poorly understood. However, it has long been hypothesised by landowners and ecologist alike, that the re-population of newly vacated territories plays a significant role in the persistence of the black-backed jackal in South African agricultural land use types (Asa and Valdespino, 1998). As a patchwork of land use and carnivore management strategies is present across the North West Province (Thorn *et al*., 2013), small jackal subpopulations in non-persecuted areas can act as source populations which maintain and re-populate newly vacant territories following the removal of territory holding individuals. In addition, the effect of removing territory holding individuals may exacerbate territorial competition and promote smaller territory sizes for a given area through territory destabilisation (Asa and Valdespino, 1998). It can therefore be argued that the current level of removal of the black-backed jackal from private game farm environments is not viable in the long term maintenance of local jackal populations.

Studies investigating the control of European badger populations residing in agricultural land-use types of the UK have shown that minimum population reduction of > 70% is required over a six week period to successfully reduce and manage this species in the long term without causing local population extinctions (Anon, 2013). However, the specific analytical method used in the DEFRA report to produce the estimation of the required 70% reduction in population size is drawn on highly variable population density data which has resulted in unreliable culling estimates that have yet to undergo peer review (Anon, 2013). Predator harvesting quotas such as this are often controversial as detailed information on absolute population size are frequently unavailable and thus inadequate for estimating sustainable offtake quotas. Aside from the blanket ban on the use of poisons and gin trapping in wild species control, little legislation exists pertaining to the black- backed jackal with respect to pest control and offtake quotas within South Africa. Therefore future studies, that estimate the absolute density and average local population size of the black-backed jackal in a wider number of game farm environments of the North West Provence of South Africa, are required to better understand the ecological implications of predator offtake and lethal management of this species. Further information on the dispersal rates of the black-backed jackal are also required in order to better understand the metapopulation dynamics relating to territorial disturbance arising from predator control methods in order to effectively manage this conflict species.

3.4.2 The abundance of *C. mesomelas* in predator feeding sites

Supplementing the diet of carnivores had a significant positive effect on the camera trap capture frequency of the black-backed jackal in this study (Figure 3.1). The relative abundance indices calculated for local populations of *C. mesomelas* under the predator feeding treatments were significantly higher than the non-managed, predator neutral, sites by an order of several magnitudes (Figure 3.1), therefore the hypothesis that predator feeding significantly increases the abundance of the black-backed jackal can be accepted with some degree of certainty. A similar increase in the abundance and density of *C. mesomelas* has been recorded by other studies investigating the effect of clumped abundant food resources on the local density and behaviour of *C. mesomelas* in the Namibian Skeleton Coast (Jenner *et al*., 2011). These studies have found that clumped food resources resulting from the seasonal abundance of seal carcasses synonymous with the breeding season of grey seal colonies (Jenner *et al*., 2011) prompted a local increase in black-backed jackal abundance which was many times higher than those recorded outside of the grey seal breeding season. It was hypothesized by the authors that a breakdown in territorial stability, in areas where food resources are abundant, is due to the reduction in territory defence, as the tolerance of transient and intruding individuals reduces the risk of the permanent loss of territory for breeding pairs. The tolerance of unrelated transient individuals can be explained by the assumptions of the resource dispersal hypothesis, where a spatially and temporally heterogeneous food supply promotes group living in non-social species. However, in contrast to the source-sink dynamics proposed by Jenner *et al* (2011) to explain the conglomeration of black-backed jackals among the seal colonies

of the Namibian coast, an increase in fecundity of the black-backed jackal and the reduction in dispersal rates of young from their natal range is a potential factor in the formation of groups of *C. mesomelas* around the vulture feeding sites of this study.

The dispersal patterns of the black-backed jackal are highly variable with some reports of jackals dispersing in as little as 12 months, to jackal offspring remaining in their natal range for many years as helper individuals for subsequent generations (Ginsberg and Macdonald, 1990, Estes, 1992, Sillero-Zubiri, 2004). It is therefore possible that the presence of a permanent and abundant food resource within a natal range may reduce dispersal rates as the local carrying capacity has been increased in line with the supplementation of food. As pup survival rates for a number of Canid species, including the black-backed jackal and the red fox, have been shown to correlate with food availability and the presence of helper individuals in the rearing of young, the formation of large conglomerations of individuals would be visually indistinguishable from an unrelated concentration of individuals drawn from a source population (Moehlman, 1979). Due to the inability to visually distinguish family groups from a conglomeration of unrelated transient individuals, the use of a genetic analysis to investigate the genetic composition, relatedness and structure of a population is required to glean further information on the group-forming behaviour seen at high food availability sites observed in this study (see chapter VII).

3.4.3 Predator control and carnivore community composition

Due to the variation in behavioural response to offal attractants between different species investigated in this study, no detailed analyses were attempted to quantify abundance or density of non-scavenging carnivore species. However, the total carnivore abundance was shown to be statistically similar between sites of the same treatment type (Figure 3.3), with a significant difference in carnivore abundance between treatment groups (Figure 3.4). This difference in carnivore abundance between treatment types shows evidence of the wider impacts of predator management on carnivore abundance which may significantly impact the density and dispersal of the black-backed jackal through a reduction in interspecies competition in areas where large carnivores are lacking. However, as no direct comparisons of abundance were able to be made between differing species, a dissimilarity matrix was used to describe the variation in community structure with respect to treatment type (Figure 3.5 + 3.6). The NMDS dissimilarity matrix used in this investigation places emphasis on species presence rather than any derived abundance estimate as the species-specific response to bait attractant, used to lure carnivores to camera trap sites, remains consistent between study sites.

A substantial dissimilarity in carnivore community composition was observed between the predator control and predator neutral treatment groups. Large carnivores, such as the leopard and the brown hyaena, were most frequently photographed in predator neutral sites, while being largely absent from sites that practised predator control. Smaller mesocarnivores such as the black-backed jackal, caracal and serval were more

commonly encountered under the predator control treatment group when compared with the predator neutral treatment group.

Both the leopard and the brown hyaena have been observed maiming and killing jackal individuals at or near kill sites (Owens and Owens, 1978), with one study investigating the dietary composition of the leopard concluding that an average of 15% of its diet consisted of the black-backed jackal (Hayward *et al*., 2006). Therefore the lack of large carnivores in predator control sites would be expected to remove the top down control imposed on smaller carnivore species through a reduction in inter-guild competition, resulting in the localised population expansion of the black-backed jackal and other generalist mesocarnivore species seen in this study.

Mesocarnivore release is a theory that has been proposed as an explanation for a number of population expansions seen in generalist mesocarnivore species including the red fox and European badger, where large apex predators have declined through habitat destruction or disturbance (Crooks and Soulé, 1999, Prugh *et al*., 2009, Roemer *et al*., 2009). The underlying mechanisms responsible for such a shift in community composition are unclear but could potentially be due to the susceptibility of large carnivores to anthropogenic disturbance (Woodroffe, 2000) and the highly plastic and generalist life history strategies synonymous with smaller mesocarnivore species (Roemer *et al*., 2009). In areas of high disturbance, generalist mesocarnivore species are more tolerant to disturbance than the larger carnivores, due to their higher

reproductive rate and ability to undertake diet switching and adaptive dispersal patterns (Yarnell *et al*., 2013). It is therefore probable that the persistence of the black-backed jackal in areas of predator control is a result of the complex interaction between the competition with large carnivore species and the territorial destabilisation resulting from disturbance originating from active conflict management techniques. To further investigate territorial destabilisation and dispersal of the black-backed jackal, studies with a focus on the genetic structure of these localised black-backed jackal populations are required.

3.4.4 Carnivore community composition in predator feeding sites

An increase in carnivore population abundance in response to an increase in food abundance is not, in itself, an unexpected result (Figure 3.3 + 3.4). The localised nature of this effect however, is marked with the potential for these sites to act as a source population for carnivores (Figure 3.5). This observation is an attractive notion for the conservation of large apex predators such as the leopard and brown hyaena, yet the potential for increased depredation rates and the increased susceptibility of high density populations to detrimental infectious diseases, such as rabies and TB, must also be considered with respect to promoting conflict within agricultural land-use types. As black-backed jackal abundances were far higher than any other treatment group examined, it is probable that the high food availability at vulture feeding sites increases the carrying capacity of the local environment to such an extent that generalist species such as the black-backed jackal are able to sustain high population

numbers and reduced dispersal rates despite the top down pressures from large competitive carnivore species.

3.5 Conclusions

Camera traps have been largely promoted as a standardised sampling platform for the monitoring of low density, individually indistinguishable, terrestrial species; with estimates of the relative abundance of mammalian species successfully used to monitor carnivore populations of animals such as the Sumatran tiger (*Panthera tigris)* and leopard (O'Brien *et al*., 2003, Hayward *et al*., 2006). These studies have shown that relative abundance estimates, derived from camera trap capture frequencies, produce accurate estimates of abundance that are directly proportional to independently derived estimates of tiger and jaguar (*Panthera onca*) densities described using methods such as faecal analysis and sign surveying sampling techniques (O'Brien *et al*., 2003, Hayward *et al*., 2006). In addition, RIAs derived from camera trap capture frequencies have been shown to be highly informative in such population monitoring programs, via calibration, using absolute density estimates derived from capture-mark-recapture studies (Silveira *et al*., 2003, O'Connell *et al*., 2011). However, due to the variation in detection probability across large temporal and spatial distances, and the variation in detection between differing species, the ecological relevance of camera trap derived abundance indices has recently been called into question (Sollmann *et al*., 2013). Relative index of abundance estimates drawn from camera trap capture frequencies have been shown to be highly inaccurate due to the imperfect distribution of detection probability in study species assessed by simulation experiments in

controlled environments (Sollmann *et al*., 2013). Variation in ecological life history strategies and sampling biases have been shown to substantially affect the reliability of this technique as an estimator of species abundance in these simulation experiments (Sollmann *et al*., 2013). Furthermore, comparisons between differing species were shown to produce highly variable correlates of abundance when tested under simulated conditions owing to the variation in behaviour, species-specific ecology, and variation in average home range sizes (Sollmann *et al*., 2013). It is therefore apparent that many limitations exist when inferring species abundance from photographic capture frequencies in camera trap based studies (Sollmann *et al*., 2013). In an attempt to reduce the variation in detection probability across heterogeneous habitat types, authors have suggested the additional use of a number of methodologies and sampling regimes to collaborate camera trap based findings. These suggestions include a longer sampling duration and the use of multiple methodologies such as sign surveys and the genetic analysis of population parameters (Sollmann *et al*., 2013).

The black-backed jackal has shown remarkable population plasticity given the localised nature of this study. The presence and relative abundance estimates for black-backed jackals detected under the predator control group raises many questions on the current level, effectiveness and appropriate use of lethal control to manage black-backed jackal numbers in game farm environments. On first consideration, these results appear counter intuitive given the nature and aims of lethal control management strategies, however, the assumption that the removal of black-backed jackals from a local population will inevitably lead to a population decrease is in itself a

simplistic argument that fails to account for breeding rate and migration and is thus applicable to hypothetical closed systems. When examining the carnivore community structure, a marked difference in species composition was observed between predator control and predator neutral treatment groups. The lack of large carnivores under the predator control treatment could greatly promote mesocarnivore survival and radiation through the net reduction in resource competition between species. The reduction in net resource competition can negate the requirement for individuals to leave their natal range in search of adequate nutrition. This effect, known as mesocarnivore release, is well established as a mechanism used to explain the large shifts in carnivore community seen under extreme disturbance. However as yet, very little evidence exists to support this theory in this system.

Particular limitations of note in this study include the duration of the sampling periods and the number of sites used to infer relative abundance estimate of the black-backed jackal under differing predator management strategies. Although previous studies have used equal, or smaller, sample sizes and durations to infer abundance indices from camera-trap-capture frequencies in order to confer both broad scale and local population trends in similar carnivore species (Thorn, 2009, Singh *et al*., 2010, Yarnell *et al*., 2013), it is suggested that future camera trap studies are required to increase the number of study sites in order to account for any potential variation in detection probability between treatment groups (Noss *et al*., 2012, Sollmann *et al*., 2013). Despite the afore mentioned limitations in the inference of abundance indices from camera trap capture frequencies, no significant difference in mean RIAs could be

detected between sites of the same treatment group within this study. Furthermore, a general linear model (GLM) examining the residual site-specific variation in relative abundance estimates of the black-backed jackal indicated no additional experimental power when site-specific variation was removed from the model via a step-wise model simplification process. It can therefore be argued that, within this data set, the variation in estimates of RIA for *C. mesomelas* resulting from an imperfect and heterogeneous detection probability between sites is considered to be broadly consistent within each treatment group and that the response detected in this data set is primarily a function of treatment type. However, additional abundance estimates, such as faecal density surveys and genetic analysis, are fundamental in order to corroborate any assumed correlates of abundance derived from these camera trap capture frequencies (Sollmann *et al*., 2013) (see chapter IV and VII). The variability in detection probability between sites may be influential on the camera trap capture frequency of the black-backed jackal, and the potential for type I statistical errors may be present when comparing the relative abundance estimates between treatments, given the number of site level repeats undertaken in this study.

Chapter IV

Faecal density counts for the quantification of jackal abundance in game farm ecosystems of the North West Province, South Africa

4.1 Introduction and aims

The accurate quantification of abundance is fundamentally important when monitoring any species in the natural environment (Wilson and Delahay, 2001, Barea-Azcón *et al*., 2007). However, as discussed previously in chapter I, many limitations are apparent when attempting to derive abundance and density estimates using ecological sampling techniques in the wild. Extrapolation of absolute abundance and density estimates from direct sampling methods can be highly informative yet subject to inaccuracies dependant on detection probability across heterogeneous habitat types (Webbon *et al*., 2004). Field sign surveys are commonly used as an indirect sampling technique to derive density and abundance estimates of focal species although they often produce estimates with wider confidence intervals when compared with direct sampling techniques such as capture-mark-recapture models (Jachmann, 1991). Sign surveys such as faecal density estimates are also often detectable across a wide range of animal densities and habitat types permitting the comparison of spatially separate study areas (Jachmann, 1991, Webbon *et al*., 2004).

The use of faecal density counts have been used to estimate the abundance and / or density of the red fox in a number of studies (Sharp *et al*., 2001, Webbon *et al*., 2004) and has been shown to be appropriate for the estimation of population parameters due to the ease of identification of faecal deposits and the constant detection between habitat types (Webbon *et al*., 2004). Faecal density counts are advantageous over direct count techniques such as spotlighting surveys because the variability in detection arising from avoidance behaviour in hunted or disturbed animals is negated (Ruette *et al*., 2003). However, biases in abundance estimates have been shown to occur due to the non-random movement patterns and territorial behaviour of the study organisms. Previous studies using faecal density to infer estimates of relative abundance have controlled for this factor by using multiple calculations of abundance using both the total encounter rate of faecal deposits per km driven as well as examining the frequency of 1 km segments of transects that contain faecal deposits. This second abundance estimate is used to reduce the overestimation of abundance arising from the non-random distribution of faecal deposits (Yarnell *et al*., 2013). Although many limitations exist with the use of faecal density estimates as a proxy for abundance, such as the unknown deposition rate, degradation and non-random spatial position of faecal pellets in the natural environment and the difficulty in maintaining a consistent sampling effort between sites, this methodology is not subject to the same biases in detection that are synonymous with camera trap derived abundance estimates. As a consequence this presents a suitable method for potentially corroborating the conclusions drawn in chapter III.

This present study aims to infer relative abundance estimates of the black-backed jackal based on faecal density counts at the study sites and treatment groups examined in this investigation. An estimate of relative abundance for jackal populations under the treatment conditions of predator control and predator feeding will be calculated and contrasted with sites that do not practice any form of predator management (predator neutral).

It is hypothesised that sites that practise lethal control will have a lower faecal abundance relative to sites that do not practise any form of predator management. In addition, it is also hypothesised that sites supplementing the diet of local carnivore populations will increase faecal abundance relative to predator neutral sites.

4.2 Methods

4.2.1 Experimental design

Faecal counts were undertaken along driven transects confined to the road networks within each site, as described by methods previously developed for use with this species (Thorn *et al*., 2010). All transects were undertaken by two experienced observers and were driven at a speed maintained between 5 and 10 km/h in order to minimise variation in detection probability and sampling effort. Due to the variability in the structure of the road networks within each site, surveying effort was maintained between sites of different size by standardising the total transect length relative to the

site area. An approximate sampling effort of 1.4 km of transect per 1 km^2 of site area was achieved (Table 4.1).

Table 4.1. *Total study area and total transect length for each site.*

Site	**Total area (km^2)**	**Total transect length (km)**
Predator feeding Site 1	46	66
Predator feeding Site 2	36	53
Predator neutral Site 1	31	45
Predator neutral Site 2	22	32.6
Predator control Site 1	10.5	15.5
Predator control Site 2	13.4	19.8

Due to the confines and conditions of the road network present at each site, the transect routes were chosen in order to maximise an even coverage of area and habitat types (Figure 4.1). All transect routes and property boundaries were recorded on a Garmin GPSmap 62 in order to ensure accurate re-sampling. Transect width was standardised at 2m from the edge of the road to minimise the variation in detection as described by the original sampling method. Once faecal pellets were observed from the vehicle, the total number of pellets; approximate age (< 24h: humid, 1 day – 1 week: desiccated/glossy, > 1 week: partially decomposed) and identification confidence were estimated (1-5). Any samples with a confidence interval of < 5 were discarded from the analysis to standardise identification confidence between observers. Identification was aided with field guides and expert advice where necessary (Walker, 1997, Stuart and Stuart, 2000). The spatial position of each faecal

pellet was recorded using a Garmin GPSmap 62. Faecal pellets were collected, coded and stored at –20^{o}C. Sites were sampled over 16 days in a concurrent stratified random order. Each site was sampled twice within a 12 month period, at approximately six month intervals, in order to account for seasonal variation in jackal abundance and faecal degradation rate. All sites were sampled in both the wet and dry season.

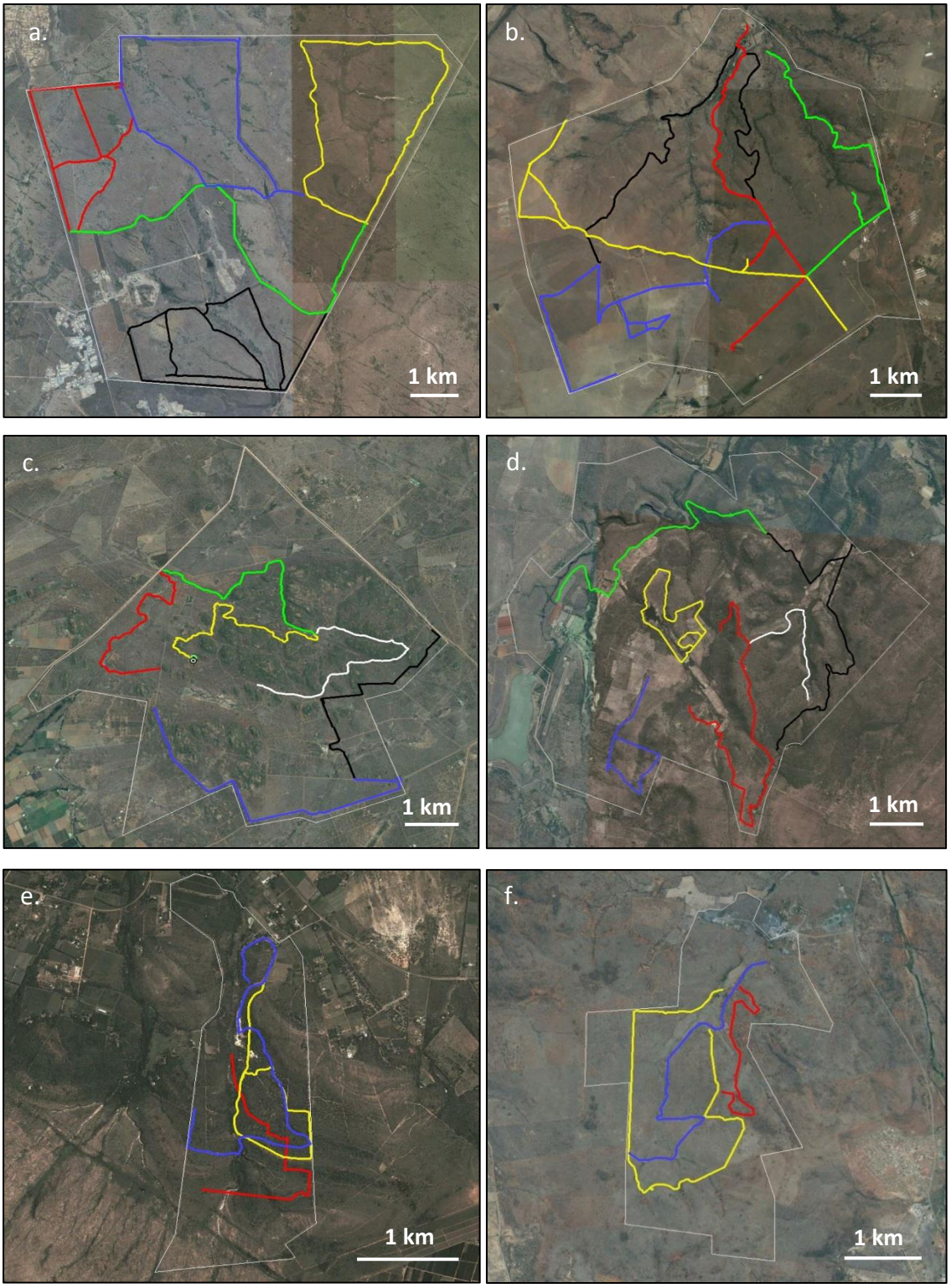


Figure 4.1. Transect routes (individually identified by colour) and property boundaries (thin white) overlaid on aerial photographs for each study site. ***a.*** *and* ***b.*** *= predator feeding sites;* **c**. and **d**. = predator neutral sites; **e**. and **f**. = predator control sites.

4.2.2 Faecal counts and the calculation of relative index of abundance estimates

All observations that had an identification confidence of less than 5 were discounted from the data set in order to minimise error originating from misidentification of faecal deposits (Yarnell *et al*., 2013). The average faecal encounter rate per km driven was calculated for each transect within each site for each of the two sampling sessions. The average encounter rate per km was calculated for all transects across all sampling sessions as an indicator of faecal density. No estimates of absolute density or abundance were attempted due to the lack of a known and standardised defecation rate of the black-backed jackal.

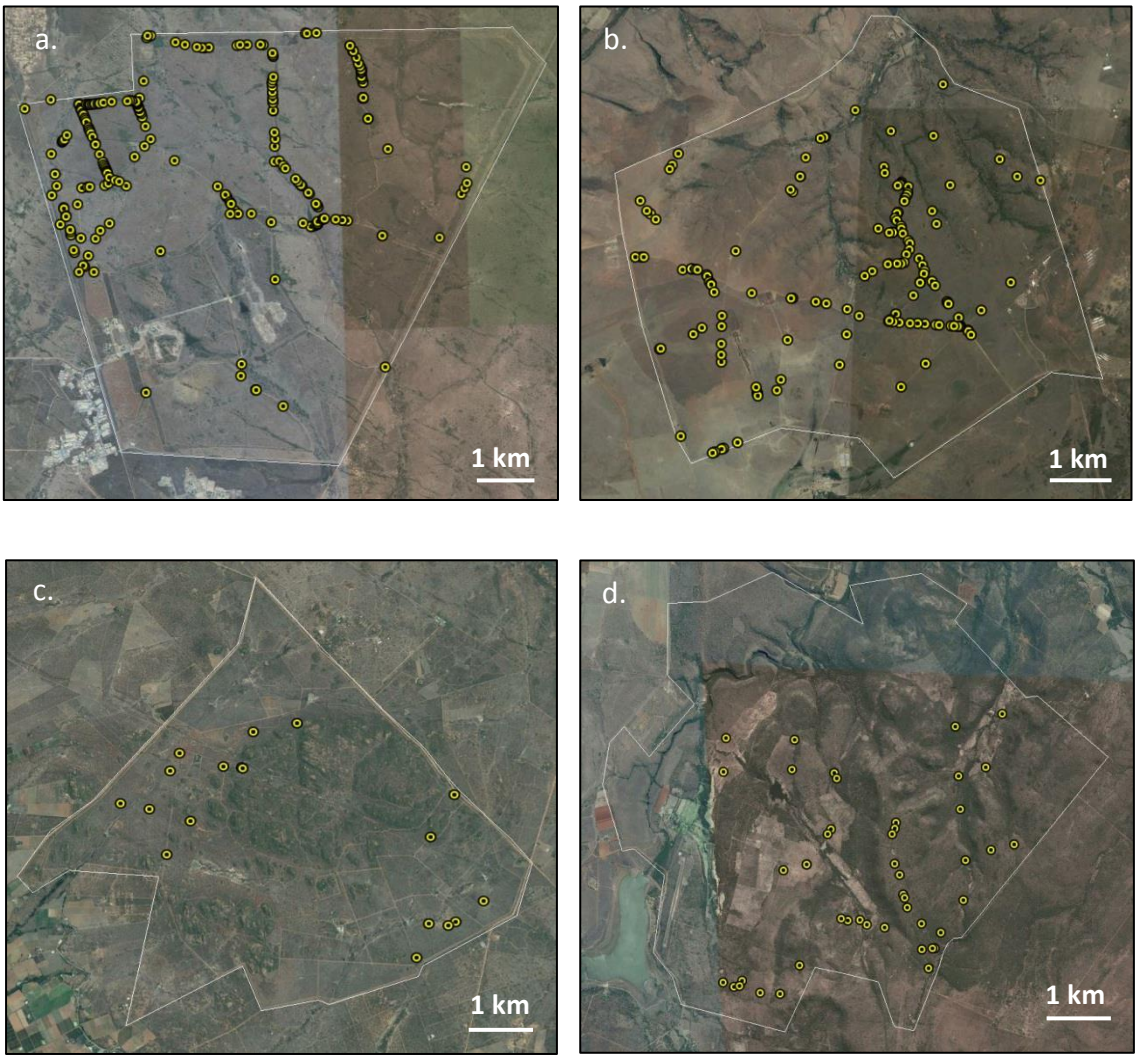
The relative abundance of jackals at each site was estimated by examining 1km segments of each transect and quantifying a presence or absence of faeces within that segment (Yarnell *et al*., 2013). Jackal faecal abundance was then calculated as the percentage of 1km transect-segments containing faecal deposits from the black-backed jackal. This method was used in order to minimise the bias in count data originating from repeated sampling of the same individual.

Transect abundance indices were pooled from sites of the same treatment type in order to investigate the effect of management on jackal abundance. Pairwise t-tests were used to compare the variation in mean proportional abundance between treatment groups (predator control; predator feeding) and the predator neutral

control group. All statistical analysis was undertaken using the program R v. 3.0.2 (Venables and Smith, 2005).

4.3 Results

A total of 525 observations were made during the study period (Figure 4.2. a-f) culminating in a count of 711 individual scat samples recorded in total across all sites and repeats. Faecal deposits from the black-backed jackal were positively identified at all study sites.



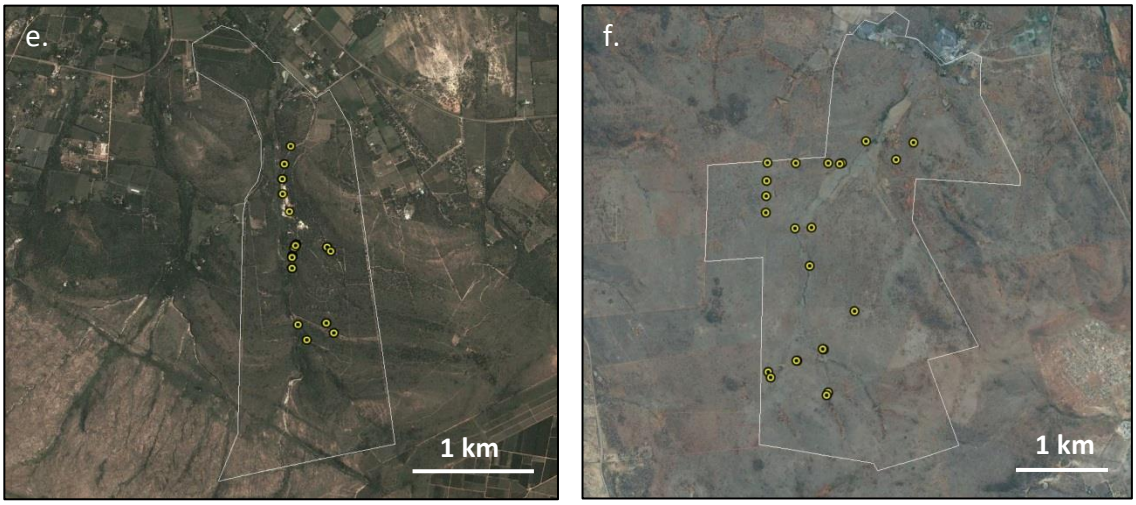


Figure 4.2. *An overlay of faecal observation points on aerial photographs of each site.* ***a*** *and* ***b*** *= predator feeding sites;* ***c*** *and* ***d*** *= predator neutral sites;* ***e*** *and* ***f*** *= predator control sites.*

4.3.1 Faecal encounter rate per km

The standard deviation and average faecal encounter rate observed per driven km are summarised for each site in Figure 4.3.

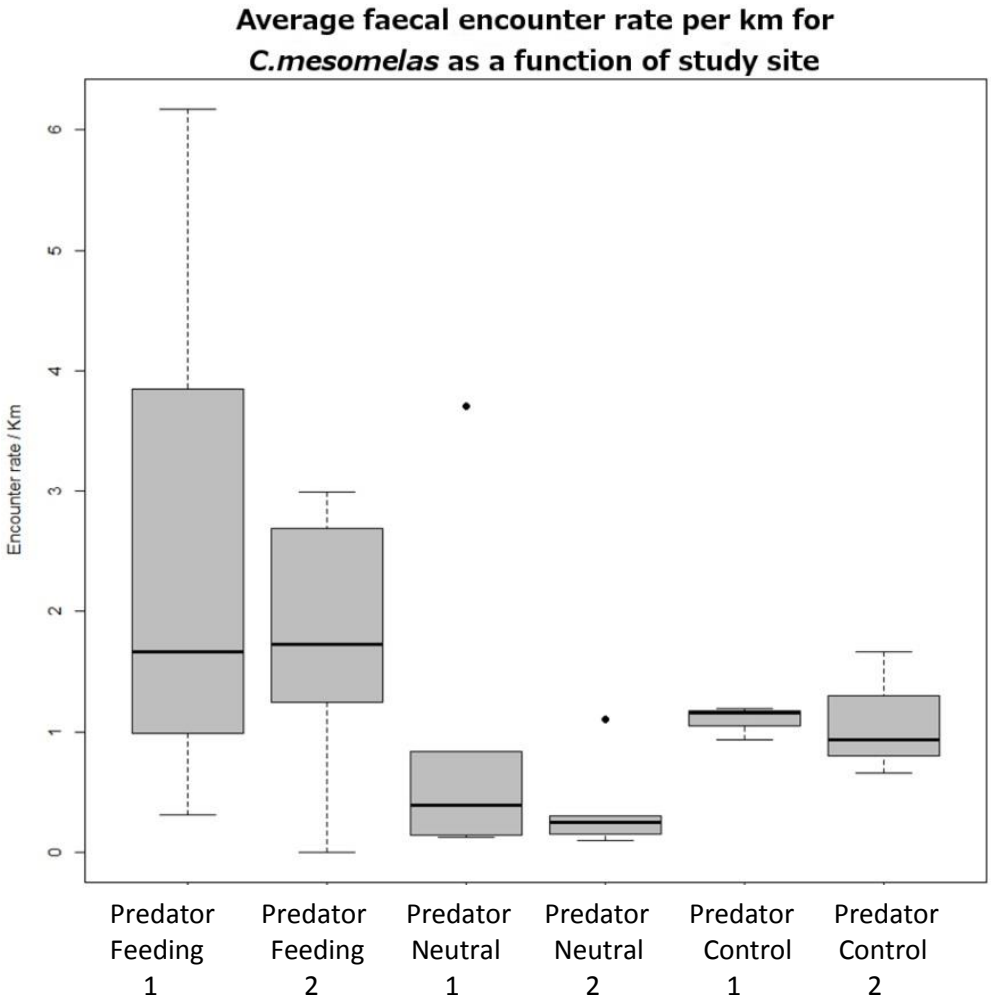


Figure 4.3. *Average faecal encounter rate per km from all transects for each site. No significant difference in average faecal encounter rate was detected between sites of the same treatment type (Welch two sample t-test p > 0.05.)*

No significant difference was detected in faecal encounter rate between pairwise comparisons of sites of the same treatment type (Welch two sample t-test: $p > 0.05$.) As no significant variation in means was detected between sites of the same treatment type, and sampling effort was maintained between all locations, faecal abundance data was pooled by treatment (Figure 4.4.)

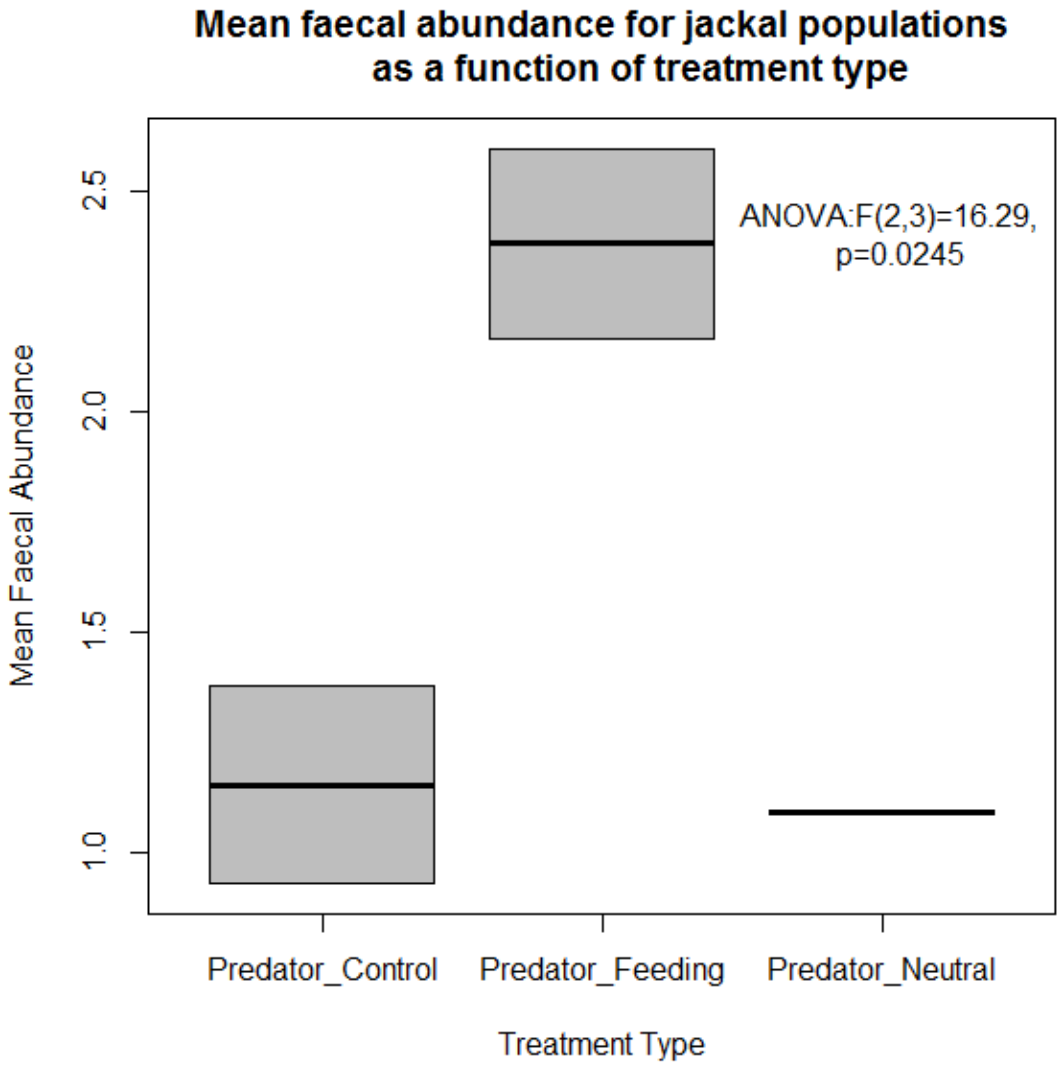


Figure 4.4. *Mean faecal encounter rate data at the three treatment types. A significant variation in means at the 95% confidence interval was detected using an analysis of variance between the three treatment types (F = 16.29, df = 1 p = 0.0245). Post Hoc Tukey HSD indicates predator feeding treatment type had a significantly higher abundance of faeces than both the predator neutral (p =0.03) and predator control (p = 0.034) treatments. No significant difference in mean faecal abundance could be detected between the predator control and predator neutral treatment types (p = 0.967).*

A significant variation in group means was detected between the treatment types following data pooling. Post Hoc TukeyHSD reveals that a significant proportion of variation in faecal encounter rate is partitioned between only the predator feeding

group and the predator neutral group. Pairwise analyses of means, using a Welch two sampled t-test, were used to detect a difference in faecal encounter rate between the experimental treatment types (predator control; predator feeding) and the predator neutral control group.

A significantly higher faecal encounter rate per km driven was seen in the predator feeding treatment group when compared with the predator neutral treatment group using the Welch Two sample t-test (df = 14.019, p = 0.0421). No significant difference in faecal encounter rate was between the predator control group and the group of farms that take no action against predator species (p = 0.2556).

4.3.2 Proportional abundance of black-backed jackal faeces

As the accuracy of faecal encounter rate may not be dependent on a linear relationship between site size and transect distance due to non-random movement patterns and territorial behaviour of the black-backed jackal, a relative index of abundance was calculated using the average proportion of 1km sections of transect occupied by one or more faecal signs at each site (Figure 4.5) and subsequently pooled by treatment type (Figure 4.6 a + b.)

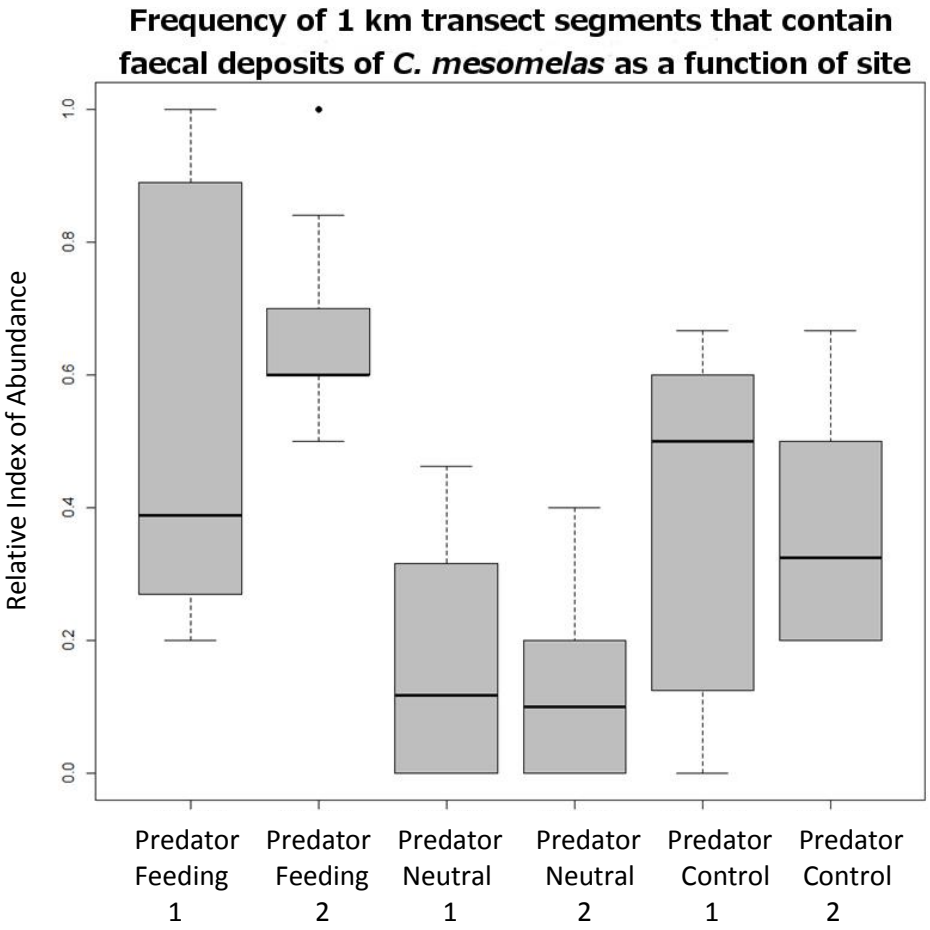
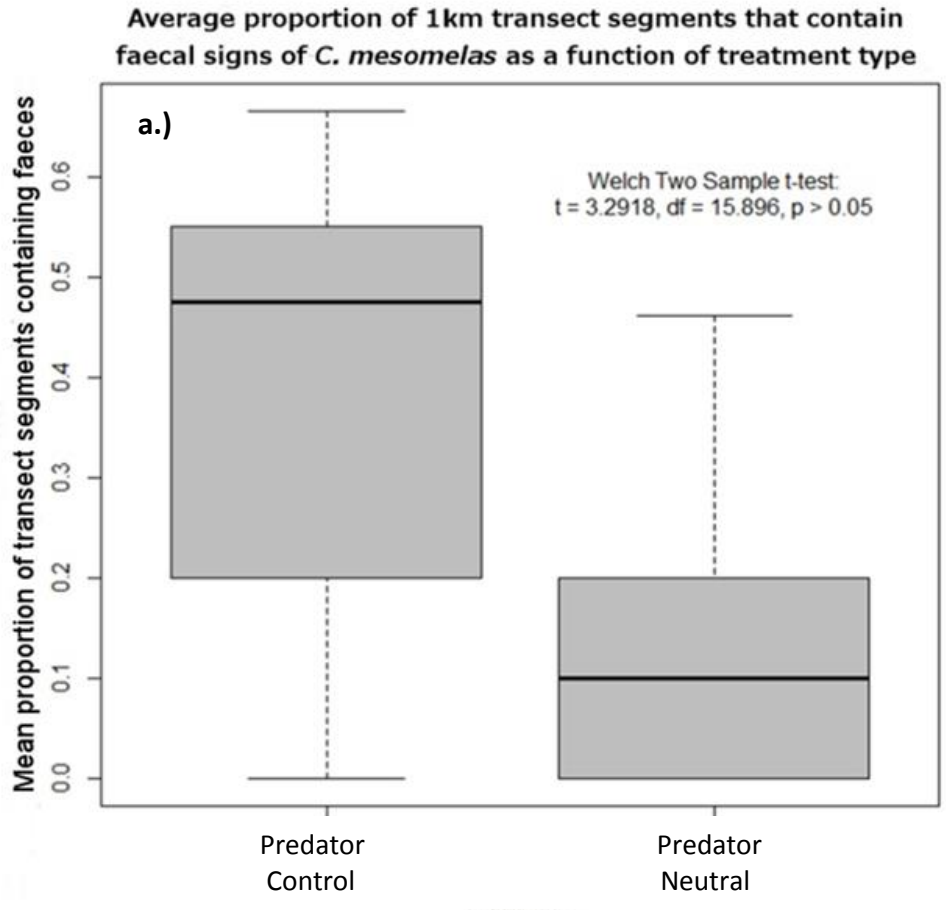


Figure 4.5. *The mean proportion of km segments occupied by faeces from C. mesomelas at each site. No significant difference in average faecal encounter rate was detected between sites of the same treatment type (Welch two sample t test p > 0.05.)*

No significant difference in means was detected at the 95% confidence interval using a pairwise analysis using a Welch's two sample t-test between sites of the same treatment type. When data was pooled by treatment type (Figure 4.7), no significant difference was detected between the predator control group and the predator neutral treatment group (Figure 4.7a). A significantly higher RIA was estimated for the predator feeding group when compared with the predator neutral treatment group (Figure 4.7b). For completion; the predator feeding group was compared with the

predator control group and a significantly higher estimation of relative abundance was detected ($t = 3.154$ $p < 0.05$.)



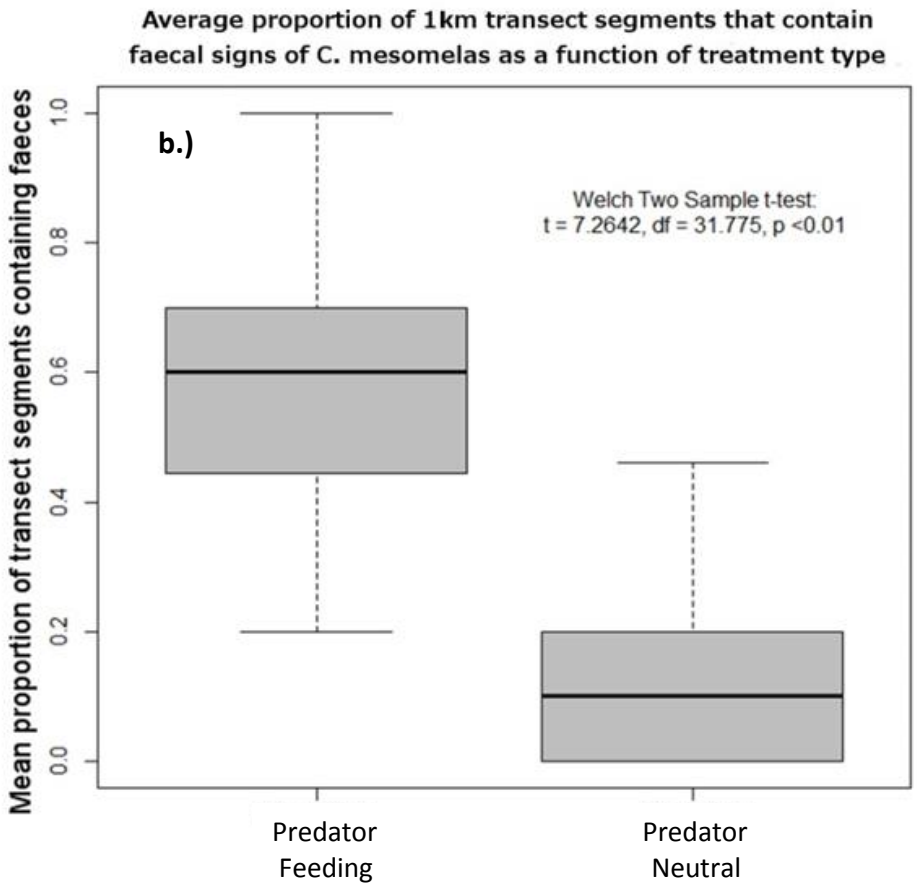


Figure 4.6. *The relative faecal abundance calculated for each of the two experimental groups, predator control and predator feeding, compared with predator neutral treatment group.* ***a)*** *No significant difference faecal abundance estimate was detected between the predator control and predator neutral treatment types (t test t = 3.218 p >0.05).* ***b)*** *The mean proportion of 1km transect segments in the predator feeding group was significantly higher than the predator neutral treatment group (t = 7.264 p <0.01).*

Both relative abundance indices, derived from faecal encounter rate and the proportion of transect segments that contain faecal samples, indicate that the relative abundance of the black-back jackal under predator feeding regimes contain a significantly higher abundance relative to predator neutral treatment group. Therefore the hypothesis that predator feeding will significantly increase faecal abundance

relative to non-managed treatment type can be accepted. No significant variation between the predator control group and predator neutral group could be detected by examining faecal encounter rate or faecal abundance estimates. Therefore the null hypothesis, that there is no difference in faecal density estimates between the control group and predator neutral group, can be accepted.

4.4 Discussion

Relative indices of abundance derived from indirect sampling techniques are of limited use to inferring absolute population estimates of a species. However, relative abundance indices are often used in making large scale spatial or temporal comparisons between areas (Edwards *et al*., 2000, MacKenzie and Kendall, 2002, Karanth *et al*., 2004) and are frequently used in management and monitoring studies of this nature. Many factors can affect the accuracy of abundance indices of the black-backed jackal derived from faecal density estimates. These include the variation in detection probability arising from spatial and temporal differences in habitat, the variation in movement patterns, territorial behaviour and the seasonal variation in deposition rate (Webbon *et al*., 2004, Yarnell *et al*., 2013). As such, this study attempted to control for the effects of seasonality and habitat heterogeneity by using both spatial and temporal repeats at the treatment and site level as well as using two different estimates of faecal abundance indices. The two analytical methods, which estimated the faecal encounter rate and the proportional abundance of faeces within transects, were used to detect any overestimation in abundance from the bias in repeated sampling of individuals from any one model. The similarity in the response

measured by both analytical methods provides strong evidence for the reliability of these sampling techniques.

From examination of the average faecal encounter rate per km and the relative abundance indices derived from the proportion of faecal transect segments containing faecal deposits, no significant difference could be detected between the predator control group and the predator neutral group at the site or treatment level (figure 4.6 + 4.7b). This potentially indicates that predator control was not significantly influential on the local jackal population when compared with sites that do not implement predator management strategies. Although no evidence is available to support the hypothesis that predator control significantly reduces jackal faecal abundance compared with predator neutral treatment types and the null hypothesis is accepted; it is not possible to state that predator control does not significantly affect the abundance of the black-backed jackal in game farm ecosystems with the data set alone without the susceptibility of type I statistical errors. However, given the similarity of the results gained from camera trap frequency analysis in chapter III, it seems reasonable to suggest that the current effort invested in the management and removal of *C. mesomelas* in the game farms studied in this investigation does not offer any significant reduction in local jackal abundance when compared with the lack of control at predator neutral sites. Therefore it can be argued that the time and resources invested in lethal control at the game farm sites in this investigation may need to be supplemented or redirected to alternate predator control strategies.

Avoidance behaviour towards anthropogenic control strategies such as cyanide guns, free shooting and poisoning is a well recorded and documented attribute in *Canid* species such as the black-backed jackal (Brand and Nel, 1997) and coyote (Séquin *et al*., 2003). The behaviour, activity and movement patterns of jackals in areas of high persecution may therefore be highly dissimilar from individuals in areas that lack persecution. As such, transient individuals who do not hold fixed territories within sites that practice lethal control may account for the number of faecal deposits recorded at such sites.

Predator feeding had a significant positive effect on the relative abundance of faeces when compared with the non-managed, predator neutral group and present comparable results to those from chapter III. It is therefore an attractive and probable conclusion from these data sets that predator feeding has a significant positive effect on the density of jackal populations due to the availability of nutritional resources and reduced inter-guild competition. However, an overestimation of relative abundance indices at supplementary feeding sites could be an artefact of increased defecation rate in response to the increase in food supplementation and is suggestive of a significant limitation to this indirect sampling technique as a standalone index of abundance.

4.5 Conclusions

Abundance indices derived from both faecal abundance estimates and camera trapping (chapter III) provides independent and mutually supportive approach to the quantification of jackal abundance which indicates a true representation of jackal abundance in all treatment groups. The hypothesis that supplementary feeding increases jackal abundance can therefore be accepted. The low accuracy inherent in indirect sampling techniques provides many limitations to the resolution and firmness of any conclusions drawn from such studies. Results from both the camera trapping study and the faecal count data support the notion that supplementary feeding significantly increases jackal abundance and that lethal control has no discernible effect when compared with sites acting as a non-managed control. The underlying mechanisms responsible for the detected responses remain unclear, albeit of great significance to the successful management of this species in the agricultural regions of South Africa. Further studies into population structure, migration and dispersal are required to ascertain the movement and mechanisms behind such observations, which are questions that lend themselves to molecular analysis. As no genetic markers were characterised for the specific use in *C. mesomelas* prior to this investigation, the following two chapters focus on the development of suitable molecular markers for their use in this species.

Chapter V

Amplification and characterization of six cross-species microsatellite loci in the black-backed jackal (*Canis mesomelas*) originating from the domestic dog (*Canis familiaris*)

5.1 Introduction and aims

As with many cryptic mammalian species, the elusive and nocturnal nature of *C. mesomelas* has partially constrained the progress of research into this species. A number of existing studies have focused on using traditional techniques such as direct observations, trapping counts, radio tracking, ear tagging, faecal density counts, sign survey counts and scaled observational studies in order to quantify the abundance and investigate the density and distribution of *C. mesomelas* (Moehlman, 1979, Ferguson *et al*., 1983, Moehlman, 1983, Hiscocks and Perrin, 1988, Loveridge and Macdonald, 2001, Loveridge and Macdonald, 2003, Walton and Joly, 2003, Loveridge and Nel, 2004, Jenner *et al*., 2011, Yarnell *et al*., 2013). However, additional studies with a focus on population genetics are likely to further contribute to, and resolve, information on the population parameters of the black-backed jackal under anthropogenic pressures. Factors such as the structure of a population, in terms of the isolation and gene flow between breeding groups; the stability of a population with respect to territory turnover and maintenance; and the diversity of a breeding population in terms of its genetic makeup are all essential population parameters that can be quantified using the single molecular technique of genetic profiling.

The selectively neutral and co-dominant nature of non-coding DNA has become fundamental in population based studies particularly through the use of microsatellite markers. The absence of natural selection at these markers (as they do not code for proteins) leads to a high degree of allelic diversity, relative to coding regions, as mutations of no affect persist. The correlation between ancestral descent and shared microsatellite alleles provides the logical assumptions on which predictive models of population heterozygosity, inbreeding and relatedness are based. Characteristics such as the bias towards the accumulation of repeat motifs, at a rate typically far above that of other genomic regions, provide a high-resolution methodology able to detect recent changes in population demography. Due to these characteristics, microsatellites are now used in a wide range of applications where shared variants can be used as a measure of shared ancestry. Microsatellite markers have frequently been used in the assessment of inbreeding (Coulson *et al*., 1999, Curik *et al*., 2003, Cain *et al*., 2014); the detection of genetic bottle-neck events in natural and captive populations (Luikart *et al*., 1998, Jones *et al*., 2004); the monitoring of conservation and reintroduction programs in the natural environment (García-Moreno *et al*., 1996, Vernesi *et al*., 2003, Storfer *et al*., 2014); the genetic diversity of livestock breeds (Yang e*t al*., 1999, Bjørnstad and Røed, 2001, Pavão *et al*., 2014); paternity testing (Jobling *et al*., 1997, Fridolfsson *et al*., 1997, Sakaoka *et al*., 2014) and forensic science (Jobling *et al*., 1997, Sanches *et al*., 2011, Wictum *et al*., 2013).

Despite their clear utility in population studies, the use of microsatellite markers is not without complications (see chapter VI). The quantity of source DNA is typically in the

pico gram range and requires artificial amplification by the process of polymerase chain reaction before it can be suitably analysed. Polymerase Chain Reaction (PCR) artefacts, such as allelic drop-out, where some allelic variants fail to amplify, and early stage PCR slippage, where the polymerase enzyme responsible for amplifying the source DNA loses position, combined with novel mutations in the primer binding region (null alleles) and identical alleles by descent (allelic homoplasy), all have the potential to introduce an additional degree of bias to any mutation model based on the assumption of Mendelian inheritance and the Hardy-Weinberg relationship between allele and genotype frequencies. Accurate genotyping of individuals requires that the primer binding efficiency and primer-binding region is consistent for every allele at each locus. In addition, DNA template concentration variability can dramatically influence PCR efficiency giving rise to allelic drop-out and negative amplification. The presence of each of these biases must be considered during the development of a microsatellite-based population study and so have been evaluated to ensure that the information generated by the use of the selected molecular markers provides a reliable representation of the population.

A common property of microsatellite markers is their conservation across related species (eg. (Primmer *et al*., 1996, Galbusera *et al*., 2000, Zachos *et al*., 2009) as their mutation rate is high within the repeat region but much lower throughout the flanking regions where the primers anneal. After the process of speciation has occurred through, for example, reproductive isolation, each new species' genome will accrue mutations independently. Point mutation events are rare, thus considerable time is

required before microsatellite primer regions are expected to diverge between closely related species. The assumption of their existing cross-species homology of microsatellite primer regions can therefore be exploited under certain conditions, to yield functional microsatellite markers in closely related non-characterised species. Cross-species microsatellite amplification is a worthwhile pursuit as it significantly reduces the cost and time spent required for the development of novel markers, requiring genome searches and clone library screening. The relatively recent splitting of lineages within the *Canis* genus (Figure 5.1) provides an expectation that a number of species are likely to possess microsatellite loci homologous to those that are, as yet, undefined for *C. mesomelas*.

In order to accurately quantify the effects of anthropogenic pressures on the population dynamics of the black-backed jackal, a suite of polymorphic molecular markers are required. However, no suitable genetic markers have been characterised and published for *C. mesomelas* that would allow for the quantification of genetic differentiation within and between populations. Therefore the aims of this study are to develop and characterize a number of simple sequence repeat loci for the express purpose of investigating the population parameters of the black-backed jackal at the population level.

5.1.1 Canidae phylogeny

The genus *Canis* includes eight currently extant species: the grey wolf (*Canis lupus),* domestic dog (*C. familiaris),* Ethiopian wolf (*C. simensis*), coyote (*C. latrans*), dingo (*C. dingo*), golden jackal (*C. aureus)*, black-backed jackal (*C. mesomelas)* and side-striped jackal (*C. adustus*). A reconstruction of the phylogeny of the Canidae family, undertaken by Wayne and Ostrander (1999), compared 2001 bp fragments of both cytochrome C oxidase I and II genes which indicated that the current classification of the genus *Canis* forms a monophyletic group within the Canidae (Vilà *et al*., 1997). All members of the genus *Canis* have an identical Karyotype of 2n = 78; however the variation in karyotype number within the family Canidae varies dramatically from 36 – 78 (Wayne and Ostrander, 1999). *Canis mesomelas* is nested alongside other wolf-like Canidae within the genus *Canis* which are morphologically distinct from the genus *Vulpus*, and the remaining South American Canidae taxa (Figure 5.1). Within the genus *Canis, C. lupus, C. simensis, C. latrans, C. familiaris* and *C. aureus* form a monophyletic group, with *C. mesomelas* and *C. adustus* forming a closely related sister clade (Figures 5.1 and 5.2.) (Wayne *et al*., 1989, Vila *et al*., 1999, Lindblad-Toh *et al*., 2005).

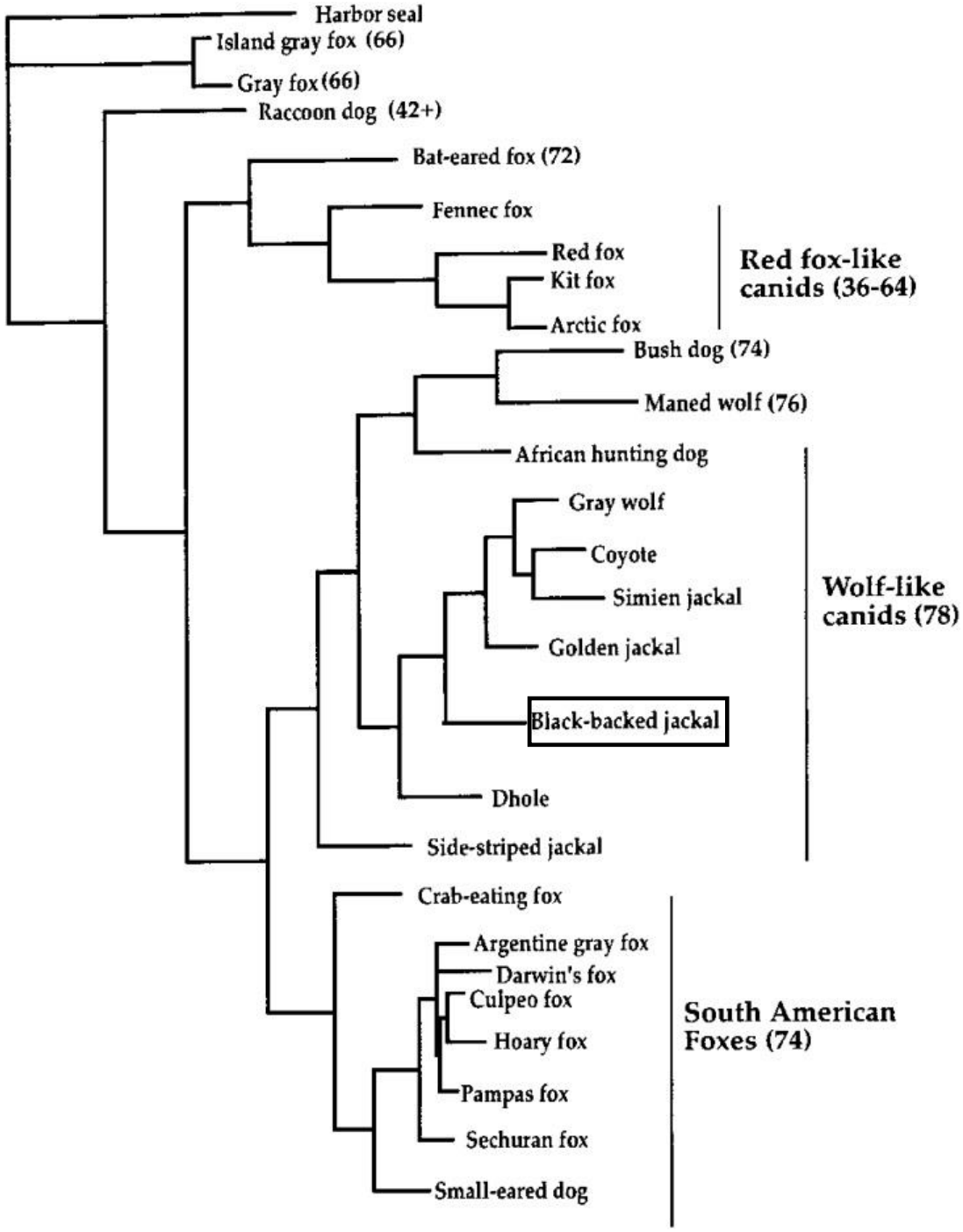


Figure 5.1. *A maximum parsimony tree based on a 2001 bp fragment of mtDNA from 26 Canid species. Karyotype number (where known) in brackets. Modified from (Wayne and Ostrander, 1999).*

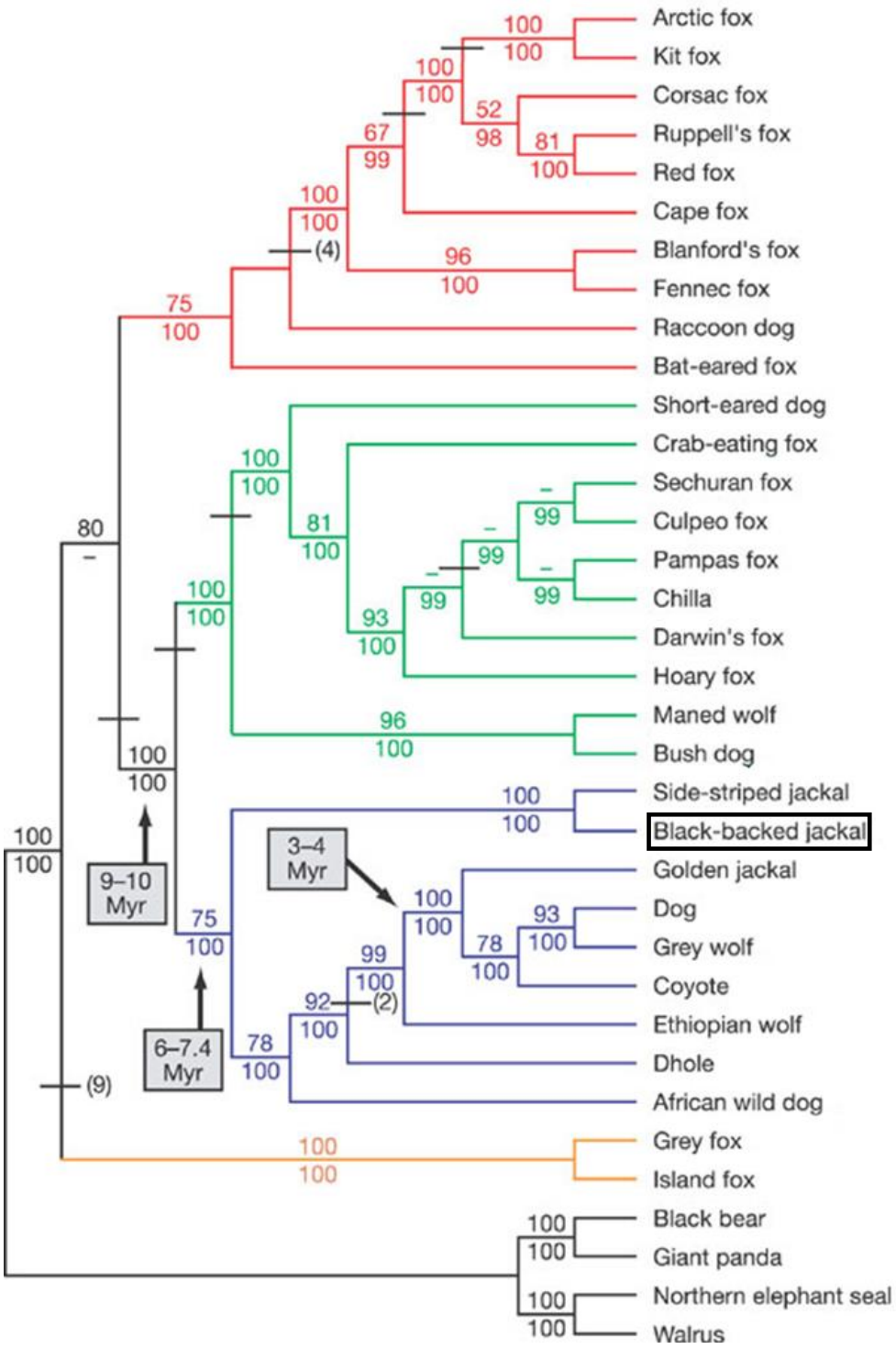


Figure 5.2. A maximum parsimony phylogenetic reconstruction of the wolf-like, fox-like and dog-like species using a 15 Kb exon and intron sequence. *Fox-like species (red), South American clade (green), wolf-like (blue) grey and island clade (orange) out group (black). Bootstrap values are indicated above tree branches. Modified from (Lindblad-Toh et al., 2005).*

Due to the commercial interest in high-resolution pedigree analysis of purebred domesticated dogs, a vast number of highly informative nucleotide repeat motifs have been described and published for *C. familiaris* (Ostrander *et al*., 1993, Ostrander *et al*., 1995). A selection of these microsatellite loci has shown to successfully amplify in other members of *Canis* genus (Wandeler and Funk, 2006, Zachos *et al*., 2009). Both *C. aureus* and *C. lupus*, have been shown to possess a number of homologous microsatellite loci to those described by Ostrander's group (Roy *et al*., 1994, Zachos *et al*., 2009). However, as yet, many of these microsatellites markers remain un-characterised at their homologous loci in *Canid* species other than the domestic dog. Therefore the suitability of a range of markers developed for pedigree analysis in the domestic dog must first be ascertained for homologous loci in *C. mesomelas* before population based studies can be undertaken.

5.2 Methods

5.2.1 Tissue sample collection

In order to assess the potential homology of microsatellite loci in *C. mesomelas*, a total of 18 individual jackals were opportunistically sampled for genetic material by taking tissue biopsies of the ear lobe. All tissue samples were collected from recently deceased individuals within the North West Province of South Africa. Tissue samples were collected over an 18 month period and primarily originated from animal-traffic collisions. All tissue samples originate from individuals positively identified as *C. mesomelas* and spatially referenced with GPS data upon collection. Tissue samples

were placed in absolute ethanol (EtOH) upon collection and stored at -20^{o}C prior to transport to the UK for analysis. All samples were imported to the UK under DEFRA licence numbers TARP/11/392 and TARP/12/404.

5.2.2 DNA template extractions from tissue

Host genomic DNA was extracted from 25 mg of ear lobe tissue fixed in absolute EtOH, using the DNeasy Blood and Tissue Kit (Qiagen cat no: 69504) and the manufacturer's protocol for tissue extraction. Cartilaginous tissue was removed from epidermal skin cells prior to proteinase K digestion at 56^{o}C. DNA was recovered in 150 µl of manufacturer-supplied PCR compatible elution buffer. All extracts underwent PCR promptly following extraction and were subsequently stored at –20 ^{o}C for future analysis. The DNA concentration of extracts was estimated using the NanoDrop 2000 spectrophotometer (Thermo Scientific).

5.2.3 Microsatellite primer selection

A total of nine microsatellite loci were selected for homology testing within *C. mesomelas* (Table 5.1) from those originally described for the domestic dog (Ostrander *et al*., 1993, Ostrander *et al*., 1995, Wayne and Ostrander, 1999, Wictum *et al*., 2013). All primers used were synthesised by Eurofins MWG Operon and purified by HPLC. The primer pairs for all nine markers were selected as they had previously shown the potential for cross species homology within the *Canid* genus (Zachos *et al*., 2009). Careful consideration was given to maintaining consistent PCR annealing temperatures

across all selected primers in order to facilitate their use in batch processing of large sample sizes. Unmodified primers were initially used for all microsatellite loci to test for homology of conserved primer binding regions within *C. mesomelas*.

Table 5.1. *Microsatellite loci, forward (F) and reverse (R) primer sequences (5' – 3'), repeat type and NCBI accession numbers (AN) where available.*

Locus	F primer	R primer	Repeat type	A N
DogP109	aactttaagc cacacttctg ca	acttgcctct ggcttttaag c	$(CA)^n$	L15666
DogP123	aactggccaa acataaacac g	ttcattaacc ctttgccctg	$(CA)^n$	L15700
DogP204	cgagagcaac ataggcatga	caaagtgctg tggcaggtc	$(CA)^n$	L15664
DogP225	agcgactatt atatgccagc g	ctcattggtg taaagtggcg	$(CA)^n$	L15644
DogP374	aggaggacag aaagacagaa gg	atggatgtat tgtgagggtg g	$(CA)^n$	L24264
DogP468	aagagcatac ccatgatgtt ca	taaaagaggg tgaaaagatg cc	$(CA)^n$	L24348
DogP502	tttgaaaggc tgtatgcatc c	gttatggcca agtactcttc ca	$(CA)^n$	L24360
VGL1541	gagctcctga tggaagagct ta	catcctgtcc gtgacttcaa	$(CTTT)^n$	NA
DogPa622	cttgtgcaat catcatcttg a	cccgaggtac ctatggct	$(CA)^n$	L27184

All primers were initially tested for amplification success on a subset of three DNA jackal tissue extracts. The potential amplification of human DNA contamination was assessed using a human DNA template of known origin. A positive amplification control DNA template was obtained from hair belonging to a pure bred collie dog. PCR amplicons were stained with NBS Biologicals "Safe View" and visualised under UV radiation on 2% agarose gels.

5.2.4 PCR conditions

Individual PCR reactions were undertaken in 25 µl volumes containing approximately 40 ng of DNA template, 1 × Invitrogen PCR buffer, 1.5mM $MgCl_2$, 1 unit of Invitrogen hot start Platinum®Taq DNA polymerase (Invitrogen), 1 unit of Qiagen Q-solution, 0.5 µl/ng BSA, 0.2mM dNTP mix and 0.2 µM primer mix. Amplification conditions used on a Techne TC-4000 thermal cycler consisted of: initial denaturation at 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 1 min, 55^{o}C for 45 seconds and 72^{o}C for 1 min finishing with a final extension stage of 72^{o}C for 5 min.

5.2.5 Allelic diversity

Allelic diversity at each locus was examined by scoring a sample of 18 individuals sampled from the North West Province of South Africa. Six randomly chosen microsatellite loci, all of which displayed successful amplification, were selected for preliminary examination from the nine markers originally screened. The six microsatellite markers were chosen in order to remain within the financial limitations of this study. In order to assess the resolution and power of this marker suite, further testing on the allelic diversity and probability of identity were performed in chapter VII. The six successful microsatellite loci were amplified using fluorescently labelled forward primers in 18 individual jackal tissue samples. PCR products were visualised under UV light using 10 µl of PCR product on a 2% agarose gel. Subsequently, 6 µl of PCR product was then sent for direct commercial genotyping (Source Biosciences: Life Sciences, UK). Forward primers were fluorescently labelled at the 5' end with either

FAM or HEX and amplicons were separated using capillary electrophoresis using an ABI Prism 3730. PCR fragment size was determined using the internal size standard Rox 500 (ABI Dye Set – 30) and GENEMARKER v. 2.1.2. All PCR products were single loaded to ensure accurate characterisation of allele polymorphisms and identification of potential PCR artefacts. Allelic drop-out estimates were calculated using CERVUS v 3.0 (Marshall, 1998).

5.2.6 Sequencing and microsatellite repeat motif characterization

To characterise the basic repeat motif at each microsatellite locus, two PCR amplicons from 14 independent homozygote individual samples were sequenced. Direct Sanger sequencing was undertaken on both of the DNA strands for each locus to ensure accurate motif characterisation. Commercial sequencing companies Source Bioscience: Life Science and GATC Bioscience were used to undertake high quality Sanger sequencing analysis on an ABI 3730xl. Consensus sequences were aligned and compiled using BIOEDIT v1.0 (Hall, 1999). Sequence repeat motifs were visually inspected and quantified using BIOEDIT v1.0 (Hall, 1999).

5.3 Results

5.3.1 Cross species amplification of microsatellite loci

All DNA extracts from *C. mesomelas* tissue provided good quality DNA templates of > 50 ng/µl; suitable for PCR analysis. Jackal DNA templates initially produced positive

PCR amplicons for all nine primer sets screened for cross-species amplification. Successful amplification of domesticated dog control templates was also seen for all loci. No visible amplification of human genomic DNA was apparent on agarose gels at any of the primers screened. Six of the original nine microsatellite primers screened by agarose gel electrophoresis were selected on the basis of their reliable PCR performance and were fluorescently labelled for further fragment analysis (Table 5.2).

Table 5.2. *Forward primer selection and fluorophore modification. Chromosome number was determined using primer-blast search query of the whole genome shotgun sequence assembly of the domestic dog.*

Locus	Chromosome	F primer sequence	Label
DogP109	4	aactttaagc cacacttctg ca	FAM
DogP123	23	aactggccaa acataaacac g	FAM
DogP204	30	cgagagcaac ataggcatga	HEX
DogP374	20	aggaggacag aaagacagaa gg	HEX
VGL1541	15	gagctcctga tggaagagct ta	FAM
DogPa622	20	cttgtgcaat catcatcttg a	HEX

5.3.2 Fragment analysis

All six loci gave repeatable and scorable allele sizes from a total sample size of 18 individuals (Table 5.3).

Table 5.3. *Number of allelic length variations in each microsatellite locus; PCR success rate and allelic drop-out estimate calculated by Cervus V 3.0 (n = 18.)*

Locus	Allele number	Product size Range (bp)	Positive PCR (n = 18)	Null allele frequency estimate
DogP109	13	126 – 192	100%	-0.0539
DogP123	10	134 – 152	100%	-0.0459
DogP204	11	198 – 222	100%	-0.0073
DogP374	7	170 – 206	94.5%	0.0231
VGL1541	7	181 - 227	100%	-0.0079
DogPa622	6	210 - 220	100%	0.0334

Accumulative PCR and fragment analysis success rate for all microsatellites was calculated at 98.15 % (n = 108). All microsatellite markers were seen to be polymorphic at all loci in *C. mesomelas* with between 6 and 13 allele variations detected per locus. No significant allelic drop-out or presence of null alleles was detected within the sampled population (Table 5.3). No significant PCR artefacts were present in any positive genotype electrophoretogram (see appendix).

5.3.3 Microsatellite repeat motif characterisation

Direct Sanger sequencing of PCR amplicons was used to determine the basic microsatellite repeat motif at each locus. Repeat sequence motifs for each allele sequenced are shown in Table 5.4. All repeat length variation was consistent with allele size variation within each locus examined. The number of repeated nucleotide subunits ranged between 6 and 13 across all loci. Four of the five di-nucleotide microsatellite loci sequenced were comprised of pure and uninterrupted $(CA)^n$ based simple sequence repeats. Locus DogP374 was found to repeatedly produce uninterrupted compound repeat motifs $(GC)^m(CA)^n$ for all three alleles sequenced. The singular tetra-nucleotide marker at locus VGL1541 comprised an uninterrupted variable $(CTTT)^n$ repeat sequence for three separately genotyped individuals.

Table 5.4. *Locus name; microsatellite composition; sequenced allele size and repeat characterisation for microsatellites amplified from 14 individual Canis mesomelas samples.*

Locus	Composition	Allele size (bp)	Repeat motif
DogP109	Pure	144	$(CA)^{14}$
DogP109	Pure	136	$(CA)^{12}$
DogP123	Pure	138	$(CA)^{18}$
DogP123	Pure	136	$(CA)^{17}$
DogP204	Pure	214	$(CA)^{16}$
DogP204	Pure	204	$(CA)^{11}$
DogP374	Uninterrupted Compound	196	$(GC)^{8}(CA)^{9}$
DogP374	Uninterrupted Compound	196	$(GC)^{8}(CA)^{9}$
DogP374	Uninterrupted Compound	196	$(GC)^{8}(CA)^{9}$
VGL1541	Pure	193	$(CTTT)^{16}$
VGL1541	Pure	193	$(CTTT)^{16}$
VGL1541	Pure	185	$(CTTT)^{14}$
DogP622	Pure	216	$(CA)^{8}$
DogP622	Pure	210	$(CA)^{5}$

5.4 Discussion

The results of this study indicate a high degree of conserved microsatellite homology between *C. familiaris* and *C. mesomelas*. In addition, the consensus of studies successfully using cross-species microsatellite loci, characterised in the domestic dog, suggests a high degree of genetic homology within the entire *Canis* genus. The predictable template amplification and low number of PCR artefacts associated with

the use of these markers in *C. mesomelas* shows potential for their informative use in population studies. The estimated low frequency of null alleles (<0.1) in the sampled population (n = 18) and the variation in number of alleles at each locus (6-13) make these selected microsatellites suitable for estimating population heterozygosity based on the assumptions of neutral Mendelian inheritance in the black-backed jackal.

Pure di-nucleotide and tetra-nucleotide repeat types are commonly used molecular markers in population genetics due to their tendency to be more polymorphic than interrupted or compound repeat types. They are also highly valued in population studies as there is a greater probability that identical allele sizes detected in a population reflect identity by descent rather than undetectable convergence by state.

Repeat motifs at loci DogP109, DogP123, DogP204 and DogP622 consisted of pure and uninterrupted $(CA)^n$ based repeats indicating a high potential for allelic polymorphisms and their suitability for use in landscape scale genetic studies. In addition locus VGL1541 contained pure and uninterrupted tetra nucleotide repeat type $(CTTT)^n$ also shown to be polymorphic in the sampled population.

The simple sequence repeat for allele 194 at locus DogP374 was shown to consist of an uninterrupted compound $(GC)^m(CA)^n$ repeat type. The probability of this repeat motif being characterised incorrectly due to chimerisation of sequences was reduced by repeat sequencing of both the forward and reverse amplicons from three individuals

for allele size 194. Compound repeat types have the tendency to be less polymorphic than pure uninterrupted repeat types (Sunnucks, 2000) as well as providing less confidence that allele size homology correspond with allele sequences homology, hence their use in population studies can be limited. Therefore additional information on estimated null allele frequencies are required from a larger sample size in order to ensure the informative use of this marker. Three of the nine molecular markers that were not taken forward for further analysis and characterisation still showed strong positive amplification in the preliminary stages in this investigation. Therefore these three markers still present the potential for the future use in population studies of the black-backed jackal after characterisation, should a suitable resolution of genetic identity not be available from the six microsatellite markers chosen in this investigation.

5.5 Conclusions

A total of six microsatellite loci designed for pedigree analysis for the domestic dog have shown successful cross species amplification with tissue samples from the black-backed jackal. The markers were shown to amplify reliably from tissue source material using end-point PCR and can be seen as highly polymorphic at all loci. Therefore these molecular markers are of potential use in the estimation of the population parameters of the black-backed jackal. However, as these markers remain largely untested in the natural environment further studies investigating the reliability of these markers are required using a larger sample size. Furthermore, as direct sampling of the black-

backed jackal for genetic source material is problematic in terms of sample size, a method for sourcing, extracting and amplifying genetic source material from a non-invasive faecal deposit s must be developed.

Chapter VI

Quantification of DNA copy number from non-invasive genetic samples of faecal deposits from *Canis mesomelas* and characterisation of microsatellite markers

6.1 Introduction

The difficulty in obtaining adequate quantities of genetic material, required to undertake a molecular study with sufficient power, is often a significant hurdle when attempting to study species in the wild (Pemberton *et al*., 1995, Morin *et al*., 2001, Broquet and Petit, 2004, Reddy *et al*., 2012). Due to this difficulty, a variety of DNA source material is commonly used for the purpose of estimating population parameters of free roaming wild species via microsatellite fragment analysis (Ernest *et al*., 2000, Morin *et al*., 2001, Reddy *et al*., 2012). Direct invasive sampling of individuals from wild populations often result in DNA templates of high yield and quality as the collection of tissue biopsies and blood samples from captured organisms ensures a good quality source of DNA of known origin (Taberlet *et al*., 1999). Additional advantages of using direct sampling methods include a low level of inherent Polymerase Chain Reaction (PCR) inhibitors adulterating the sample and a low risk of sample cross contamination (Taberlet *et al*., 1999). However, the financial cost, sampling effort and ethical considerations associated with direct sampling can counter the benefits and jeopardise the success of a study. These disadvantages associated with direct sampling for genetic material have motivated the development of a range

of non-invasive sampling techniques (Taberlet *et al*., 1999, Piggott and Taylor, 2003, Broquet and Petit, 2004, Broquet *et al*., 2007) that focus on collecting DNA from indirect sources such as associate host cells found with shed guard hairs, saliva and faeces (Gagneux *et al*., 1997, Frantz *et al*., 2003, Broquet *et al.*, 2007, Taberlet and Luikart, 2008). Non-invasive and indirect sampling can be advantageous in that much about the populations past demography and diversity can still be gleaned, but without the requirement to capture and handle study animals. Accurate non-invasive genetic sampling has therefore become of fundamental importance when attempting to study cryptic, endangered or easily disturbed species in the wild (Piggott and Taylor, 2003).

6.1.1 Faeces as a genetic source material

The faecal deposits of wild animals offer a useful source of spatially identifiable host DNA that can prove highly valuable in the study of cryptic mammalian species(Piggott and Taylor, 2003). The host's intestinal cells, shed into the mucosal coating of a faecal pellet as it passes through the digestive tract, can be isolated and extracted yielding suitable DNA templates for genotyping analysis (Davison *et al*., 2006). A plethora of disadvantages are apparent with the use of non-invasive genetic sampling of faecal deposits which include the indirect link between sample and individual (Broquet and Petit, 2004, Broquet *et al*., 2007), false positive amplification from contamination (Broquet and Petit, 2004, Broquet *et al*., 2007), severe fragmentation of target DNA through exposure to the environment (Taberlet *et al*., 1999, Parsons, 2001, Piggott and Taylor, 2003), sample adulteration from naturally occurring PCR inhibitors (Taberlet *et al*., 1997, Broquet et al., 2007) and the often poor recovery of genetic material and

resulting stochastic allele amplification (Navidi *et al*., 1992, Taberlet *et al*., 1999, Broquet and Petit, 2004).

6.1.2 Environmental degradation of genetic source material

Prolonged exposure to the environment can have a detrimental impact on the quality of host DNA template through weathering, mechanical shearing and UV degradation (Taberlet *et al*., 1999, Broquet and Petit, 2004). Environmental and microbiological degradation of target DNA over time presents a significant problem in the successful recovery and amplification of species specific DNA from non-invasive sources (Piggott, 2005). The storage of raw material at – 20^{o} C is a standardised practice in order to temporarily reduce microbial and environmental action within the sample. However, repeat freeze-thaw cycles between transportation and long-term storage continue to induce significant DNA template damage within a sample (Morin *et al*., 2001, Frantz *et al*., 2003). The degradation of biological samples prior to analysis has been a problem frequently addressed in the field of clinical and medical diagnostics. As such, a number of procedures have been developed within these fields to increase the longevity of biological samples requiring analysis. Roche Diagnostics have developed a Stool Transport and Recovery (S.T.A.R.) buffer designed to be used in a clinical environment with the purpose of halting microbial action in human faecal samples requiring clinical analysis for disease-causing agents. S.T.A.R. buffer (cat no: 03335208001) has been designed to halt microbial action and preserve all DNA in vitro, in a chemically inert environment, for up to five days at room temperature. A previous study investigating the genetic identification of the European pine martin (*Martes martes*) and red fox

using faecal samples collected in the wild, was successful in transporting and extracting DNA from source material using this buffer (O'Reilly *et al*., 2008). In this study Real-time TaqMan and SYBR green qPCR assays were used to detect amplification of species specific mtDNA isolated from faecal matter after undergoing extraction using S.T.A.R. buffer. Although no absolute quantification was undertaken in this study, positive amplification was seen in both TaqMan and SYBR green assays when using <1 µl of purified faecal material.

6.1.3 PCR inhibitor compounds in faecal samples

The adulteration of DNA samples by organic compounds often reduces the success of DNA amplification via PCR (Morin *et al*., 2001, Hajkova *et al*., 2006). Organic PCR inhibitors, such as humic acids, are prevalent in the environment and primarily arise from the decomposition of organic matter (Inbar *et al*., 1990). Organically derived PCR inhibitors can alter the binding properties of Taq polymerase to dsDNA during PCR by altering the reaction pH and interfering with Mg^{2+} co-factor interactions (Tsai and Olson, 1992, Harry *et al*., 1999). This action reduces the efficiency of the polymerase enzyme activity and thus reduces or halts the DNA amplification rate during a PCR. A number of studies have used organic solvents such as chloroform and phenolic compounds to extract or reduce the concentration of organically derived PCR inhibitors from a range of adulterated sources such as faeces and soil prior to extraction of DNA (Moore *et al*., 1999).

6.1.4 DNA template quantity

Previous studies have attempted to use faeces as a reliable source of DNA templates in order to study the population structure of species such as the brown bear (*Ursus arctos*), the pine martin (*Martes martes*) and the European otter (*Lutra lutra*) using microsatellite analysis (Taberlet *et al*., 1997, Bellemain and Taberlet, 2004, Hajkova *et al*., 2006, O'Reilly *et al*., 2008, Mullins, 2010). The success of these studies has been reliant on recovering an adequate quantity of non-degraded DNA from faecal samples collected in the field. The variability in DNA template quantity associated with indirect genetic sampling can significantly increase genotyping error rates, resulting in a heavy bias in predictions of genetic diversity based on standardized mutation models in diploid organisms (Navidi *et al*., 1992). Consistent failure in the amplification of a single allele at a given locus, due to an inadequate DNA template amount, can result in false calling of heterozygous genotypes as homozygotic individuals and can lead to a misrepresentation of individual and population genetic diversity. This error, known as allelic drop-out, is an artefact of a failed PCR and can be predominantly negated by ensuring an adequate DNA template concentration sufficient to undertake a PCR (Navidi *et al*., 1992). Micro-Checker is a software package developed to aid in the identification of error rates arising from allelic drop-out as well as the miss-typing of allele sizes due to inconsistent stutter patterns arising from low template quantities (van Oosterhout *et al*., 2004).

The low initial DNA concentration present in raw faecal samples has proven to be problematic when using faeces as a source material for genetic analysis (Hajkova *et al*.,

2006, Broquet *et al*., 2007). However, a selection of studies to date, have shown the successful use of non-invasive sampling to accurately estimate a population's genetic diversity using precautions such as replication of analysis; the adherence of good working practice in the laboratory; robust experimental design and quantification of adequate template quantity via the use of quantitative PCR (qPCR) (Taberlet *et al*., 1997, Ernest *et al*., 2000, Morin *et al*., 2001, Taberlet and Luikart, 2008).

6.1.5 Quantitative Polymerase Chain Reaction

Quantitative Polymerase Chain Reaction (qPCR) is a method of PCR developed to quantify and trace the amplification rate of DNA as the PCR progresses in real time. Double stranded DNA binding dyes, as well as target specific oligonucleotide reporter dyes, labelled with a fluorophore and quencher are two common approaches to labelling and detecting newly synthesised strands of DNA during the PCR. DNA binding dyes, such as *Sybr Green* and *Eva Green*, have an affinity for double stranded DNA (dsDNA) and produce fluorescence upon their binding through laser excitation. Therefore an increase in dsDNA during PCR should lead to an increase in detectable fluorescence due to the binding of these dyes to newly synthesised strands of DNA. However, binding dyes have little to no target DNA specificity (Kutyavin *et al*., 2000) and are highly inaccurate due to the variability in sensor capabilities and difficulty in detecting fluorescence when low concentration or impure initial DNA templates are used (Morin *et al*., 2001). This method is therefore unsuitable for the use in quantifying low template copy numbers from non-invasively sampled DNA originating from faecal and hair source material.

Fluorescent reporter dyes are short, modified oligonucleotides which contain a fluorophore and a fluorescence quencher in close proximity. During the annealing phase of a PCR cycle, these inert oligonucleotides bind to a specific complementary sequence on the target DNA which is flanked by the primer binding sites used in the PCR reaction. During the extension stage of a PCR cycle the modified oligonucleotide is dislodged from the dsDNA by the progression of the Taq polymerase along the complementary strand, resulting in the cleaving and spatial separation of the fluorophore from the quencher. Upon dissociation, the absorbance of fluorescence by the quencher on the fluorophore is diminished resulting in detectable and quantifiable fluorescence intensity. As the PCR reaction progresses and newly synthesised DNA strands are formed, the fluorescence intensity increases due to the rapid dissociation of the probe and the quencher during each cycle. The fluorescence intensity using reporter probes is highly reflective of the absolute DNA copy number throughout a reaction due to the direct link between fluorophore dissociation quantity and DNA strand replication number. In addition, fluorescent reporter probes have advantages over binding dyes in their high target specificity, flexibility in oligonucleotide design and high affinity to low concentration or impure DNA templates.

Quantitative PCR has a number of applications originating from the ability to detect fragment amplification during PCR cycles in real time. The absolute and relative quantification of the initial number of copies of the DNA template of an unknown concentration can be calculated relative to amplification rates of genomic templates of known concentrations or known DNA copy number (Deagle *et al*., 2006). Standard

amplification curves are created using an isolated DNA template in solution from a single organism, or recovered from a clone library containing plasmids ligated with the target DNA fragment. Both DNA binding and reporter dye methods can be used for this purpose. However, due to the high potential for non-target microbial DNA to be contaminating the faecal samples, and low target DNA concentration, reporter probe methods provide the essential qualities required for absolute quantification of host specific DNA from faecal samples.

Due to the benefits of increased sample size associated with non-invasive faecal sampling, this study aims to define a suitable and robust non-invasive faecal sampling methodology for recovering adequate concentrations of host DNA for the purpose of studying the population dynamics of the black-backed jackal in the wild. To ensure having an adequate DNA template quantity for accurate genotyping analysis, absolute quantification of target DNA will be undertaken using qPCR and a custom modified TAMRA Taqman reporter probe. In addition, this study aims to employ non-invasive sampling in order to achieve the required sample size to accurately characterise all selected microsatellite markers for their use with *C. mesomelas.*

It is hypothesised that faecal-derived DNA templates have a significantly lower total DNA concentration, absolute copy number and purity than DNA from tissue source material. In extenuation, faecal-derived DNA templates are hypothesised to be recoverable in adequate DNA template copy numbers to accurately amplify target

microsatellite loci without significant allelic drop-out effects. In addition, this study will employ non-invasive sampling in order to accurately characterise all selected microsatellite markers for their use with *C. mesomelas.*

6.2 Methods

6.2.1 Genetic source material collection

Two faecal samples were initially collected from captive jackals at Colchester zoo in order to undertake a pilot study into a novel DNA extraction technique (detailed below). A further sixty-five independent faecal samples were then collected from the six separate sites described in chapter II, within the North West Province of South Africa. The samples were geographically referenced using a Garmin GPS 62S upon collection. All samples collected showed little to no signs of environmental degradation and possessed an un-desiccated mucosal coat.

Stool Transport and Recovery (S.T.A.R.) buffer (Roche diagnostics: 03335208001) was used to minimise microbial degradation post-collection and prevent sample desiccation during transport and storage. Upon collection, approximately 1 g of faecal matter was removed from the outer most layer of the faecal pellet, using a sterile and previously unused razor blade, and subsequently deposited into 5 ml of S.T.A.R. buffer within a 15 ml collection tube. The outer most layer of the faecal pellet was targeted to maximise the number of shed host cells recovered from the mucosal coat. Samples

were shaken until a homogenous solution was formed. Variation in stool consistency required additional S.T.A.R. buffer to be used to ensure homogenisation of some samples. To reduce the potential for cross sample contamination, a new and sterile razor blade was used to collect each independent sample. Sample solutions were labelled and stored at -20 ^{0}C prior to transport to the UK for further extraction and analysis. Samples were imported into the UK under DEFRA licence numbers: TARP/2012/404 and TARP/2011/392.

6.2.2 DNA Extraction

Template DNA was extracted from collected samples using a Qiagen DNeasy spin column modified with a novel chloroform DNA extraction procedure for use in this investigation.

Raw samples were defrosted in batches of 10 at 4^{o}C for 2 hours prior to extraction. Samples were again homogenised by vortexing then 10ml of each sample was pipetted into a clean and sterile 15 ml collection tube. To remove inherent organic PCR inhibitors from raw faecal samples, a chlorophorm extraction protocol was followed, recommended for the use with samples stored in S.T.A.R. buffer.

One ml of ≥99.8% chloroform + ethanol (GC) was added to the 10 ml of S.T.A.R. buffer / faecal solution and mixed gently until emulsified. Samples were then centrifuged at

1000 × g for 3 min. The supernatant was then carefully removed and stored in a clean 15 ml collection tube. 400 µl of supernatant was then added to 120 µl of Qiagen DNeasy buffer ATL and 20 µl of proteinase K. Samples were vortexed and incubated at 56^{o}C for 15 min. Samples were vortexed regularly throughout the incubation. Samples were removed from the water bath and 200 µl of Qiagen buffer AL was added to the solution. Samples were immediately vortexed for 5 seconds and incubated at 56^{o}C for a further 10 min. Samples were removed from the water bath and 200 µl of absolute EtOH was then added to the solution. Samples were immediately mixed by vortex for 10 seconds. Samples were then pipetted into a Qiagen DNeasy spin column and centrifuged at 6100 × g for 1 min. The column flow-through was discarded and 500 µl of Qiagen buffer AW1 was added to the column membrane. Columns were centrifuged at 6100 × g and washed with buffer AW1. The flow through was discarded and 500 µl of Qiagen buffer AW2 was added. Columns were centrifuged at 1400 × g for 3 min and allowed to dry at room temperature for 5 min. DNA was eluted using 75 µl of warmed Qiagen elution buffer (54^{o}C) added to the membrane and allowed to stand at room temperature for 5 min. Samples were then centrifuged at 6100 × g and the DNA eluted into a 1.5 ml micro-centrifuge tube. The DNA concentration and elution quality was quantified using a Thermo Scientific NanoDrop 2000 spectrophotometer. All samples were measured in triplicate to ensure adequate analytical power.

6.2.3 DNA template amplification

All PCRs were prepared in a laboratory free from *Canid* DNA and contaminants of *Canid* origin. DNA extracts and raw samples were stored in separate locations at -20^{o}C. All reactions were run with negative and positive controls. All positive control DNA was extracted from a pure-bred collie dog via the standard Qiagen cheek swab extraction protocol.

Using the aforementioned extraction protocol, DNA templates were primarily isolated from the faeces of two captive black-backed jackal individuals from Colchester zoo as part of the pilot study into the suitability of this method. Nine microsatellite loci with alleles < 300bp in length were chosen. DogP109, DogP123, DogP204, DogP225, DogP374, DogP468, DogP502, VGL1541 and DogPa622, were tested for PCR amplification using their associated primers (chapter V, Table 5.1). Individual PCR reactions were undertaken in 25µl volumes containing a final working concentration of 5 µl of DNA template extract of approximately 10ng/µl, 1 x Invitrogen PCR buffer, 1.5mM $MgCl_2$, 1 unit of Invitrogen hot start platinum taq, 1 unit of Qiagen Q-solution, 0.5µl/ng BSA, 0.2mM dNTP mix and 0.2µM primer mix. Reactions were undertaken on a Techne TC-4000 thermal cycler under the amplification conditions of: initial denaturation at 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 1 min, 55^{o}C for 1 min and 72^{o}C for 1 min finishing with a final extension stage of 72^{o}C for 5 min. Samples were held at 4^{o}C prior to being visualised under UV light on a 2% agarose gel stained with SafeView (NBS biologicals). Gel electrophoresis was undertaken using a 50ml gel in 1 ×TAE buffer for 22min at 100V.

6.2.4 Microsatellite fragment analysis and allelic diversity

Sixty five DNA extracts recovered from the total sample of independently isolated faecal samples were used to examine the variation in allelic diversity at six microsatellite loci. The loci DogP109, DogP123, DogP204, DogP374, VGL1541 and DogPa622 were chosen for consistency and preferential amplification during PCR pilot studies. The forward primer associated with each microsatellite locus was modified with a fluorescent probe (chapter V, Table 5.2). The PCR products were initially visualised under UV light using 10µl of PCR product on a 2% agarose gel. Subsequently, 6µl of PCR product was then sent for direct commercial genotyping (Source Biosciences: Life Sciences, UK,). Amplicons were separated using capillary electrophoresis using an ABI Prism 3730. PCR fragment size was determined using the internal size standard GSRox 500 (ABI Dye Set – 30) and GENEMARKER v. 2.1.2. PCR amplicons were dual loaded for fragment analysis with contrasting HEX and FAM labelled probes in order to increase efficacy. A total of 16.6% of the samples were re-analysed on an ABI Prism 3730 using the internal size standard GSRox 500 to ensure accurate size calling of PCR amplicons. The allelic drop-out and null allele frequency for each microsatellite locus was estimated using Micro-Checker and CERVUS v 3.0. These programs employ a number of iterative algorithms that assign a probability to the consistent calling of allele sizes estimated from a data set and can be used to identify large allelic drop-out rates in PCR products from diploid organisms.

6.2.5 Identification of selection on individual microsatellite loci

The assumption of selective neutrality in non-coding molecular markers is fundamental in the application of microsatellites to population based studies. While individual markers themselves may not be under direct selection due to their functional inactivity, the frequency of such markers in a population may be influenced by a number of selection based factors. Individual markers in close proximity on the same chromosome may result in a bias in inheritance due to alleles at different loci being inherited together at a greater frequency than expected under random assortment. However, the large genetic distance between each microsatellite loci examined in this study (Chapter IV) makes linkage an unlikely source of error in this marker set.

An additional selection based factor that can influence inheritance of specific microsatellite alleles within a population is genetic linkage to functional genes under selection. If particular microsatellite alleles are in physical linkage with functional mutations, then their population frequencies will reflect the affect of natural selection as opposed to neutral population dynamics, thus confounding the interpretations of the results. Therefore individual loci that show extreme levels of genetic differentiation with respect to the entire marker set are often assumed to be subject to selection.

Identifying selection at microsatellite loci is based on the assumption that the effect of inbreeding at different loci should be equal given the demographic history of the

population in question as inbreeding has a genome wide effect. As inbreeding and differentiation are strongly linked to the heterozygosity at each locus, outlying F_{ST} values can be identified by plotting locus F_{ST} values against the heterozygote frequency of the total population using a null distribution generated from the data.

The program Lositan (Tiago Antao, 2008) is an open source program used to detect selection in a range of molecular markers based on the F_{ST}-outlier method (Beaumont and Nichols, 1996). All sixty five individual genetic profiles were used in the analysis collected from the six study sites. The program was run with the default settings using the infinite alleles model with 50,000 iterations at a confidence interval of 0.95.

6.2.6 DNA template quantification

The average absolute copy number of host specific DNA recovered from faecal samples was ascertained to ensure reliable DNA template quality for accurate genotyping analysis. A real time quantitative PCR using a FAM modified TaqMan® probe and TAMRA based BlackBerry® quencher (Table 6.1) was used to quantify the absolute DNA copy number in a sub-sample of four tissue and ten faecal extracts. The TaqMan® probe used in this study had been designed for the quantification of cellular copy number in the domestic dog by targeting a highly conserved 287bp fragment of the β-actin housekeeping gene.

Table 6.1. *β-actin forward and reverse primer sequences and custom TaqMan® probe design for Canis familiaris.*

Oligonucleotide	Sequence	Tm ^{o}C
β-actin Forward Primer	5′-CCTgCggCATCCATgAAA-3′	54
β-actin Reverse Primer	5′-ggggTgCgATgATgTTgATgTT-3′	55
TaqMan® Probe	FAM-AggACCTCTATgCCAACACAgTgCTgT-TAMRA -3′	61

The conservation of the β-actin gene is expected to be high within the *Canid* genus due to its functional importance in cellular structural maintenance and thus suitable for quantifying the DNA copy number in *C. mesomelas*. However, to ensure the cross-species compatibility of this probe in *C. mesomelas*, the fragment of the β-actin gene containing the TaqMan® probe binding site was first isolated and screened for use in the black-backed jackal.

6.2.7 TaqMan® probe homology in *Canis mesomelas*

To validate the homology of the TaqMan® probe binding site in *C. mesomelas*, the probe primers were used to amplify the homologous locus in two high quality jackal tissue extracts using a gradient PCR protocol (n = 27) (Table 6.1). The gradient PCR protocol consisted of an initial denaturation of 94^{o}C for 5 min followed by 35 cycles of 94^{o}C for 1 min, 53^{o}C; 53.2^{o}C; 53.7^{o}C; 54.5^{o}C; 55.5^{o}C; 56.7^{o}C; 57.8^{o}C; 59.0^{o}C and 60^{o}C for 1 min and 72^{o}C for 30 sec. A final extension stage was used at a temperature of

72^{o}C for 5 min. PCR conditions consisted of 25µl reactions comprised of 1 × Promega environmental PCR mastermix, 0.4pmol/µl β-actin fragment primer mix and 2µl of 1:10 dilution template DNA. Positive amplicons were visualised under UV light on a 2% agarose gel stained with Ethidium Bromide.

One optimal PCR product from each of the two individuals examined on a 2% agarose gel was then reclaimed using a Qiagen QIAquick gel purification kit (cat nos. 28704 and 28706). Purified PCR products were cloned into chemically competent *E.coli* using a TOPO TA® Cloning kit, PCR®2.1-TOPO® TA vector and manufacturer based chemical cloning protocol. *E. coli* was then spread onto ampicillin selective plates to identify positive uptake of the plasmid. *Lac-Z* gene expression was up-regulated using 100mM IPTG (isopropyl-β-D- 1-thiogalactopyranoside) to aid in x-gal blue – white colony screening for successful insert uptake. *E coli* were then grown for 24 hours at 37^{o}C.

Ten positively screened colonies were selected and removed from the agar plate using sterile pipette tips. Colony PCR was undertaken on each colony stab using the vector based M13 primers in order to ensure successful insertion of the β-actin fragment within the recovered plasmid. PCR conditions consisted of an initial denaturation stage of 94^{o}C for 10 min followed by 30 cycles of 94^{o}C for 1 min, 55^{o}C for 1 min and 72^{o}C for 1 min. A final extension stage of 72^{o}C for 10 min was used. The final concentration used in a PCR consisted of 1 x Promega environmental master mix, 0.25pmol/µl β-actin forward and reverse primer, 0.5µM BSA and up to 25µl DI H_2O with 1 colony stab as

DNA template. 10ml of amoxicillin selective nutrient broth was subsequently inoculated with each colony stab pipette tip and incubated on a mechanical shaker at 37^{o}C for 24 hours. Colony PCR amplicons were visualised under UV light on a 2% agarose gel stained with ethidium bromide.

Ten ml of amoxicillin selective nutrient broth was subsequently inoculated with each colony stab pipette tip and incubated on a mechanical shaker at 37^{o}C for 24 hours. Two nutrient broth inoculations were selected due to the success of their corresponding colony PCR. Plasmids were recovered from *E. coli* in the nutrient broth solutions using a Qiagen mini prep recovery kit. Plasmid recovery was screened on a 1.2% agarose gel stained with ethidium bromide under UV light. To accurately quantify the length and composition of the insert sequence, 5µl of a 1:5 dilution of mini prep solution, was sent for commercial Sanger sequencing. Both forward and reverse strands of the plasmid were sequenced using the M13 insert flanking primers by the commercial sequencing company GATC bioscience. Sanger sequencing was undertaken on an ABI 3730xl. Forward and reverse consensus sequences were compiled, trimmed and analysed using CLC Genomics Workbench v6.8.

6.2.8 DNA copy number standards

To accurately estimate the absolute copy number of host DNA within the extracted tissue and faecal samples, a set of DNA template standards of known copy numbers were created. DNA plasmids containing the β-actin fragment insert were recovered

from the selected *E. coli* using a Qiagen DNA mini prep plasmid recovery kit. The average DNA concentration of the mini prep elution was estimated using a NanoDrop 2000 spectrophotometer and five replicate samples. The total plasmid copy number per µl of mini prep elution was calculated from the combined molecular mass of the recovered plasmid and insert based on an average nucleotide mass of 650 Daltons and a total plasmid length of 3908 bp.

The plasmid copy number was then standardised to 1 x 10^7 copies/µl by diluting 4.13µl of mini prep elution in 495.87µl of DI H_2O. To reduce a qPCR bias from pipetting error, no less than 5µl of DNA template was pipetted during the qPCR. A 1:5 dilution of the standard was created by pipetting 100µl of standard @ 1 x 10^7 copies/µl into 400µl of DI H_2O. From this, six 1:10 serial dilutions were created from 2 x 10^6 to 2 x 10^{-1} by diluting 50µl of stock into 450µl of DI H_2O.

6.2.9 Absolute DNA template quantification via qPCR

Ten faecal sample extracts and four tissue sample extracts were used in the quantification of DNA template copy number by qPCR using a *Canid* specific reporter probe. Each extract was run in triplicate with a negative and positive control. qPCR was performed on an ABI Fast 7000 real time thermal cycler. Copy number quantification and analysis was undertaken using ABI software 7000 V2.0.5.

Seven standards of known fragment copy number were used to calculate sample template copy number and cycle threshold (C_t). Each standard was run in triplicate to ensure copy number accuracy. A total of 96 reactions were run with the following PCR conditions: 50^{o}C for 2 min followed by 95^{o}C for 10 min then 40 cycles of 95^{o}C for 15 seconds and 60^{o}C for 1 min. The detection of TaqMan® probe dissociation and subsequent fluorescence took place at the end of each annealing cycle. The following PCR reagents were used in 25µl reactions with a final reaction concentration of 1 × Promega environmental master mix 900µM β-actin forward primer, 900µM β-actin reverse primer, 0.25µM probe, 5µl of DNA template and up to a 25µl reaction volume with RNA free DI H_2O.

The ability to resolve population sub-structuring and individual identities using the novel microsatellite data set was assessed using API-CALC v 1.0 (Ayres and Overall, 2004). The probability that two identical copies of a constructed genotype from a population are from distinct individuals is the average probability of identity (PI_{ave}), and was calculated using all microsatellite genotype profiles generated in this study. As little is yet known about the potential for population sub-structuring, and the degree of relatedness between *C. mesomelas* individuals within a population, PI_{ave} was estimated for a range of F_{ST} values.

6.3 Results

6.3.1 DNA template recovery quality and concentration

The average total DNA concentration recovered from faecal source material was 30.249ng/µl. The total DNA concentration (ng/µl) was quantified by spectrophotometry in 65 faecal extracts and compared with the average total DNA concentration in the 18 tissue samples examined in chapter V (Figure 6.1.) The total DNA recovered from faecal source material was significantly less than DNA recovered from tissue source material (Welsh t-test: t = -6.52, p <0.001).

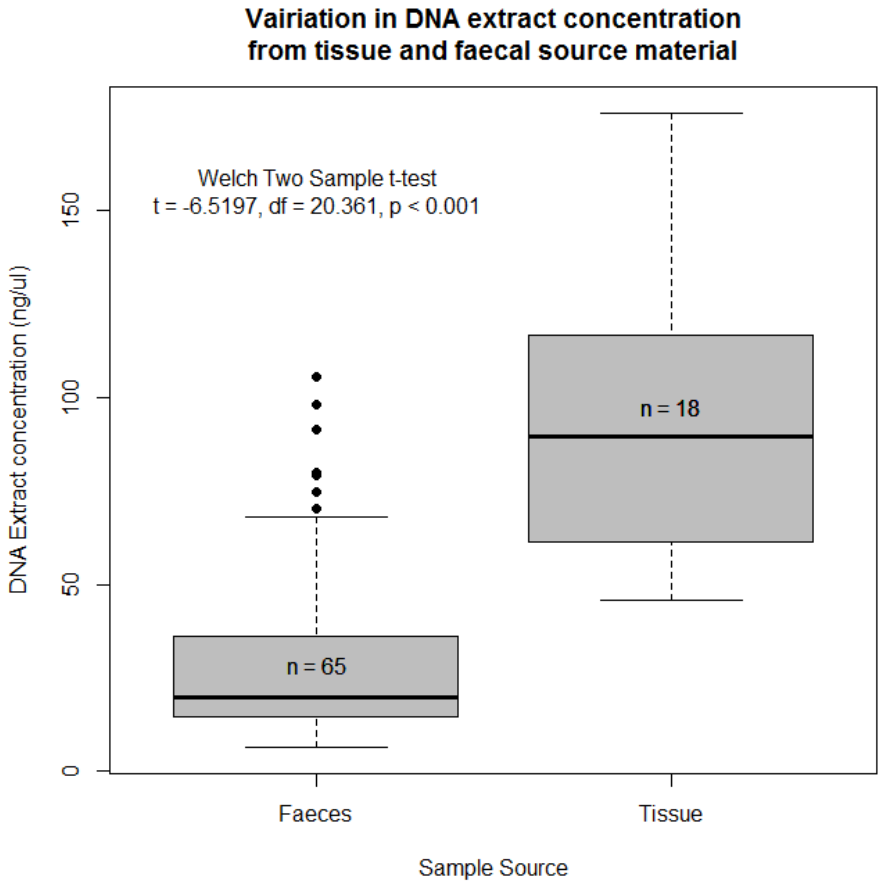


Figure 6.1. *Box plot contrasting the variation in DNA extract concentration as a function of genetic source material.*

A total of 7 of the 65 faecal DNA extracts analysed had a total dsDNA concentration greater than the 95 % of the standard deviation of the sampled group.

Upon examination of DNA extract purity, the absorbance ratio between 260 and 280 nm shows that DNA extracts from faecal source material had a significantly lower 260:280 ratio than DNA reclaimed from tissue source material (Figure 6.2.)

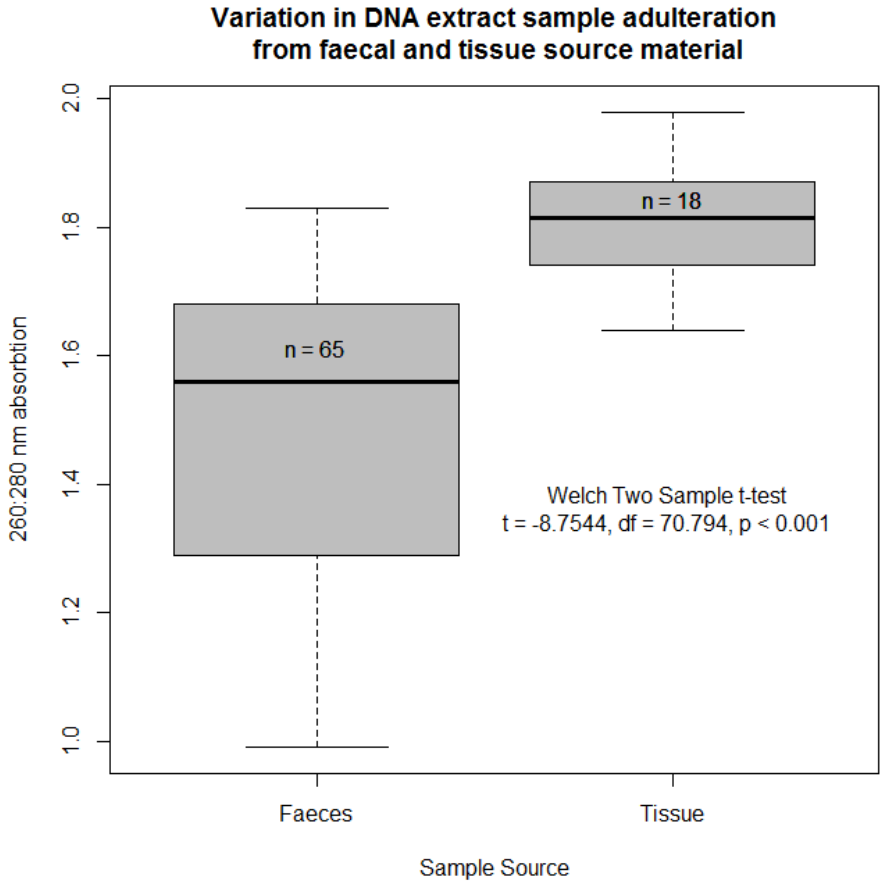


Figure 6.2. *Box plot contrasting the variation in DNA extract purity as a function of genetic source material.*

DNA templates from faecal samples are of consistently lower concentration and purity than DNA templates isolated from tissue biopsies when measured using

spectrophotometry. Despite this, however, the total DNA concentration extracted from faecal samples still proved to be adequate for accurate genotyping by PCR. However, the presence of adulterants in the faecal extract solution and contamination by prokaryotic and non-host DNA may significantly impact PCR efficiency and reduce amplification success.

6.3.2 Template amplification and microsatellite characterisation

All six microsatellite loci chosen for initial examination were successfully amplified from DNA faecal extracts collected from 2 captive jackal individuals at Colchester zoo. DNA fragments were visually screened for PCR success on an agarose gel. No amplification in the reaction negative control was apparent. Positive amplification was seen in both controls with template DNA from a domestic dog.

Sixty-five faecal extracts gave positive amplification of one or more microsatellite loci using end-modified fluorescently-labelled primers when visualised on a 2% agarose gel. PCR amplicon size was then estimated using capillary electrophoresis. Null allele estimates and allelic diversity for each locus are summarised in Table 6.2. No significant false allele calling, due to stutter, was detected via MICROCHECKER V. 2.2.3 (van Oosterhout *et al*., 2004). The expected and observed allele frequencies were highly similar for all loci examined. However, the total observed heterozygote individuals at locus DOGP123 was much lower than expected under the assumption of

random assortment, given the number of alleles present within the sampled population.

Table 6.2. *Allelic variation and estimated null allele frequency calculated for all loci examined using a recovered DNA template from faeces. Null allele frequency, H_{Obs} and H_{Exp} was estimated using CERVUS v3.1.2. No significant evidence for allelic drop-out or null alleles was detected by MICROCHECKER v2.2.3. and repeat type specific analysis at 95%. H_{Obs} = observed heterozygote frequency; H_{Exp} = expected heterozygote frequency. H_O = total number of observed heterozygotes, H_E = total number of expected heterozygotes, n = 72.*

Locus	Allele number	Null allele frequency estimate	H_{Obs}	H_{Exp}	Total H_o	Total H_E
DOGP109	12	-0.0160	0.824	0.818	11	7.854
DOGP123	14	-0.1038	0.971	0.824	2	10.254
DOGP204	11	+0.0409	0.727	0.800	10	7.302
DOGP374	9	-0.0221	0.667	0.675	21	19.877
VGL1541	10	-0.0509	0.818	0.759	12	10.489
DOGP622	6	-0.0653	0.727	0.663	17	21.211

No significant evidence for large allelic drop-out or null alleles was detected within the sampled population by analysis either via MicroChecker or Cervus. The allele frequencies for each of the six loci examined are shown in the appendix.

Sampling effort was plotted as a function of accumulative allelic diversity to ensure an adequate detection of allelic variation within the sampled population (Figure 6.3).

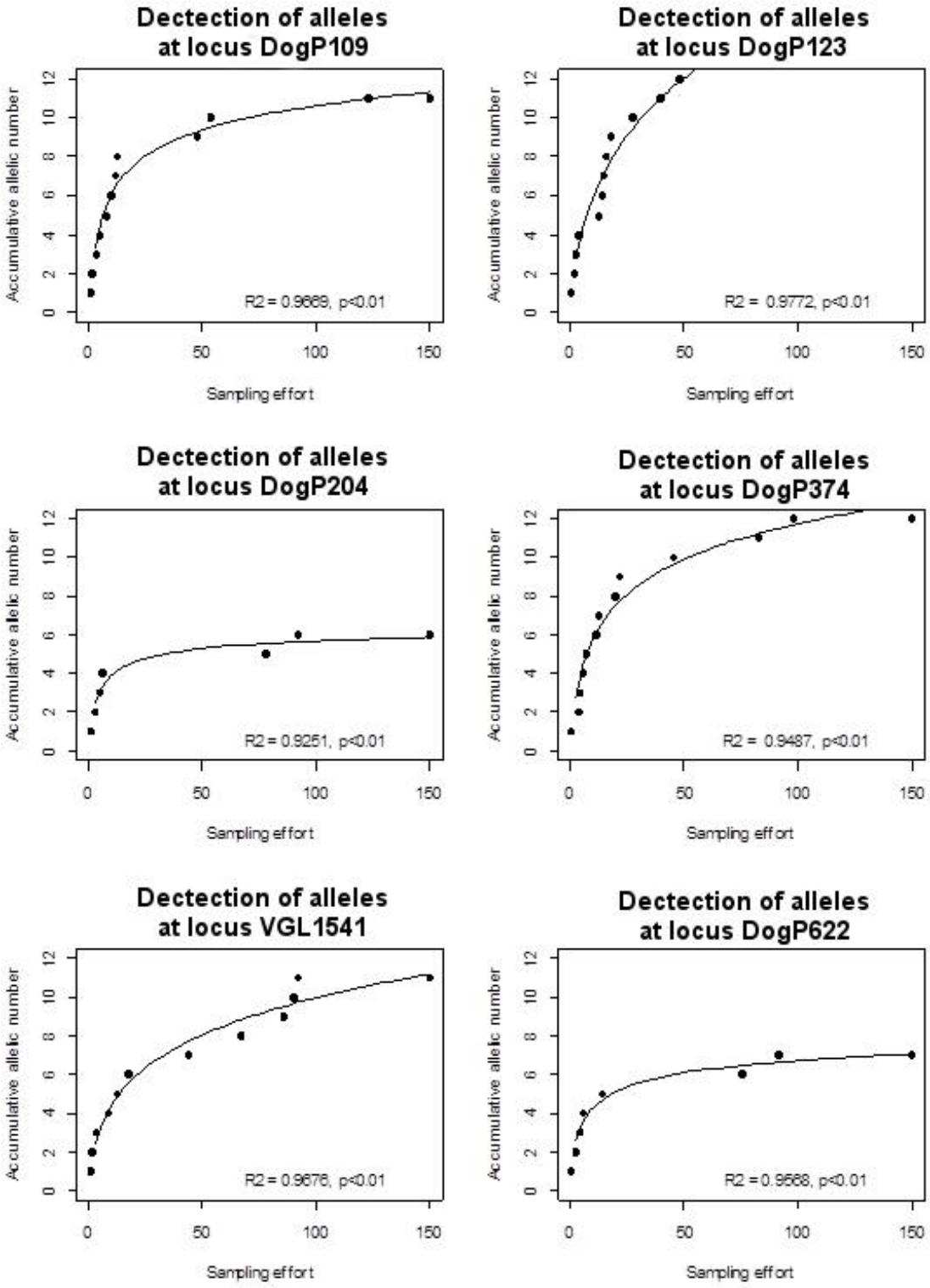


Figure 6.3. *Allele detection frequency as a function of sampling effort for each of the six loci examined. The partial detection of all alleles at locus DogP374 is probably due to the highly polymorphic nature of this locus.*

6.3.3 Identification of selection at microsatellite loci

No significant identification of selection could be identified for any microsatellite marker when examined using the program Lositan (Figure 6.4). No significant outlier values (Simulated F_{ST} – Sample F_{ST} > 0.95) could be ascertained after 50,000 simulations (Table 6.3).

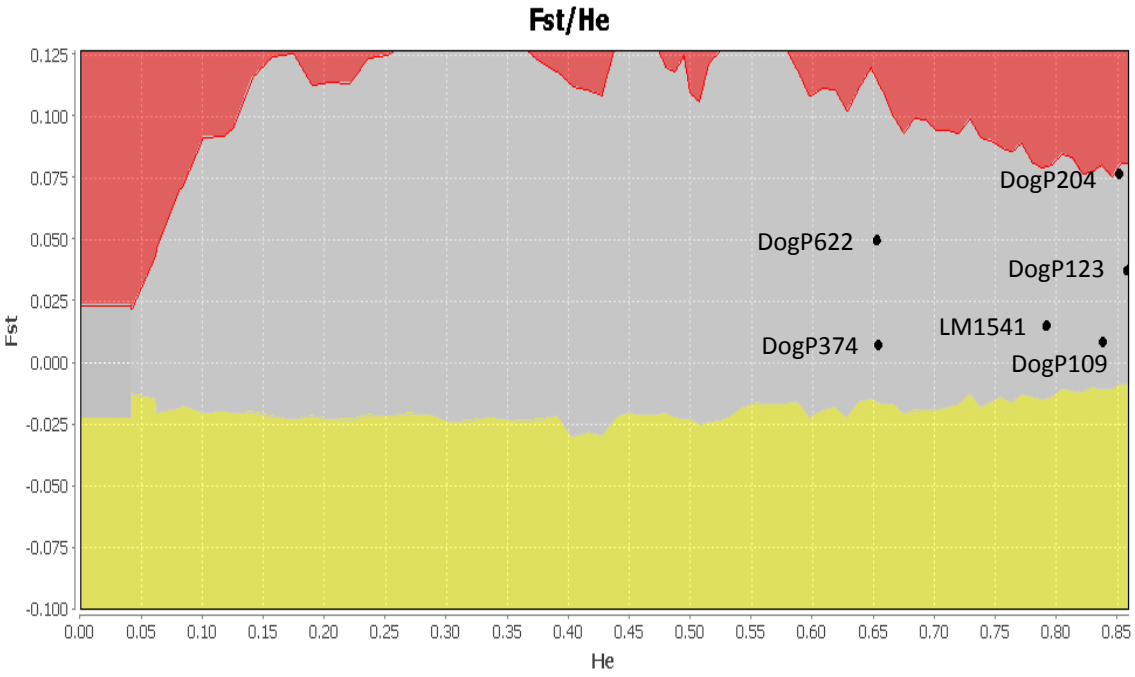


Figure 6.4. *Output from the program Lositan plotting FST against heterozygosity for each locus. Black = candidate markers, Yellow = candidate under balancing selection, red = candidate under positive selection grey = neutral selection. N = 65, markers = 6, Pop = 2, significance level = 0.95, number of simulations = 50, 000, False Disc. Rate = 0.1, Data set F_{ST} = 0.033119, Simulated F_{ST} = 0.016917.*

Table 6.3 *Locus specific output from the program Lositan depicting Heterozygosity (He), FST, P and outlier identification for the six microsatellite markers examined in this study.*

Locus	He	F_{ST}	P(Simulated F_{ST}<sample F_{ST})	Outlier
DogP109	0.857753	0.037563	0.829848	No
DogP123	0.837919	0.008534	0.44698	No
DogP204	0.851054	0.076675	0.964232	No
DogP374	0.653747	0.007199	0.515967	No
LM1541	0.791551	0.015102	0.568372	No
DogP622	0.652439	0.049867	0.843072	No

All markers were identified as being void of significant selection relative to the marker set. F_{ST}/He simulations placed candidate markers within the bounds of neutral selection (grey) at the 95% significance interval. Marker DogP204 showed a level of heterozygosity and F_{ST} value that places it on the boundary of acceptability. However an extension of the simulation run could find no additional signal placing this marker as an outlier.

6.3.4 β-actin probe homology

All 27 PCRs from the two tissue samples produced positive amplicons using the β-actin primers. All amplicons were within the expected size range of 500 bp when analysed on an agarose gel. Gradient PCR provided the optimum annealing temperature as 55^{o}C for the use of the β-actin primer pairs on the DNA templates originating from *C. mesomelas*.

Following plasmid recovery, the insert fragment in the vector was sequenced using the flanking M13 primer pair to ensure the correct sequence insert and accurate characterisation of the primer and probe annealing sites. Both the primer binding sites and TaqMan probe target site remained conserved and intact within *C. mesomelas* (Figure 6.5).

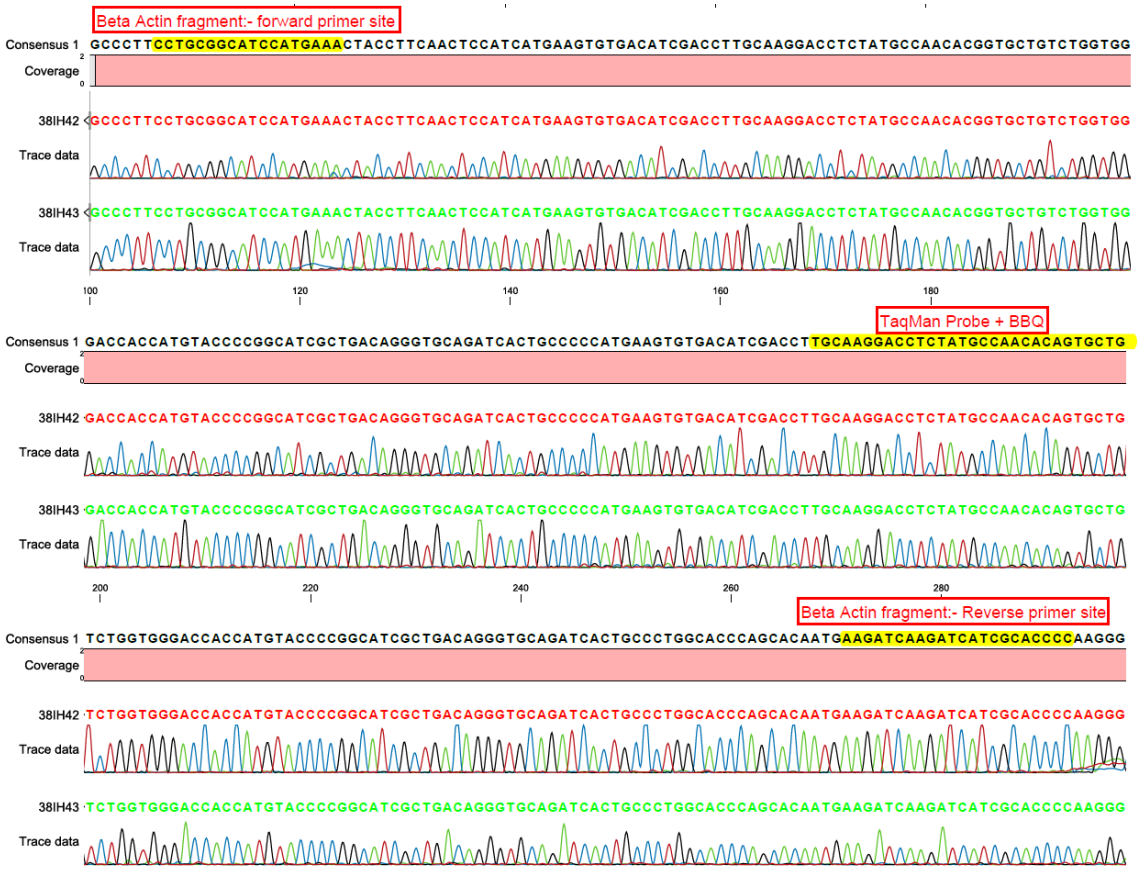


Figure 6.5. *Post vector trim consensus sequence compiled of the parallel and antiparallel strands for the plasmid insert. The consensus sequence shows the conservation of the amplicons and probe target site within Canis mesomelas.*

In order to ensure the correct amplification of the DNA fragment in *C. mesomelas* using the β-actin primers designed for *C. familiaris,* the NCBI nucleotide data base was queried with the NCBI nucleotide BLAST algorithm for a corresponding fragment sequence to reveal a 94% coverage similarity with the β-actin DNA sequence within *C.*

familiaris. In addition, a 1bp deletion and an 8bp mismatch are apparent within the sequence acquired from amplicons originating *in C. mesomelas* when compared with the original sequence from *C. familiaris* (NCBI Reference Sequence: NM_001195845.1). Sequence mismatches were seen at regular intervals both post and prior to the deletion (Figure 6.6).

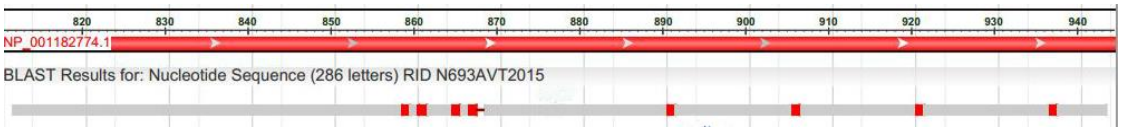


Figure 6.6. *Sequence mismatch analysis for recovered β-actin template from C. mesomelas compared with the beta actin fragment in Canis familiaris. A one base pair deletion was detected with 8 additional base pair mismatches (red) when the two sequences are compared.*

6.3.5 Faecal source material DNA template quantification

During the qPCR, no amplification was seen within the three negative controls. All four DNA extracts from tissue source material showed positive amplification in all three repeats. Of the ten faecal samples run in triplicate, six samples showed clear and positive amplification in all three repeats (Figure 6.7). After removing the failed reactions from the analysis, the qPCR efficiency was calculated at 103.461% with a R^2 value of 0.935 (Figure 6.8). Approximately 3.7 cycles separated the cycle threshold (C_t) value for each of the tenfold standard dilution. The average number of copies of host DNA recovered from tissue source material was significantly greater than the average DNA copy number recovered from faecal source material (Figure 6.9).

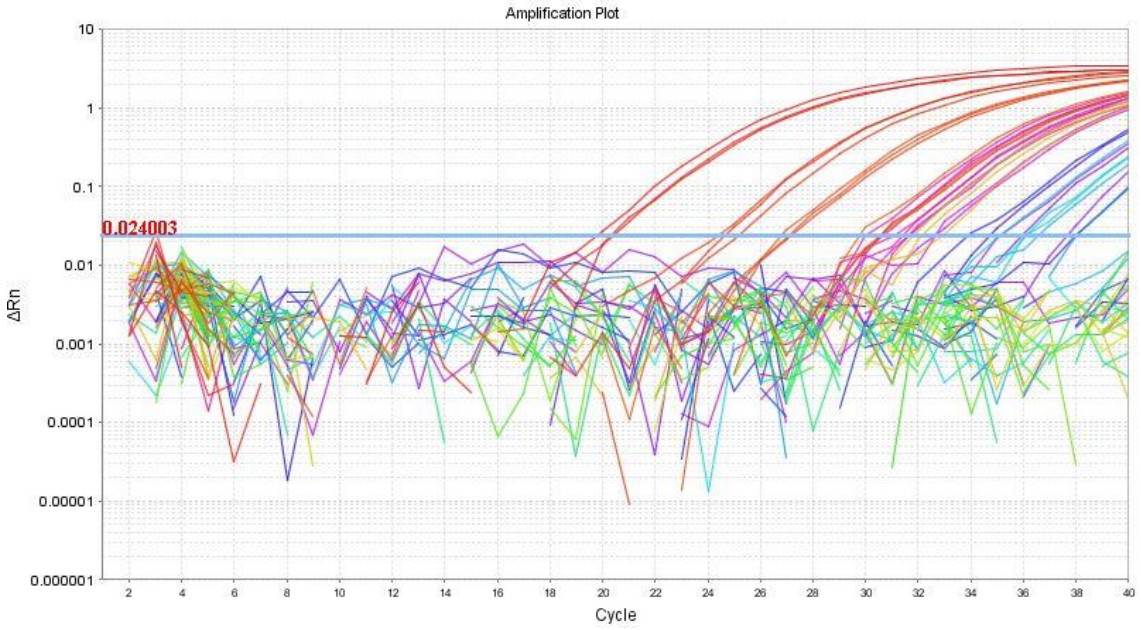


Figure 6.7. *Real time qPCR – TaqMan amplification plot of the β-actin fragment and cycle threshold (Ct = 0.024003) from faecal and tissue DNA template source material isolated from C. mesomelas. Red = known plasmid DNA concentrations. Pink = Unknown DNA concentration from tissue sources; yellow; purple; blue; orange and lilac = Unknown DNA concentration from faecal sources.*

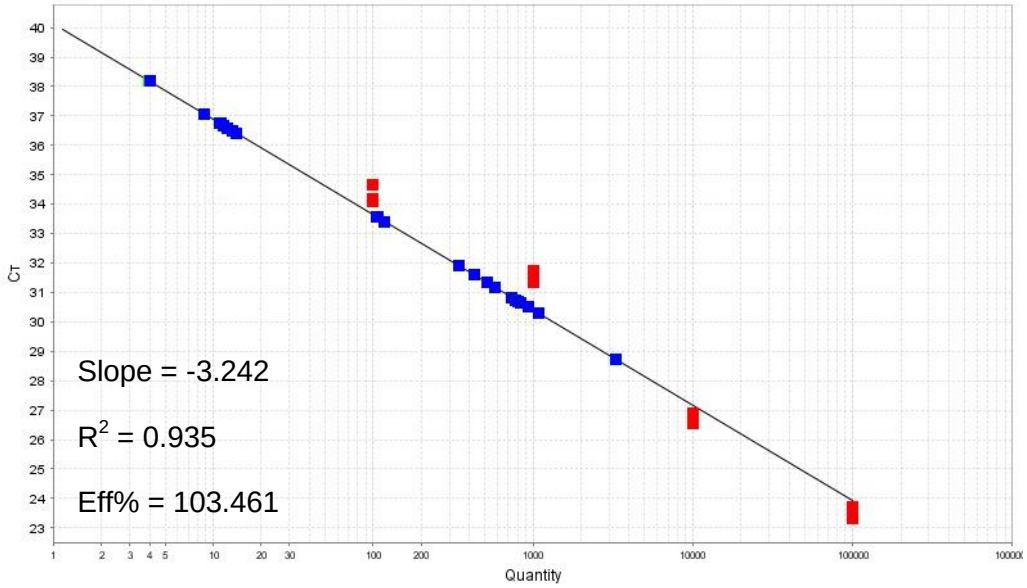


Figure 6.8. *qPCR standard plot showing PCR cycle threshold (Ct) as a function of DNA quantity. Red = known plasmid concentration, blue = Unknown sample concentrations.*

The average total amount of DNA in 1ul of faecal and tissue extract was estimated using the known copy numbers (Figure 6.7 : red) as 81.1 and 1723.6 copies respectively (Figure 6.9)

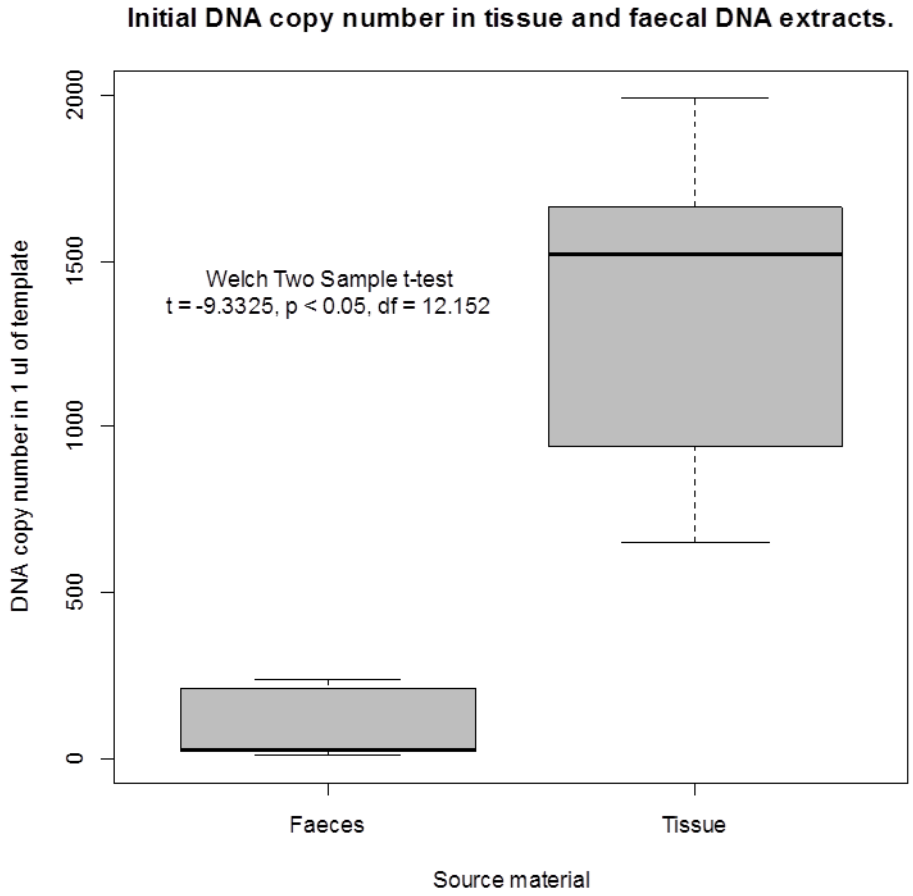


Figure 6.9. *The total number of copies of host DNA recovered and isolated from faecal and tissue DNA source material. Significantly more DNA is recovered from tissue when compared with the faecal DNA recovery using the extraction protocol in this investigation.*

The difference in detection cycle threshold (C_t) was examined between the two source material extracts. Amplification of the β-actin target fragment was seen consistently earlier in the PCR cycles than the amplification of target DNA in faecal extracts, by a maximum range of eight cycles (Figure 6.7 and 6.10).

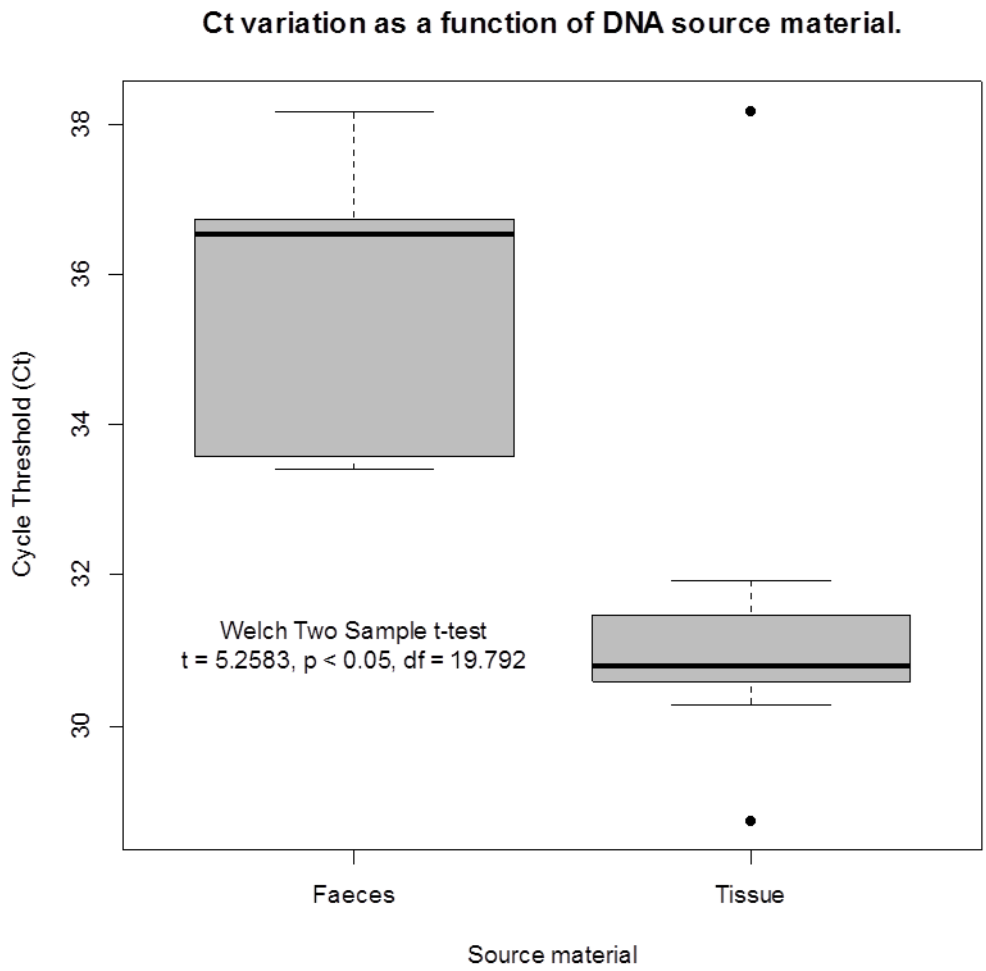


Figure 6.10. *Variation in qPCR Ct values for DNA templates originating from faecal and tissue source material. Faecal DNA source material exhibited significantly later amplification than DNA templates from tissue source material. n = 18.*

6.3.6 Microsatellite characterisation

A consensus of allelic diversity was made by combining the genotype data originating from 18 tissue samples and 65 faecal samples. Null allele frequency estimates H_O, H_E and total PCR success for all samples is summarised in Table 6.4.

Table 6.4. *Total allelic variation and estimated null allele frequencies calculated for all loci examined using recovered DNA template from all tissue and faecal samples. Null allele frequency; H_{Obs} and H_{Exp} were estimated using CERVUS v3.1.2. Total observed (H_O) and expected (H_E) homozygote frequency was calculated using MICROCHECKER v2.2.3. In addition, no significant evidence for allelic drop-out or null alleles was detected by the program MICROCHECKER, n = 18.*

Locus	Allele number	Null allele frequency estimate	H_{Obs}	H_{Exp}	Total H_o	Total H_E	Genotyping success
DogP109	15	-0.0016	0.846	0.853	13	9.719	92%
DogP123	13	-0.0830	0.981	0.852	2	12.06	93%
DogP204	14	+0.0130	0.824	0.850	10	8.517	74.7
DogP374	13	-0.0040	0.647	0.670	28	25.943	92%
VGL1541	11	-0.0394	0.843	0.794	14	13.249	86.6%
DogP622	7	-0.0151	0.706	0.703	23	25.064	93%

The range of allelic diversity across all six markers, for all samples typed, was between 7 and 15. No locus showed null allele frequency estimates of > 0.1.

6.3.7 Average probability of identity (PI_{ave})

Individual locus heterozygosity, expected average probability of identity for each locus, and total accumulative observed and expected $PI_{ave,}$ across all loci was estimated using API-CALC v 1.0 across a range of F_{ST} values (Figure 6.11). The microsatellite markers examined in this study are seen to be highly informative of identity in non-inbred

subpopulations under simulated F_{ST} values of < 0.75. As inbreeding is known to reduce the probability of identity through the duplication of alleles resulting from mating between related individuals with commmon ansestors, a range of F_{IS} values were plotted against PI_{ave} at an F_{ST} value of 3% (Figure 6.12).

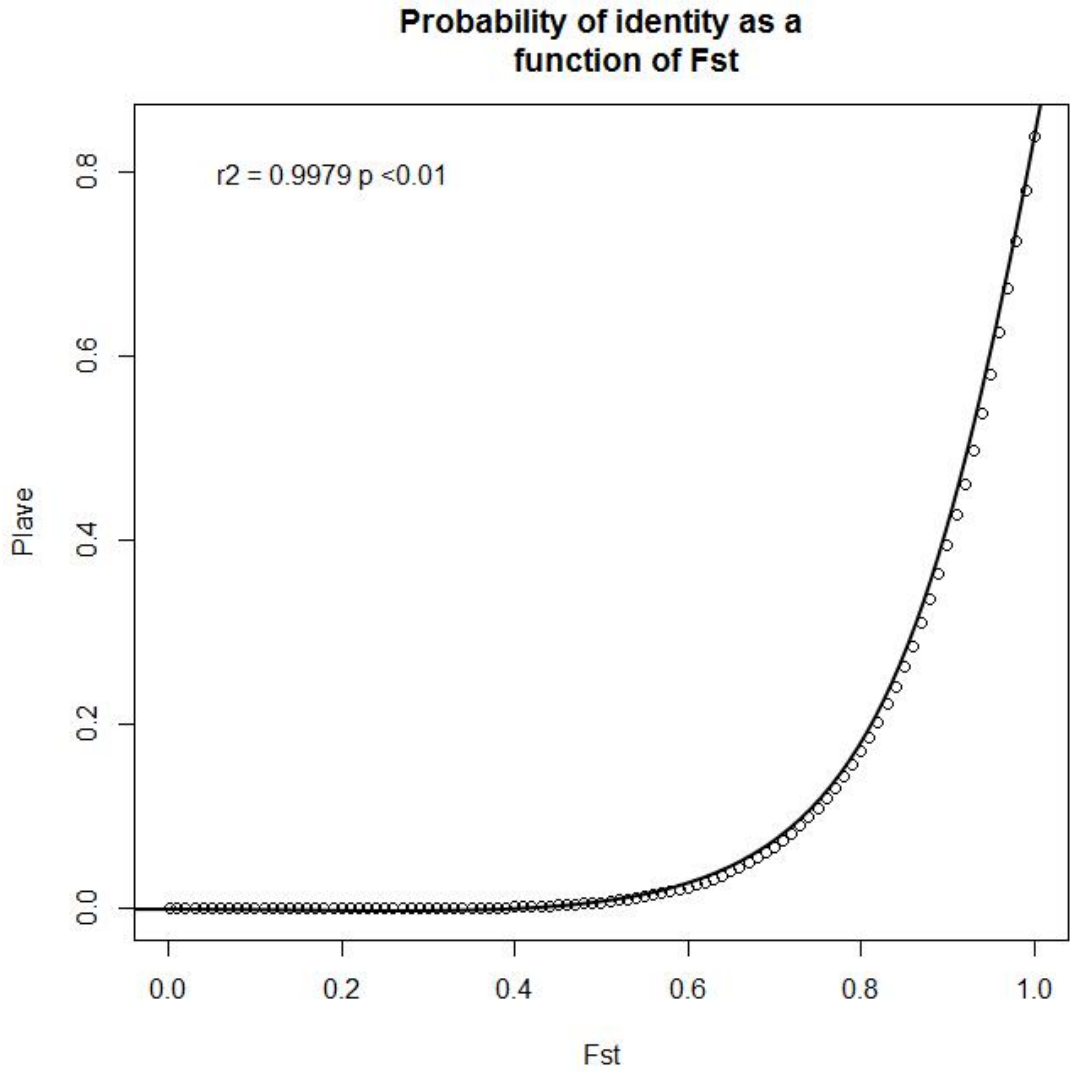


Figure 6.11. *Total probability of identity as a function of increasing simulated F_{ST} values across all loci. Whole genotype profiles constructed from the cross species microsatellite loci used in this study are highly informative of identity in non-inbred jackal subpopulations. The probability of identity increases dramatically with simulated F_{ST} values of greater than 0.7. R^2=0.9979.*

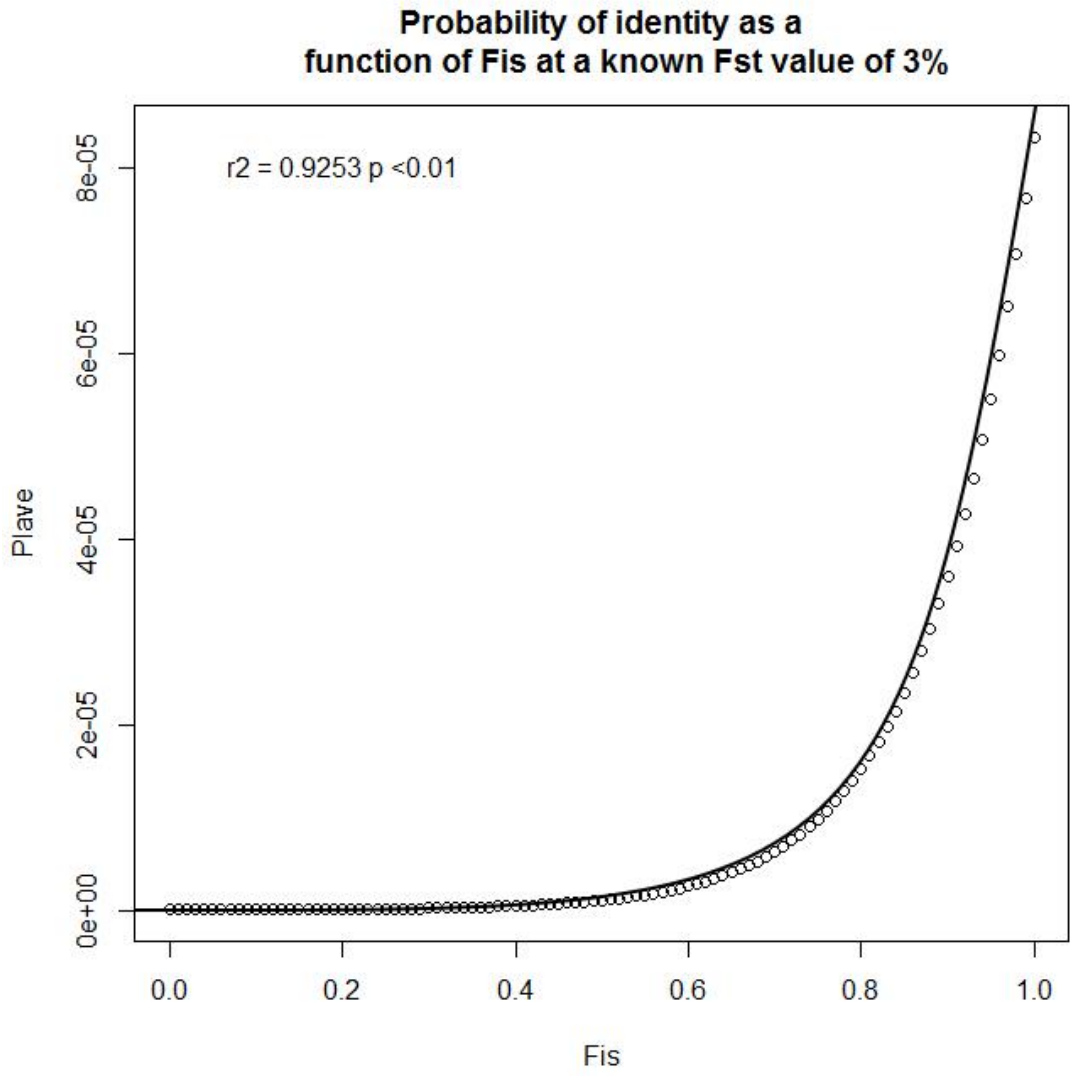


Figure 6.12. *Total probability of identity as a function of increasing simulated FIS values across all loci at known F_{ST} value of 0.03. Whole genotype profiles constructed from the cross species microsatellite loci used in this study are highly informative of identity in relatively inbred jackal populations with an ecologically relevant level of substructuring (see chapter VII). The probability of identity remains highly informative in inbred populations with F_{IS} values < 0.6 %.*

Cumulative and individual observed and expected PI_{ave} for a population with assumed F_{IS} and F_{ST} values of 0 are shown in Table 6.5.

Table 6.5. *Probability of identity for each locus examined calculated using API-Calc V 1.0. All loci are suitably informative to estimate inbreeding and relatedness at the population level. Total accumulative probability of identity = 1.96E-08.*

Locus	Expected PI	Observed PI	Expected Heterozygosity	Cumulative Expected PI	Observed Multi-Locus PI
DogP204	0.012506	0.021622	0.912356	1.25E-02	0.081081
DogP109	0.023469	0.030631	0.885244	2.94E-04	0.012613
VGL1541	0.039977	0.043604	0.840089	1.17E-05	0.002883
DogP123	0.084741	0.137297	0.78	9.94E-07	0.001081
DogP622	0.130236	0.157838	0.688089	1.29E-07	0.001081
DogP374	0.151657	0.136216	0.658133	1.96E-08	0.001081

The most informative marker studied in this investigation was DogP204 with an expected probability of identity of 0.912356. This indicates that the probability of two identical genotypes sampled at this locus originate from two distinct individuals is approximately 1%. The addition of the next most informative marker, DogP109 indicates that the probability that two identical genotype profiles for these two markers being from two distinct individuals is 0.000294%. The use of a combination of all six microsatellite markers characterised in this investigation indicates that the probability of two identical genotypes obtained from a non-inbreeding population belong to different individuals is 1.96×10^{-8}, otherwise expressed as an error in

identification rate of 1 in 51 million samples. This allows us to be confident that each six-locus genotype is unique to an individual.

6.4 Discussion

6.4.1 DNA collection storage and extraction

Although the concentration of DNA recovered from faecal source material was significantly lower than that of tissue source material, the collection, storage and extraction protocol developed for this study successfully yielded DNA templates from faecal material that were of adequate concentration to undertake successful PCR studies (Taberlet *et al*., 1996, Ewen *et al*., 2000). The targeting of the outer layer of the faecal pellet during collection, in an attempt to maximise the number of shed host rectal cells collected, may go some way to account for the total DNA recovered from faecal source material in this study. However, the concentration of total DNA recovered from the faecal material was equal to, or greater than, the average expected DNA concentration recovered from other low yield DNA source material such as plant matter (Keb-Llanes *et al*., 2002) when quantified using spectrophotometry. Yet the presence of adulterated DNA from non-target eukaryote and prokaryote organisms present in faecal samples may account for the values quantified by spectrophotometry.

The presence of non-host specific DNA in sample extracts presents a potential limitation to DNA quantification by spectrophotometry as no clear distinction can be made between host specific and non-specific DNA. Therefore the preliminary indication of total DNA yield is unlikely to be a true representation of the target DNA concentration for a given extract. Many factors such as individual variation in jackal diet, duration of exposure to the environment prior to collection, microbial degradation and deposition time, are likely to highly influence the microbial biomass of a faecal source material. Thus further absolute quantification of host specific DNA was required to ensure an adequate recovery of host DNA templates.

After collection, samples were stored at -20^{o}C in S.T.A.R. buffer for up to 1.5 years and were still able to yield DNA templates of detectable concentration. This supports the manufacturer's claim that S.T.A.R. buffer can keep samples in a biologically inert environment for prolonged periods of time at -20^{o}C. This is advantageous for any sampling regime in remote locations that is unable to transport samples rapidly at room temperature for immediate analysis. However freezer storage is essential for long term storage of samples in order to minimise any additional sample degradation outside the capabilities of the buffer.

The purity of the DNA extracts from faecal source material was significantly lower than DNA extracts derived from tissue source material. It is highly probable that this is due to the presence of adulterants of organic origin that have persisted from the raw

sample through the sample purification and DNA extraction processes. Although large organic compounds are highly soluble in the organic solvents used in the sample purification process, the high concentration of humic acids and other adulterants (Hajkova *et al*., 2006) and the variable chemical composition of faecal material may result in sample contamination despite the process of solvent purification. Organic compounds that have failed to be removed during the purification process may be subsequently carried over in the DNA extraction and therefore contaminate the final DNA elution. Although the chemical composition of the sample adulterants is unknown, the stochastic amplification of microsatellite loci is of potential concern in samples contaminated with PCR inhibitors. As the mean 260:280 nm absorption ratio for faecal-derived DNA samples was 1.58, the concentration of adulterants in the final DNA elution is thought to be of low enough concentration to avoid significant PCR inhibition using this extraction and purification technique. Undesired ethanol carry-over during the extraction protocol has the potential to induce positive bias in absorbance ratios when examined by spectrophotometry. However, as the evaporation of residual ethanol from the spin column membrane prior to elution was undertaken to minimise ethanol carry over, the potential for substantial ethanol contamination in the final elution was negated. Previous studies investigating the prokaryotic community structure of faecal and soil samples have used a more robust phenol:chloroform:isoamyl alcohol extraction procedure combined with a bead beating process aimed to increase the overall DNA yield from a sample (Griffiths *et al*., 2000, Griffiths *et al*., 2011). However, the benefits in reducing inhibitor compounds, seen with such robust extraction processes, may prove counterproductive when

targeting DNA from fragile eukaryotic cells present in low copy numbers due to mechanical shearing and template fragmentation (Seutin *et al*., 1991).

6.4.2 Microsatellite amplification from faecal source material

The amplification of microsatellite loci from DNA extracts derived from faecal source material was initially assessed in a pilot study using faeces collected from captive jackal individuals. All nine microsatellite markers selected for screening were successfully amplified and visualised on an agarose gel. The success of this preliminary investigation in producing clear and distinct PCR fragments provided proof-of-concept for successful isolation of host DNA from faecal matter. The lack of amplification of fragments outside of the expected microsatellite size range is indicative of the host-specific nature and binding site specificity of the microsatellite primers selected. The lack of any significant amplification from DNA templates derived from human samples again indicates the species specificity of the primers. Furthermore, the lack of primer site homology in human derived DNA templates reduces the potential for human contamination during the sample collection. This negates the possibility of human contamination inducing genotyping errors in subsequent fragment length analysis.

Due to the successful amplification of all nine microsatellite markers, six of the nine loci were selected at random for further characterisation with respect to the financial and effort based constraints of this study. However, the three remaining loci remained available for further characterisation should the six selected markers prove

insufficient. The amplification of six microsatellite fragments from sixty-five faecal-derived DNA templates revealed a high mean amplification success rate of > 88.8% across all loci examined. The PCR success rate, lack of large PCR artefacts (see appendix) and low statistical chance of allele size calling error due to stutter, provides a preliminary indication of successful amplification of low template copy number samples from faecal extracts. Upon detailed analysis of sample size, sequence homology (see chapter V for sequence analysis) and expected allele frequencies, no evidence could be found for a high frequency of allelic drop-out arising from low template copy numbers and miss calling of allele sizes. This again indicates an adequate quantity of host-specific DNA is being recovered from non-invasively sampled faecal source material.

The null allele frequency in the sampled population was estimated at less than 0.1 at all six loci examined. These values are consistent with the indicator values described for templates of high purity derived from tissue in chapter V. Analysis using Micro-Checker (van Oosterhout *et al*., 2004) reveals that Locus DogP123 showed a large discrepancy between the total numbers of expected and observed homozygote individuals within the sampled population, yet no substantial effect of allelic drop-out was ascertained. In continuation, the observed heterozygote frequency did not differ substantially from the calculated expected frequency at locus DogP123 when analysed using Cervus and API-CALC software. This discrepancy can be partially accounted for by the highly polymorphic nature of this locus and described by incomplete sampling of all alleles present throughout a population as indicated in Figure 6.3. The discrepancy

seen between the total number of observed and expected homozygote individuals at locus DogP123 is therefore more likely to be an artefact of sample size rather than inconsistent amplification of alleles via PCR. Little discrepancy is seen in observed and expected allele frequencies when examined by the program Cervus (Marshall, 1998), however the inability for Cervus to calculate allele frequencies based on incomplete data sets (Kalinowski *et al.*, 2007) is a limitation in estimating expected homozygotic genotype frequencies within a population.

6.4.3 β-actin probe cross species homology

Both the β-actin primer annealing sites and Taqman© probe annealing site within *C. mesomelas* were shown to be homologous to that of *C. familiaris* by plasmid sequence analysis (Figures 6.5 and 6.6). A single base pair deletion and 8 sequence mismatches were seen within the amplified β-actin fragment from *C. mesomelas,* when compared with the amplified fragment from *C. familiaris* from which the probe and primers were designed. The 1 bp deletion and 8 independent bp substitutions detected in the jackal template seems to have little effect on the probe and primer efficiency as no mutations were detected within the probe and primer annealing sites; however the presence of such genotypic inconsistencies in *C. mesomelas* is indicative of the 3-5 million year evolutionary divergence of *C. mesomelas* and *C. familiaris* from their common ancestor. The ability to identify homology of the translated protein sequence to known putative conserved domains of the sugar-kinase actin superfamily leaves little doubt in the homology and conservation of the β-actin fragment within *C. mesomelas.*

With a range of allelic diversity from 6 to 14 and negligible null allele and allelic drop-out effects; the DNA storage, extraction and amplification procedure designed by this investigation was considered suitable for amplification of DNA templates from faecal source material collected in the field. The prominent amplification of DNA templates, devoid of significant PCR artefacts and inconsistent stutter patterns, is indicative of accurate primer homology and suitable recovery of target DNA. However, in order to ensure sufficient DNA is recovered from faecal source material, the absolute mean DNA copy number, recovered from faecal source material, required quantification by qPCR.

6.4.4 qPCR template copy number quantification

The accumulative efficiency of the qPCR assay across all reactions was 103.461% which lies within the acceptable reaction efficiency of 95-105% for accurate detection of fragment amplification (Figure 6.8 + 6.9). The predictive power of the standard dilutions for estimating the DNA copy number in unknown samples was sufficient with an R^2 value of 0.935. These factors indicate the recovery, quantification and serial dilution of the purified plasmid fragment used as the template standards was performed efficiently, thus accurate estimates of unknown DNA template copy number can be ascertained with sufficient statistical power.

Four of the six faecal samples examined via qPCR failed to indicate significant amplification of the β-actin target fragment in any of the three repeats examined. The

failure of amplification in these samples may be due to inadequate initial DNA template concentration or a failure of the assay itself. The sensitivity of qPCR to reaction inhibition by chemical adulterants is significantly greater than that of standard end point PCR due to the additional requirement of successful probe annealing and dissociation. In addition, the total length of the β-actin amplicon is approximately 80bp longer than the largest microsatellite examined, thus the success rate of qPCR should be considered a conservative estimate when compared with amplification of smaller microsatellite loci from faecal templates using standard endpoint PCR protocols.

The amplification of the six remaining faecal samples showed positive amplification with C_t detection values of < 34 cycles (Figure 6.7). The average DNA copy number recovered from the faecal template was 81.1 copies/µl with a standard deviation of 96.6 copies/µl. This indicates that recovery of significant quantities of host specific DNA from faecal source material is possible; however, the absolute number of DNA copies recovered from faecal source material can be highly variable. The large difference in initial template copy number between faeces and tissue is undoubtedly due to the indirect nature of the faecal sample collection and the associated low quantity and quality of host DNA harvested when compared with pure tissue biopsies taken from captured animals. Microbial and mechanical degradation of host DNA will also play a factor in low initial DNA template concentration.

When compared with tissue derived templates, the delay in amplification cycle threshold of faecal derived templates is highly indicative of reaction inhibition. It is therefore highly probable that the discrepancy seen in the C_t values of tissue and faecal derived material is a direct result of reaction inhibition due to the presence of chemical adulterants carried over from the extraction process. The presence of high concentrations of inhibitor compounds in a PCR assay can drastically increase the chances of stochastic allele amplification due to the failure to fully amplify sufficient quantities of DNA for detection within the cycle limit. Although some degree of inhibition is apparent within the PCRs examined in this study, the lack of significant allelic drop-out in microsatellite frequencies collected from the same faecal derived DNA templates indicates the sensitivity of the qPCR assay to inhibition and thus provides a conservative proxy for microsatellite amplification success.

This analysis of absolute template quantification using a highly specific reporter probe method, combined with the statistical analysis for allelic anomalies arising from faecal derived DNA templates, strongly supports the use of jackal faecal samples as a source of genetic material in population studies using the genetic marker-set described in this investigation. The variability in template quantity recovered from faecal samples may be a hindrance in a subset of samples collected in the field, therefore it is advised that a crude visual screening process as proposed by Hogan *et al* (2008) is undertaken prior to fragment analysis to avoid sampling templates of insufficient template quantities or incorrect species origin (Hogan *et al*., 2008).

6.4.5 Microsatellite characterisation and identification of selection

The complete consensus genotype data from all DNA source material was required to maximise the sample size needed to accurately characterise the microsatellite set developed for *C. mesomelas* in this investigation. Again, the full consensus dataset of 72 individuals indicated no significant presence of null alleles or allelic drop-out in the sampled population or misidentification of allele sizes due to inconsistent stutter patterns in the electrophoretogram. The cross-species amplification of these microsatellite loci, which have been specifically refined for pedigree analysis in the domestic dog, has proven consistent in both faecal and tissue derived DNA templates from *Canis mesomelas.* The genetic homology of primer binding sites between *C. familiaris* and *C. mesomelas* must therefore be highly conserved over an estimated 3-5my evolutionary divergence separating these two species from their common ancestor (Wayne *et al*., 1989, Vila *et al*., 1999, Lindblad-Toh *et al*., 2005).

The effort invested in the development and characterisation of microsatellite loci required to undertake accurate pedigree analysis in the domestic dog is substantial and has driven the creation of highly informative, polymorphic and unlinked markers (Ostrander *et al*., 1993). The consistency in amplicon size and lack of erroneous stutter patterns in amplicons (see Appendix) observed from samples taken from *C. mesomelas* may again be in part due to the stringent screening process originally undertaken to characterise these markers in the domestic dog (Ostrander *et al*., 1993).

The total probability of identity, PI_{ave}, across all loci was used to quantify the ability of the microsatellites to uniquely identify individuals from non-invasive tissue samples. The total PI_{ave} indicates a low probability of shared genotype profiles occurring within the sample population based on the assumption of a random sampling of a non-structured population (Table 6.5). The least informative single locus marker was identified as locus DogP622 with an observed PI_{ave} of 0.1578. This result is concurrent with the low allelic diversity associated at this marker when compared with the rest of the marker set. The most informative marker was DogP204 with a PI_{ave} of 0.0216, closely followed by DogP109 with an observed PI_{ave} value of 0.0306. These two microsatellite markers were the most polymorphic of the analysed marker set with 14 and 15 alleles respectively. However, although Locus DogP123 was also seen to be highly polymorphic with an allelic number of 13, the large proportion of heterozygote individuals recorded at this locus was thought to increase the expected PI_{ave} to 0.13828 when F_{ST} and F_{IS} were set at 0. The comparatively high PI_{ave} value, combined with the unexplained deficit in expected number of homozygote individuals at this marker, is of potential concern with respect to the validity and reliability of this marker when used with *C. mesomelas*. However, the full six-locus microsatellite profile is estimated to have a cumulative PI_{ave} of 1.96×10^{-8} (Table 6.5). This means that the probability that two profiles matching at all six markers originating from two distinct individuals is less than 1 in 51 million.

As population sub-structuring will have a significant effect on the probability of shared genotypes within a sample, accumulative probability of identity was calculated for a

range of F_{ST} values. A sharp increase in the accumulative probability of identity for the six microsatellite loci examined was seen when simulated F_{ST} values were greater than approximately 0.75. As values of F_{ST} are rarely greater than 0.2 in wild populations (Pritchard *et al.*, 2000, Allendorf *et al.*, 2012), it is therefore apparent that the microsatellite set developed in this investigation consists of informative molecular markers capable of accurately differentiating individuals in populations subject to partially reduced gene flow and migration. However, in populations that are highly inbred, the ability for these markers to resolve individuals can be substantially reduced. Within highly inbred populations the proportion of homozygotes increase at the expense of heterozygotes due to the recombination of alleles identical by descent and subsequent duplication alleles through the mating between related individuals carrying alleles inherited from the ancestral population. Therefore molecular markers have a reduced efficiency in individual identification in highly inbred populations when Inbreeding coefficients are high (F_{IS}) as individuals are more likely to share genotypes that are identical by descent from common ancestors rather than identical by random assortment.

No significant effect of selection could be found acting on the marker set chosen in this investigation. However, locus DogP204 did show a high level of genetic differentiation in the populations studied compared with the remaining five markers. It is therefore reasonable to assume that the cross species marker set, characterised to investigate the population parameters of the black-backed jackal in this study, meet the

assumption of neutral Mendelian inheritance and are suitable to infer population parameters in this species.

6.5 Conclusions

This study presents a robust and verified method for the collection, storage, purification and extraction of host specific DNA templates derived from faecal source material from the black-backed jackal. Although the quantity of DNA recovered from non-invasively sampled faecal source material is lower than that of directly sampled tissue derived source material, the absolute mean quantity of genetic material was more than adequate to undertake efficient PCR and avoid significant effects of allelic drop-out. Inhibitor compounds are of potential concern to the progression of the PCR reaction. A preliminary visual gel screening for PCR success is therefore recommended in order to reduce additional costs in genotyping samples that have failed to PCR, most likely due to PCR inhibitors.

Due to the stringent examination of microsatellite behaviour, adherence of good working practice in the laboratory, robust experimental design and quantification of adequate template quantity via qPCR; it can be said with reasonable confidence that non-invasive sampling of species specific faecal derived DNA templates presents a feasible and accurate method to facilitate the estimation of population parameters of the black-backed jackal in the North West Province of South Africa. In addition, strong evidence is presented for the conserved cross-species amplification and

characterisation of the microsatellite set developed for use with *Canis mesomelas* in this investigation.

Chapter VII

The population structure of the black-backed jackal

7.1 Introduction and aims

Small isolated populations experiencing little immigration will suffer a loss of genetic diversity over time due to the effects of genetic drift. Naturally occurring physical and geographic barriers to gene flow have long been known to inhibit the dispersal of a species, and have been the fundamental basis for divergence and speciation (Mayr, 1942, Dobzhansky, 1970). However, barriers to gene flow can also result from anthropogenic factors such as habitat fragmentation and land use change, the presence of which can be identified by analysing the variation of allele and genotype frequencies within a sampled population using non-selective molecular markers such as microsatellites (Riley *et al*., 2006). The genetic analysis of populations in the natural environment has become increasingly popular in ecological based studies, as the ability to describe a population in terms of genetic isolation and breeding structure provides valuable information for both the management and conservation of species in the wild.

The perception of *C. mesomelas* as a pest species within the livestock industry of South Africa has prompted a number of studies to investigate the population parameters of the black-backed jackal using techniques such as behavioural observations (Jenner *et*

al., 2011), radio tracking (Msuha, 2009), dietary analysis (Merwe *et al*., 2009, Yarnell *et al*., 2013) and camera trap based frequency estimates (Thorn, 2009). However, although the data produced from such sampling techniques provide highly valuable information on the ecology of this species, the use of a genetic approach can provide detailed information on the population structure of the black-backed jackal that would not be obtainable through traditional sampling and monitoring methods such as these. Thus far, no study has attempted a molecular based approach to answer ecological questions about the black-backed jackal within or outside of nationally protected areas of South Africa. Therefore, this study aims to use microsatellite derived genotype and allele frequencies to investigate the potential for both inbreeding and population structure of the black-backed jackal as a result of the anthropogenic predator management strategies used in the private game farms of South Africa.

The social dynamics of the black-backed jackal are complex with reports of both highly aggressive territorial defence between territory-holding individuals and neighbouring residents (Moehlman, 1983, Estes, 1992), as well as the tolerance of trespassing individuals by territory-holding pairs during periods of high food availability (Hiscocks and Perrin, 1988, Jenner *et al*., 2011). It is therefore hypothesised that the continual removal of territory-holding individuals in areas of nominal food availability would permit unrelated transient individuals and mated pairs to take up residence in newly vacated territories, thereby altering the genetic makeup of the subpopulation by promoting gene flow at the population level. In areas of high food availability that are void of any lethal control, it is hypothesised that limited dispersal would occur at the

site-level, resulting in sub-structuring at the population level. This may, in part, be due to a reduction in territorial defence and competition between both related and unrelated individuals in response to the high abundance of food at each site. However, a causal link between competitive behaviour and genetic diversity was not assessed in this study. In predator neutral areas, void of both predator control and predator feeding, territories would be expected to remain stable with some degree of offspring dispersal (Nel *et al*., 2012, Elmhagen *et al*., 2014), thus measurements of relatedness and genetic diversity were examined to obtain information of the causal factors that drive territorial stability.

Sixty five genotype profiles were constructed from individual faecal samples collected across all study sites in this investigation. Twenty seven individuals were typed from predator feeding site one with an amplification success rate of 70.7 %. Nineteen individuals were typed from Predator feeding site 2 with an amplification success of 70.4 %. Six individuals were typed from predator neutral site 1 with a 100 % amplification success rate. Six individuals were sampled from predator neutral site 2 with an amplification success of 100 %. Three individuals were genotyped from predator control site one with 100 % amplification success rate and four individuals were typed from predator control site 2 with an amplification success rate of 95.83 %.

7.2 Methods

7.2.1 Sample collection and DNA extraction

Genetic material used in this study was sourced from faecal and tissue samples collected between 2012 and 2014 within the six study sites outlined in chapter II. Twenty seven individuals were sampled from the predator feeding site 1 with a total of 162 PCR reactions and a PCR success rate of 87.03 %. Nineteen individuals were also sampled from the predator feeding site 2 with a total of 144 PCR reactions and a PCR success rate of 87.5 %. A total of six individuals were sampled from the predator neural site 1 with a total of 36 PCR at a 94.44 % success rate and six individuals were also sampled from predator neutral site 2 with a total of 36 PCR reactions and a success rate of 75%. Four individuals were sampled from predator control site one with a total of 24 PCR reactions and a success rate of 91%, and a total of three individuals were sampled from predator control site 2 with a total PCR success rate of 91%. Host genetic material was collected; stored, isolated and extracted using the S.T.A.R. buffer + chlorophorm extraction protocol; the development of which is outlined in chapters V and VI. Fresh faecal samples (<24h since deposition) were collected opportunistically within the faecal sampling transects outlined in chapter IV. Faecal sampling transects were used to ensure a fixed sampling effort between sites. Tissue samples were collected opportunistically upon discovery of a recently deceased individual within each site. GPS locations of each sample origin were recorded using a GARMIN GPSMap 62.

7.2.2 Microsatellite selection and PCR conditions

Six *Canid*-specific polymorphic microsatellite markers, characterised in chapters V and VI for use with *Canis mesomelas,* were employed. Individual PCR reactions were undertaken in 25µl volumes containing 40ng/ml DNA template, 1 x Invitrogen PCR buffer, 1.5mM $MgCl_2$, 1 unit of Invitrogen hot start Platinum®Taq DNA polymerase (Invitrogen), 1 unit of Qiagen Q-solution, 0.5µl/ng BSA, 0.2mM dNTP mix and 0.2µM primer mix. The amplification conditions used on a Techne TC-4000 thermal cycler consisted of: initial denaturation at 94^{o}C for 2 min, followed by 35 cycles of 94^{o}C for 1 min, 55^{o}C for 45 seconds and 72^{o}C for 1 min finishing with a final extension stage of 72^{o}C for 5 min. Forward primers were fluorescently labelled at the 5' end with either FAM or HEX and amplicons were separated using capillary electrophoresis using an ABI Prism 3730. PCR fragment size was determined using the internal size standard Rox 500 (ABI Dye Set – 30) and sized using GENEMARKER v. 2.1.2.

7.2.3 Test for Hardy-Weinberg proportions

The genotype frequencies of the total sampled population of the black-backed jackal were tested for concordance with Hardy-Weinberg equilibrium in order to identify the potential for disruptive factors to gene flow arising from any of the predator management strategies examined in this study. If any predator management strategy promotes a significant disruption to gene flow, and thus affects the random union of gametes within the population as a whole, then a significant deviation from the Hardy-Weinberg proportions would be expected, specifically excess homozygosity. Tests for

deviation from Hardy-Weinberg equilibrium were undertaken using GENEPOP v. 4.2.1 (Raymond and Rousset, 1995). Deviations were tested for significance using the Markov chain algorithm (batches = 50, iterations / batch = 1000.) The inbreeding coefficient, F_{IS}, was also calculated at the population level using GENEPOP v. 4.2.1 in order to test for evidence of inbreeding within the total population. If predator management does not affect dispersal, and thus random mating occurs within the total population, then F_{IS} values would be expected to be not significantly different from 0. Global F_{IS} values for each locus were tested for significance using the Markov chain algorithm (batches = 20, iterations / batch = 5000.) Genotype frequency concordance with Hardy-Weinberg proportions was then tested within each sampling site using GENEPOP v. 4.2.1. employing the Hardy-Weinberg exact test and a multi locus test for heterozygote frequency. Deviations were tested for significance using the Markov chain algorithm (batches = 50, iterations / batch = 1000.) The inbreeding coefficient, F_{IS}, was calculated for each sampling site using GENEPOP v. 4.2.1 in order to test for evidence of site and treatment specific inbreeding.

7.2.4 Population structure and differentiation

Population sub-structuring and differentiation would be expected at the population level if any predator management strategy resulted in barriers to gene flow between sites. This would describe population differentiation through drift between subpopulations which results from reduced migration and dispersal at the treatment level. If no barriers to gene flow were apparent between the sites investigated in this study, and the sampled population was therefore continuous, then a correlation

between spatial distance and genetic diversity would be expected between study sites and no significant evidence of differentiation between study sites would be apparent.

Total population fixation index (F_{ST}) and allele frequency correlation estimates between all six sampled subpopulations (Weir and Cockerham, 1984) were calculated using the program GENEPOP v. 4.2.1. Evidence for genetic isolation by distance was assessed by plotting a pairwise genetic distance matrix and a pairwise spatial distance matrix. Pairwise F_{ST}, significance values and Bonferroni corrections were undertaken using the program FSTAT v2.9.3.2 (Goudet, 1995). A Mantel test for dissimilarity was performed between the two matrices using the program R v. 3.0.2 (permutation = 999 model = "strata"). Population differentiation between the six study sites was tested using the exact G test and tested for significance using the Markov chain algorithm (Dememorisation = 1000, batches = 100, iterations / batch = 1000.)

In order to establish the degree of free migration and isolation between the sampled subpopulations with unbalanced and limited sample sizes, the program STRUCTURE v2.3.4 (Pritchard *et al*., 2000) was used which employs a Bayesian clustering algorithm to estimate these two factors by correlating microsatellite allele frequency dissimilarities between individuals both with and without prior knowledge of sample location. The program STRUCTURE uses a Bayesian approach which demands that prior information is explicitly stated regarding the number and location of samples. In the absence of explicit knowledge, uniform prior distributions on these parameters are

assumed. As such the inclusion of sample location is specifically recommended when determining low levels of population structuring under small spatial scales, where a significant F_{ST} value has been determined (Pritchard, 2010). This addition to structure was developed specifically to detect weak population structuring over small geographical scales and is of specific relevance to the analysis of this data set. However as this method may be subject to overestimation of K, a second method not requiring the use of sampling locations was also used. Discrepancies between these two approaches were used to identify the influence of the prior probability distributions on the analysis.

The number of sample origins is estimated as K and the probability of an individual originating at a sampled origin is graphically represented by vertical lines in the output created by the program distruct v1.1 (Rosenberg, 2004). Individuals were assigned a probability of belonging to a cluster. The admixture model, accounts for the possibility of admixture within clusters, as opposed to pure distributions of genotypes, while being robust to the absence of admixture. This was employed to detect any indication of subtle population structure using the genotype data in this study. K was estimated between 1 – 7 using a burn-in of 10,000 runs, Markov Chain Monte Carlo simulation (MCMC) run length of 100000 with 15 iterations per simulation.

Accurate identification of the number of distinct, genetically consistent groups within the sampled population was estimated using both the rate of change in the log

probability of the data between successive estimates of the number of populations, termed delta K (ΔK) (Evanno *et al*., 2005) and via the more conservative estimator of K described by Pritchard *et al* (2005) in the original software instructions for the STRUCTURE program. By examining the rate of change in the log probability of the data (ln(PD)) between estimates of K, the program STRUCTURE has been shown to accurately predict the upper most level of population clusters within the data set examined (Evanno *et al*., 2005). The html based program Structure Harvester was used to calculate ΔK and compile graphical outputs from structure analyses (Earl, 2012).

7.2.5 Relatedness between individuals within sites

In order to investigate the dispersal and family group formation of the black-backed jackal in game farm ecosystems, the program COANCESTRY V.1.0.1.2 was used to estimate the average individual pairwise relatedness. Continued territory turnover and territorial destabilisation resulting from lethal removal of the black-backed jackal would be expected to reduce relatedness in response to immigration of individuals into newly vacated territories. If the large conglomerations of black-backed jackal individuals at predator feeding sites, indicated by the results of chapters III and IV, is the result of immigration in response to high food availability, then relatedness between individuals would be expected to be low as territorial stability is decreased relative to predator neutral sites. Conversely, if predator feeding results in decreased dispersal rates relative to predator neutral sites, then a high degree of relatedness would be expected between individuals from the same family lineage. In continuation, Wang's relatedness estimator (2007) was used to account for the potential for an

inbreeding effect within the sampled population. The Wang estimate for pairwise relatedness examines nine levels of relatedness including first and second cousins mating, and produces a likelihood of two random genes being identical by descent, while accounting for the variation in F_{ST} between sites. Results are presented as a pairwise comparison in a histogram format using the program R v.3.1.1.

7.3 Results

7.3.1 Deviation from Hardy-Weinberg equilibrium

A high degree of allelic variation was seen at the six microsatellite loci examined. Between 6 and 15 alleles were recorded for each locus within the entire sampled population. When the sampled individuals from all six sites were clustered into one data population, a significant deviation from the Hardy-Weinberg equilibrium was apparent (Chi^2 = Infinity, DF = 12, $P < 0.01$). The Hardy-Weinberg probability test for each locus within the total sampled population showed a high degree of variability, with both positive and negative F_{IS} values calculated as being significantly different from zero (Table 7.1).

Table 7.1. *Weir & Cockerham's F_{IS} estimates calculated for each locus for the total samples population. Significant values are denoted by *.*

Locus	P-val	S.E.	W&C F_{IS}
DogP109	0.0069	0.0033	0.0215*
DogP123	0.0001	0.0001	-0.0791*
DogP204	0.0814	0.0169	0.0740
DogP374	0.8060	0.225	0.0448
VGL1541	0.5325	0.0208	0.0189
DogP622	0.2391	0.0091	-0.0156

The Hardy-Weinberg exact test and a multi-locus test for heterozygote frequency revealed five of the six sampled populations did not deviate from Hardy-Weinberg expectations at the 5% significance level (Table 7.2). The singular site "predator feeding site 1" showed a significant deviation from Hardy-Weinberg expectations (Chi^2= 33.4919, Df = 12, P= 0.0008) and multi-locus excess of heterozygote individuals (P = 0.016, S.E. = 0.0022).

Table 7.2. *F_{IS} estimates calculated for each locus within each locality and multi-locus deviation from expected Hardy-Weinberg proportion Chi 2 results. Significant values are denoted by **

Site	DogP109	DogP123	DogP204	DogP374	VGL1541	DogP622	Chi^2	Df	P-val
Predator feeding 1	-0.0066	-0.1566*	0.0395	0.2626	0.1200	-0.1343*	33.4919	12	0.0008*
Predator feeding 2	0.2308	-0.2203*	0.0164	-0.0488	0.0405	-0.1029	18.3459	12	0.1056
Predator neutral 1	-0.0204	-0.2500	-0.3158	-0.0323	-0.3953	-0.2821	6.2296	12	0.9041
Predator neutral 2	0.2	-0.1321	0.2857	0.2105	-0.0526	-0.4706	10.2587	12	0.5933
Predator control 1	-0.2	-0.0909	-0.1429	-0.5	-0.0909	0.2727	1.5243	12	0.9999
Predator control 2	0.1818	-0.1429	-0.5	0.2941	0.0526	0.5	2.7242	12	0.9972

No significant deficit of heterozygote individuals was detected globally or at any site when tested by locus, and across the combined six loci (P > 0.05 Table 7.3). However,

an excess of heterozygosity was recorded at locus dogP109 (-0.0066), dogP123 (-0.1566*) and dogP622 (-0.1343*) at predator feeding site 1.

Table 7.3. *Observed (Ho) and expected (He) number of heterozygotes at each locus within each population.*

	DogP109		**DogP123**		**DogP204**		**DogP374**		**VGL1541**		**DogP622**	
Pop	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**
feeding 1	19	18.878	24	20.816	15	15.600	11	14.837	17	19.267	19	16.796
feeding 2	10	12.903	18	14.838	9	9.143	12	11.457	11	11.449	5	4.548
neutral 1	5	4.909	6	4.909	5	3.909	4	3.889	6	4.455	5	4
neutral 2	4	4.909	6	5.364	3	4.091	3	3.727	4	3.818	5	3.545
control 1	3	2.600	3	2.8	2	1.800	3	2.200	3	2.800	2	2.600
control 2	3	3.571	4	3.571	3	2.200	2	2.714	3	3.143	4	2.858

7.3.2 Population structuring and inbreeding within subpopulations

The total proportion of variation in allele frequency explained by sample site isolation (F_{ST}) is approximately 3 % (Table 7.4), however a large degree of variation is apparent when each locus is examined independently.

Table 7.4. *Weir and Cockerham fixation statistics for each locus and combined across six loci between all localities.*

Locus	F_{IS}	F_{ST}	F_{IT}
DogP109	0.0835	-0.0070	0.0771
DogP123	-0.1788	0.0074	-0.1701
DogP204	-0.0024	0.0834	0.0812
DogP374	0.1005	-0.0015	0.0991
VGL1541	0.0223	0.0062	0.0284
DogP622	-0.1823	0.1146	-0.0468
All:	**-0.0253**	**0.0307**	**0.0062**

Significant genetic differentiation was apparent between sample sites when examining the variation in allele frequencies between sites using the exact G test (Chi^2 = 53.768, df = 12, p = 3.00604 x 10^{-7}). P-values (Fisher's method) across all loci for each population at each study site are summarised in the Appendix.

Pairwise F_{ST} estimates were calculated for each possible population pair and summarised across all loci to examine isolation by distance to test for evidence of a continuous population (Table 7.5).

Table 7.5. *Multi locus F_{ST} estimates for genetic distance following standard ANOVA as in Weir and Cockerham (1984). Bonferroni Correction p < 0.0033. Significant values denoted by **

Population	Predator feeding 1	Predator Feeding 2	Predator neutral 1	Predator neutral 2	Predator control 1	Predator control 2
Predator Feeding 2	0.00667	-	-	-	-	-
Predator neutral 1	0.01	0.00333 *	-	-	-	-
Predator neutral 2	0.00333 *	0.00333 *	0.44	-	-	-
Predator control 1	0.94667	0.4	0.89333	0.53	-	-
Predator control 2	0.24667	0.01333	0.49333	0.26	0.48333	-

Pairwise F_{ST} estimates were plotted against site distance matrix tested for correlation by estimating a mantel statistic (Figure 7.1). Pairwise F_{ST} gave no indication of population structuring due to isolation by distance alone as no significant correlation between pairwise F_{ST} distance estimates and site distance was apparent (Mantel statistic r = -0.1017, p= 0.336, based on 999 permutations). The significant F_{ST} values between predator feeding and predator neutral populations indicate reduced gene flow and genetic barriers within the total population.

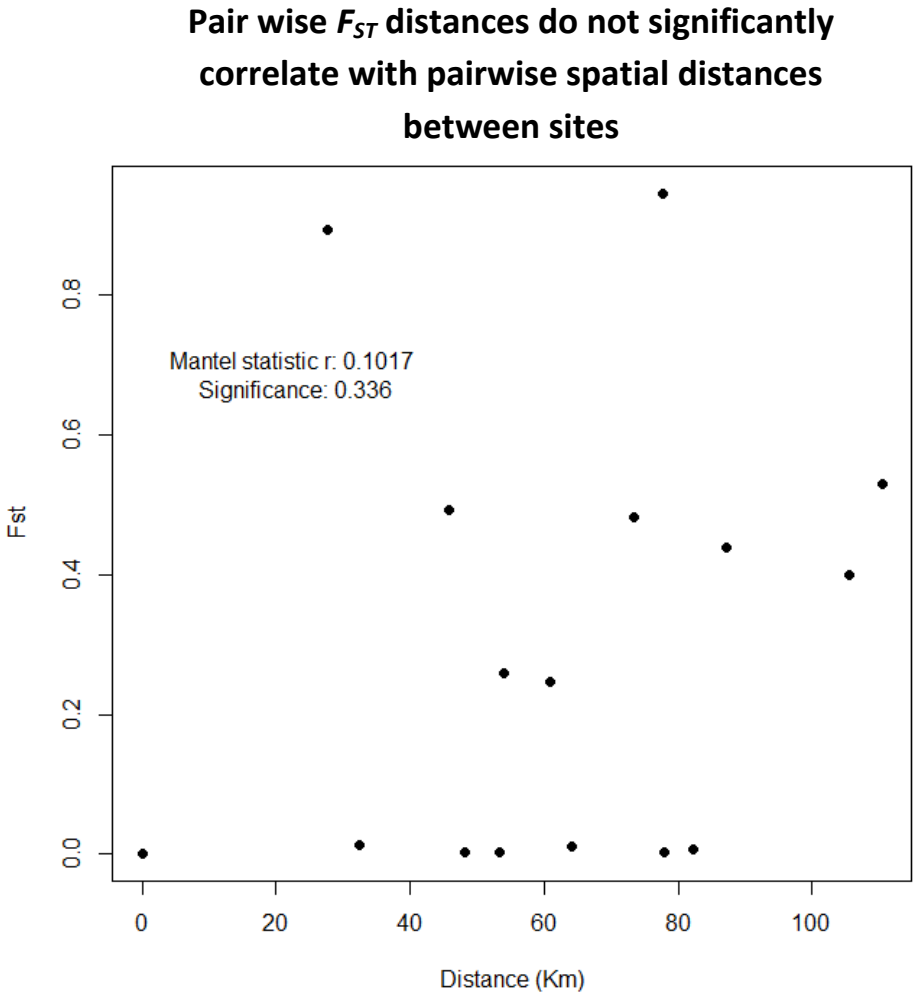


Figure 7.1. *Pairwise F_{ST} distances as a function of spatial distance. Mantel statistic = 0.1017, p= 0.336.*

7.3.3 Analysis population structure

As subjective assignment of individuals to specific subpopulations can be highly error-prone in the absence of observing migration and mating events, STRUCTURE was employed as an objective attempt to partition the total population into clusters according to genetic identity. STRUCTURE was therefore used to determine the number of ancestral populations from the total sampled population across all six study-sites. This analysis was undertaken whenever a positive estimate of F_{IS} was found. The question as to whether the population studied is actually a conglomerate of

several subpopulations needs to be addressed to differentiate inbreeding from a possible Wahlund effect using iterative structure finding algorithms.

The probability of assignment of an individual to an ancestral population was estimated for all individuals at all sample sites using correlated alleles genetic admix model and prior knowledge of sample location (Figure 7.2). The most probable number of genetic clusters detected from the entire data set was K = 2. The number of clusters was recognised as the K value that presented the lowest consistently estimated variation in the probability of the data (ln Pr(Data|*K*)) with the smallest consistent variation between runs (Pritchard *et al*., 2000). The non-random segregation of K demonstrates the genetic structure associated with sampling location.

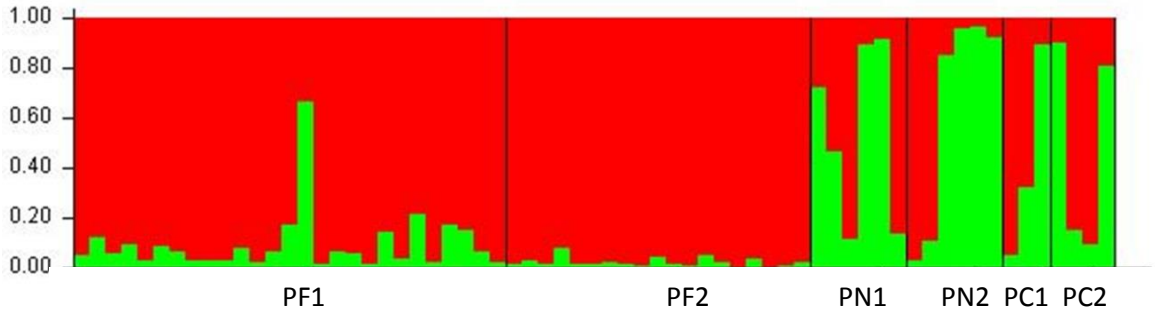


Figure 7.2. *STRUCTURE graphical output using prior sample location information to test for ancestral population origins. K = 2. Predator feeding site 1 = PF1; predator feeding site 2 = PF2; Predator neutral site 1 = PN1; Predator neutral site 2 = PN2; Predator control site 1 = PC1; Predator control site 2 = PC2. The probability of an individual originating from an ancestral population is represented by individual vertical lines, with the colour proportional to the probability of belonging to a predicted ancestral population. Burn-in period = 10000. MCMC = 100000. K range = 1 – 6.*

However, when K was estimated using the maximum hierarchical inference of K using the change in log probability of successive K values (ΔK) as outlined by Evanno *et al*

(2005), the number of genetically homogeneous clusters had a higher estimate of k = 3 (Fig 7.3 and 7.4).

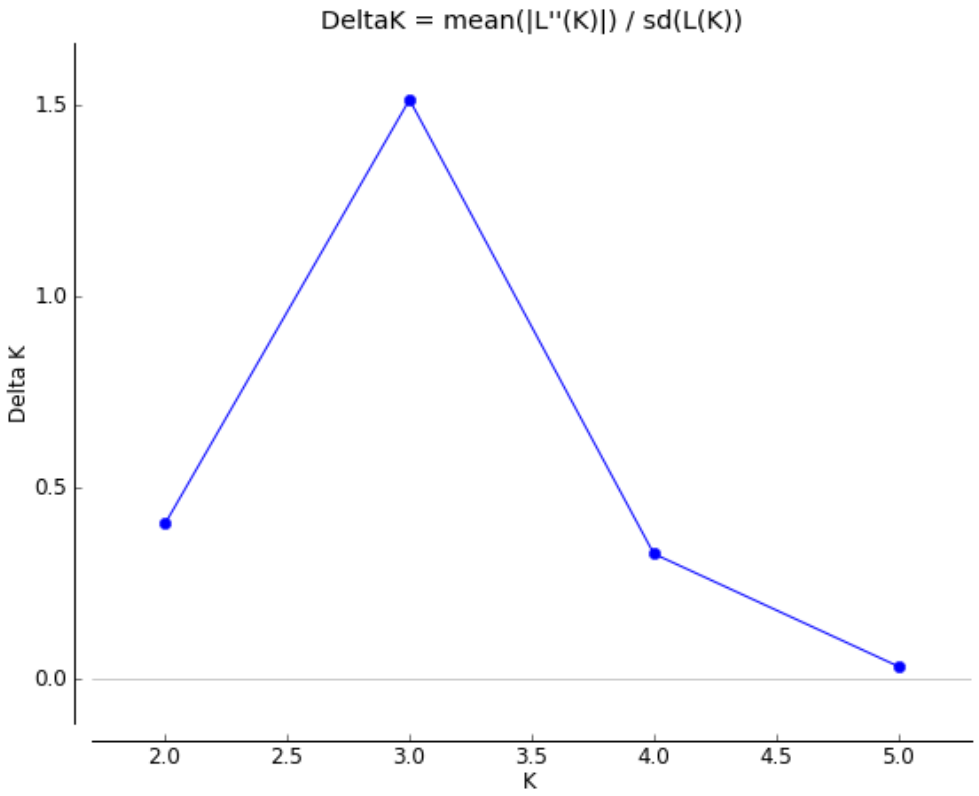


Figure 7.3. *Estimate of ΔK to infer the maximum number of hierarchical structured populations as outlined by Evanno et al (2005). Prior knowledge of sample location used. K = 3, Burn-in period = 10000. MCMC = 100000. K range = 1 – 6.*

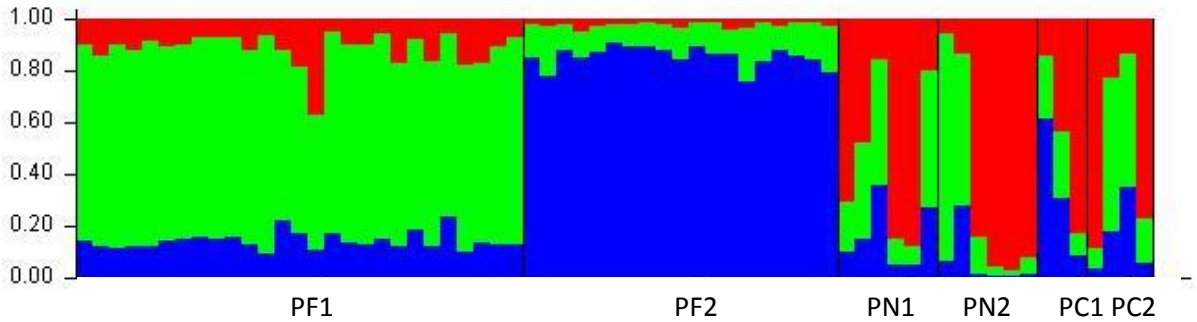


Figure 7.4. *STRUCTURE graphical output using sample location information to test for ancestral population origins. K = 3. Predator feeding site 1 = PF1; predator feeding site 2 = PF2; Predator neutral site 1 = PN1; Predator neutral site 2 = PN2; Predator control site 1 = PC1; Predator control site 2 = PC2. The probability of an individual originating from an ancestral population is represented by individual vertical lines, with the colour proportional to the probability of belonging to a predicted ancestral population. Burn-in period = 10000. MCMC = 100000. K range = 1 – 6.*

Genotype data was also analysed without the use of prior knowledge of sampling location to determine if any signal of genetic structuring could be detected at lower levels of resolution. K was estimated as 2 using the minimum consistent value of (ln Pr(Data|*K*)) outlined by Pritchard *et al* (2000) (Figure 7.5) and k = 3 when analysed using the ΔK estimator outlined by Evanno *et al* (2005) (Figure 7.6 and 7.7).

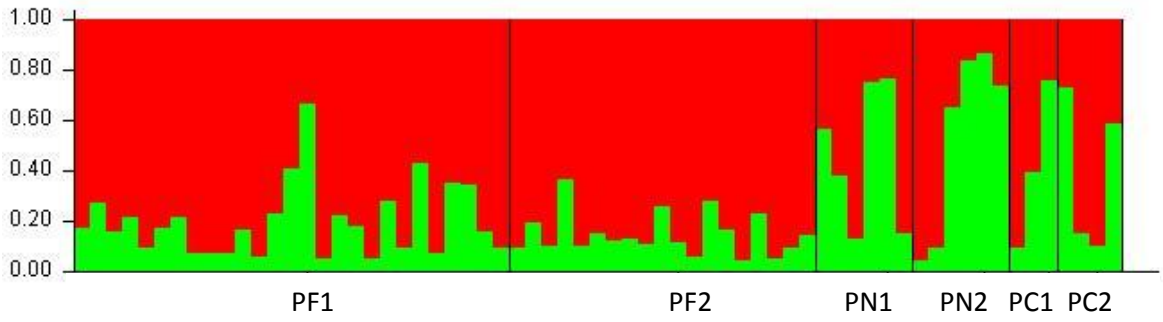


Figure 7.5. *STRUCTURE graphical output with no assumptions of sample origin to determine K. K = 2. Predator feeding site 1 = PF1; predator feeding site 2 = PF2; Predator neutral site 1 = PN1; Predator neutral site 2 = PN2; Predator control site 1 = PC1; Predator control site 2 = PC2. Burn-in period = 10000. MCMC = 100000. K range = 1 – 6.*

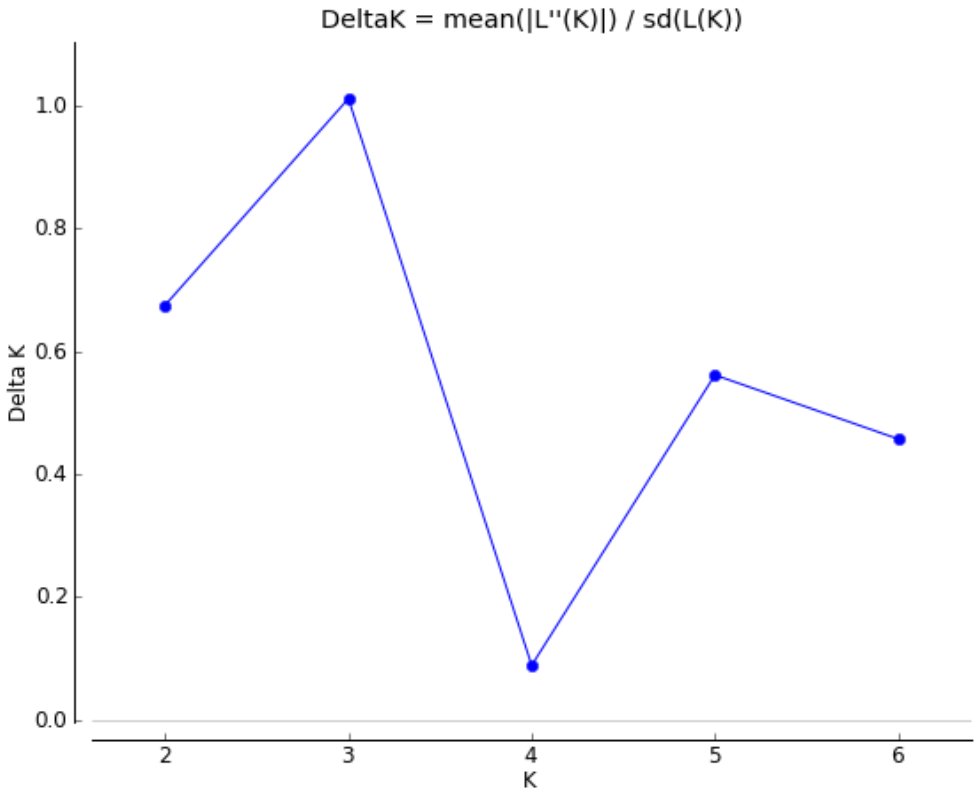


Figure 7.6. *Estimate of ΔK to infer the maximum number of hierarchical structured populations as outlined by Evanno et al (2005). No prior knowledge of sample location assumed. K = 3, Burn-in period = 10000. MCMC = 100000. K range = 1 – 6.*

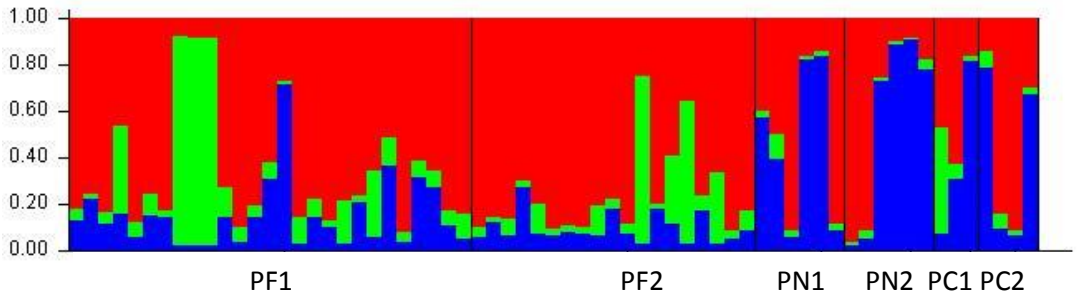


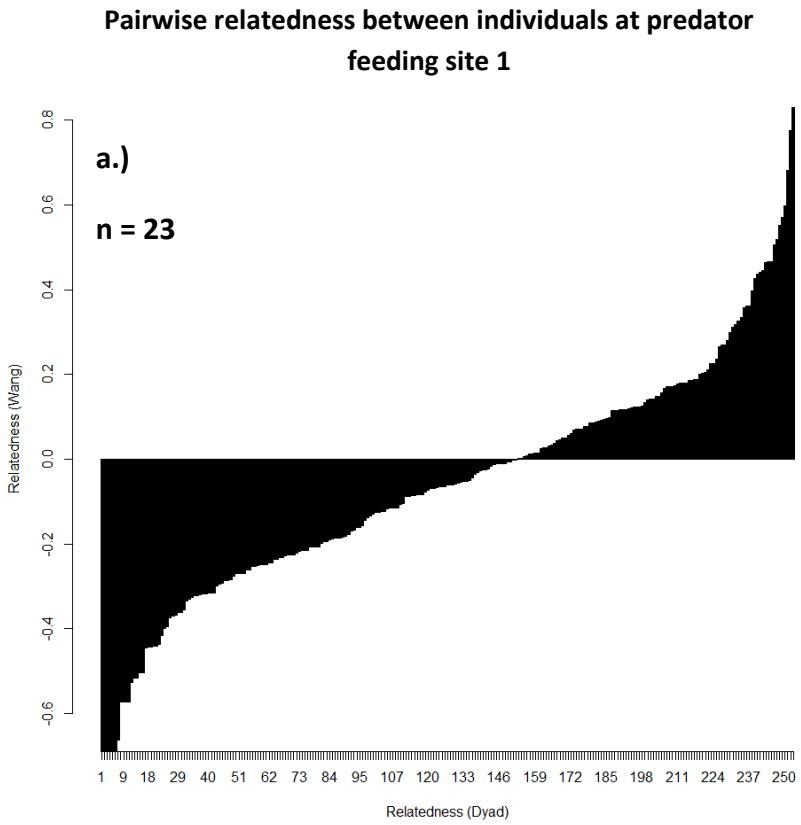
Figure 7.7. *STRUCTURE graphical output with no assumptions of sample origin to determine K. K = 3. Predator feeding site 1 = PF1; predator feeding site 2 = PF2; Predator neutral site 1 = PN1; Predator neutral site 2 = PN2; Predator control site 1 = PC1; Predator control site 2 = PC2. Burn-in period = 10,000. MCMC = 10,0000. K range = 1 – 6.*

Predator feeding sites 1 and 2 were shown to be marginally dissimilar in individual population assignment probability using both the Pritchard and Evanno method of estimating K. A single individual at predator feeding site 1 was presented as having a high probability of belonging to an alternate ancestral population than the other individuals sampled within that site, additionally it was indicated that a barrier to gene flow exists between the predator feeding and the remaining four sites. Individuals for the remaining four sites had shown variable clustering with variable ancestral population assignment probability. A high degree of genetic admixture is therefore inferred across all predator neutral and predator control sites. In addition, 66.6% of individuals sampled at predator neutral site 1 were assigned to population 2 with a high degree of certainty, indicating a population sub-structuring and genetic dissimilarity between the remaining five sites.

Both the Pritchard and Evanno methods for estimating the number of genetically homogenous groups in a population indicated the K is > than 1 both with and without the inclusion of prior knowledge of sampling locations in the analysis. These analyses indicate that the population structuring, highlighted by an inbreeding coefficient of approximately $F_{ST} \approx$ 3% is partitioned into the supplementary feeding sites. However, the exact estimates of K varies between the Pritchard method and Evanno method with k = 2 and 3 respectively.

7.3.4 Relatedness between individuals within sites

The program COANCESTRY V.1.0.1.2 was used to calculate individual and population pairwise relatedness estimates. Relatedness, in this case, is underlined by the principle that simulated individual genotypes, randomly formed from alleles in a population, will share alleles Identical By Descent (IBD) at a frequency proportional to the allele frequency in a population. If alleles identical by descent occur *within* individuals more often than *between* individuals, the population is shown to be inbred. When alleles IBD occur between individuals at a higher proportion than expected by chance, then the individuals are related proportionally to the allele frequency (rarity) in that population: relatives share the same alleles with greater probability than expected by chance (i.e., independently inherited). Just how much greater the probability is, reflects the degree of relatedness. Because the estimation of relatedness is dependent upon the accurate estimation of the population allele frequencies, a large population is required for sampling. For situations presented here, where the population is not expected to be a homogenous one, the assumption of random mating is not appropriate. The Wang estimate of relatedness (Wang, 2007) incorporates the inbreeding co-efficient calculated for each population using the allele frequencies of that population, in order to prevent an over-estimation of relatedness between individuals due to unrepresentative allele frequencies used in the calculation. As the sampled population is assumed to be in Hardy-Weinberg equilibrium at the genotypic level, but showed a significant degree of variation in heterozygote excess within sites across specific loci, the Wang estimator of relatedness was used to estimate relatedness between individuals for this data set (Figure 7.8 a - f).



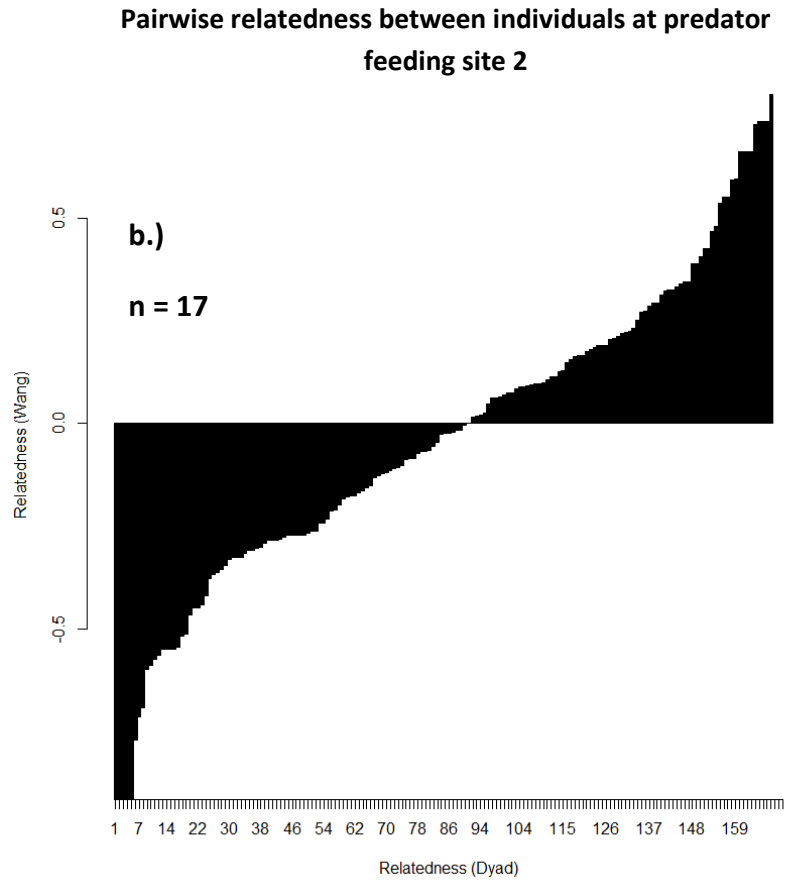
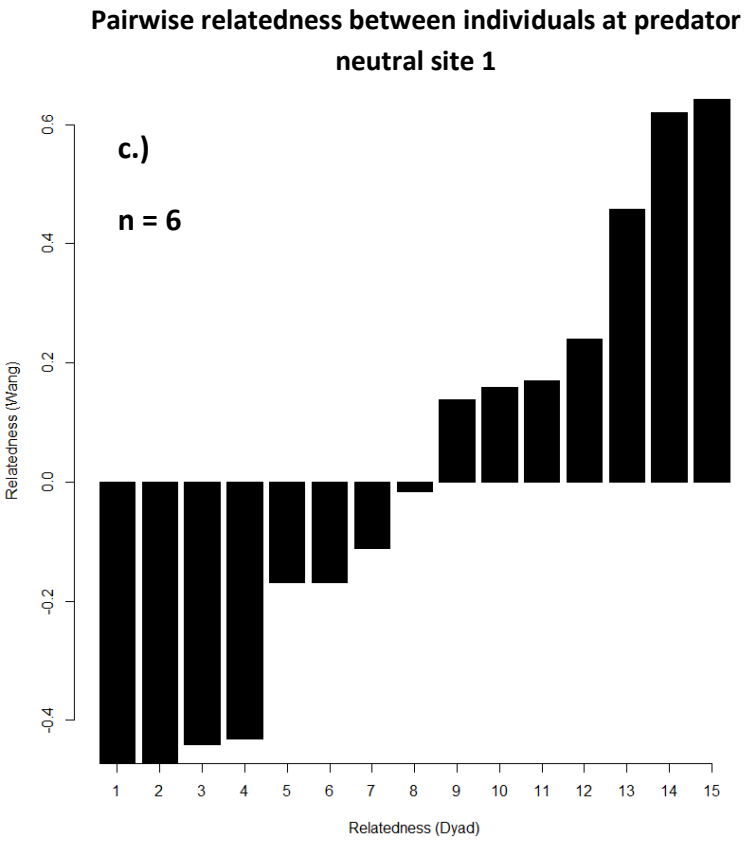


Figure 7.8 a:b). *Pairwise estimates of relatedness between individuals within the predator feeding sites.*



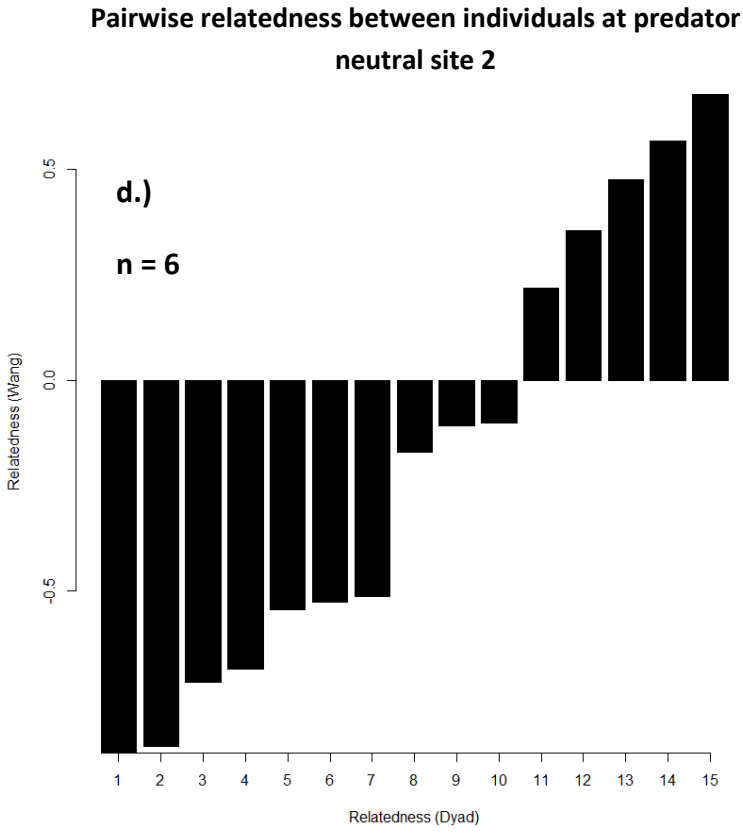
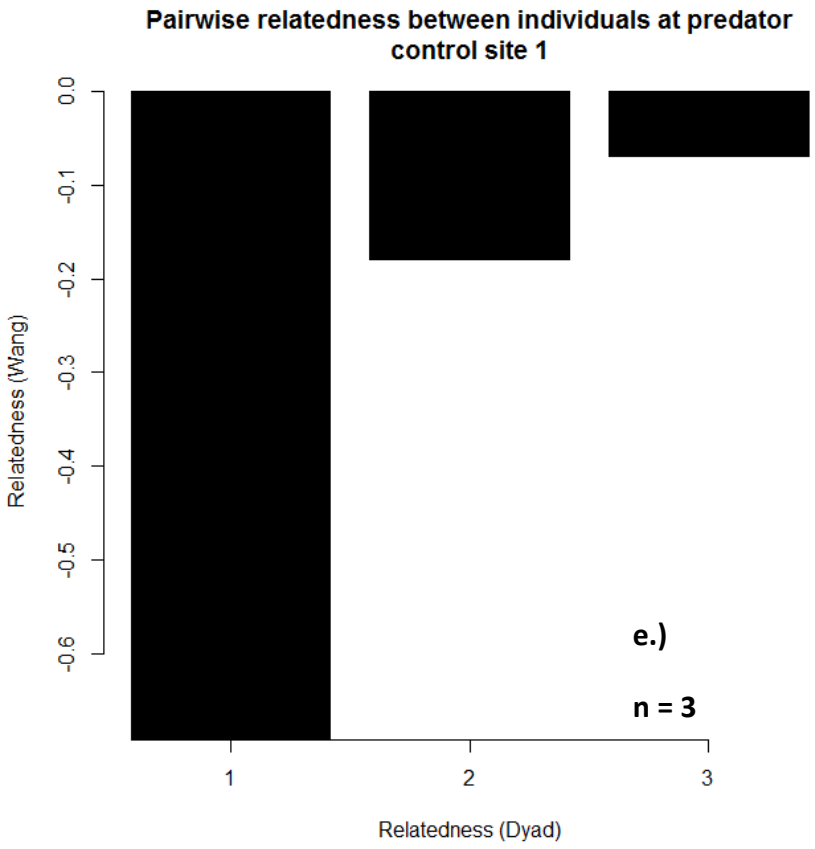


Figure 7.8 c:d). *Pairwise estimates of relatedness between individuals within the predator neutral sites.*



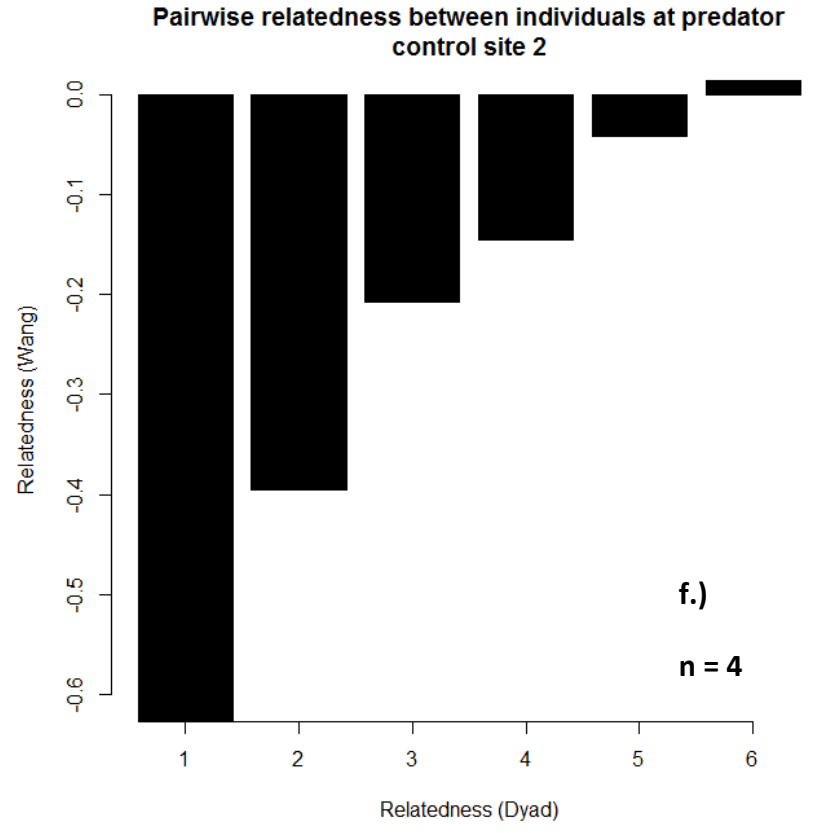


Figure 7.8 e:f). *Pairwise relatedness between sampled individuals within each predator control site.*

Six incomplete microsatellite profiles were removed from the analysis in order to undertake an accurate estimation of relatedness. Both predator feeding sites had a proportion of individuals that were highly related at the full sibling level and greater, as well as a number of highly unrelated individuals. This correlation was also seen at the predator neutral sites with a proportion of individuals related up to full siblings. The majority of individuals sampled at the predator control sites were shown to be less related than expected and excessively heterozygous at different alleles and singular loci; this is indicative of subpopulation interbreeding and genetic admixture.

Although some degree of population structuring is apparent within the total population, relatedness between individuals within sites was calculated using the allele frequencies of the entire sampled population in an attempt to account for the small sample sizes at predator control sites (Figure 7.9). A departure from the results using the allele frequencies of the subpopulation to estimate relatedness is seen, with a shift towards related individuals at all sites. However, as structuring is apparent within the total population, the relatedness estimates may be subject to a bias due to the variation in allele frequencies between subpopulations as well as small and unbalanced sample sizes between sites.

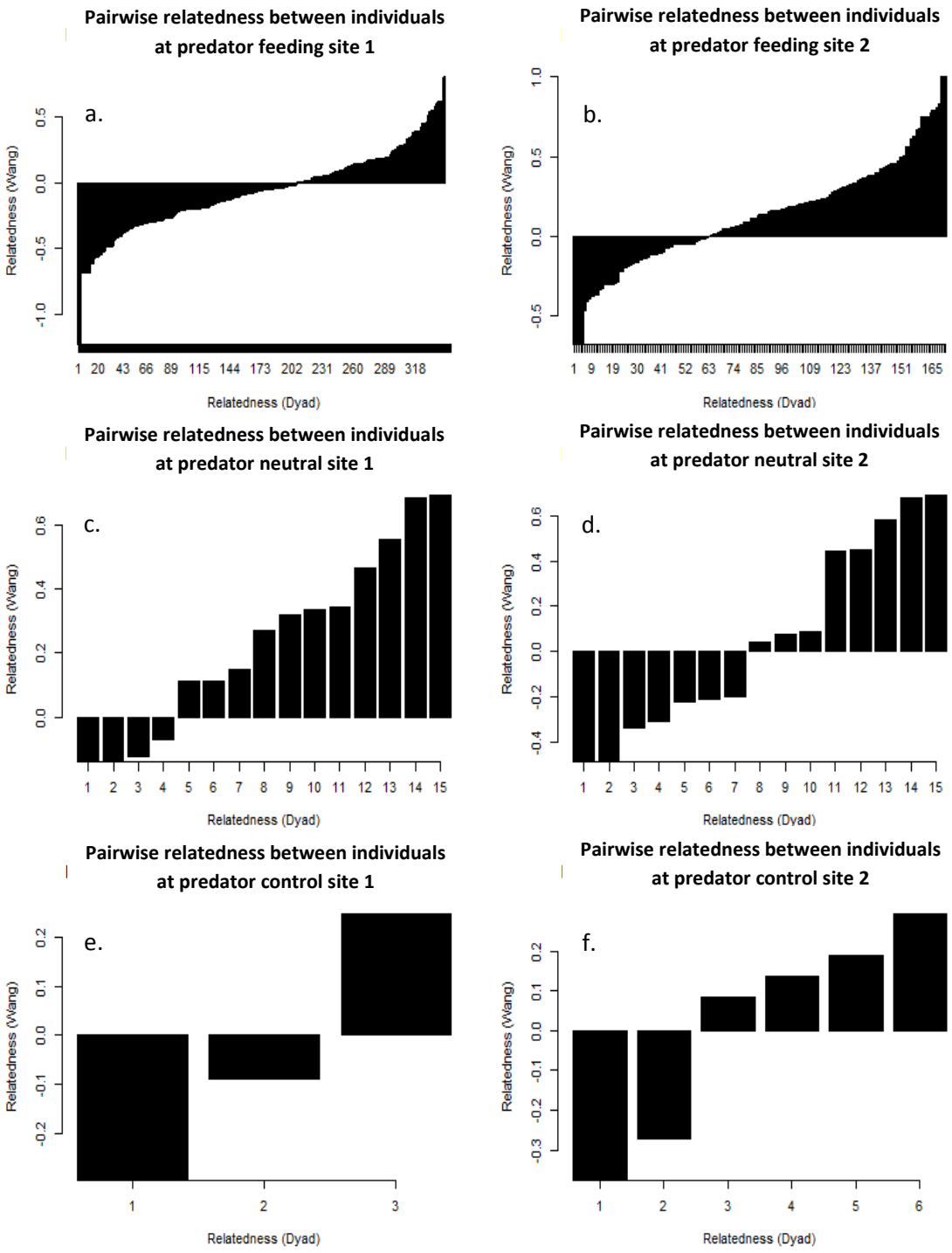


Figure 7.9 *Pairwise relatedness between sampled individuals within each site using the allele frequencies of the total sampled population. An increase in bias towards individual relatedness is apparent at all sites when compared with the analysis using the allele frequencies of the sampled subpopulation. a = Predator feeding 1, b = predator feeding 2, c = predator neutral 1, d = predator neutral 2, e = predator control 1, f = predator control 2.*

7.4 Discussion

This study investigated the genetic diversity of the black-backed jackal using six microsatellite loci and six sampling locations comprised of private game farms in the North West Province, South Africa. The aim of this study was to determine the effect of three predator management strategies on the population dynamics of the black-backed jackal by examining the population structure, inbreeding coefficient and relatedness.

This study found that when examining the entire sampled population of the black-backed jackal, the observed genotype frequencies deviated significantly from those expected under the Hardy-Weinberg equilibrium given the observed allele frequencies within the total population (Chi^2 = Infinity, DF = 12, $P < 0.01$). This indicated that the total sampled population cannot be described as one large randomly mating population. An excess of heterozygosity at the population level (F_{IS} = -0.0253) indicated gene-flow and potential outbreeding within the total population.

Black-backed jackal subpopulations at five of the six sites were shown to be in Hardy-Weinberg equilibrium. Given the deviation from Hardy-Weinberg expectations noted at the population level, and the maintenance of Hardy-Weinberg proportions at the majority of sites within this study, evidence of population sub-structuring is apparent. The variation in allele frequencies between subpopulations gives rise to a deviation from the expected Hardy-Weinberg proportions when examined at the total

population level, indicating barriers to gene-flow and the non-random association of gametes within the population. However, predator feeding site 1 showed a significant deviation from Hardy-Weinberg expectations (Chi^2= 33.4919, Df = 12, P= 0.0008) and a multi-locus excess of heterozygosity (P = 0.016, S.E. = 0.0022). The level of heterozygote excess at predator feeding site 1 is indicative of outbreeding and/or inbreeding avoidance within this site. The remaining five sites also showed an average inbreeding coefficient of less than zero for a number of loci indicating potential outbreeding, however all values were not significantly different from zero. The lack of a sufficient sample size from both predator control sites and predator neutral sites is likely to have resulted in an unreliable estimate of the coefficient of inbreeding across the total population given the degree of population sub-structuring described by the analysis of allele frequencies within and between sites.

The analysis of allele frequencies within the sampled subpopulations relative to the total sampled population indicates population structuring with an F_{ST} value of 0.0307. Significant subpopulation differentiation was also calculated within the total sampled population (Chi^2 = 53.768, df = 12, p = 3.00604 x 10^{-7}). No evidence of spatial correlation with genetic isolation by distance could be ascertained using the mantel test within the total population when examining the variation in allele frequencies within and between subpopulations. The fixation statistic for the total population (F_{ST}), the significant level of subpopulation differentiation expected through genetic isolation by distance (Mantel test, Figure 7.1), are indicative of a population that is not continuous across its range and thus is subject to barriers to gene-flow across the

study area. It is assumed that in a genetically homogeneous population where dispersal is not spatially limited, gene flow reduces as the distance between individuals increase. The isolation by distance model (Write 1943) is a modification of the island model which considers short distance dispersal as opposed to assuming random and equal migration between any given island. The IBD model is therefore used to infer population parameters by comparing genetic distance with liner geographic distance under the assumptions of equal and unimpeded dispersal in all directions from a point of origin. In this model it is assumed that Euclidean distance is equal to the biological distance between sites, analogous to Writes islands, and provides the null model to which departures arising from barriers to gene flow can be tested. As the model significantly differs from the assumptions of isolation by distance, it is reasonable to assume that the sampled population does not fit the continuous population model and thus some form of barrier to gene flow is acting on the total population. It is not possible to infer what form of barrier to gene flow is in action from this analysis, nor identify which sampling sites are isolated from the rest, yet it does provide evidence that a barrier to gene flow is in effect. One major drawback surrounding the use of Euclidian distance without the inclusion of biological distance between sites is that any barrier to gene flow identified may be a result of geographic and anthropogenic factors such as mountains, road networks and river systems which limit dispersal between study sites and are not of interest in inferring treatment responses.

The program IBD (Bohonak, 2002) is capable of comparing isolation by distance matrices while accounting for the third variable of biological distances between sites.

This program is often used when investigators wish to assess the explanatory power and biological relevance of alternative dispersal pathways in addition to the Euclidean distance model. For example it is often the case when the IBD model holds true when biologically relevant distances between sampling sites are accounted for. However the difficulty in accurately quantifying landscape scale biological distances and connectivity makes studies using biologically relevant distances uncommon. In this study, no data was recorded or available which would permit the use of biologically relevant distances in this analysis.

Further analysis of the population structure at the site level using a Bayesian clustering algorithm with and without prior knowledge of sampling location partitioned the population structuring between the predator feeding sites 1 and 2 and the remaining four sites. The partitioning of the population between the two predator feeding sites was ambiguous, with the Pritchard estimator of K indicating that both sites were part of the same ancestral population, whilst the Evanno estimator of K indicating barriers to gene flow between the two sites. However both estimators of K gave a consensus that both supplementary feeding sites were genetically isolated from the remaining study sites. The STRUCTURE analysis also gave no support of the hypothesis that barriers to gene flow are present between any of the predator control and predator neutral sites, as no clear distinction could be formed between these sites (Figures 7.2, 7.4, 7.5 and 7.7).

As subpopulation structuring was observed within this data set, and a lack of inbreeding was seen at the site level, relatedness between individuals was examined in order to further investigate the genetic isolation, immigration and the potential for inbreeding avoidance within each sample site. The analysis of relatedness between individuals within each site revealed that individuals within predator feeding sites and predator neutral sites had an approximately equal proportion related and unrelated individuals with a slight bias towards unrelated individuals (Figure 7.8 a – d). This slight bias towards unrelated individuals is concurrent with population mixture within these sites, yet the non-significant excess of heterozygosity suggests that genetic admixture is not a feature of these subpopulations. However, the majority of individuals sampled at both predator control sites were unrelated within their site of origin (Figure 7.8 e and f) indicating a higher rate of immigration compared with predator feeding and predator neutral sites. Although a deviation from a mean relatedness of zero was observed within these predator control sites, Wang's estimator of relatedness is sensitive to small sample sizes as the estimator of relatedness is proportional to the frequency of alleles IDB within a subpopulation. Therefore while evidence exists of a departure from a mean relatedness of zero is apparent at the predator control sites, the possibility of a departure as an artefact of sample size and / or a variation in allele frequencies between sites, shown in Figure7.4, may be in effect.

The proximity of all study sites (<100 km) and the lack of any substantial geographic barriers to migration indicates that the population structuring and subpopulation differentiation seen within the total population is likely due to the predator

management strategies implemented at the site level. However, the excess of heterozygosity seen at the population level indicates outbreeding within the total population (Chi^2 = 53.768, df = 12, p = 3.00604 x 10^{-7}, table 7.4), which implies regular mating between unrelated or distantly related individuals. This level of outbreeding is concurrent with the lack of structure between the predator control and predator neutral sites which can result from increased dispersal rates and territorial destabilisation. As no clear distinction could be made between any of the predator control or predator neutral sites based on either the allele and genotype frequency and identity, nor from the STRUCTURE analysis, it is likely that the individuals sampled from these four sites comprise a larger metapopulation with little evidence for barriers to gene flow. Therefore there is no evidence to support the hypothesis that territorial stability results in strong population sub-structuring between these sites.

Although the sites under predator neutral and predator control treatment types could not be distinguished in terms of a variation in allele frequencies and thus free migration is apparent between these sites (Figures 7.2, 7.4, 7.5, and 7.7), a distinct difference in the relatedness between individuals within each predator control site was observed (Figure 7.8). At both predator control sites, all individuals were shown to be unrelated, however, both the predator neutral sites had approximately equal proportions of related and unrelated individuals. The lack of related individuals within the predator control sites is consistent with the continual removal of black-backed jackal individuals, resulting in territorial destabilisation, which potentially permits the immigration of unrelated individuals from the larger metapopulation and increases

territorial turnover. This disturbance to the spatial organisation of a species due to disturbances such as culling is termed the perturbation effect (Carter *et al*., 2007). A level of heterozygote excess was seen at both predator control and predator neutral sites indicating a degree of outbreeding which could be explained by territorial destabilisation and the resulting perturbation effect arising from predator removal. The perturbation effect has received substantial attention in a number of studies including the long term population response of the European badger to culling trials undertaken in the UK (Tuyttens *et al*., 2000, Delahay *et al*., 2006, Carter *et al*., 2007) and is an attractive explanation to the results recorded in this study. However, due to the limitation in sample size from predator control and predator neutral sites, and the discrepancy seen when using the allele frequencies of the total population to estimate relatedness when compared with the results using the allele frequencies of the subpopulation, these results must be treated with a degree of caution. Therefore further studies are required to obtain genetic material from distinct individuals in order to undertake an analysis of sufficient power.

A number of studies have suggested that many *Canid* species, including the black-backed jackal, undergo behavioural modification in order to avoid frequently implemented predator control strategies such as free shooting and cyanide guns (Brand and Nel, 1997). These behavioural modifications can include a shift in activity patterns to reduce contact with humans and learned avoidance of traps and snares (Brand and Nel, 1997). While this study does not contest that behavioural modification occurs in response to predator control, the lack of related individuals at

both of the predator control sites indicates that the current level of removal results in the repopulation of territories through immigration rather than the persistence of individuals in areas of persecution through a modification in behaviour and predator control avoidance.

The population being structured due to barriers to gene flow between both predator feeding sites presents support for the hypothesis that predator feeding reduces territorial stability and thus dispersal of the black-backed jackal. Population structuring of approximately 3% ($F_{ST} \approx 0.03$) was calculated within the total population, which indicates a degree of genetic isolation between some of the sample sites. This genetic isolation was partitioned into both of the predator feeding treatment sites, as clear barriers to gene-flow were identified between the two predator feeding sites and the remaining treatment groups (Figures 7.2 – 7.7). When attempting to investigate a true value of K using the Pritchard (2010) and Evanno *et al* (2005) methods, K was estimated to be between 2 and 3, respectively (Figures 7.3 and 7.5). This discrepancy between estimators largely concerned the lack of consensus for the genetic isolation between the two predator feeding sites. It is widely regarded by many that the Pritchard method of estimating K is highly informal and has a tendency to underestimate the true value of K (Evanno *et al*., 2005, Pritchard, 2010, Pritchard *et al*., 2000). This is due, in part, to STRUCTURE taking a conservative estimate of K when the ln Pr(Data|*K*) reduces between estimates of K. However, it is also assumed that the Evanno method for estimating K has the potential to overestimate K in small data sets due to the assumptions used to infer the maximum likely number of clusters produced

for Δk (Pritchard, 2010, Evanno *et al*., 2005). Therefore no clear consensus on the true estimate for K can be made further than K = 2-3 without subsequent investigation.

The difference between estimates of K is not an un-expected result when trying to partition a small percentage of inbreeding (F_{ST} 3%) across limited spatial and temporal distances. As all study sites were within the known maximum dispersal distance of the black-backed jackal (Estes, 1992) and the of supplementary feeding regimes had been in existence for up to five years prior to this investigation, it is likely that this discrepancy between estimates of K are a result of the limited resolution of the study. However, the inference of K > 1 is in itself evidence of reduced dispersal rates within supplementary feeding sites and is of particular interest into the management of this species.

Studies of the black-backed jackal in the Namibian coast (Jenner *et al*., 2011) concluded that high and abundant food resources resulted in a territorial destabilisation where territory-holding individuals tolerated the presence of transient and unrelated individuals under the model of the resource dispersion hypothesis (Hiscocks and Perrin, 1988, Johnson *et al*., 2002). The results of this study indicate the presence of unrelated individuals within both predator feeding sites. However the population structuring observed within this data set and the presence of both highly related and highly unrelated individuals within these study sites indicate that a simple territorial destabilisation and transient tolerance do not offer a sufficient explanation for the relative abundance observed at these sites (chapters III and IV). The number of highly related individuals can be explained by the reduction in dispersal rates of

offspring due to a reduction in competition for resources combined with the immigration of unrelated individuals in line with the resource dispersion hypothesis. Although the black-backed jackal is a non-social *Canid* (Estes, 1992) which is not known to undertake pack-forming behaviour (Ferguson *et al*., 1983), a proportion of offspring of territorial holding individuals are commonly seen to remain within their natal range for a number of years to act as helper individuals by supplying the subsequent generation of offspring with food prior to their own dispersal (Ferguson *et al*., 1983, Estes, 1992, Jenner *et al*., 2011, Nel *et al*., 2012). The conditions that dictate whether offspring disperse from their natal range as soon as they reach sexual maturity or remain to undertake alloparenting are unclear (Estes, 1992), however a small number of observational studies have suggested that a complex dominance hierarchy develops between offspring from the same litter (Dreyer and Nel, 1990, Estes, 1992, Nel *et al*., 2012), with dominant individuals choosing to disperse or force subordinate dispersal dependant on the current availability of resources (Moehlman, 1979, Hiscocks and Perrin, 1988). Under favourable conditions it has been suggested that dominant offspring disperse readily from their natal range in order to establish territories in close proximity to their parental territory. This permits subordinate individuals to remain within their natal range at the cost of forgoing reproductive rights in order to act as helper individuals for subsequent generations (Moehlman, 1979, Moehlman, 1983, Estes, 1992). Conversely, under non-favourable conditions, high dispersal rates are thought to result from competition for resources both between siblings and between offspring and parents (Moehlman, 1983, Estes, 1992). It is therefore likely that the high and abundant food source seen at both predator feeding sites is a significant factor in the dispersal rates of related jackals at these sites. In an anthropogenically modified

environment, where food availability and thus the carrying capacity is extremely high, it is possible that dispersal is negated as territorial stability between both related and unrelated individuals is greatly reduced. As there is little evidence of inbreeding within both predator feeding sites, forms of inbreeding avoidance must be in action in order to reduce the mating between related individuals, which are present. It is likely that the immigration of unrelated individuals is tolerated to some degree by resident territory holding black-backed jackals which promotes or maintains genetic diversity at these sites.

The difficulty in identifying and partitioning genetic structure between the two supplementary feeding sites themselves raises important questions about the effect of supplementary feeding of carnivores. It would be simplistic to assume that each predator feeding site is undergoing divergence following reduced gene flow and dispersal, when considering the estimator of K derived from Evanno *et al* (2005). This would support the hypothesis that predator feeding reduced dispersal and migration resulting in genetically distinct populations at high abundances. However, as it has been cautioned by the authors of STRUCTURE that estimates of K should be conservative, as the possibility of gene flow between feeding sites is a real possibility given the spatial distribution of the sites. It could be argued that, given the relatively recent introduction of supplementary feeding at both sites and the substantially higher jackal abundance recorded at both feeding sites, the resulting similarity in genetic composition of populations at both sites are a result of a founder effect, with individuals at both sites sharing a common ancestor. The apparent lack of genetic

divergence may itself be due to the small number of generations through which both populations have yet to undergo divergence. Such inferences on the mechanisms of genetic partitioning between predator feeding sites are somewhat speculative given the difficulty in resolving K, thus further studies are required to better resolve genetic partitioning in the case of supplementary feeding sites. It is recommended by this author that future studies may wish to specifically examine the genetic diversity within and between additional supplementary feeding sites across the North West Province to test for similar structuring at all sites. In addition, the option to use an additional number of microsatellite markers remains, with the inclusion of the additional three markers partially examined in chapter V. The use of these extra markers may result in a higher resolution estimate of K and accurately partition genetic structure between these two and additional supplementary feeding sites.

7.5 Conclusions

Evidence from this study suggests that both predator feeding and predator control have significant effects on the population dynamics of the black-backed jackal. The genotype frequencies of the total sampled population deviate significantly from those expected under the Hardy-Weinberg equilibrium thus predator management strategies may induce disruptive pressures on the sampled population which impact gene-flow, mating and migration. An excess of heterozygosity at the population level presents evidence of outbreeding i.e. the mating between unrelated individuals, which is concurrent with high dispersal rates and migration between sample sites. Indeed, when examined at the subpopulation level, no significant barriers to gene flow and

population structuring were observed between the predator control and predator neutral sites. Yet when relatedness between individuals within sites was examined, a difference in the proportion of individuals that were highly related was observed. As relatedness was shown to be minimal between individuals sampled at the predator control sites when compared with predator neutral sites, there is a strong possibility that predator control results in territorial destabilisation and increased territorial turn over by promoting immigration through the vacuum effect (Carter *et al*., 2007), and thus is responsible for altering the spatial organisation and dispersal patterns of the black-backed jackal.

When considering the variation in allele frequencies within and between sites a small degree of population structuring was apparent. This is indicative of subpopulations that show a partial degree of genetic isolation from the remaining total population. The use of a STRUCTURE clustering algorithm, that iteratively processes the most likely population assignment for each individual by minimising the variation in allele frequencies between hypothetical clusters, indicates that this structure is mainly partitioned into the predator feeding sites. It is therefore likely that a small degree of genetic isolation is apparent between the predator feeding sites and the remaining total population. Yet given the presence of both highly related and unrelated individuals at predator feeding sites 1 and 2, and the excess heterozygosity observed at predator feeding site 1, it is likely that forms of inbreeding avoidance are in effect. Given the degree of alloparenting behaviour and philopatry recorded with the black-backed jackal (Jenner *et al*., 2011, Nel *et al*., 2012, Moehlman, 1979, Sillero-Zubiri,

2004), and the partitioning of population structure in the predator feeding sites combined with the presence of unrelated individuals and the negative inbreeding coefficient of these subpopulations; it is highly likely that predator feeding results in both the reduction in individual dispersal from natal ranges within or at close proximity to the feeding site, and the tolerance of unrelated transient or trespassing individuals due to the reduction in territorial stability resulting from high food availability.

While no attempt was made to identify the gender of individuals at either site during this investigation and thus the assumptions of sex bias dispersal were not included in the analysis, the conclusions drawn from this study remain reliable estimates of population structuring and indicators of gene flow between sites. However, the potential impact of a sex bias dispersal in the black-backed jackal should not be overlooked when attempting to interpret the results of this study. Species in the family Canidae have a well-documented relationship between body size and reproductive behavioural characteristics. The smaller of the Canidae species, such as the red fox and the bat eared fox, are mainly monogamous but may switch to a polygynous mating strategy under stress (Lamprecht, 1979, Gittleman, 1996). These smaller species (< 6kg) often have a skewed sex ratio towards female individuals and female alloparents with a male bias dispersal (Gittleman, 1996). Medium sized canids (6.0 -15.0 kg) such as the black-backed jackal, golden jackal and coyote are highly monogamous for the duration of their partners life, have equal sex ratios within populations, exhibit both male and female helping behaviour and disperse in equal sex ratios (Moehlman, 1989, Gittleman, 1996, Ginsberg and Macdonald, 1990). The largest of the Canidae, such as

the African wild dog and grey wolfs, have a monogamous mating system with slight polyandry, have a population sex bias in favour of males and have a female sex bias dispersal (Gittleman, 1996). However, despite the aforementioned assumption of an equal sex ratio dispersal in the black-backed jackal and other similar sized canids, it has been noted by previous studies that a bias in dispersal between genders can be induced in some of the Canidae especially in response to varying levels of stress, disturbance and resource availability (Goltsman *et al*., 2005).

A common feature of many social carnivore species is a lack of dispersal among female individuals with the majority of individuals choosing to remain within their natal range after reaching sexual maturity (Van Horn *et al*., 2004, Gompper *et al*., 1998, Biek *et al*., 2006). This life history strategy is thought common in social carnivores and often results in a clustering of highly related female individuals in close proximity. A lack of female dispersal has often been linked to social group formation in the carnivores, inferring an evolutionary advantage arising from cooperative behaviour, such as hunting and defence, as well and as the group benefits inferred from maternal care and kin selection. It is often assumed that male bias dispersal is an evolutionary response to such social group formation in order to reduce fitness costs associated with high levels of inbreeding (Biek *et al*., 2006, Gompper *et al*., 1998).

As the black-backed jackal is most frequently encountered to display a non-social, monogamous life history strategy (Estes, 1992, Rowe-Rowe, 1982) it is reasonable to

assume that sex bias dispersal is not a factor in inbreeding avoidance in undisturbed environments. However, given the high abundance of jackals recorded at the supplementary feeding sites, the slight skew towards related individuals and the lack of inbreeding at the site level, it is possible that sex bias dispersal plays a significant factor in reducing inbreeding depression at the supplementary feeding sites. As the black-backed jackal has evolved a generalist life history strategy, and thus is able to exhibit substantial plasticity in response to environmental perturbations and disturbance, it is not unreasonable to suggest that a shift in favour of a sex bias dispersal may be a common feature of jackal populations in close proximity to abundant but intermittent food resources.

The link between resource dispersion and group formation in non-social mammals is often described by the resource dispersion hypothesis. The potential for animals such as the black-backed jackal to alter life history strategies in response to resource availability and potentially exhibit a strong sex bias dispersal at high population densities adds an extra layer of complexity to the RDH model that requires more detailed investigation in itself. However, given the success of the DNA extraction protocol from faeces developed for this investigation, further studies using sex typing markers on the genetic samples collected from both predator feeding sites would shed considerable light on the dispersal patterns and population sex ratios in this investigation. At present, no sex typing markers exist for the black-backed jackal, however a number of STR markers have been characterised for the X chromosome of the domestic dog (Neff *et al*., 1999). Given the close homology of the cross species

markers and probes developed here for the black-backed jackal from the domestic dog template genome, it is likely that such sex typing markers would show a similar homology between species. Further trials investigating the reliability, allelic drop out and false negatives using captive animals of known gender would first be required before undertaking such an analysis.

The variation in sample size between sites and the potential for anomalies arising from PCR artefacts and mistyping, present potential limitations to the conclusions of this study. However, as the results of chapter VI and VII show that the molecular markers and extraction protocol used to collect data in this study are robust, it is highly likely that the results of this study are informative representations of the population dynamics of the black-backed jackal. Although the sample size acquired from the predator control sites are likely a limiting factor to the conclusions of this study, the tools developed and tested in the field to source genetic material and amplify informative molecular markers provides a stable platform from which future studies can proceed. It is prudent, therefore to accept the results found to be significant, but consider the high probability of making a type II error regarding the non-significant results. Therefore future studies, with a focus on sample collection from a wider array of study sites and microsatellite loci, would be necessary in order to corroborate the findings of this study.

Chapter VIII

"We have become, by the power of a glorious evolutionary accident called intelligence, the stewards of life's continuity on earth. We did not ask for this role, but we cannot abjure it. We may not be suited to it, but here we are."

Stephen Jay Gould

In 'The Flamingo's Smile: Reflections in Natural History' (1985)

Final discussion

8.1 Thesis overview

As resource availability and territorial stability are cited as highly influential factors in the ecology of the black-backed jackal, the principle aims of this project were to investigate the effects of lethal predator control and predator feeding on the population dynamics of the black-backed jackal in privately owned game farms of the North West Province of South Africa. The black-backed jackal is commonly targeted for population control in privately owned game farms across South Africa in response to the substantial level of depredation and disease transmission attributed to this species. However, a deficit of data pertaining to the ecology of this species outside of the nationally protected areas of South Africa has limited insight into the effects of predator control programs, thus the mechanisms by which this species persists under persecution remains unclear. The black-backed jackal was chosen as the study

organism for this investigation in order to provide information that may better inform the management of this species and to help resolve the ongoing human-carnivore conflict synonymous with the black-backed jackal. As the management strategies and game farming techniques used within the game breeding farms studied in this investigation are common throughout the game breeding sector of South Africa, this study offers a valuable insight to the population dynamics of the black-backed jackal that is potentially transferable to the majority of agricultural landscapes throughout the species range. In addition, this investigation offers a platform for future studies wishing to compare the population parameters of the black-backed jackal in undisturbed areas and investigations into human-carnivore interactions in agricultural settings.

The additional aims of this project were to develop and characterise a new and novel suite of genetic markers capable of providing robust information on the genetic composition of the black-backed jackal. In continuation, a suitable technique for the non-invasive recovery of genetic material from faecal deposits of the black-backed jackal was required in order to provide a sufficient quantity of DNA templates to undertake such an analysis.

8.2 The effects of predator control on the population dynamics of the black-backed jackal

The findings of this project indicate that the current level of lethal predator control undertaken at the study sites in this investigation does not significantly reduce the

abundance of the black-backed jackal when compared with predator neutral sites (Figures 3.1, 3.2, 3.3, 4.3, 4.4, 4.5 and 4.6), and thus is an ineffective and inefficient conflict resolution strategy in the game farm environments of South Africa. The corroborative results from the two sampling techniques using camera trapping (Chapter III) and faecal density analysis (Chapter IV) were used to estimate the abundance of the black-backed jackal, and the concurrent results from two spatially randomised sites per treatment type (Figures 3.1, 4.3 and Table 3.1) makes it highly unlikely that these results are an artefact of site-specific effects.

As no significant reduction in abundance of the black-backed jackal could be deduced at predator control sites when compared with sites that lacked any form of predator management (Figures 3.2, 4.4, and 4.6) two explanations remain for the continued persistence of the black-backed jackal within the sites that practice lethal control. Either black-backed jackals persist within strongly defined territories and undergo behavioural modification in order to avoid predator control strategies (Brand and Nel, 1997), or that black-backed jackals are dispersing from stable source populations into newly vacated territories within lethal control sites as described by the vacuum effect resulting from territorial destabilisation (Tuyttens *et al*., 2000, Carter *et al*., 2007). The presence of gene flow between the predator control and predator feeding study sites (Figures 7.2, 7.4, 7.5, 7.7, Tables 7.2 and 7.5) and the lack of related individuals at predator control sites (Figure 7.8e and 7.8f) is indicative of the latter argument, which indicates that not only is lethal control ineffective in controlling the local black-backed jackal population, but is potentially counterproductive in the management of this

species. The social perturbation effect, as described with reference to the European badger (Carter *et al*., 2007), is a term used to describe the significant alteration of the territorial behaviour, dispersal and movement patterns of individual badgers in response to culling attempts. Female badgers have been observed moving into newly vacated setts in attempts to avoid breeding suppression following the removal of long standing badger setts (Tuyttens *et al*., 2000). In addition, the mixing of individuals from both sexes between badger setts in an attempt to increase extra-group mating has been recorded, following culling attempts of the European badger, giving rise to an increase in local density, range size and a reduction in territorial stability (Delahay *et al*., 2006). Given the degree of gene-flow between populations of the black-backed jackal at the predator control and predator neutral sites in this investigation, and the lack of related individuals within lethal control sites, it is highly likely that similar perturbation effects are in action with respect to the black-backed jackal populations sampled from the predator control sites in this investigation. Due to the lack of any apparent subpopulation structuring between all predators neutral and predator control sites it is likely that individuals are free to disperse into areas under predator control from the larger metapopulation in response to the vacuum effect. It has also been suggested that the effect of removing territory holding individuals may exacerbate territorial competition and promote smaller territory sizes for a given area via the decreased maintenance of territory boundaries in response to culling (Asa and Valdespino, 1998). This decrease in territorial defence and the resulting immigration and gene flow recorded between the predator neutral and predator control sites is problematic with respect to a number of similar mesocarnivore species that are in direct conflict with livestock owners and native wildlife. The management of non-

native vertebrate predators in the USA, such as the invasive red fox (*Vulpus vulpes regalis*) and feral cats (*Felis catus*) have shown that the random offtake of adult individuals is not sufficient to reduce predation in these areas (Harding *et al*., 2001). This study goes on to report that the specific targeting of a cross section of individuals from different partitions of the age structure of these populations is required to successfully manage these species with respect to reducing depredation rates (Harding *et al*., 2001). A similar targeted approach to culling the black-backed jackal in game farm ecosystems of South Africa may be required in order to significantly reduce population numbers and resolve the conflict arising from depredation synonymous with this species.

The number of faecal deposits successfully sampled from both areas of predator control and predator neutral sites for genetic material has resulted in small sample sizes and thus is a significant limiting factor to the conclusions of this investigation. The relatively small sample of individuals at predator control and predator feeding sites compared with the number of individuals sampled from both predator feeding sites is testament to the abundance of the black-backed jackals at the feeding sites described by both camera trapping and faecal density estimates. Thus future studies that include a larger array of predator control sites are required to gain sufficient genetic material to further investigate the effects of social perturbation and relatedness in response to culling of the black-backed jackal to support the findings of this investigation.

The lack of large carnivores at predator control sites relative to the remaining study sites (Figures 3.4, 3.5 and 3.6) is also of importance to the dynamics of the black-backed jackal and associated conflict. The destabilisation of the ecosystem due to changes in community composition may result in increased densities of mesocarnivores such as the black-backed jackal and caracal due to the reduced trophic limitations imposed by large carnivores through the process of mesocarnivore release (Prugh *et al*., 2009). While not conclusive, the amount of gene-flow (F_{ST} = 0.0307, Figures 7.2, .7.4, 7.5, 7.7 and Tables 7.4 and 7.5) and outbreeding seen at the population level (F_{IS} = -0.253, Figure 7.2-7) and between predator control and predator neutral sites (Table 7.2) may be indicative of a population undergoing expansion. The top-down model of ecosystem stability places an emphasis on the ability of carnivores to limit prey population sizes and consequently play a key role in the structure and function of an ecosystem (Leopold *et al.*, 1947, Estes, 1992, Terborgh *et al*., 2001, Treves and Karanth, 2003). Inter-guild competition and the controlling effect of large carnivores on the density of smaller sympatric carnivore species is thought to extend tropic interactions of carnivores across a multitude of food webs (Leopold *et al.*, 1947, Ripple and Beschta, 2006). The resulting trophic cascade from a reduction or loss of any carnivore species is therefore thought to have far reaching ecological implications to both ecosystem stability and the ecosystem services that scavenger species such as the black-backed jackal provide (Leopold *et al*., 1947, Ripple and Beschta, 2006). Evidence based on the decline of indicator species suggests that a continued loss of carnivore diversity will alter key environmental processes, affecting ecosystem function, stability and productivity (Naeem and Li, 1997, Daily *et al*., 1997, Tilman, 1999, Daily *et al*., 2000, Hooper *et al*., 2005, Dobson *et al*., 2006, Wardle *et al.*, 2011,

Hooper *et al*., 2012). Thus the removal and or destabilisation of the black-backed jackal may have far reaching implications for both the South African game farm industry and the ecotourism industry alike.

8.3 The effects of predator feeding on the population dynamics of the black-backed jackal.

The results of this study indicate that predator feeding corresponds with a substantial and significant increase in the abundance of the black-backed jackal when compared with sites that do not supplement the diet of carnivores (Figures 3.2, 4.3, 4.4, 4.6). The high degree of population plasticity, in terms of abundance noted between the predator feeding and predator neutral sites, indicates that food availability plays a significant role in the population dynamics of this species. Under the assumptions of the resource dispersion hypothesis (Carr and Macdonald, 1986, Johnson *et al*., 2002), the tolerance of large numbers of individuals at high and abundant food resources is expected. This is consistent with the results recorded within the studies of the black-backed jackals surrounding the seal colonies of the Namibian cost (Hiscocks and Perrin, 1988, Jenner *et al*., 2011) and runs in parallel to the results seen when examining dingo populations around clumped food sources in Australia (Newsome *et al*., 2013). However, due to the population structuring seen at these sites (Figures 7.1 and 7.2) and the barriers to gene flow between the supplementary feeding sites and the remaining study sites (Figures 7.1, 7.2 and Tables 7.4 and 7.5), it is unlikely that immigration, due to the increased carrying capacity that is synonymous with increased

food availability, is solely responsible for the abundance of the black-backed jackal recorded at the predator feeding sites.

The observation that the two predator feeding sites, typically being 70 km apart, were genetically similar whilst separate from predator control and predator neutral sites on the same spatial scale can be explained by a number of factors. A failure to detect very weak structure between the two feeding sites may be an artefact of the relatively short time divergence has been in operation since the employment of supplementary feeding programs at each site. This coupled with a form of founder effect may have resulted in the apparent gene flow between the two supplementary feeding sites. However, the discrepancy between estimators of structure (Figures 7.2 and 7.4; 7.5 and 7.6,) presents the argument for a degree of genetic isolation between the two predator feeding sites when the Evanno method for estimating K is considered. This supports the hypothesis that supplementary feeding reduces dispersal and prevents gene flow from the supplementary feeding sites to the wider metapopulation. When considering the more conservative estimate of K, following the guidelines in Pritchard *et al* (2005), gene flow is not considered to be limited between study sites. This is likely due to the lack of resolution in the microsatellite loci and the relatively short period of time for which divergence has been in operation. The similarity in genetic homogeneity between supplementary feeding sites may therefore be due to a form of founder effect from which independent populations subsequently underwent divergence.

As dispersal decreases, a spatially restricted accumulation of individuals that share close relatedness will become apparent, which form spatially and genetically separate groups of individuals within a population (Perrin and Mazalov, 2000). Despite the lack of close inbreeding within the predator feeding sites the genetic sub-structuring within the predator feeding sites indicates that a degree of philopatry is therefore likely with a proportion of adult offspring failing to disperse from their natal range. This is comparable to the social dynamics of the red fox, with groups of related individuals form with adult offspring comprising subordinate group membership in order to provision future generations with food (White and Harris, 1994, Baker *et al*., 2004). The red fox was shown to form strict dominance hierarchies when populations are at high densities, with subordinate individuals forgoing the opportunity to mate (Baker *et al*., 2000, Baker *et al.*, 2004). However, given the outbreeding seen at both supplementary feeding sites, it is likely that such group formation in the black-backed jackal is less strict, with evidence of polygynandrous mating systems in effect. Together this suggests that the effects of excess food availability can be described as having a dual effect on the dynamics of the black-backed jackal, by promoting the immigration and tolerance of new individuals into predator feeding sites, while preventing dispersal of individuals from their natal range due to the increased carrying capacity and reduction of dispersal pressures. The ability of the black-backed jackal to switch from a monogamous life history strategy, where gender dispersal rates are thought to be equal, towards a sex bias dispersal may be sufficient to account for the lack of inbreeding recorded at supplementary feeding sites. The ability for the black-backed jackal to exhibit a male bias dispersal may be an evolved inbreeding avoidance strategy where population densities are high. While no data was collected on the sex ratio of

the populations studied in this investigation, the potential for the black-backed jackal to exhibit a plasticity in sex bias dispersal is not only interesting from an evolutionary perspective, given the assumptions of the resource dispersion hypothesis and the evolution of group living organisms, but is potentially of relevance in terms of the dispersal of individuals from such sites into neighbouring farms once the carrying capacity of the location is reached.

The population structuring at these sites is in direct contrast to the finding of previous studies which concluded that territorial instability and immigration is solely responsible for the high density of jackals seen at clumped and abundant food resources (Jenner *et al*., 2011). This presents a substantial challenge to the understanding of the population dynamics and dispersal of the black-backed jackal. Thus further studies into the genetic diversity of jackals in non-anthropogenically altered environments, such as the Namibian coast and the East African population, would be required to clarify whether or not this result is solely limited to anthropogenically altered game farm ecosystems of South Africa, where clumped and abundant food resources are available all year round, which in turn leads to higher population densities and further conflicts with humans.

The disposal of game and livestock carcasses, resulting from the meat and hunting trade, at a single location within farm boundaries is a common management strategy practised in a high proportion of functioning breeding and hunting farms within South

Africa (Tainton, 1999). The resulting population expansion of the black-backed jackal as a result of high and abundant food resources has the potential to affect the disease prevalence at such sites, as high density populations are susceptible to disease transmission and may present a significant threat to the health and safety of both livestock and humans (McKenzie, 1993, Loveridge, 1999). The transmission of rabies is of concern in such situations as frequent outbreaks are recorded across South Africa, with the black-backed jackal being cited as a significant vector for this disease (Loveridge and Macdonald, 2001, Zulu *et al*., 2009).

8.4 Microsatellite characterisation: A suite of genetic markers for the black-backed jackal

As no molecular markers were available to investigate the population dynamics of the black-backed jackal prior to this investigation, a suite of six cross-species molecular markers were developed for use in this investigation. The markers showed a high degree of allelic variation (Figure 6.4, Tables 5.3, 5.4 and 6.4) and were shown to be of significant value in the inference of population parameters of this species (Figures 6.1 and 6.12, Tables 5.3, 6.4 and 6.5). These markers have been rigorously tested for signs of allelic-drop out and PCR artefacts (Table 6.3), and have been proved to be suitable for their use within this species (Table 5.3, 5.4 and 6.4). This not only provides a valuable insight into the dynamics of the black-backed jackal, which was not previously possible using traditional ecological sampling techniques, but provides a solid foundation from which future investigations can be based. Due to the close evolutionary history of the *Canid* genus there is high confidence that these markers

provide a method for the high resolution analysis of the black-backed jackal and are potentially transferable to other *Canid* species of ecological interest. This is advantageous as the cost associated with developing microsatellites using clone libraries and screening techniques can be substantial.

8.5 Non-invasive genetic sampling: Recovery of DNA from faecal deposits of a cryptic species

The study of the population parameters of species using a genetic approach provided information on the dynamics of a species that was not readily available, if at all possible to obtain, from traditional ecological sampling and monitoring programs. A majority of species that would benefit from such analysis of their population parameters are often endangered and at low densities and / or are cryptic in their life history strategies. Therefore the availability of genetic material from low density or cryptic species is often a limiting factor in the success of such studies. The effort required to capture and sample individuals of a species of interest remains high and often results in sample sizes insufficient for meaningful analysis. The ability to sample genetic material indirectly from faecal pellets is therefore of significant interest to the wider field of conservation genetics. The simple procedure and relatively safe extraction protocol developed in this investigation for the isolation and purification of DNA from host individuals, is a methodology that is potentially transferable to a multitude of species of conservation and ecological interest. In absolute terms the total DNA copy number recovered from faecal samples using quantitative PCR leaves little doubt about the effectiveness of this method.

The use of S.T.A.R. buffer and a chlorophorm extraction protocol (page 130) negates the use of dangerous extraction protocols using Phenol:chloroform:isoamyl alcohol, which is inherently dangerous to use and can cause significant risk to life if mis-handled. In addition, the stability and reduction in microbial degradation of samples that S.T.A.R. buffer offers does not limit its use to faecal pellets alone. The use of this transport buffer and extraction protocol provides a basis from which numerous low DNA templates with high organic adulterations can be stored, transported and extracted. Preliminary studies undertaken by the author for the extraction of DNA templates from both brown hyaena pastes and faeces have also shown substantial promise.

8.6 Future work

As a substantial amount of data now exists from this study pertaining to the population parameters of this species under the conditions of both high food availability and lethal control, future studies investigating the effect of the removal of food availability and the cessation of lethal control at these study sites would provide high resolution data regarding the response of this species to anthropogenic disturbance.

As a large response to predator feeding was seen in this study with a degree of population structuring apparent at these sites, further investigation into the dispersal of black-backed jackal from these sites would be of interest to the farming community.

This could be achieved by sampling faecal deposits within sites at several spatial scales and linear distances from central feeding sites. Furthermore, as the tools for the genetic analysis of the population parameters of the black-backed jackal are now available, future studies investigating the population dynamics of this species both outside of protected areas in the undisturbed environment of the Namibian coast, and inside the protected areas of South Africa with a range of apex predators, would provide a suitable contrast to the data collected in this investigation that would shed considerable light on the complex social dynamics of this species across an ongoing climate of persecution and land use change.

In order to examine the potential for plasticity in gender dispersal of the black-backed jackal in response to disturbance, a genetic approach using sex typing markers could be employed to sex type a large number of jackal individuals from a multitude of sites. However, at the time of writing, no sex typing markers for the black-backed jackal have been developed. Given the close genetic homology of the black-backed jackal and the domestic dog, observed in the microsatellite markers used in this study, it is likely that known molecular markers on the X chromosome of the domestic dog could be used to efficiently sex type black-backed jackal individuals. When used in addition to the faecal DNA collection and extraction protocol used in this investigation, these methods present a highly possible method for investigating the variation in sex bias dispersal of the black-backed jackal in South African game farm environments.

8.7 Conclusions

To conclude, the black-backed jackal shows remarkable population plasticity across a highly disturbed and anthropogenically modified environment. At present, no significant threat of extinction persists with this species, yet the effects of predator management strategies employed within the game breeding farms of South Africa are having a detectable effect on both the local abundance and population dynamics of this carnivore species. It is therefore apparent that given the important role the black-backed jackal plays in the ecosystem as a scavenger species, and the concurrent ecosystem services this species provides in terms of nutrient cycling, careful consideration should be given to the application of appropriate predator management strategies in order to insure the longevity of both the black-backed jackal and the game breeding and ecotourism industries of the North West Provence.

Estimates of abundance derived from camera trap capture frequencies and faecal density analysis require a long sampling duration with temporal repeats in order to achieve sufficient analytical power (O'Connell et al., 2011, Noss et al., 2012, Sollmann et al., 2013). This places an inherent limitation on the total number of sites that can be sampled given the logistical constraints of sampling multiple sites across the North West Provence. Because of these limitations a total of six study sites were sampled in this investigation using two temporal repeats. Given the limitations inherent in this investigation, namely the small number of site replicates, it is difficult to draw large scale generalised conclusions based on this experimental data set alone as the six sites

chosen for this investigation may not fully represent the effects of predator control across the entire game farming industry of the North West province.

Evidence from this investigation does suggests that both lethal control and resource availability have a detectable effect on both the abundance and genetic diversity of the black backed jackal within the study sites examined in this investigation. While this provides an interesting insight into the ecology and population plasticity of this generalist mesocarnivore species, the wide range of management practices implemented in the private game breeding industry of South Africa, and the mosaic of land use types across the study area, makes these results arguably no more than detailed observations at individual sites. While these sites are potentially not representative of the ecological effects of carnivore management in all game farm environments, the management strategies employed are typical of those used in many private farms that comprise the game breeding industry of South Africa. Therefore conclusions drawn by readers of this study pertaining specifically to the effectiveness of lethal control must be treated with caution until further investigations can be undertaken using additional sites which represent a larger sample of game farm environments of South Africa. Due to this, the analysis on a collaborative camera trap dataset is currently being undertaken at the time of writing, which will increase the number of site replicates to 18.

In addition to increasing the number of site replicates to strengthen the analytical power of the study, future manipulative experiments would be of significant use in determining viable offtake quotas with regard to culling and age structure targeting in this species. The access to species stocking density and accurate depredation rates over a long term basis would be invaluable in correlating depredation rate to jackal density throughout any manipulative culling trial. The ability to estimate recolonization rate after an intensive large scale culling event in private farmland across the North West Province would be highly influential in establishing viable culling efforts and implement legislation pertaining to this species.

Many methods of predator control are available to reduce the impact and conflict resulting from livestock depredation that offers a potential alternative to lethal control. A number of private game farms have implemented the use of predator-proof fencing in order to reduce livestock depredation. However, although methods such as predator-proof fencing and other appealing non-lethal methods to control depredation and resolve conflict are available, the effects of habitat fragmentation and isolation on the genetic health of the predators and wild game species of South Africa should also be considered before recommendations can be made about the wide scale implementation of such methods.

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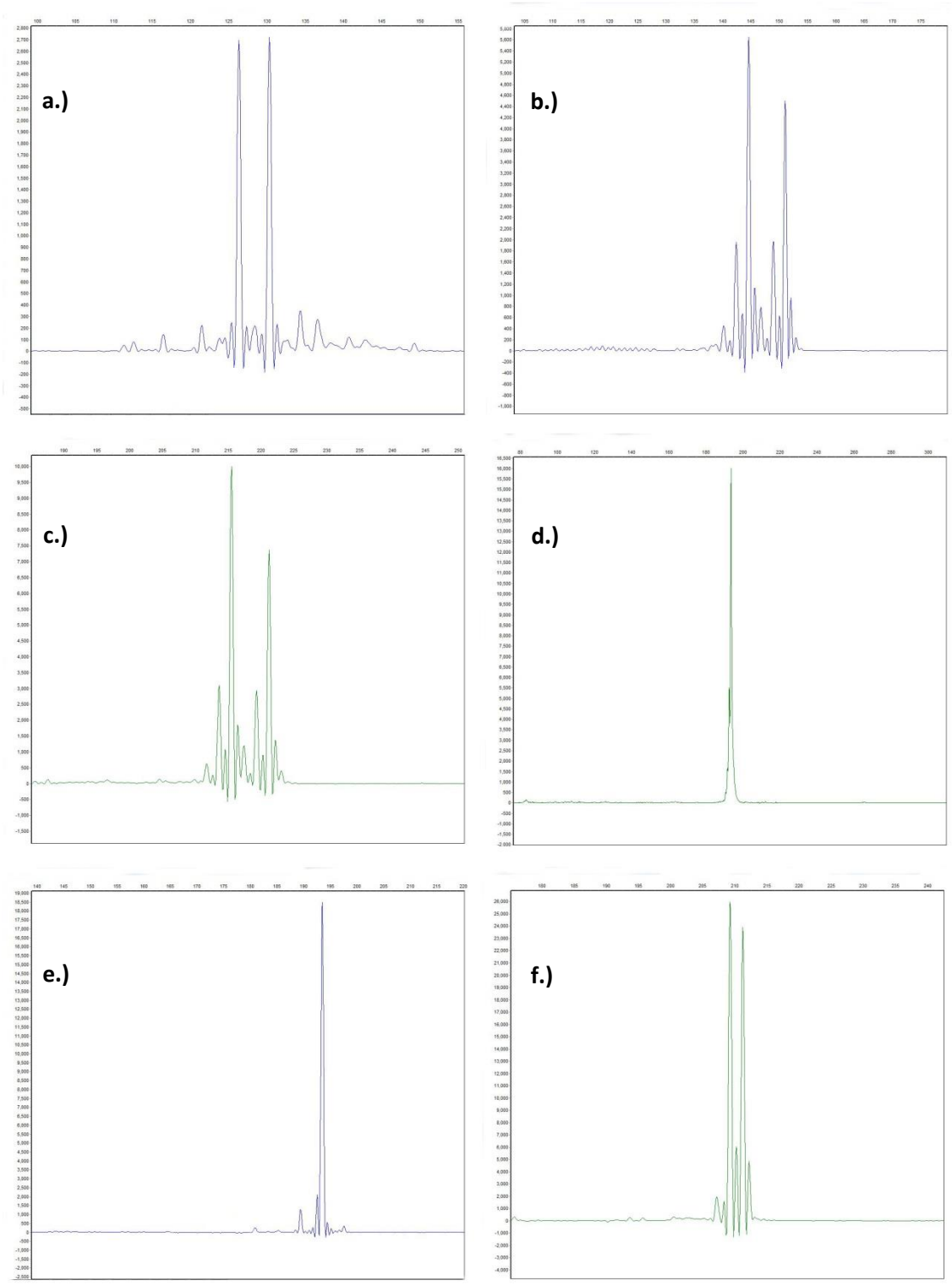
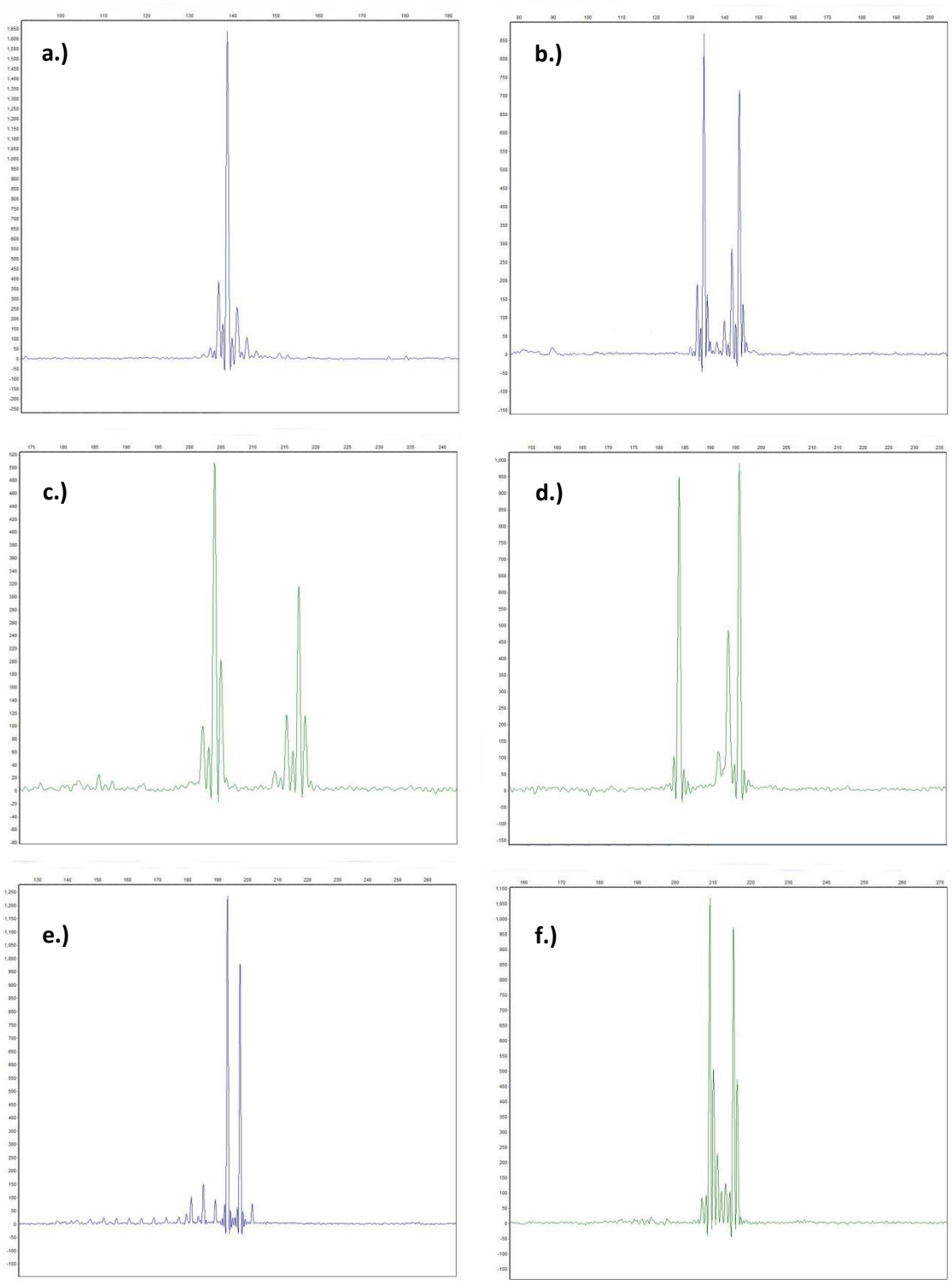
Appendix



Example genotype electrophoretograms for six molecular markers amplified from a single black-backed jackal individual, using tissue as a genetic source material. No significant stutter or PCR artefacts are present. a = DogP109, b = DogP123, c = DogP204, d = DogP374, e = VGL1541, f = DogP622.



Example genotype electrophoretograms for six molecular markers amplified from a predominantly heterozygous black-backed jackal individual, using faeces as a genetic source material. No significant stutter or PCR artefacts are present. a = DogP109, b = DogP123, c = DogP204, d = DogP374, e = VGL1541, f = DogP622.

Allele counts and frequencies for all loci

Locus DogP109			
Allele	Count	Frequency	Frequency with null
126	1	0.0096	0.0097
130	1	0.0096	0.0097
132	2	0.0192	0.0194
134	3	0.0288	0.0293
136	17	0.1635	0.1679
138	26	0.25	0.2532
140	15	0.1442	0.1565
142	13	0.125	0.1120
144	16	0.1538	0.1451
146	7	0.0673	0.0697
148	1	0.0096	0.0097

Allele frequency analysis for each locus DogP109 using faeces as a genetic source material.

Locus DogP 123			
Allele	Count	Frequency	Frequency with null
132	1	0.0096	0.0096
134	30	0.2885	0.3465
136	16	0.1538	0.1553
138	10	0.0962	0.1005
140	9	0.0865	0.0900
142	14	0.1346	0.1441
144	11	0.1058	0.1112
146	3	0.0288	0.0291
148	2	0.0192	0.0193
150	4	0.0385	0.0390
152	2	0.0192	0.0193

Allele frequency analysis for each locus DogP123 using faeces as a genetic source material.

Locus DogP 204			
Allele	Count	Frequency	Frequency with null
198	1	0.0098	0.0099
200	1	0.0098	0.0099
202	2	0.0196	0.0198
204	32	0.3137	0.2998
206	1	0.0098	0.0099
208	14	0.1373	0.1368
210	10	0.098	0.0925
212	7	0.0686	0.0711
214	11	0.1078	0.1144
216	6	0.0588	0.0607
218	9	0.0882	0.0925
220	5	0.049	0.04
222	3	0.0294	0.0299

Allele frequency analysis for each locus DogP204 using faeces as a genetic source material.

Locus DogP 374			
Allele	Count	Frequency	Frequency with null
143	1	0.0098	0.0099
171	2	0.0196	0.0198
183	9	0.0882	0.0818
187	1	0.0098	0.0099
189	1	0.0098	0.0099
191	7	0.0686	0.0607
193	54	0.5294	0.5572
195	21	0.2059	0.1956
201	1	0.0098	0.0099
203	3	0.0294	0.0299
205	1	0.0098	0.0099
209	1	0.0098	0.0099

Allele frequency analysis for each locus DogP374 using faeces as a genetic source material.

Locus VGL151			
Allele	Count	Frequency	Frequency with null
177	3	0.0294	0.0298
181	11	0.1078	0.1142
185	11	0.1078	0.1142
189	15	0.1471	0.1366
193	40	0.3922	0.4218
197	7	0.0686	0.071
201	9	0.0882	0.0924
205	3	0.0294	0.0298
225	2	0.0196	0.0198
237	1	0.0098	0.0098

Allele frequency analysis for each locus VGL151 using faeces as a genetic source material.

Locus DogP 622			
Allele	Count	Frequency	Frequency with null
208	14	0.1373	0.1368
210	48	0.4706	0.495
212	2	0.0196	0.0198
214	16	0.1569	0.1482
216	20	0.1961	0.1956
218	2	0.0196	0.0198

Allele frequency analysis for each locus DogP622 using faeces as a genetic source material.

Locus	p values (fishers method)
DogP109	0.47175
DogP123	0.10634
DogP204	3.00E-05
DogP374	0.17834
LM1541	0.1966
DogP622	4.00E-05
All	**All: Chi2= 53.768 (df= 12), P-value= 3.00604e-0**

Exact G test for genetic differentiation p values (Fishers method) across all loci.

Microsatellite sequences

Locus: 109 Allele: 144 Repeat motif: 14(CA)

Sequence:AACTTTAAGCCACACTTCTGCATATTAACTTTAATCTGTATACATAAGTTAAATAAG
CTTAAACTACTTTAAAAAATTTTTGTCAAAGGACTATANACACACACACACACACACACACA
CAGCTTAAAAGGGAGAGGCAAGT

Locus: 123 Allele: 136 Repeat motif: 17(CA)

Sequence:AAGGCCAAACATAAACACGCACACACACACACACACACACAGACACACACACAAA
TAATCAGTGACAGAATAGGACAGTTTGGTAATCGGGCAAATGCAATCAGTGCTTTGCCTTACT
AAAATCTAAATTTTTCTT

Locus: 204 Allele: 204 Repeat motif: 11(CA)

Sequence:CGAGAGCAACATAGGCATGAGAACACAAACAGTAAAAACAGTGTGCTTACTAGG
GCTCATTTTCTAATATGCTAAATCTTGTATATTTTAATTTAGAAACCAAAGGAAGAGAAAAGCA
TCTTGTAGATTAGACAAAGGAAACTTCCTGTTAACACACACACACACACACACACACCCTGTGT
GTTGACCTGCCACAGCACTTTG

Locus: 374 Allele: 196 Repeat motif: 8(GC)9(CA)

Sequence:AGGAGGACAGAAAGACAGAAGGCTTAGAGAAAGTGTTTATTAAAACATGCGCGC
GCGCGCGCGCGACACACACACACACACACAGGTTTATGGCACCCCCATTACCTCCTGTGTGTA
GCATTATCCCTGTGCCAGGCACTGTGTACAGGGCTGTACACACCTCCTTCTTATCCTTCCACCCT
CACAATACATCCAT

Locus: 541 Allele: 193 Repeat motif: 16(CTTT)

Sequence:GAGCTCCTGATGGAAGAGCTTACTTGAGTTTTATTCTTAACTTTCTTTCTTTCTTTCT
TTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCTTTCATTTGGGTTAGGTATTATCTG
TGCTTGAGTATCAGGAGTTGCTCTCTAGACCTTCATGGAAGAAAATTTTGAAGTCACGGACAG
GATG

Locus: 622 Allele: 216 Repeat motif: 8(CA)

Sequence:GAACACGTTAGTAGTAGAACTTATTACACACTCAGAAATTTTCCCAGATAAAACCT
TTGGTGTGGGACCCACACACACACACACACAATACGTATGTACTTACACAAAATTTTCTTATCTTTC
TCTATGTGTTTCGTTTTATATTTTGCGTATTTTTGTGGTGCAAGTACAATATCTAACAAAGTACG
TCACACATAATATCGGTATCCATGGAGCCC

Example Camera Trap Photographs

Example photographs from ScoutGuard camera traps of Canis mesomelas and Hyaena brunnea at predator feeding sites.

Example photographs from ScoutGuard camera traps of Canis mesomelas at predator neutral sites.

Example photographs from ScoutGuard camera traps of four Canis mesomelas individuals at a predator control site, including aggressive interaction between individuals.

Example photographs from ScoutGuard camera traps of Panthera pardus at predator neutral site 1.

Example photographs from ScoutGuard camera traps of Panthera pardus at predator neutral site 2.

Example photographs from ScoutGuard camera traps of Hyaena brunnea at a predator feeding site (Left) and a predator neutral site (Right).