The molecular ecology of Australian wild dogs: hybridisation, gene flow and genetic structure at multiple geographic scales

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"Sometimes the Dingo is married, and other times a bachelor; while sometimes he goes in for a club. All depends on the quantity of meat available and what size it is. When it is small, he hunts by himself – then he is sure of having enough to go round. When big enough for two, he picks up Mrs Dingo; when extra, such as emus or kangaroos, the whole club turns out."

- Robert Kaleski, in *Australian Barkers and Biters* (1933)

Abstract

Advances in molecular ecology, particularly over the past two decades, have allowed studies of populations to extend to increasingly broad geographic and temporal scales without sacrificing detail. Limitations on sample numbers and types are decreasing, as efficiency and techniques for extracting DNA from sub-optimal sources (such as hair or scats) improve. In this thesis I use microsatellite DNA markers to produce the first study of population genetics in Australian wild dogs, including dingoes (*Canis lupus dingo*), feral domestic dogs (*C. l. familiaris*) and their hybrids. Dingoes are unique among the Australian vertebrate fauna because they were transported to the continent approximately 5,000 years ago. They have therefore not been in the ecosystem on evolutionary timescales, but have been present much longer than other introduced species. Dingoes are Australia's apex predator, and have spread across habitats from deserts to tropical forests, but are currently under threat of extinction due to widespread hybridisation with domestic dogs. The conservation of dingoes is a management priority in some areas, but in others they are actively persecuted to protect livestock from predation.

The research areas addressed in this thesis are: the type of genetic samples best suited to different questions in research on wild dogs; the locations of pure dingoes; the patterns of gene flow among individuals and groups; and the degree of variability in spatial ecology across their range. Research outcomes are also placed into the context of how they can inform the management of wild dogs. Comparison of three non-invasively collected DNA sources with each other and with an invasively collected source (DNA swabs) showed that non-invasive samples, particularly scats, can be an appropriate source of DNA for monitoring based on identification of individual. The costs and time involved in quality control of non-invasive samples, however, make them a less attractive option for large-scale or population-level studies, which require more genetic markers. I therefore employed tissue samples sourced from culling programs to examine aspects of gene flow at four geographic scales. At the continental scale, I assessed the level of interbreeding between dingoes and domestic dogs, using both established methodology and a Bayesian clustering approach. Both methods provided similar

results, showing increased hybridisation in coastal areas, particularly the southeast of Australia, but fewer hybrids in the inland areas with lower densities of human settlement. Because population structure may affect approaches to control of wild dogs and conservation of dingoes, I then examined the scale and pattern of genetic subdivision and relatedness in three regions: the Tanami desert in the northern central region of Australia; south Queensland on the east coast; and across the western third of the continent. Wild dogs showed unexpected patterns of population structure, with variations in the geographical extent and separation of clusters. Relationships and spatial ecology of wild dogs in the Tanami desert appeared to be strongly affected by human activity, particularly the presence of artificially abundant food resources. The wild dogs sampled in south Queensland and Western Australia showed distinct genetic clusters in the absence of geographic barriers, showing that Australian wild dogs display cryptic genetic subdivision at a similar scale found in wolves in vastly different habitats. The findings of this study reinforce the variability found in Australian wild dogs previously demonstrated by studies of diet and movements, and provide a novel and comprehensive overview of gene flow both among wild dogs and between dingoes and domestic dogs.

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Some notes on thesis structure

This thesis has been prepared as a series of papers, and as such contains some repetition so that each chapter contains sufficient information to stand as an individual work, although none of the chapters have yet been published. All data chapters, with the exception of Chapter 5, were prepared as co-authored papers. Chapter 5 was prepared as a single-authored paper. The details of the contributions of co-authors are listed on the following page, and other collaboration is noted in the acknowledgements of each chapter. Where collection of samples was performed with or by collaborators, this is reported in the methods of each chapter in the passive voice.

The taxonomy of domesticated species is an area still under debate and often without formal consensus (Gentry *et al.* 2004). Throughout this thesis, I have use the phylogenetically appropriate taxonomy *Canis lupus dingo* and *Canis lupus familiaris* for dingoes and dogs respectively, to reflect the monophyly of the group and in adherence to the biological species concept.

Dogs and dingoes live in various situations that are relevant to their ecology. Therefore, four main terms are used in this thesis to differentiate them, and are defined as follows:

- **Dingoes:** *wild dingoes with a high level of genetic purity. Unless explicitly stated, this does not include Asiatic dingoes or other morphologically similar pariah dogs.*
- **Domestic dogs:** *domesticated pet or working dogs owned by humans*
- **Wild dogs:** *inclusive of dingoes, domestic dogs or their hybrids which are living without human ownership in the wild*
- **Free-roaming dogs:** *domestic dogs that roam away from human settlements, but which do not live in the wild*

Contributions of collaborators

Chapter 2: Stephens D, Fleming PJS, Ballard G, Berry O
Contributions: Pen and field trials were conducted by P Fleming, J King and D Jenkins. Field collection of samples was performed jointly by D Stephens, G Ballard, P Fleming and T Newsome. All laboratory work and analysis were performed by D Stephens. P Fleming contributed some text, approximately equivalent to four paragraphs.

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Contributions: Laboratory work for Western Australian samples was performed by Y Hitchen. A Wilton provided data for the reference samples.

Chapter 4: Parts of the methods and results were included in a manuscript prepared by T Newsome under the authorship Newsome T, Stephens D, Ballard G, Fleming PJS, Dickman CR, but the introduction and discussion and were re-written for this thesis.
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Chapter 6: Stephens D, Berry O, Woolnough AP, Rose K
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Chapter 1

Introduction

1.1 Project overview

The spread of invasive species across the globe has repeatedly caused devastating losses of biodiversity (Mooney & Cleland 2001). This is especially the case in Australia, which has the highest number of native mammal extinctions worldwide, partly due to the introduction of foxes, cats, d ogs (both domestic dogs *Canis lupus familiaris* and dingoes *C. l. dingo*) and other exotic species (Short & Smith 1994; Dickman 1996; Johnson 2006). The management of invasive species is often hampered by incomplete understanding of their ecology in the new environment, and research is complicated by their capacities for prolific breeding and rapid dispersal (Fuller *et al.* 1996; Phillips *et al.* 2007; Whitney & Gabler 2008). Dingoes are a particularly complicated issue for management, because they have become an ecologically important apex predator since their introduction ~ 5,000 ya, and are also a declared pest due to their predation on livestock, but can be difficult to study due to their elusive nature.

Molecular genetic techniques have allowed important discoveries in a variety of scientific disciplines, from medicine to computing, and the study of ecology in particular has benefitted from the ability to track gene flow, pedigrees, population structure and demographic changes, which can be difficult or impossible to observe directly (reviews in Moritz 1994; Jones & Arden 2003; Manel *et al.* 2003; Waits & Paetkau 2005). Molecular ecological analysis is well suited to investigations of Australian dingoes, which are cryptic, crepuscular and wide-ranging, making them difficult to study directly (Fleming *et al.* 2001; Fleming *et al.* 2006). Despite this, few studies of dingo genetics have been made (exceptions in Wilton *et al.* 1999; Wilton 2001; Savolainen *et al.* 2004; Elledge *et al.* 2008).

In this study, I demonstrate the application of molecular techniques to assist dingo management, by providing both detailed and broad-scale ecological information about dingoes. The overarching aim is to provide novel and comprehensive ecological data that are relevant to management of wild dogs in Australia. Because the management of dingoes involves consideration of both

mitigating their predation on livestock and conserving pure dingoes where practical, I include analyses of the optimal methods for genetic sampling, the extent of hybridisation between dingoes and domestic dogs, and the patterns of genetic structure and relatedness to infer their movement and breeding habits. This thesis presents the first range-wide genetic study of Australian dingoes.

1.2 The history of dingoes in Australia

Dingoes are medium-sized carnivorous canines (average weight 15-16 kg; Corbett 2001; Fleming *et al.* 2001) that are most commonly yellow or black and tan, with a lean frame, broad skull and bushy tail. The oldest archaeological evidence of dingoes was found in Thailand, dating from approximately 5,500 ya (Higham *et al.* 1980). Dingoes appear to have originated in south Asia, and to have lived as semi-domesticated dogs in both Asia and Australia as guardians, companions, and as a fresh food supply on voyages (Breckwoldt 1988; Clutton-Brock 1995). Recent phylogenetic analyses have shown they are within the same lineage as domestic dogs, having been selectively bred from wolves, but not selected to the extent of modern dog breeds (Vilà *et al.* 1997; vonHoldt *et al.* 2010). Dingoes are most closely related to other ancient Asiatic breeds, including the New Guinea singing dog, chow chow and akita (vonHoldt *et al.* 2010).

Dingoes were transported to Australia approximately 5,000 years ago, probably by Asiatic seafarers (Gollan 1984; Savolainen *et al.* 2004). It is probable that only a few individuals founded the Australian dingo population, because genetic diversity in the breed is low compared to other dog breeds (Wilton *et al.* 1999; Savolainen *et al.* 2004; vonHoldt *et al.* 2010). After their arrival in Australia, dingoes spread across the mainland swiftly, probably with human assistance (Corbett 2001). Their presence had a significant impact on the native fauna, and dingoes have been implicated, possibly in combination with humans, in the mainland extinction of the Tasmanian devil (*Sarcophilus harrisii*), the Tasmanian hen (*Gallinula mortierii*) and the indigenous top-order predator, the marsupial thylacine (*Thylacinus cynocephalus*) - leaving the dingo as Australia's largest terrestrial predator, after humans (Corbett 2001; Johnson & Wroe 2003; Wroe *et al.* 2007).

The arrival of European settlers in 1788 caused great upheaval to the life of dingoes, both directly through human activities and from the introduction of modern domestic dogs (Fleming *et al.* 2001). Aborigines quickly traded camp dingoes for domestic dogs, probably due to the latter's biddability, preference for human company and selectively bred hunting instincts (Breckwoldt 1988). Colonial reports of the dingo emphasised its ferocity and untrustworthiness, but the antagonism towards dingoes began in earnest with the establishment of the sheep industry that was critical to the early Australian economy (Parker 2007). Dingo predation on sheep resulted in the dingo becoming a declared pest in 1885 (Allen & Sparkes 2001), and control programs were begun, including shooting, trapping, bounties on dingo scalps, and the importation of strychnine for poisoning (Fig. 1.1; Breckwoldt 1988). Dingoes are efficient predators of sheep and calves, and often engage in surplus killing of sheep as a reaction to their mobbing and circling behaviour (Fleming *et al.* 2001). As a result of seemingly killing 'for fun' and the wary nature of dingoes, they became intensely disliked and mistrusted among graziers, which still affects the way many people view the dingo (Cathles 2001; Hytten 2009).

Fig. 1.1 A succesful dingo hunt in Queensland, 1940. Source: State library of Queensland, accesion number 83-1-7

One striking example of an attempt to protect livestock from dingoes was the construction of an 8,370 km dingo-proof fence through South Australia, Queensland and along the New South Wales border (Bauer 1964). Much of the fence is still employed to exclude wild dogs, and although its length has been reduced to a more manageable 5,614 km, it is still the longest fence in existence (Glen & Short 2000). McKnight (1969) speculated that the scale at which pest exclusion fencing is undertaken is unique to Australia, as the fences act as a psychological symbol of dividing the productive pastoral areas near the coast from the barren, intimidating desert areas of inland Australia. The effectiveness of the fence in stopping wild dogs has varied according to the intensity of maintenance since the beginnings of its construction in the early 1900s (McKnight 1969; Breckwoldt 1988).

Despite the negative portrayals of dingoes, there was also some admiration for their cunning and hardiness, and some were deliberately crossed with working dogs to improve their stamina and intelligence (Kaleski 1933; Arnstein *et al.* 1964; Breckwoldt 1988). Although there is no documentation of dingoes being crossed with Australian kelpies, despite their similar looks in some lines, reports of a genetic legacy of dingoes in Australian cattle dogs are more reliable (Arnstein *et al.* 1964). Some dingoes were also taken in as pets, although many were later found to be unsuitable because of their strong predatory drive and skill in escaping captivity (Kaleski 1933). The admiration for the dingo as a wild animal and affection for it as a family pet, in contrast to perceptions of it being a savage killer by other members of the public, to this day incite a mixed reaction to culling of wild dogs (Hytten 2009). Management of dingoes and wild dogs are complicated by these social issues, particularly as successful management requires cooperation of landholders across tenure boundaries (Fleming *et al.* 2006).

1.3 Dingo biology and ecology

The current knowledge of dingo ecology has been gained largely through observation in captivity and in the field, and through the use of radio and, more recently, satellite tracking collars. These studies have focused on dingo spatial ecology, social behaviour, and diet. Dingoes have been revealed to be highly adaptable in many aspects of their ecology, including their movement patterns,

social cohesion (in the form of packs which may include family members and alloparental helpers) and foraging strategies. Studies of forays and dispersal have found dingo home ranges from 22 km^2 in Kosciusko National Park, New South Wales (McIlroy *et al.* 1986) up to 156 km^2 in eastern Victoria (Robley *et al.* 2010). Reports of individual forays, however, have shown that wild dogs can move up to 230 km (over 9 days; Robley *et al.* 2010), although they often return to their home ranges. These studies have shown high variability of dingo movement patterns between habitats, life stages and the sexes.

Social behaviour and pack dynamics of dingoes in captivity and in their natural habitat have been studied intensively (Corbett 1988; Catling *et al.* 1992; Thomson 1992a; Thomson *et al.* 1992a). Like other canids, such as wolves and coyotes, dingoes often form packs, consisting of a breeding pair, their offspring, and helpers (Corbett 2001). However, dingoes have flexible pack structure, with their degree of social interaction most strongly correlated with the type of prey available (Corbett & Newsome 1987; Thomson 1992b). This flexibility mirrors the situation in coyotes (Bowen 1981) and some wolves (Harrington *et al.* 1983). When more difficult and larger prey, such as kangaroos, are the primary food source, dingoes may form stable packs to aid hunting. When the predominant prey is easy to catch, such as rabbits or sheep, dingoes are usually more successful hunting alone (Marsack & Campbell 1990). The quality of the environment in food and water resources also influences pack dynamics, with lower quality habitat associated with smaller packs covering larger home ranges (Thomson 1992c).

Social flexibility has two major implications for how wild dogs are managed in Australia. First, it invites caution when transferring findings about wild dog ecology between regions. Second, where pack structure is present, control programs that eliminate some individuals may have unpredictable effects by disrupting stable social systems (Thomson *et al.* 1992a; Glen *et al.* 2007). The latter issue may manifest as an increase in dingo numbers or livestock predation despite culling, as socially enforced restrictions on breeding to the alpha pair are removed and more pups are born for the same number of adult dingoes (Corbett 1988; Wallach *et al.* 2009). Predation may also increase if juvenile dingoes no longer have the knowledge or assistance necessary to take large prey, and instead turn to predation on easily-caught sheep or calves (Fleming *et al.* 2006).

1.4 Hybridisation between dingoes and domestic dogs

Dogs and dingoes hybridise readily, and evidence of interbreeding has been found in every population studied (Fig. 1.2). Considering the ubiquity of domestic dogs in human settlements throughout Australia introgression could place the dingo, as a unique evolutionary lineage, at risk of extinction (Rhymer & Simberloff 1996; Fleming *et al.* 2001; Allendorf & Luikart 2007). Several studies have characterised the extent of introgression between domestic dogs and dingoes in specific regions, and have demonstrated a trend toward higher levels of hybridisation in more densely settled areas (Fig. 1.2). However, considering the size of the Australian continent, these purity studies have been patchily distributed, restricted largely to the central-north and the southeast of Australia, and covered a small fraction of the dingo's distribution. As yet, no comprehensive picture of the extent of hybridisation in Australia has been generated to identify regions at greatest and least risk of the extinction of dingoes.

In addition to the unique evolutionary legacy represented by dingoes, there are key behavioural differences between dogs and dingoes that may potentially lead to important ecological impacts. The key concerns about the outcomes of hybridisation are changes to predatory behaviour and increased reproductive rate, either due to the removal of social inhibition on breeding (e.g. by mating suppression or infanticide by an alpha breeding pair) or increased mating opportunity due to dogs' extended oestrus (Claridge & Hunt 2008; Glen 2010). It is not yet clear whether feral domestic dogs and hybrids perform the same ecosystem functions as Australian dingoes, so if dingoes are replaced by hybrids, behavioural differences may have an unpredictable effect on the native fauna or local livestock (Allen & Fleming 2004; Claridge & Hunt 2008).

Cultural importance of dingoes as Australian wildlife icons, to indigenous cultures as dreaming animals, and their value as tourism commodities also factor into their conservation value. As an intermediate form within the continuum of development from wolves to domestic dogs, dingoes can also provide valuable insights into the physical and behavioural changes associated with the domestication process (e.g. Smith & Litchfield 2010a,b).

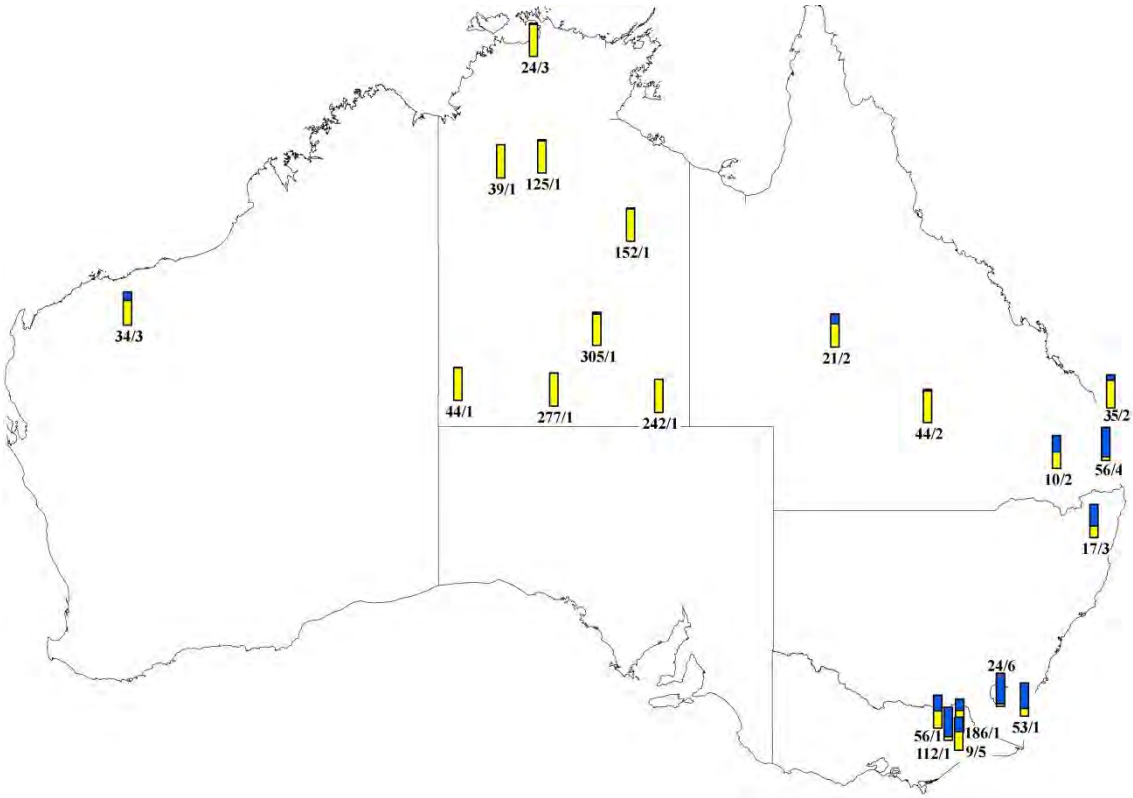

Fig. 1.2. Location and results of previous studies of dingo purity. Bars represent the proportion of pure dingoes found in each population tested. Yellow sections represent the proportion of dingoes, and blue sections are the proportion of hybrids and domestic dogs. Where more than one close location was sampled within the study, graphs are shown in the approximate centre of the sampling sites. Numbers below the graphs show the number of individuals tested at left, and the reference for each study at right (*n*/ref.). References are: 1 – Newsome & Corbett 1985; 2 – Woodall *et al.* 1996; 3 - Corbett 2001; 4 – Elledge *et al.* 2008; 5 – Robley *et al.* 2010; 6 – Claridge *et al.* 2009. References 1-3 used skull morphometrics to determine purity, 5-6 used molecular methods, and for reference 4 the average of the morphometric and molecular results is presented.

Without knowledge of the extent and locations of hybridisation, conservation strategies are unlikely to be effective, and the uniqueness of the dingo may be lost. The conflict between dingo conservation and wild dog control to protect livestock is complicated by the absence of a reliable field test for dingo purity, particularly as some breed crosses and backcrosses are difficult or impossible to distinguish from pure dingoes morphologically (Newsome & Corbett 1985; Elledge *et al.* 2008). A better approach may therefore be to manage dingo populations to minimise future introgression, rather than at the individual level.

1.5 Current management of dingoes

Wild dogs are highly successful predators of sheep, goats and cattle, costing the Australian agricultural industry an estimated AU$48.5 million per year (Gong *et al.* 2009). As a result, they are declared pests in all mainland states (but not territories), and are subject to concerted efforts to control their abundance in agricultural regions. In addition to the direct costs from stock losses, expenses are incurred from diseases transmitted to stock and the expenditure on control programs (Allen & Fleming 2004). The main methods of dingo control are shooting, trapping and poisoning. Bounties are still used in some areas, despite little evidence of their effectiveness (Harden & Robertshaw 1987; Glen & Short 2000; Allen & Sparkes 2001). Although poison baiting and is used routinely, its effectiveness in reducing wild dog numbers is often difficult to gauge. The success of annual poisoning is currently measured using relative changes in dog activity, by counting paw marks in sand traps (Fleming *et al.* 1996; Engeman & Allen 2000). Such activity indices are assumed to predict dog numbers, but can be confounded by dogs moving over the trap multiple times, or misidentification of another species' paw marks (Wilson & Delahay 2001).

Despite the pest status of wild dogs, dingoes are often not controlled in national parks or other government land. Pure dingoes cannot be reliably identified in the field (Elledge *et al.* 2008), therefore it is not possible to separate protection of dingoes from that of feral domestic dogs and hybrids. Effectively, then, all wild dogs are uncontrolled on government reserves. This is often a contentious issue for adjoining landholders, as wild dogs may breed or take refuge in national parks, then migrate onto stocked land and destroy livestock, thus negating control efforts (McIlroy *et al.* 1986). Questions over the distances which wild dogs move from uncontrolled to agricultural land is also a source of tension between management groups, with blame apportioned to perceived sources of reinvasion, which are sometimes quite distant (Hogstrom 1986).

1.6 Potential of molecular ecology to assist management

The rapid pace of technical and analytical development in molecular genetics has allowed many unique insights into the ecology of previously intractable species. Genetic analysis has the potential to complement existing ecological analysis of

wild dogs by providing insight into their patterns of gene flow, which in turn illuminates their movement and reproductive behaviours. The information that can be gained ranges from the short-term behaviour of individuals to processes operating at the landscape scale over many generations. Larger sample sizes are often available for genetic analysis than most other approaches, and, depending on the method of collection, wild animals need not be handled at all. Adding genetic analysis to the current state of understanding allows more information to be gained about individual animals, such as familial relationships to other sampled animals or whether an individual is a dingo or a dingo-dog hybrid, which are not always apparent from observation alone. The ability to extend genetic analyses from interactions between subspecies at the continental level down to interactions between individuals makes it a particularly attractive and efficient approach to understanding multiple aspects of wild dog ecology.

1.6.1 Non-invasive DNA sampling of wild dogs

Two largely unexploited sources of DNA that could be used for research are non-invasively collected DNA, including scats and hair, and carcasses from control programs. The improvement of molecular techniques has enabled the use of DNA sources such as hair, saliva, feathers and scats for a variety of applications (Goossens *et al.* 1998; Kohn *et al.* 1999; Segelbacher 2002; Sundqvist *et al.* 2008). The successful identification of individuals from such samples has been used in studies as diverse as identifying predators of endangered species (Ernest *et al.* 2002; Banks *et al.* 2003), estimates of population size, density, or the minimum number present for cryptic animals (Taberlet *et al.* 1997; Ernest *et al.* 2000; Eggert *et al.* 2003; Boulanger *et al.* 2004; Romain-Bondi *et al.* 2004) identifying the species of origin for DNA samples (Reed *et al.* 1997; Fernandes *et al.* 2008) and finding hybrids within populations of endangered taxa (Adams *et al.* 2003; Adams *et al.* 2007).

Non-invasive DNA sampling could be useful for management of wild dogs by monitoring of population fluctuations over time, particularly to record the effectiveness of poison baiting. By sampling controlled populations repeatedly and applying mark-recapture statistical analysis, the impact of control efforts could be assessed. A DNA-based approach to data collection can provide identification of

individual dogs, avoiding the confounding of activity and abundance that can occur with counts of spoor. The use of non-invasive DNA may also be useful to test for dingo purity in sensitive conservation areas where handling or culling of dingoes is undesirable.

1.6.2 The extent of dingo-dog hybridisation

Identification of dingo hybrids has previously been performed using the measurements of skull characteristics that discriminate between pure dingoes and domestic dogs (Newsome & Corbett 1982, 1985). The main drawbacks of this method are that the measurements cannot be carried out until an animal is dead, the ability to identify backcrossed dingoes is poor, and the method is inaccurate for juvenile skulls (Wilton *et al.* 1999; Daniels & Corbett 2003; Elledge *et al.* 2006). This method also has limited accuracy, as Newsome and Corbett (1985) reported that ca. 10 % of known hybrids that were crossed in captivity were classified dingoes on the basis of cranial analysis. More recently, microsatellite genetic markers have been employed to characterise the extent of introgression between dogs and dingoes (Wilton *et al.* 1999; Wilton 2001). These methods circumvent the problems associated with morphometric analysis, and they are much easier to apply to large sample sets. The development of DNA markers and admixture analysis (Pritchard *et al.* 2000) provide new opportunities to assess the proportion of admixture between domestic dogs and dingoes on a broad scale (Randi & Lucchini 2002; Koblmüller *et al.* 2009; and Norén *et al.* 2009 provide examples of this approach to testing hybridisation between other canids), but have not yet been applied to determine the current status of hybridisation across Australia.

1.6.3 The scale of movement and population structure in wild dogs

The genetic structure and movement of wild dogs is valuable information for planning the scale of management strategies and identifying whether there are unique dingo lineages that may be of particular conservation interest. Collaring studies have provided valuable information on the movement of dingoes, but are often hampered by the high cost of collars limiting sample sizes, and the loss of data caused by irretrievable collars or upload failure of GPS collars (Johnson *et al.* 2002; Uno *et al.* 2010). The development of reliable measures of dispersal and

spatial ecology from genetic data and the appropriate statistical models has received much attention, and has shown good congruence with field and simulation results (Berry *et al.* 2004; Evanno *et al.* 2005; Lowe & Allendorf 2010).

Genetic analysis of population structure can complement the information gained from collaring individual animals by providing a broader view of population behaviour. By targeting control across the entire usual movement area of animals, the potential for rapid reinvasion is reduced, because they would need to move across rather than within population boundaries (Hampton *et al.* 2004). Given the variability found in home ranges and movements of wild dogs (section 1.3), finding potential causes for their genetic structure could also assist in predicting the extent of management units across the variety of habitats within their range.

1.7 Project aims

The main objective of this study is to provide the first analysis of the molecular ecology of Australian wild dogs at the continental scale, and to provide ecological data relevant to specific management objectives. Specific aims of this thesis are:

1) To determine the feasibility of using non-invasive DNA sources to monitor individual wild dogs (Chapter 2).
2) To establish the extent of dingo-domestic dog hybridisation throughout the Australian continent (Chapter 3).
3) To determine the scale at which wild dogs exhibit kin-level and population-level structure, the geographic extent to which human activities impact this structure, and whether this is consistent across regions (Chapters 4-6).
4) To determine the role of topographic relief and environmental variables in dictating the population structure, and hence characterising the extent and limitations to movement, of wild dogs (Chapters 5 and 6).

Chapter 2

Comparison of non-invasive DNA sample sources for monitoring wild dogs in Australia

2.1 Abstract

The efficacy of non-invasive population monitoring varies widely among both species and habitats. For studies involving estimation of population parameters, biases associated with both collection and analysis of DNA need to be determined before studies begin. Capture-mark-recapture (CMR) estimators, in particular, have assumptions that must be met to obtain accurate estimates of population parameters. Here I investigate the feasibility of using non-invasive DNA for population monitoring for wild dogs in New South Wales, Australia. Explorations of potential biases, the likelihood of obtaining sufficient field samples and comparison of genotype quality from different sample types are included. Field trials of sample collection techniques (hair, scats and saliva) suggested that dogs have varied reactions to hair traps, and that scats are most likely to provide enough samples for population estimation. Comparison of polymerase chain reaction (PCR) success rates and genotyping error rates among the three sample types showed that saliva and fresh scats proved the most likely to yield reliable genotypes, but field-collected scats and hairs were more error-prone. Incorporating real time PCR screening in this study allowed the identification and removal of six fox scats, and predicted a reliable genotype in 97.4% of samples by logistic regression. I found the collection of fresh scats followed by a real-time PCR screening protocol to be the most practical approach to non-invasive monitoring of wild dogs. This approach can be applied to monitoring wild dog numbers before and after lethal control programs, as well as potentially testing for hybridization between dingoes and domestic dogs.

2.2 Introduction

Estimates of abundance, density and other population parameters are critical to the management of wildlife populations. Capture-mark-recapture, capture-recapture or capture-resight methods (here collectively termed CMR) may provide

robust estimates of abundance and other parameters, and a variety of available models allow for many field contingencies (Pollock *et al.* 1990; Krebs 1999; Schwarz & Seber 1999). Non-invasive (*sensu* Long *et al.* 2008) collection of DNA samples from wild animals can provide information on individual genotypes for CMR analysis (e.g. Boulanger *et al.* 2002). There is great potential for using non-invasively collected DNA in a CMR framework to estimate the abundance of cryptic species if accurate, unbiased data are available (Schwartz & Monfort 2008). However, the accuracy of individual identification from non-invasive DNA, and its appropriateness for use in CMR analysis, can be compromised at both the collection and laboratory stages.

For DNA testing, microsatellite markers are the most commonly used to identify individuals in non-invasive DNA studies due to their high variability and the ability to amplify multiple markers in the same reaction. Errors in individual identification using these markers (genotyping) can cause incorrect genotypes or unacceptable levels of missing data (Taberlet *et al.* 1996). Each analysis of a microsatellite marker has a chance of either failing to amplify an allele of a heterozygous pair ('allelic dropout') or displaying an incorrect allele due to errors in the amplification process ('false alleles') (Taberlet *et al.* 1996). An assessment of error rates is recommended for all new non-invasive genetic sampling studies (Waits & Paetkau 2005).

Two essential requirements pertain to the marks used in CMR: that marked animals retain marks (Caughley 1980) and that individual marks are not misidentified or overlooked (Pollock *et al.* 1990). Errors in genotyping are equivalent to marks being misidentified, and the small amounts of DNA in many non-invasively collected samples make them particularly prone to error or bias in the 'marks' (Taberlet & Luikart 1999; Valière *et al.* 2007). These errors may incorrectly identify a new animal that is actually a recapture, causing positive biases in CMR estimators. Conversely, if individuals are not correctly differentiated, the population estimates will be negatively biased (the 'shadow effect' of Mills *et al.* 2000). To adjust for genotyping errors and the shadow effect, Knapp *et al.* (2009) proposed GUAVA statistics, and tested the method on wolf (*Canis lupus*) populations by contrasting DNA obtained from faeces with that from blood, resulting in ~89% genetic consensus. For CMR analysis, Lukacs & Burnham

(2005) proposed a closed-model estimator that incorporated measured genotyping error, but which is inappropriate for low (or zero) detection probabilities.

In designing non-invasive studies, not all sources of DNA will be equally useful. One persistent problem is that of multiple individuals caught in the same 'trap' as individual animals cannot be differentiated in the non-invasive samples, spurring the development of single-capture devices (e.g. Bremner-Harrison *et al.* 2006; Pauli *et al.* 2008). Many non-invasive DNA studies have focused on the utility of scats or hairs (reviewed in Broquet *et al.* 2007), although urine, saliva and sloughed skin have also proved useful in certain contexts (Amos *et al.* 1992; Hausknecht *et al.* 2007; Inoue *et al.* 2007). Two advantages of using scats over other sample types are potentially higher sample numbers, as they persist in the environment for some time, and ease of collection. Previous studies have shown a range of success and error rates in amplifying DNA from scats, attributed to variables such as diet (Murphy *et al.* 2003), season (Maudet *et al.* 2004), or time until collection (Piggott 2004; Santini *et al.* 2007). Amplification of DNA from scats can also be affected by polymerase chain reaction (PCR) inhibition - where co-purified substances reduce the efficiency of the polymerase enzyme - and as a consequence higher concentrations of DNA extract may actually reduce the success of PCRs (Kohn & Wayne 1997; Palomares *et al.* 2002). Accordingly, pilot studies to validate the accuracy and cost-effectiveness of experiments for population monitoring are recommended for all new species and habitats under study (Valière *et al.* 2007).

When attempting to survey large carnivores, some scats from the red fox (*Vulpes vulpes*), feral cat (*Felis catus*) and wild or feral dog (*Canis lupus* ssp.) can be confused in the field, particularly when partial or degraded (Triggs 1996). Assigning samples to species before genotyping can reduce the processing of non-target samples. Protocols for identifying species using real-time PCR have already been developed for dogs, red foxes, domestic cats, and several other mammals (Berry & Sarre 2007; Moran *et al.* 2008; O'Reilly *et al.* 2008). New species can be incorporated into single-tube identification tests, with the development of species-specific primers and characterization of melt temperature profiles. This method of real-time PCR identification has advantages over sequencing-based identification

of species: it can provide the same information for less time, cost, and sample handling by reducing identification of species, quantification of DNA and testing for PCR inhibition to one step. This also reduces the potential for contamination and exhaustion of limited amounts of DNA extract (Berry & Sarre 2007; O'Reilly *et al.* 2008).

In Australia, wild dogs (feral domestic dogs *Canis lupus familiaris*, dingoes *C. l. dingo* and their hybrids) are of management interest because of their predation on livestock (Fleming *et al.* 2001), the potential for disease transmission to livestock, wildlife and humans (Grainger & Jenkins 1996; King *et al.* 2010), their potential role in mitigating the impacts of foxes and cats on native species (Glen *et al.* 2007; Johnson *et al.* 2007), attacks on people and pets (Peace 2002) and concerns regarding the conservation of dingoes (Elledge *et al.* 2006). Large investments are made in programs to reduce wild dog numbers in livestock grazing areas but little monitoring is undertaken to ensure that control is effective (Fleming *et al.* 2001; Hone 2007), usually because of the high cost and time investment required to monitor population size and change (Witmer 2005).

Because wild dogs are difficult and relatively expensive to trap, and are usually at low densities, physical capture, recapture or resighting is often difficult or impossible (Corbett 2001). Trapping wild dogs is also socially unpopular due concerns over both the humaneness of traps and objections to releasing trapped dogs, which may go on to kill livestock. In some areas, indices based on the occurrence of animal prints have been used to monitor control efficacy (e.g. Fleming *et al.* 1996; Engeman & Allen 2000), but these methods often confound activity with abundance, are subject to misidentification errors when wild dogs and foxes co-occur, and the relationship between the indices and population size is uncertain (see Caughley 1980).

If effective protocols can be developed, non-invasive DNA monitoring may allow measurements of population fluctuations after control activities such as poison baiting (Fleming *et al.* 2006; Piggott *et al.* 2008). In conjunction with abundance indices and collaring data, non-invasive DNA can be a valuable additional method for monitoring wild canids and other vertebrate predators (Rosellini *et al.* 2008; Sarre & Georges 2009). Non-invasive monitoring may also assist in dingo conservation by allowing managers to genetically test for pure

dingoes (as distinct from dingo-domestic dog hybrids) on a useful proportion of the population, leading to better information for management decisions. In this paper, I address precision and bias of non-invasive sampling for CMR estimators for estimating population parameters of wild dogs in north-east New South Wales, but the ideas can be generalized to other animals and locales. I also combine real-time PCR measurement with a protocol for identification of species, based on melt-curve analysis (Berry & Sarre 2007), to identify and exclude samples from non-target species or with low quantities of DNA. Specifically, I address the following questions: 1) can non-invasive samples be collected in an unbiased manner suitable for CMR analysis?; 2) which non-invasive DNA source can provide the most reliable genotypes?; and 3) can quantitative PCR using melt-curve species identification improve the efficiency of processing wild dog samples?

2.3 Methods

2.3.1 Study area

Field sampling of wild dogs was undertaken on transects at six (labelled 1-6) sites in north-eastern New South Wales, Australia (approximately 31º 30'S, 152º 00'E). Transect sites were paired into control and treatment sites: the treatment sites were baited with wild dog poison once per year in winter; the control sites were not baited but had similar habitat to their associated treatment site. Each pair of sites was up to 50km apart in a direct line. Sites were in forested areas adjacent to grazing land, with grassy, scrubby or rocky substrate. The local climate is temperate to sub-tropical, with a winter wet season mid-year and mean monthly rainfall from April to July ranging from 45 to 59mm (Australian Government Bureau of Meteorology 2010).

2.3.2 Sample collection in captivity

A pen trial was conducted at the Australian National University's facility near Canberra, and a paddock trial in a 1 ha exercise pen at a private dingo conservation facility. In the pen trials, 8 individually-penned young dogs were used, comprising 4 pure dingoes and 4 domestic dogs bred for experimental purposes from mixed stock including kelpie and red cattle dog. The 3-6 month-old dogs were obtained from a registered supplier of dogs for laboratory use. A buccal swab was collected

from each captive animal and stored in 4 ml of lysis buffer (Longmire *et al.* 1997). Hair, saliva and scat samples were also collected and stored in airtight plastic bags with silica beads for transportation to the laboratory.

Hairs were collected on a 'roll block': a block of wood 300 mm long, 100 mm wide and 10 mm thick with strong adhesive tape sticky-side up. Hair samples were obtained by touching the sticky area of the block to the back of the dog or dogs (Fig. 2.1a). Each block had hairs from 1-8 different dogs. Saliva samples were collected from a block of wood the same size as the roll block, but with fine-grade sandpaper on the upper surface and baited with chicken mince, which dogs licked to deposit saliva cells (lick block; Fig. 2.1b). Faecal samples were collected from each dog on the same day as defecation. Additionally, roll blocks with a drop of Canine Call lure (Russ Carman, Pennsylvania, USA) were placed in each pen and, after placement, responses of each dog were observed through windows. The dogs were observed from the time they appeared at the door of their kennel at the back of the pen until they returned to the kennel or went to sleep. Observations were ceased after 5 mins of inactivity. Later a lick block was placed into each pen to collect saliva cells. The lick block was likewise exposed for the period of interest plus 5 mins and the behaviours of each dog towards the block were recorded.

In the paddock trial fresh faecal samples were obtained from 8 dingoes at the private facility and a pair of lick and roll blocks were placed in an exercise paddock where mated pairs of dingoes were allowed to run for 10-15 mins as part of their daily exercise.

2.3.3 Sample collection at field sites

Concurrent with the captive trials, I assessed the performance of sample collection devices in the field. I placed sample collection devices on six 25-km transects which were monitored in July 2007 and April-May 2008. Aerial baiting for wild dogs was carried out with 1080 (sodium fluoroacetate) poison in meat baits in June 2007 and May 2008 at sites 1, 4 and 6 as part of normal control operations. I randomized three types of DNA 'trap' for the collection of hair and one for saliva for three nights along each transect. In 2007, sites 1-4 were monitored, and in April-May 2008 sites 2, 3, 5 and 6 were monitored. Collection devices included roll and lick blocks as used in captive trials affixed to the ground via a tent peg (Fig.

2.1a-b) and two types of upright hair trap constructed from PVC pipe over metal star posts secured with wire at the top of the post (Fig. 2.1c-d). I covered any exposed wire with electrical tape to reduce the risk of injuries. The two upright trap types differed in the type of sticky surface used: either sheets of sticky paper (Fig. 2.1c) or vertical double-sided tape strips (Fig. 2.1d). These traps were designed to elicit a rubbing response from dogs, and were baited using a small amount of Canine Call in 2007 and not used in 2008 (600 trap nights total).

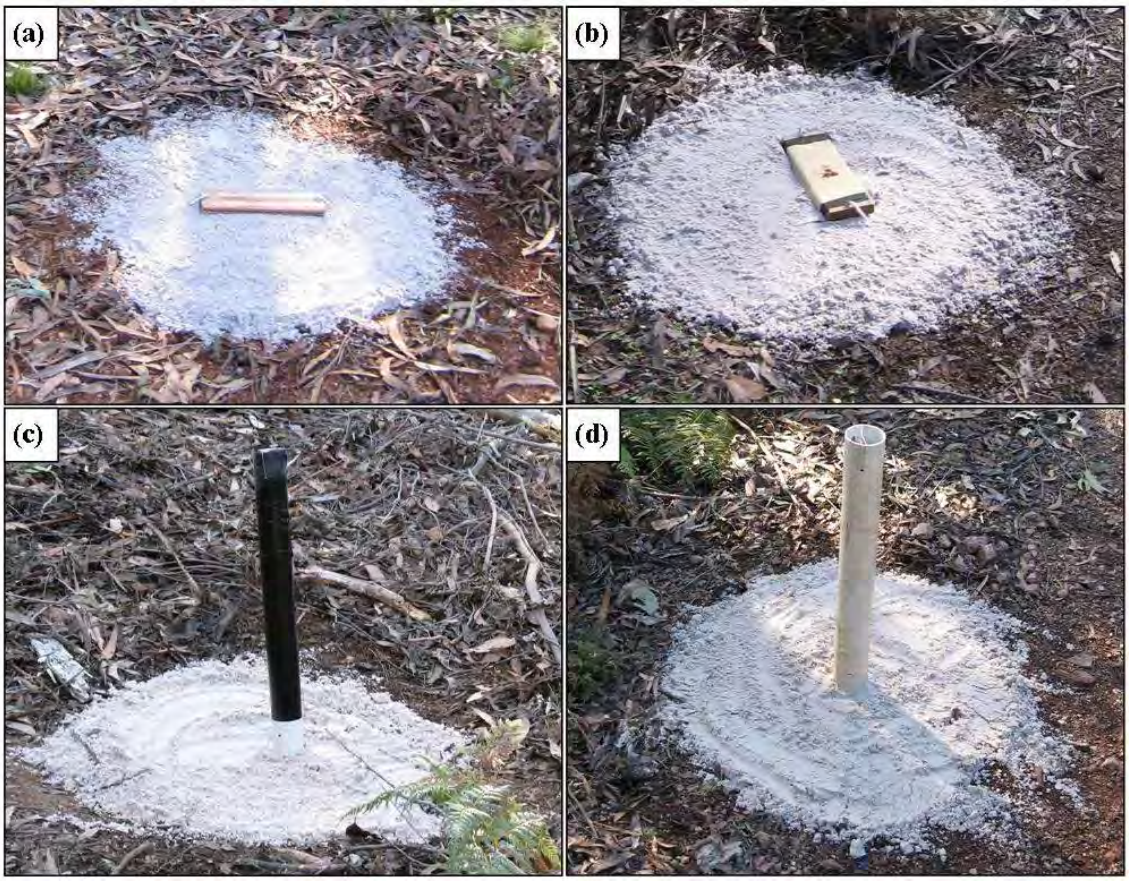

Fig. 2.1 DNA collection devices trialled in captivity (a-b) and in the field (a-d). Sand plots were 1 m diameter. (a): a roll block with two strips of sticky tape, designed to collect hairs. (b): a lick block baited with chicken mince and covered with sandpaper to collect saliva cells. (c) and (d): upright hair collection traps intended for dogs to rub against the posts. (c) is covered with a solid sheet of sticky paper, (d) has strips of tape running vertically along the length of the post.

I baited roll blocks using a small amount of 'Canine Call' in 2007 (300 trap nights), and alternately with Canine Call and 'Kiss my Ass' lure (Outfoxed Pest Control, Victoria, Australia) in April-May 2008 (210 trap nights each). I baited lick blocks with one of five food lures (anchovette paste, canned sardines, commercial

dog food, condensed milk, and a home-made milk-based fox lure, 60 trap nights per lure) which I also randomized along the length of the transect in 2007, and did not use in 2008. I checked traps each morning and replaced the tape on hair traps if it was no longer sticky, or replaced lure on lick blocks if it had been taken.

I surrounded all traps with flattened sand to approximately 1 m in diameter to record paw prints of animals that had been close to the trap, and reduce DNA testing of non-target samples. All transects also had 1 m wide sand plots every 1 km across the access trails as part of a separate study, which were monitored before and during the trap monitoring sessions for animal prints to confirm that dogs were in the area.

One or two people collected scats while walking the length of three transects in April-May 2008 (sites 1, 2 and 5; 'session 1') and one transect at site 5 in July 2008 (session 2). In other wild canids, reproductive cycles have been shown to affect the deposition of scats which may bias collection data (Ralls *et al.* 2010). The majority of dogs in northeastern NSW, however, are dingo-dog hybrids (Chapter 3), which display only a weakly seasonal pattern and may breed throughout the year (Jones & Stevens 1988; Catling *et al.* 1992), making potential biases from scats, at minimum, less pronounced in Australian wild dogs than in other wild canid taxa.

2.3.4 DNA extraction and amplification

I stored all samples for 1-2 months at room temperature with silica crystals for desiccation until DNA extraction, except for saliva samples, which were stored at -20 ˚C. Desiccation of samples was chosen in preference to storage in ethanol to comply with requirements for transportation of samples from the study site to the laboratory. To minimize the opportunity for sample contamination I carried out all DNA extractions and PCR preparations using aerosol-resistant pipette tips in a dedicated laboratory where no tissue, blood or post-PCR material had been processed. One or more negative controls were included with each set of PCRs performed in a session to check for systematic contamination. I performed all PCR preparations in a sterile PCR set-up cabinet. Laboratory benches and the PCR cabinet were subject to nightly UV sterilization. I extracted DNA from hair, saliva and buccal samples using the Qiagen DNeasy blood and tissue kit (Qiagen Inc., CA,

USA), according to the protocol for tissue samples. I used 1- 22 hair follicles ($\bar{x}$ = 5.91, SE = 0.78, *n* = 30) in extractions. For saliva samples, I cut out dampened areas of the sandpaper for use in extractions. For each scat I scraped a small portion from the outside with a fresh scalpel blade, and extracted DNA using either the QIAamp® DNA stool mini kit (Qiagen Inc.) according to the protocol of Roeder *et al.* (2004), or the Mo Bio PowerSoil™ DNA isolation kit (Mo Bio Laboratories Inc., CA, USA), using its recommended protocol.

I amplified five PCR replicates per DNA extract, using nine microsatellite loci in two multiplex reactions. I used multiplexing to improve the efficiency of this pilot study with limited resources, as the objective was to provide comparisons of samples rather than absolute yields. In full studies for CMR estimation, however, individual amplification of loci is likely to yield higher volumes of DNA. I used loci AHT109 (90-130 bp; Holmes *et al.* 1993), FH2079 (260-300 bp; Francisco *et al.* 1996), CXX410 (100-150 bp; Ostrander *et al.* 1995), CXX30 (140-170 bp; Ostrander *et al.* 1993) (multiplex 1), FH2293 (210-300 bp), FH2247 (180-260bp; Mellersh *et al.* 1997), CXX460 (120-160 bp; Ostrander *et al.* 1995), AHT103 (70-110 bp; Holmes *et al.* 1995), and VIAS-D10 (100-160 bp; Primmer and Matthews 1993) (multiplex 2). Reactions contained 5 µl of Qiagen Multiplex PCR solution (Qiagen Inc.), 1 µl Qiagen Q-Solution, 1 µl DNA, 0.2 µM of each primer and made up to 10 µl total volumes with DNAase/ RNAase-free water. PCR conditions were: 15 mins 95° C activation, then 35 cycles of 30 s at 94° C, 90 s at 60° C and 60 s at 72° C, followed by 30 mins final extension at 60° C. Fragments were run on an ABI 3730 capillary sequencer. Allele sizes were analysed using GeneMarker® software (SoftGenetics, LLC., PA, USA), and I scored all peaks without reference to other replicates from each sample.

2.3.5 Real-time PCR

I used the species identification method of Berry and Sarre (2007) on scat samples to determine: 1) if the sample was from a fox or a dog; 2) whether PCR inhibitors were present, using serial dilution, and; 3) the concentration of mitochondrial DNA. I examined real-time PCR reactions on all scat samples using a Rotor-Gene 6000 thermocycler (Corbett Life Science, NSW, Australia). Reactions contained 10 µl Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen, CA, USA), 5 µl water,

0.8 µl bovine serum albumin and 0.2 µM each of fox, dog and universal forward primers (Berry and Sarre 2007). I ran each sample using full (4 µl DNA) and 1/5 dilutions of the template to check for PCR inhibition. I considered samples to display PCR inhibition if the 1/5 dilution showed a higher DNA concentration after PCR than the undiluted sample. I included positive controls of dog and fox DNA in each experiment for comparative melt temperatures, as well as 1-3 no-template controls.

For quantification of DNA, I ran 10 two-fold serial dilutions of DNA in seven replicates to develop a standard curve, from 1/16 to 1/8192 dilution (8 ng/µl to 16.27 pg/µl). I included reference samples with known concentration in each subsequent PCR experiment. I measured DNA concentrations of standards using a NanoDrop™ 1000 Spectrophotometer (Thermo Scientific, MA, USA). I used the average of five measurements to calculate standard curves, except for concentrations < 2 ng/µl, which were extrapolated from the dilution series. Cycling conditions were as in Berry and Sarre (2007), with the exceptions that I used 40 cycles instead of 35, and ran the melting profile from 72° C to 85° C. I analysed real-time PCR results using Rotor-Gene software (version 6.0, Corbett Life Science). Cycle threshold (Ct) values, the point at which sample amplification exceeds a consistent arbitrary threshold, were calculated by the software and used to compare DNA concentration against genotyping success. Lower Ct values indicate higher DNA concentrations, as the fluorescence will exceed background levels during an earlier PCR cycle.

2.3.6 Data analyses

I calculated probability of identity (PI) and probability of identity for siblings (PI_{sibs}) using Genalex v6.1 (Peakall & Smouse 2006), based on allele frequencies calculated from 39 tissue, buccal and high quality scat samples collected near the field site and from the captive dogs. PI_{sibs} was used in preference to PI as the all of the captive dingoes used were from one of two litters, and at least two of the domestic dogs used were full sibs. I calculated 'PCR success' as the proportion of successful PCRs averaged across all extracts of each sample type (Arandjelovic *et al.* 2009). I used a Kruskal-Wallis test, followed by pairwise Mann-Whitney U tests with Bonferroni correction, to compare sample types. I performed tests for

difference between PCR success of each sample type using PASW statistics 18 for Windows (SPSS Inc., IL, USA).

I constructed consensus genotypes from five replicates for each sample according to the rules that each allele was accepted if it was observed at least twice for heterozygotes and three times for homozygotes. If more than two alleles were scored at a locus across the replicates the entire locus was coded as missing data. I accepted consensus genotypes if at least seven loci were included, as this was the minimum number of loci required to retain a PI_{sibs} of < 0.01 assuming the worst-case scenario of only the least informative markers being included in the consensus (see results). I defined 'genotyping success' as the successful construction of a consensus genotype for each extract. I calculated rates of allelic dropout from the consensus genotypes using Gimlet v1.3.3 (Valière 2002). I performed a binary logistic regression of Ct values and achievement of a 7-locus consensus genotype in PASW statistics 18 for Windows (SPSS Inc.) using the default parameters.

2.4 Results

2.4.1 Behavioural responses in captivity

None of the four penned dingoes licked or rolled on the sampling blocks, but two sniffed both blocks. The domestic dogs showed greater interest, with two licking the lick blocks clean and the other two sniffing them. No domestic dogs rolled on roll blocks. Fourteen scats, eight saliva samples and 26 hair samples were collected from the dogs for comparison of DNA quality among sample types.

None of the dingoes in the exercise yard at the captive facility rolled or licked the blocks, and no dingoes showed any response to lick blocks. Only four of the eight animals registered any response to the blocks: two male dingoes stopped near a roll block and rolled on the ground approximately 1.5 m from it, and two dingoes (one male and one female) altered stride and sniffed and focused on a roll block as they ran past.

2.4.2 Field results

Sand plot monitoring performed on the transects concurrent with non-invasive sampling showed evidence of dog activity at all sites. The least dog activity was at

site 4 in 2007, where only one print was observed in 75 trap nights. No samples were collected on the upright hair traps or lick traps. One dog print was observed at an upright trap at site 3, and dog prints were observed at two lick traps at site 3, but no lure was removed. Non-target prints were observed at the lick traps including birds, possums, cats and a fox, and a quoll was observed taking a lure using a remote camera. Processing time was highest for this type of trap due to regularly replacing lures removed by non-target animals.

On the transects, roll blocks attracted the most interest from dogs, and were the only traps to provide samples (Table 2.1). Only seven samples from two sessions were collected, and in both instances there were 2-3 times more investigations by dogs than samples left. At site 3 in July 2007 one of the activated traps had hair but no prints, although both of the other activated traps had dog prints surrounding the trap. In April-May 2008, 67 scats were collected without prior clearing of the transect, and nine were collected in July 2008.

Table 2.1. Wild dog visitations to roll blocks in field trials for two monitoring sessions in north eastern New South Wales, Australia. Dog prints were recorded from circular plots of flattened sand approximately 1 m in diameter around traps. Any set of dog paw marks at a trap was counted as one 'print'. 'Activated traps' indicates traps that yielded hair samples. Sites 5 and 6 were not monitored in the first session; sites 1 and 4 were not monitored in the second session (marked with dashes).

	Jul 2007			Apr-May 2008		
Site	Trap Nights	Dog prints	Activated traps	Trap Nights	Dog prints	Activated traps
1 (baited)	75	0	0	-	-	-
2 (control)	75	2	0	100	9	0
3 (control)	75	7	3	100	3	0
4 (baited)	75	0	0	-	-	-
5 (control)	-	-	-	100	1	0
6 (baited)	-	-	-	120	11	4

2.4.3 Genotyping

The number of samples collected for each sample type and a summary of the success and allelic dropout rates are reported in Table 2.2. The hair samples included 26 samples from captive dogs and four collected from roll blocks in the

field. The PI and PI_{sibs} for nine loci were 1.2×10^{-9} and 4.8×10^{-4} respectively. This is well below the recommended maximum PI_{sibs} of 0.01 for studies estimating population size (Waits *et al.* 2001), and allows for failure of the two most informative loci whilst still maintaining an adequate PI_{sibs} (0.005). Consensus genotypes were therefore included if at least seven loci were accepted. The buccal swabs were amplified with only three replicates, but are included as a comparison to a high quality DNA source.

All the non-invasive DNA sources showed high among-sample variation in PCR success (Fig. 2.2), and the Kruskal-Wallis test showed difference among the distributions ($P < 0.001$). Pairwise Mann-Whitney U tests with Bonferroni correction showed that scats from the first collection session had significantly lower PCR success than all the other sample types, and both the hair and saliva were significantly less successful than the buccal samples ($\alpha = 0.003$).

Table 2.2. Number and quality of genotypes obtained from various DNA sources. Field-collected scats were of unknown age. Session 1 (s1, April-May 2008) included scats from three transects; session 2 (s2, July 2008) scats were collected from only one of those transects. Only 61 of the 67 'session 1 field scats' were analysed, as 6 were identified as fox scats by real-time PCR and excluded from further analysis (section 2.4.3).

Sample type	n	% PCR success (Standard deviation)	No. consensus genotypes obtained (% genotyping success)	% Allelic dropout across loci
Buccal swab	9	98 (5)	9 (100)	1
Hair	30	40 (33)	7 (23)	36
Saliva	8	77 (20)	7 (88)	23
Captive scats	14	70 (34)	8 (57)	8
Field scats (s1)	61	19 (25)	6 (10)	6
Field scats (s2)	9	63 (35)	4 (44)	13

By matching consensus genotypes of field-collected scat samples, with missing data ignored, I found that on the transect that was monitored in both sessions, one dog was sampled once in the first collection session and four times in the second session. One other dog was identified twice in the first session. The minimum numbers of dogs at this transect were therefore two for the first session and one for the second session, and one dog was detected in both sessions. The

other two transects had a minimum of one and two dogs respectively in April-May 2008, with no recaptures.

2.4.4 Real-time PCR as a predictor of amplification success from scats

From the 90 field-collected and captive scats analysed, six were assigned by melt-curve analysis to be from foxes and were removed from the data set. Five out of the 42 samples with mtDNA concentrations greater than 15 pg/µl showed evidence of PCR inhibition (12%), i.e. the 1/5 dilution had a higher DNA concentration than the undiluted sample.

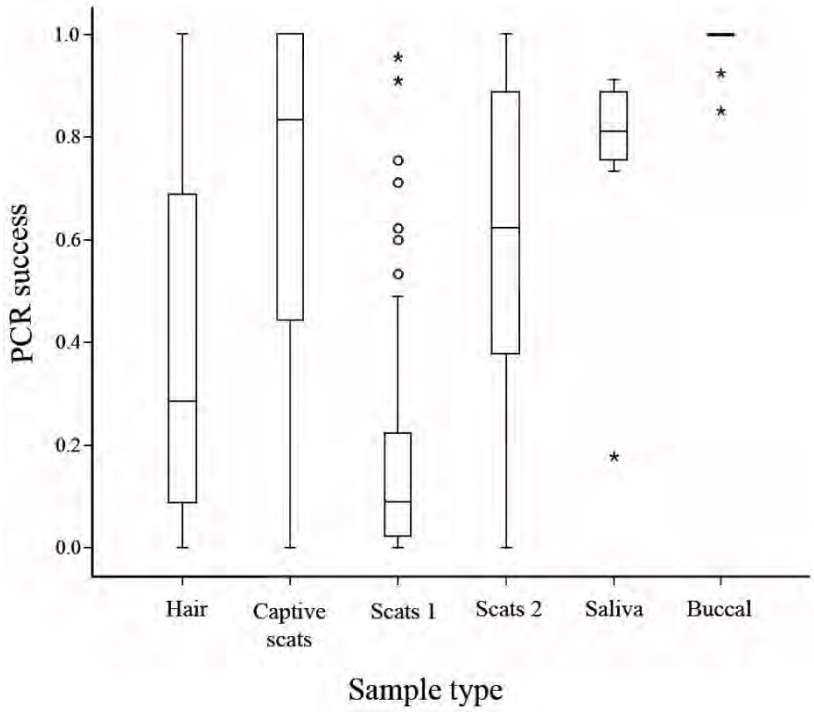

Fig. 2.2. Summary boxplot of polymerase chain reaction (PCR) success by DNA sample type. PCR success was calculated from the average proportion of successful PCRs for each sample type. Scats 1 refers to scats collected in field session 1 (Apr-May 2008), scats 2 were collected in session 2 (Jul 2008). Circles represent values 1.5-3 times the interquartile range from the box. Values more than three times the range are represented by asterisks.

The standard curve for determination of absolute concentrations had an r^2 value of 0.985, with slope -3.6 and amplification efficiency of 91%. The ability to obtain a consensus (i.e. informative) genotype ('genotyping success') showed a close relationship with the Ct score obtained from real-time PCR analysis (Fig. 2.3). The logistic regression model correctly predicted the genotyping success in 97.4%

(76/78) of cases, excluding six samples that contained no detectable target DNA. The exceptions were a sample near the threshold of predicted success (Ct = 18.55), which produced a consensus genotype despite predicted failure, and the sample with the lowest Ct score (13.25), which failed to provide a consensus genotype. Examination of the fluorescence curve for the sample with Ct = 13.25 showed that although the fluorescence exceeded background levels at an early PCR cycle, the amplification did not proceed exponentially after that point and so the final level of fluorescence was low. The probability of obtaining a consensus genotype from the logistic regression was $P = (e^{19.186-1.064\times}) / (1+ e^{19.186-1.064\times})$. Samples with Ct values below 17.87 (> 0.74 ng/µl DNA) all yielded a 7-locus consensus genotype, and samples with values above 18.1 (< 0.58 ng/µl DNA) all failed to yield consensus, with the exceptions of the samples noted above.

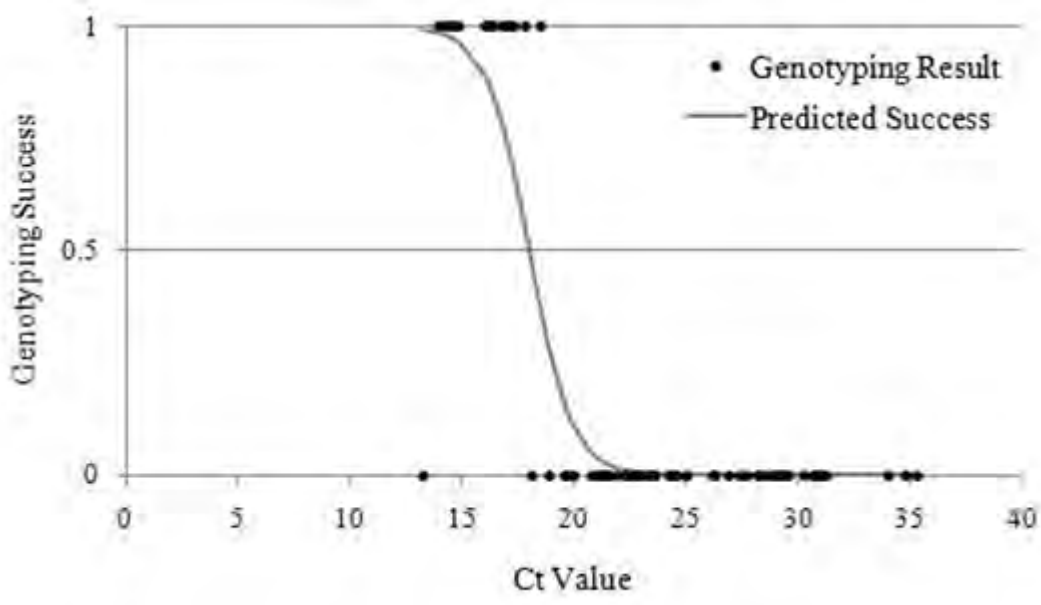

Fig. 2.3. Relationship between the cycle threshold (Ct) values from real-time PCRs and genotyping success. Only results from undiluted real-time PCR samples were used. Samples with a consensus genotype are designated 1, those without as 0. The solid line shows the predicted genotyping success as a function of Ct from logistic regression.

2.5 Discussion

2.5.1 Sample collection

In captivity, there were marked individual differences in behaviour of penned dogs towards all the DNA traps, from complete avoidance (zero probability of detection) to great interest. This was immediate evidence of unquantifiable heterogeneity or

unequal catchability that is probably also expressed in the field, which is supported by evidence of one wild dog scratching around a trap, without rolling, in the field study. As the captive subject animals were all young, roll responses may not have been fully developed, which introduces possible sources of variation by time and heterogeneity. Cubaynes *et al.* (2010) showed that failure to account for individual heterogeneity in detection probability caused the population size of wolves to be underestimated by up to 70% (average 27%). This bias renders attempts to use CMR from active traps unreliable, as even though heterogeneity is identified, an appropriate model cannot be applied to the differences in behaviour (Link 2003). Cubaynes *et al.* (2010) proposed including parameters to account for high and low survival and detection probabilities to help negate this bias, which would also seem appropriate for Australian wild dogs. Whether these additional parameters would be sufficient to account for the heterogeneity found in this study, however, will require further examination. Roll blocks may be more appropriate for indices or occupancy estimation because only the presence of dogs is recorded, not the number using the block (Caughley *et al.* 1980; MacKenzie *et al.* 2006). However, occupancy estimates may be also biased because non-detection does not mean absence (MacKenzie *et al.* 2006).

Evidence of dog activity during field sampling sessions showed that an absence of dogs was not responsible for the low success of the traps, although the dogs still may have been in low numbers. I consider that avoidance of the sand because of neophobia was unlikely to have contributed to the low number of field samples collected, because dog prints were recorded on some sand piles 1-2 nights before traps were deployed (nine prints were recorded at site 2 before trap deployment). This indicates that any effect of neophobia was more likely to be caused by the traps themselves, or actions related to setting the trap, than the sand used to monitor visits.

Lick blocks and upright hair traps provided no samples and so were not tested after the first sampling session. In addition the upright traps required more materials and construction time than the other trap types so were not cost or time efficient. Roll blocks were the most successful trap type in the field, but still yielded few samples. In at least one instance, the pattern of paw and scratch marks indicated that the dog had attempted to dig up and presumably remove a roll

block, but did not appear to have rolled on the trap and no hair sample was left. The variety of behaviours exhibited after dogs choose to investigate traps may add to the potential sources of capture heterogeneity observed in the captive trials.

The low incidence of rolling behaviour in the field may have been linked to the location of the study; substrate in the area is mostly rocky, and may deter rolling. All the activated roll traps at site 6 were on soft macropod grazing lawns. Limited trials in the Tanami Desert in central-northern Australia showed more roll blocks were used (11 visitations and four trap activations in 75 trap-nights, data not shown), but further work is required to test the effect of substrate type on rolling behaviour. Many dogs in the Tanami desert also use human refuse sites as a food source, so may also be less wary of anthropogenic items and scents (Chapter 4).

The problem of having multiple visits to a hair trap, although not observed in this study, also remains an issue for using hair samples in non-invasive genetic sampling studies (Roon *et al.* 2005; Pauli *et al.* 2008). Variations in trap design to ensure only one animal can access the trap until reset (e.g. Pauli *et al.* 2008 for American martens, *Martes americana*) is likely to increase any neophobic response if applied to dogs, and result in fewer samples. For this and the above reasons, I believe that scat collection is the best method to provide enough samples for DNA-based analysis of wild dog populations based on field and captive trial results. However, dog scent posts are unsuitable for scat collection because these are important for communication and might not be used equally by dominant and subordinate dogs, resulting in the possibility of non-detection of subordinate dogs (Fleming *et al.* 2001). Whether scat deposition by dogs varies by season, reproductive condition or gender also must be assessed during ongoing monitoring, as this has been shown to occur in kit foxes (*Vulpes macrotis mutica*; Ralls *et al.* 2010), and may be another source of bias in population estimates.

2.5.2 Comparison of sample DNA quality

The quality of sample types was highly variable, with genotyping success rates from 10% (session 1 field-collected scats) to 88% (saliva). The variability of success within sample types was also high (Fig. 2.2). The allelic dropout rates found were comparable with other assessments for canids (e.g. 11% in Creel *et al.*

(2003) and 18% in Lucchini *et al.* (2002), both for wolf scats). The hair samples showed the highest incidence of allelic dropout, and poor results for hair samples have also been reported in comparisons between hair and scats for chimpanzees (*Pan troglodytes*; Morin *et al.* 2001) and bobcats (*Lynx rufus*; Ruell & Crooks 2007). Other studies, however, have shown hair to be a preferable sample source (Broquet *et al.* 2007), so recommendations for sample collection do not appear to transfer well across species or environmental conditions. Allowing more than one dog to roll on some blocks in the captive trial reduced genotyping success, but having mixed samples is a likely scenario for field collection of hairs in studies with high capture rates (Roon *et al.* 2005). Mostly fine hairs were left on roll blocks that were likely to have low quantities of DNA, which may also have contributed to the low success and high error.

Saliva samples had a high success rate and PCR products were obtained from all eight samples tested, but were difficult to collect in the field. Lick blocks, as used in this study, yielded few samples, may be subject to capture heterogeneity among individuals, and have high non-target capture which increased processing time and cost. Development of devices that can account for these potential problems may provide high-quality DNA for population monitoring.

Scat collection yielded adequate samples in the field, and all three categories of scats had lower levels of allelic dropout than either hair or saliva (Table 2.2). There was, however, a large difference in the genotyping success and the number of samples collected between the two field sessions. On the transect that was monitored in both sessions, 30 scats were collected in session 1, and only nine in session 2. Before session 1 the transects had not been cleared for 9 months, but there were only 2 months between sessions 1 and 2 for scats to accumulate. The difference in sample numbers may be because poison baiting in the district between the sessions reduced dog numbers, because scats from the second session had less time to accumulate between sampling than for the first session, or a combination of these factors.

The inclusion of older scats from session 1 could have decreased both the PCR and the genotyping success (Piggott 2004). The fresh scats from captive animals, in contrast, showed low incidence of allelic dropout and the second-highest genotyping success of the non-invasive samples, indicating that the use of

fresh scats has potential for enabling successful non-invasive DNA programs. This also highlights the importance of ensuring minimal time lag between scat deposition and DNA extraction to improve genotyping success. Although this study focused on relative PCR and genotyping success, the absolute success of scat DNA amplification could be increased by using more DNA per reaction, reducing multiplexing of primers, or screening for more robust loci, further increasing the utility of non-invasive monitoring.

The greatest seasonal influence on the differences in quality of scats between the sessions was probably a week of heavy rain that immediately preceded sampling session 1. In addition to degradation of DNA, the rain could have removed the outer epithelial layer from the scats, which is expected to contain target DNA. Low genotyping success in carnivore scats was also associated with the wet season by Farrell *et al.* (2000), who reported 28% success in obtaining a DNA sequence from wet-season scats, compared to 66% success for samples collected in the dry season. Palomares *et al.* (2002), however, obtained PCR product from Iberian Lynx (*Lynx pardinus*) scats that had been submerged for several days. The low sample sizes for the second session scats (and saliva) hamper robust interpretation of the results from these data; in many situations, however, a trade-off will exist between sample size and sample quality. Procedures such as clearing or chalking collection areas prior to sampling can be used to increase the success of scat amplification, but will reduce overall sample numbers (Piggott *et al.* 2008).

2.5.3 Real-time PCR screening of scats

In this study, real-time PCR was a good predictor of microsatellite genotyping success, despite the real-time PCR being amplified from mitochondrial DNA and the genotyping success determined from nuclear markers. The predictive equation obtained from the logistic regression could be applied in several ways. For a conservative approach, the maximum Ct value with a successful genotype could be used as a cut-off score for the decision to perform further PCRs. In this study, such an approach would have excluded 57 failed samples, reducing the number of PCRs and fragment analyses performed by 73% with no loss of data, and significant savings in resources. Quantification could also be used to adjust DNA

concentrations to increase the chance of success in downstream applications. Alternatively, the approach of Morin *et al.* (2001) could be used to target more repeats at low quality samples by assigning them to categories based on DNA concentrations. Using a sample-specific approach to adjusting the treatment of samples can be more effective in improving PCR and genotyping success than methods that use average success to select a processing procedure for all samples (e.g. Piggott *et al.* 2006).

The species identification function of the real-time tests were also valuable, as 8% of the scat samples were excluded for returning a melt-curve profile positive for fox DNA, after morphological screening. The actual number of fox scats may have been higher, but would not have been detected in samples without sufficient PCR product. Misidentification of scats to species in the field can be frequent (Janecka *et al.* 2008), and many studies have used mitochondrial sequences or gel electrophoresis of PCR products to test for species identity before attempting individual identification of scats (e.g. Paxinos *et al.* 1997; Farrell *et al.* 2000; Lucchini *et al.* 2002). The screening procedure employed here provides the same information as these methods with the use of fewer consumable resources, as electrophoresis and sequencing reagents are not required. This procedure can therefore greatly reduce the time and cost for such a project, and in turn increase the feasibility of undertaking large-scale non-invasive DNA studies.

2.5.4 Management implications

I found that both sample collection and laboratory analysis of non-invasive wild dog samples have the potential to introduce bias or violate assumptions of CMR analysis for population monitoring. Scats showed the greatest potential for individual identification according to both field and laboratory criteria, and provided the most abundant source of samples and fewest sources of error for CMR estimates (Table 2.2). In addition, implementation of a real-time PCR screening method for scats can improve efficiency and decrease resource expenditure in this context. The potential for collection of sufficient scats for mark-recapture estimation will depend on local dog density and the size of the area under study, but in most cases valuable minimum number alive estimates can still be obtained (Krebs 1999). DNA sourced from biased trapping methods can still

provide additional information for CMR models, site occupancy estimation, and minimum known alive indices of wild dog abundance before and after control actions (Tuyttens 2000; MacKenzie *et al.* 2006). If genotypes of sufficient quality are obtained, other management issues such as the migration of dogs into baited sites or hybridization between domestic dogs and dingoes could be addressed simultaneously (Elledge *et al.* 2006).

2.6 Acknowledgements

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Chapter 3

Death by sex in an Australian icon: a continent-wide survey reveals extensive hybridisation between dingoes and domestic dogs

3.1 Abstract

Hybridisation between domesticated animals and their wild counterparts can lead to the breakdown of adaptive gene combinations, the loss of genetic diversity, the extinction of wild populations and changes to ecosystem function. Hybridisation between domestic dogs (*Canis lupus familiaris*) and dingoes (*C. l. dingo*) is the primary threat to the survival of the Australian dingo outside of captivity. To characterise the extent and spatial pattern of dingo-dog hybridisation throughout Australia, I obtained genotypes from 3,637 wild dogs and 88 captive domestic dogs using 23 microsatellite DNA markers. Bayesian clustering and a log of odds method were used to determine the proportion of dingo ancestry in each animal tested, with good concordance between the two methods. All regions sampled had some degree of introgression, but the extent varied substantially. Overall, 46% of the free-roaming dogs sampled were classified as pure dingoes by a Bayesian clustering method. The south-east of Australia showed a very high proportion of hybrids, with 99% of animals sampled being hybrids or feral domestic dogs. In contrast only 13% of the animals tested from central Australia were hybrids. Surprisingly, almost all wild dogs showed some dingo ancestry, indicating that either dogs raised in captivity have poor survivorship in Australian environments or that the majority of hybridisation is with roaming dogs that then return to their owners. Overall, many pure wild dingoes remain in Australia, but the speed and extent to which hybridisation has occurred in the approximately 220 years since the introduction of domestic dogs indicates that the process may soon threaten the existence of pure dingoes.

3.2 Introduction

A common consequence of the expansion of human settlements is the interaction between domestic and wild animals. Well understood sources of conflict between

these groups include predation, competition, and interbreeding (Mooney & Cleland 2001). Gene flow from domestic to wild species can be a significant threat to the integrity of wild lineages, as shown for mink (Kidd *et al.* 2009), cats (Oliveira *et al.* 2008; Randi *et al.* 2001), salmon (Hutchings 1991; Einum & Fleming 1997), and others. In many cases hybridisation is detrimental to at least one taxon because of maladaptation by disruption of adaptive gene complexes, lost reproductive opportunities, or the loss of one taxon due to asymmetry in population sizes (Rhymer & Simberloff 1996). Hybridisation may, however, be beneficial in instances where a stable hybridised population has greater fitness than either parent, or if the increase in genetic diversity expands adaptive potential. The progression of hybridisation in vulnerable populations is important to monitor because it may not be obvious in cases where the parent taxa are morphologically similar. Due to widespread introgression with domestic dogs (*Canis lupus familiaris*), Australian dingoes (*C. l. dingo*) are among the taxa that are under threat of extinction from hybridisation with an introduced domestic animal.

Dingoes are medium-sized generalist predators that have been historically or are currently present in all regions of mainland Australia. Dingoes are an ancient breed of dog that form a discrete clade within the most basal domestic dog lineage (vonHoldt *et al.* 2010), and were transported to Australia from East Asia approximately 5,000 years ago by seafarers through the south-east Asian archipelago (Gollan 1984; Savolainen *et al.* 2004). Dingoes have replaced the extinct Tasmanian tiger (*Thylacinus cynocephalus*) as Australia's mainland apex predator, and are thought to fulfil an important ecosystem role by moderating densities of native herbivores and introduced mesocarnivores (Johnson *et al.* 2007). In particular, the suppression of smaller predators such as foxes and cats might have significant beneficial effects on native biodiversity (Dickman *et al.* 2009; Johnson & VanDerWal 2009; Letnic & Koch 2010).

Domestic dogs have been present in Australia since the arrival of European settlers in 1788 (Fleming *et al.* 2001), and interbreeding between dogs and dingoes has been observed both directly and through the identification of hybrid offspring by skull morphometrics and DNA testing (Elledge *et al.* 2008; Newsome & Corbett 1982, 1985). These studies in several different regions suggest hybridisation between dogs and dingoes has been rapid and widespread across mainland

Australia. The high number of domestic dogs in Australia and the regular contact between dogs and dingoes facilitate the potential swamping of the dingo's gene pool, so that pure dingoes may soon no longer exist in the wild (Daniels & Corbett 2003). This could lead to the loss of a taxon that is unique in both its place in the ecosystem and within the history of domestication.

In addition to the potential loss of an iconic taxon, hybridisation may lead to the disruption of adaptive gene combinations and a decrease in fitness (Tufto 2010). The domestication of dogs and selection humans have imposed on them may have led to smaller brain size and skull morphology differences described by Newsome *et al.* (1980), which may reduce survival of hybrids. Alternatively, given the likely few progenitors of the dingoes extant at the arrival of Europeans (Savolainen *et al.* 2004), hybridisation may enhance survival by increasing genetic diversity (Seehausen 2004), or have no long-term impact, as natural selection evolves a new, but similar, dingo adapted to Australian environments. The effect that hybridisation may have on the behavioural ecology of dingoes and hybrids remains unexplored (discussed in Claridge & Hunt 2008; Claridge *et al.* 2010, Glen 2010), and a reliable test for the introgression of dog genes into dingo populations is required for research into the presence of these effects.

The dingo is an excellent case study in the ability to detect complicated hybridisation patterns in a large terrestrial vertebrate. Although hybridisation has also been reported in other wolf-like canids, such as between wolves (*Canis lupus* ssp.) and dogs (Randi & Lucchini 2002; Muñoz-Fuentes *et al.* 2010), and between wolves and coyotes (*Canis latrans*; Koblmüller *et al.* 2009; Wilson *et al.* 2009), it has been at low levels. The regular contact between dingoes and dogs, the presence of multiple introgression sources, the difficulty in confidently identifying pure dingoes for a genetic 'baseline' (e.g. Newsome *et al.* 1980), the relatively low level of genetic difference between dogs and dingoes (e.g. compared to interspecific hybrids), the acknowledged use of dingo genes in established domestic breeds (e.g. Australian Cattle Dog; Arnstein *et al.* 1964) and the likelihood of multiple generations of backcrossing and hybrid interbreeding are all challenges to predicting accurately the proportion of dingo ancestry in wild dogs.

Despite maps showing the suspected range of hybridisation between dingoes and other dogs (e.g. Chapter 1; Newsome & Corbett 1985; Corbett 2001; Fleming *et al.* 2001) the extent of hybridisation is currently poorly known

throughout most of their range, although it is an important consideration for both conservation and their management for livestock protection. All Australian States and Territories have legislation and policies for the management of dingoes (Fleming *et al.* 2001), yet implementation is hampered by lack of knowledge about the distribution and abundance of pure dingoes. In some regions, the identification of a wild dog as a hybrid, domestic dog or a pure dingo can mean the difference between mandated destruction and active conservation.

This study is the first continent-wide survey of the extent of hybridisation in Australian dingoes. I focus in particular on the western third of the continent, which had been little studied compared with south-eastern and central Australia. I expect (after Newsome *et al.* 1980 and Corbett 2001) the majority of south-eastern Australia to have high levels of hybridisation and central Australia to have lower levels, in line with smaller regional studies of skull morphometrics (e.g. Newsome & Corbett 1985; Woodall *et al.* 1996). I employ both Bayesian clustering and log-of-odds methods for identification of hybrids. Bayesian clustering analysis using microsatellites has been successfully used to reveal hybridisation in other canids, including wolves and coyotes in North America (Roy *et al.* 1994; Adams *et al.* 2007) and wolves and domestic dogs in Europe (e.g. Andersone *et al.* 2002; Randi & Lucchini 2002). I also evaluate the performance of these methods with a comprehensive set of simulated hybrid crosses.

3.3 Methods

3.3.1 Sample collection and laboratory analysis

Tissue samples, mostly ear tips, of approximately 5 mm^2 were collected between 2007 and 2009 from dogs culled by land managers or government-employed dog control agents, or used in behavioural research. Samples were stored at room temperature in 3 ml of lysis buffer (Longmire *et al.* 1997). Map coordinates or descriptions of the collection locality were recorded for each specimen. Specimens were obtained from every mainland Australian state, totalling 3,941 wild dogs and 92 captive dogs. The majority (n = 86) of the captive dogs were collected from Yuendumu (22.253º S, 131.801º E) in central Australia. The captive dogs were used for creation of *a posteriori* reference populations (see details below), but

were not included in other analyses because they were unlikely to have exchanged genes with the wild population.

DNA was extracted using a manual glass-fibre method (Ivanova *et al.* 2006) on 96-well plates. A 1/3 dilution of extracts was used for amplification to mitigate oversized fluorescence peaks. Twenty-four microsatellite loci were amplified in 5 multiplex PCR reactions. Primer combinations are listed in Table 3.1. PCRs were carried out in 10 µl, consisting of: 5 µl Qiagen Multiplex PCR solution (Qiagen Inc. Valencia, CA, USA), 1 µl Qiagen Q-Solution, 1 µl DNA, 0.2 µM of each primer and DNAase/RNAase-free water. PCRs were run with 15 minutes at 95 ^{o}C for polymerase activation, followed by 35 cycles of 30 s at 94 ^{o}C, 90 s at 60 ^{o}C and 60 s at 72 ^{o}C, with 30 minutes final extension at 60 ^{o}C. Fragments were run on an ABI 3730 capillary sequencer, and analysed using GeneMarker® software (SoftGenetics, LLC.). To determine the level of error in genotyping, 48 specimens were tested twice to create consensus genotypes, and the error rate calculated with the software Microchecker (Van Oosterhout *et al.* 2004).

3.3.2 'Average 3Q' tests of dingo purity

Historically, dingo hybrids were identified through analysis of skull morphology (Newsome *et al*. 1980; Newsome & Corbett 1982, 1985). However, significant limitations of this method exist, including that it cannot be used on either live or juvenile animals, that it fails to identify accurately backcrossed animals (Wilton *et al.* 1999; Daniels & Corbett 2003; Elledge *et al.* 2006), and that the dingoes initially used to differentiate from other dogs were assumed not to be hybrids based only on their remote acquisition and colour morphs (Newsome *et al.* 1980). Measurements may also be affected by non-heritable factors, such as levels of nutrition during growth (Newsome & Corbett 1982). Genetic analysis offers a more direct means to identify hybridisation. Suitable microsatellite genetic markers have been developed for the purpose of dingo hybrid testing (Wilton *et al.* 1999; Wilton 2001), and have been successfully applied on relatively small spatial scales (Elledge *et al.* 2008; Robley *et al.* 2010).

Table 3.1. Microsatellite loci used in this study. For capillary sequencing analysis the multiplexes 2/3 and 4/5 were combined into panels.

Locus	Multiplex	Reference
AHT103	1	Holmes *et al.* 1995
FH2247	1	Mellersh *et al.* 1997
m13c19	1	A. Wilton, unpublished
FH2257	1	Mellersh *et al.* 1997
CXX434	1	Ostrander *et al.* 1993
CXX460	1	Ostrander *et al.* 1995
FH2199	1	Francisco *et al.* 1996
AHT109	2	Holmes *et al.* 1993
m13tt	2	A. Wilton, unpublished
FH2313	2	Mellersh *et al.* 1997
CXX30	2	Ostrander *et al.* 1993
CXX109	3	Ostrander *et al.* 1993
FH2079	3	Francisco *et al.* 1996
CXX410	3	Ostrander *et al.* 1995
CXX402	3	Ostrander *et al.* 1993
CPH2	4	Fredholm & Winterø 1995
AHT125	4	Holmes *et al.* 1993
CXX406	4	Ostrander *et al.* 1993
LEI008	4	Mellersh *et al.* 1994
FH2346	5	Mellersh *et al.* 1997
FH2293	5	Mellersh *et al.* 1997
VIAS-D10	5	Primmer & Matthews 1993
FH2138	5	Francisco *et al.* 1996

All specimens were analysed for dingo purity with the 'average 3Q' method of Wilton (2001). Categorisation of dingo ancestry relied on a reference sample set of allele frequencies and private alleles from 90 dogs and 60 dingoes considered pure from a known breeding history, skull morphometric testing, or appearance. New genotypes were compared to the respective dingo and dog allele frequencies to establish the relative probability that a dog is from pure dingo ancestry and not that of a ¾ dingo. This comparison provides an 'average 3Q score', the log of the probability ratio ($\log(P_{dingo}/P_{3/4\ dingo})$) (Elledge *et al.* 2008; Wilton *et al.* 1999). The probability was then averaged across loci for comparison across samples with

different numbers of successfully scored loci. Other hybrid crosses were further inferred from the dog and dingo reference alleles, e.g. a 50% dingo DNA profile was created by averaging the frequency from dogs and dingoes for each allele (described below). Individuals were then assigned to a category most likely to represent their percentage of dingo ancestry.

The presence of alleles diagnostic for dog ancestry ('doglike' alleles) was used in conjunction with the 3Q scores to assign individuals to one of 7 categories. The categories used were: (1) Pure dingo (3Q score > 0.1, no doglike alleles); (2) probable pure dingo (0.05 < 3Q < 0.1, no doglike alleles); (3) probable hybrid, >75% dingo (0 < 3Q < 0.05); (4) 65-75% dingo (-0.1 < 3Q < 0); (5) 50-65% dingo (-0.25 < 3Q < -0.1); (6) less than 50% dingo (-0.5 < 3Q < -0.25) and; (7) domestic dog (3Q < -0.5). As the 90 dogs and 60 dingoes used for reference in this method are required for the development of the test, I refer to them as '*a priori*' reference populations.

The fragment analysis equipment used in this study was different from that used to analyse the fragment data from the original reference groups. Because different equipment and choice of fluorophores for each locus can cause size differences in microsatellite data (Pasqualotto *et al.* 2007), I adjusted microsatellite sizes to match the reference groups of Wilton (2001) using a combination of running 20 samples on both fragment analysers to compare size differences, and matching the pattern of allele frequencies between the data sets. If a locus could not be matched to the *a priori* reference group (e.g. inconsistent sizes when both samples were run on each fragment analyser) it was discarded.

3.3.3 Bayesian tests of dingo purity

The reference sample-based method of Wilton (2001) has been criticised because of uncertainty whether dingo reference samples are genuinely free of domestic dog DNA (Daniels & Corbett 2003). While the level of such impurities is unlikely to be high because large allele frequency differences exist between the reference groups, it may still bias the results by under-estimating the amount of dog ancestry in hybrids. A further potential problem is that the dingo reference samples may not adequately represent the extent of genetic diversity throughout the Australian continent (Elledge *et al.* 2006; Bray *et al.* 2009). An alternative method less reliant

on *a priori* reference samples is the use of a model-based clustering algorithm that seeks to identify long-interbreeding clusters of individuals. Such methods are widely used to detect population structure, but have also been applied to characterise admixture, e.g. between domestic cats and wildcats in Europe (Beaumont *et al.* 2001).

I used the program Structure (v2.3.3; Pritchard *et al.* 2000) to determine the contribution of dingo and domestic dog ancestry to each individual's genome. Structure clusters individuals in order to maximise conformance to Hardy-Weinberg and linkage equilibria, thereby identifying clusters of interbreeding individuals, and attributes all or part of each individual's genome to a pre-defined, but adjustable, number of clusters (Pritchard *et al.* 2000). Each analysis performed in this study was run for 200,000 iterations with 20,000 burn-in runs, which was sufficient for the parameters to reach convergence, using the admixture and correlated allele frequency models (Falush *et al.* 2003).

The Bayesian framework underlying Structure permits prior identity information, such as the sampling location, to assist in modelling. Running Structure with no prior information caused confounding of clusters between geographical variation and hybridity (data not shown). To refine the clustering process, I employed a 'learning samples' approach (Pritchard *et al.* 2010), where I identified *a posteriori* dog and dingo reference samples to establish two reference clusters, which provide allele frequency estimates to assign the unknown samples to either cluster. These reference individuals were sampled for this study and were different individuals from those used by Wilton (2001). First, all specimens identified by the average 3Q method as category 1 (pure dingo; n = 361) or 7 (domestic dog; n = 113, including the 96 Yuendumu dogs and 27 dogs caught during the study) were separated from the rest of the sample. Principal coordinates analysis (PCoA) was then used to identify the dogs and dingoes most genetically distinct from each other and therefore least likely to be introgressed. The PCoA was performed on a genetic distance matrix (described in Smouse & Peakall 1999) between all individuals in categories 1 and 7, as implemented in Genalex v6.4 (Peakall & Smouse 2006). Although the *a posteriori* domestic dog reference sample is not representative of all dog breeds, it is likely to represent the types of breeds and crossbreeds that come into contact with dingoes, which is

more suitable for the purpose of this study. The samples were also screened with Structure for the level of Q-assignment to each of the dingo and dog 'parent' populations, and only samples with ≥ 95% assignment to the appropriate population were retained for use in the reference population.

The individuals in the *a posteriori* reference groups were then added to Structure with the USEPOPINFO flag turned on and updating allele frequencies from the reference populations. Structure was re-run with all specimens (with the USEPOPINFO flag turned off for all non-reference specimens) with the cluster number of k = 2 .These settings ensure only the allelic information from the *a posteriori* reference groups are used to assign the remainder of the sample, and does not use data from the non-reference samples to inform the model.

3.3.4 Additional analyses

Heterozygosity (H_o and H_e) statistics and the number of alleles were also calculated from the *a posteriori* reference groups to determine any differences in genetic diversity between the dogs and dingoes with Arlequin v3.5 (Excoffier & Lischer 2010). Related samples Wilcoxon signed-rank tests were used to determine if the difference between groups was significant, implemented using PASW statistics 18 for Windows (SPSS Inc.).

3.3.5 Spatial patterns of dingo purity

Results of purity tests were geographically mapped using ArcGIS 9.3 (ESRI Inc., Redlands, CA, USA), including ordinary kriging analysis to interpolate to areas not sampled and to display multiple specimens that were sampled at the same site. A prediction map was generated, using the mean purity results when there were multiple points at the same location. The spherical model in ArcGIS was used with between 2 and 50 neighbours included. Dogs sampled in captivity were not included in the interpolation.

3.3.6 Simulations of hybrid populations

Because the true ancestry of wild animals is unknown, and therefore the accuracy of the reference assignments and clusters difficult to evaluate, I simulated hybrid crosses with the program Hybridlab (Nielsen *et al.* 2006) to test the performance

of both methods in detecting various hybrid and backcrossed individuals. Fifty randomly selected individuals from each of the *a posteriori* reference samples were used as the 'parent' populations, and all simulated hybrid groups also contained 50 individuals. The crosses (detailed in Table 3.2) were then analysed with the clustering and average 3Q methods. The settings in Structure to analyse the simulated data were the same as described above for the real data, with all the *a posteriori* reference samples that were not used in the simulation included as learning samples. The means and 95% confidence intervals of each cross were calculated, where I used the estimate of the proportion of ancestry in the dingo cluster for the clustering method, and the average 3Q score was used for the average 3Q method. Both were then compared to the theoretically expected content of dingo ancestry (Table 3.2).

Table 3.2. Simulated hybrid groups generated from *a posteriori* dingo and dog reference populations (F_0 dingoes and F_0 dogs). Each simulated group contained 50 individuals. Crosses were generated between the groups listed in the columns 'Parent 1' and Parent 2'. The numbers in parentheses for the F_3 and F_4 hybrids refer to the crosses in previous generations, as identified from the first column (No.). The expected inheritance of ancestry is averaged (and therefore approximate) in the F_2 – F_4 generations (crosses 2-10) due to independent assortment of chromosomes.

No.	Generation	Parent 1	Parent 2	Expected dingo DNA (%)
1	F_1	F_0 dingo	F_0 dog	50
2	F_2	(1)	F_0 dingo	~75
3	F_2	(1)	F_0 dog	~25
4	F_2	(1)	(1)	~50
5	F_3	F_0 dingo	(2)	~87.5
6	F_3	(2)	(1)	~62.5
7	F_3	(1)	(3)	~37.5
8	F_4	(6)	(7)	~50
9	F_4	F_0 dingo	(5)	~93.75
10	F_4	F_0 dog	(7)	~18.75

3.4 Results

3.4.1 Distribution of samples

After the removal of specimens without spatial coordinates, those with fewer than 14 loci successfully amplified, and those with evidence of sporadic contamination (more than 3 alleles at a locus for more than 4 loci), 3,632 wild dog specimens and 88 captive dogs were available for analysis (Fig. 3.1). Locus FH2175 was removed from the data set because it could not be reliably scored due to an inconsistent repeat pattern. Locus FH2293 was not used in average 3Q method tests because it could not be consistently matched to the original *a priori* reference population alleles. The number of loci used was therefore 22 for the average 3Q method tests and 23 for the clustering analysis. The error rate determined from the replicated genotyping was 0.000 (negligible).

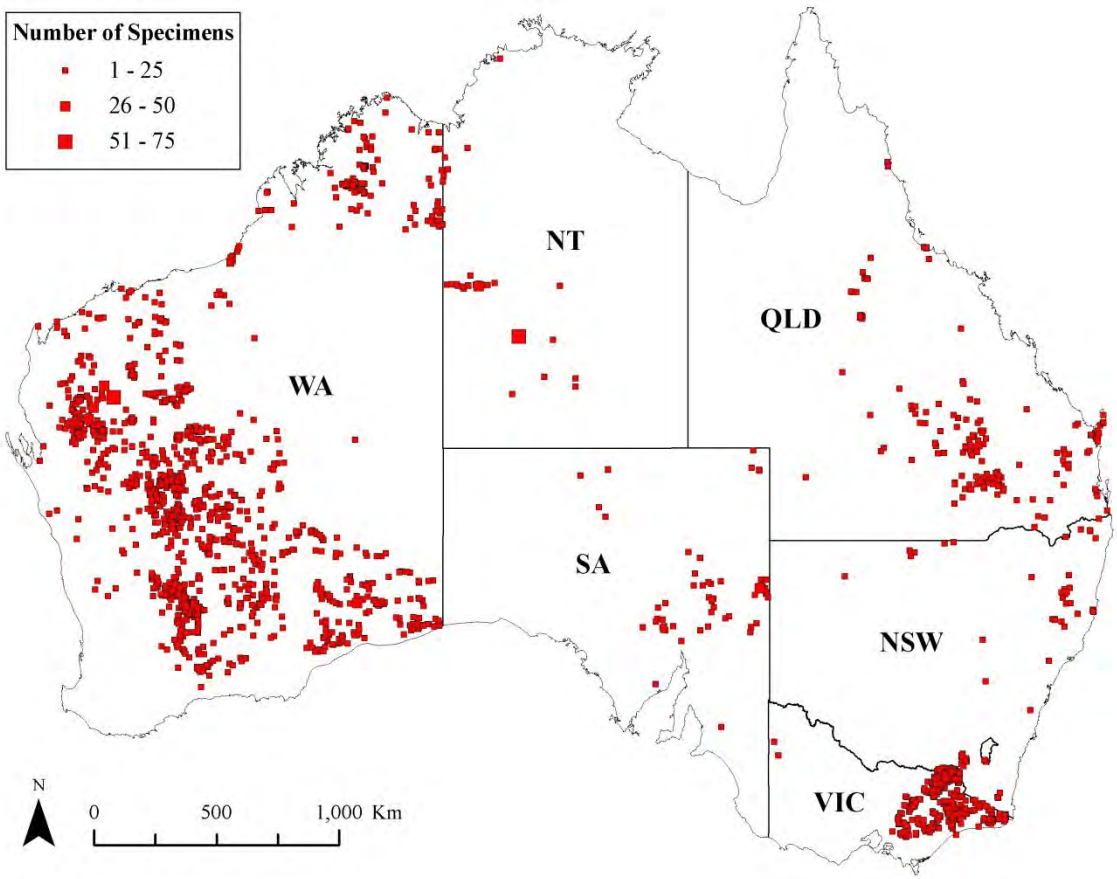

Fig. 3.1. Locations of specimens collected for this project, including only those with a successfully determined genotype. The states and territory included are Western Australia (WA, n = 2,284), Northern Territory (NT, n = 128), South Australia (SA, n = 148), Queensland (QLD, n = 356), New South Wales (NSW, n = 95) and Victoria (VIC, n = 626). Specimens collected in the Australian Capital Territory were classified as 'NSW'.

3.4.2 Construction of a posteriori *reference groups*

PCoA revealed that the specimens identified with the average 3Q method as being pure dingo formed a single, well-defined cluster (Fig 3.2). The horizontal outlier from the dingo group in Fig. 3.2 (x = -0.19) is probably separated from the other dingo specimens because only 15 loci were successfully typed for this individual. Specimens with a coordinate 1 score of > 0.15 were used as a preliminary group of reference 'pure' dingoes. Individuals with PCoA scores < -0.5 were used as the *a posteriori* reference population for domestic dogs in construction of simulated hybrid crosses as they were most dissimilar to pure dingoes. This population consisted of 86 captive animals and 16 dogs caught in the wild, from Western Australia (10 specimens), Victoria (5) and Queensland (1), which clustered with the other domestic dogs in the PCoA. The dingo reference group contained 240 samples from WA, 23 from QLD, 14 from SA, 1 from NSW and 44 from NT (Fig. 3.2 inset). After specimens with < 0.95 population assignment in Structure were removed, the final sample numbers were n = 322 for the dingo reference group, and n = 102 for the dog group.

The expected and observed heterozygosities and the mean number of alleles per locus from the *a posteriori* reference groups were all significantly lower in the dingo than the dog group (Wilcoxon sign rank test, $P < 0.001$ for all tests). H_e, H_o and mean number of alleles for dingoes were 0.47 (± 0.34 S.D.), 0.38 (± 0.28) and 9.23 (± 7.89) respectively, and for dogs were 0.76 (± 0.19), 0.69 (± 0.17) and 11.78 (± 7.93).

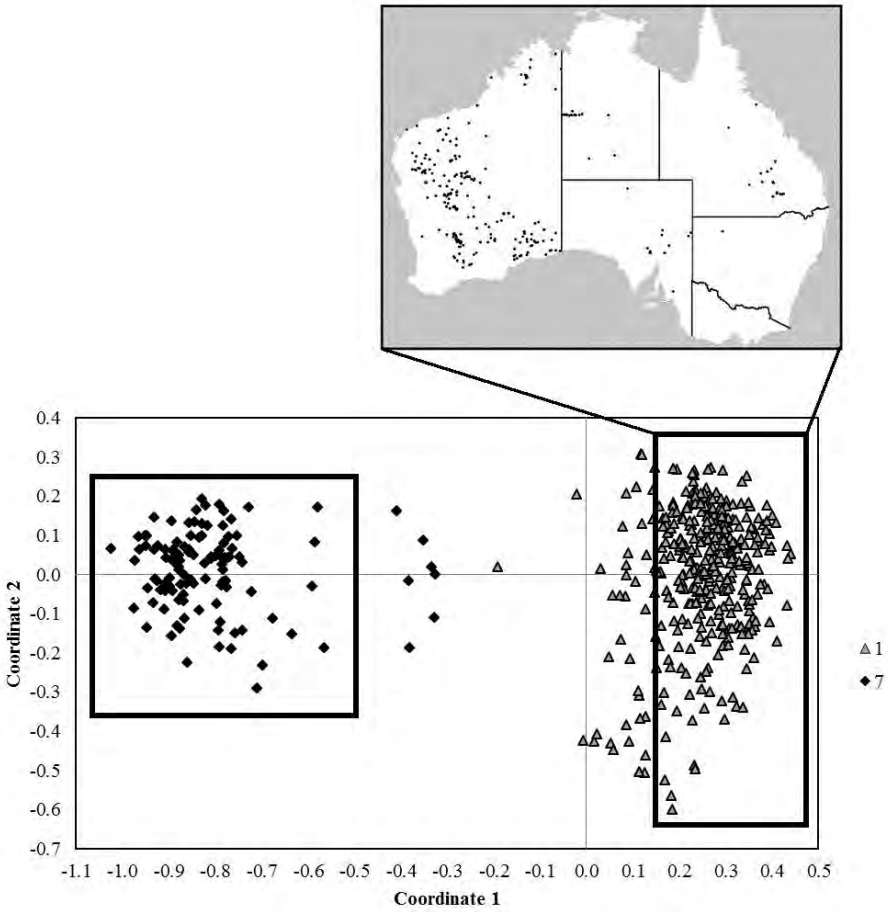

Fig. 3.2. Principal coordinates analysis of specimens identified as being either 'pure dingo' (category 1) or 'domestic dog' (7) from the average 3Q method results. Clusters enclosed by rectangles were used for the *a posteriori* reference dingo (coordinate 1 > 0.15) and domestic dog (coordinate 1 < -0.5) groups. The horizontal dimension accounts for 66.2% of the variation, and an additional 8.1% is explained by the vertical dimension. Inset map shows the geographic distribution of dingo reference specimens (• = one specimen).

3.4.3 Hybridisation levels and spatial distribution of dingoes

Using the average 3Q method, 1,695 (47%) of the wild dogs were 'pure' dingoes (category 1 or 2). Analysis using the clustering method showed 1,664 specimens with cluster assignment ≥ 0.90 for dingo ancestry (46% of the sample) (Fig. 3.3). Assignment of ≥ 0.90 was chosen as an arbitrary cut-off for the classification of 'pure' dingoes using the Bayesian method. The clustering method showed equal or fewer pure dingoes in all states except WA than the average 3Q method (Fig 3.3(b) and (d)), but hybrids from the former results generally were assigned a higher percentage of dingo ancestry. The highest proportion of pure dingoes was found in the Northern Territory (88%), with intermediate proportions in WA, SA and QLD,

but very few dingoes were detected in NSW and VIC (1% in each of these states according to the clustering method).

3.4.4 Performance of clustering methods using simulated hybrids

Both methods showed strong agreement between the expected purity scores and the average purity of individuals that was identified from analysis (Fig. 3.4). The maximum deviation from the expected mean was 2.5% for the Bayesian clustering method, on cross 6 (mean deviation over all crosses was 0.95%). All of the expected values were within the 95% CI of observed results for both methods. Between 2% and 58% of the individuals within each simulated cross were incorrectly classified using the *a priori* method (outside of the ranges shown in Fig. 3.4a; $\bar{x}$ = 26%, SD=21.35). Using the *a posteriori* methods 2-26% were outside of the expected value ± 10% ($\bar{x}$ = 13.67%, SD=9.05). The percentages of misclassifications were roughly equal above and below the range (6.7% under and 8% over).

3.5 Discussion

3.5.1 Distribution of dingoes and hybrids

This study is the first range-wide assessment of hybridisation between dingoes and domestic dogs. The ubiquity of domestic dogs in human settlements has provided many contact points where introgression with dingoes has occurred.

Hybrids are most prevalent in coastal areas (Fig. 3.3), which have the longest duration and highest density of human settlement, and hence more opportunities for contact between dogs and dingoes. These densely populated areas also have the most intensive culling programs, which might disrupt dingo social structure (Wallach *et al.* 2009) and therefore facilitate hybridisation. Although dingoes remain in relatively high proportions in the central and western parts of the Australian continent, no area is free from hybrids. This finding places the pattern of hybridisation in the most extreme category under the scheme of Allendorf *et al.* (2001) (type 6: anthropogenically mediated complete admixture).

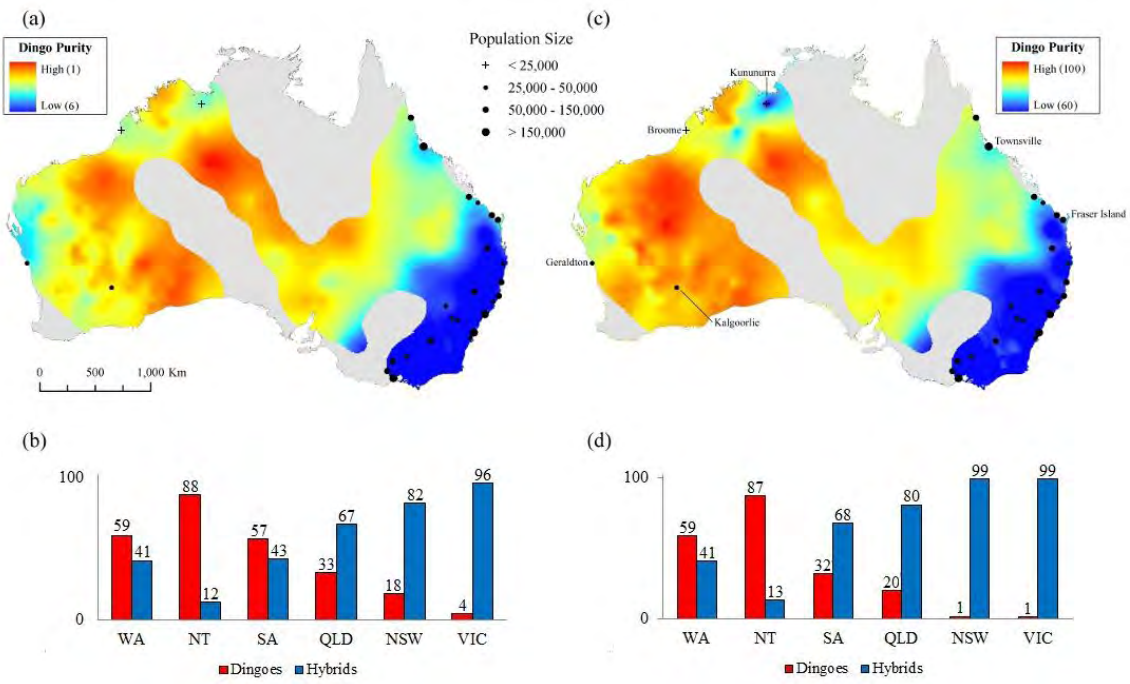

Fig. 3.3. Ordinary kriging of purity results and proportions of dingoes and hybrids by state. State abbreviations are described in Fig 3.1. For kriging maps red-yellow patches represent areas with a high level of dingo purity, blue regions contain individuals with lower purity categories. Unsampled areas are shown in grey. (a) Average 3Q method results with categories 1-7 interpolated. Major towns (>25,000 residents, excluding capital cities) and their population sizes are shown for association with hybridisation. Two smaller towns (+; Broome and Kununurra) are also shown because they occur near higher levels of hybridisation than surrounding regions. (b) Percentage of dingoes and hybrids in each Australian state, based on results from the average 3Q method purity tests: dingoes are categories 1 and 2, hybrids categories 3-7. (c) Ordinary kriging on the percentage of dingo ancestry from the clustering method results. Results below 60% dingo ancestry are grouped as few specimens were less than approximately half dingo. Towns referenced in the discussion are labelled. (d) Percentage of dingoes and hybrids in each state as determined by the clustering method. Dingoes are defined as specimens with ≥0.90 Q-assignment to the dingo cluster.

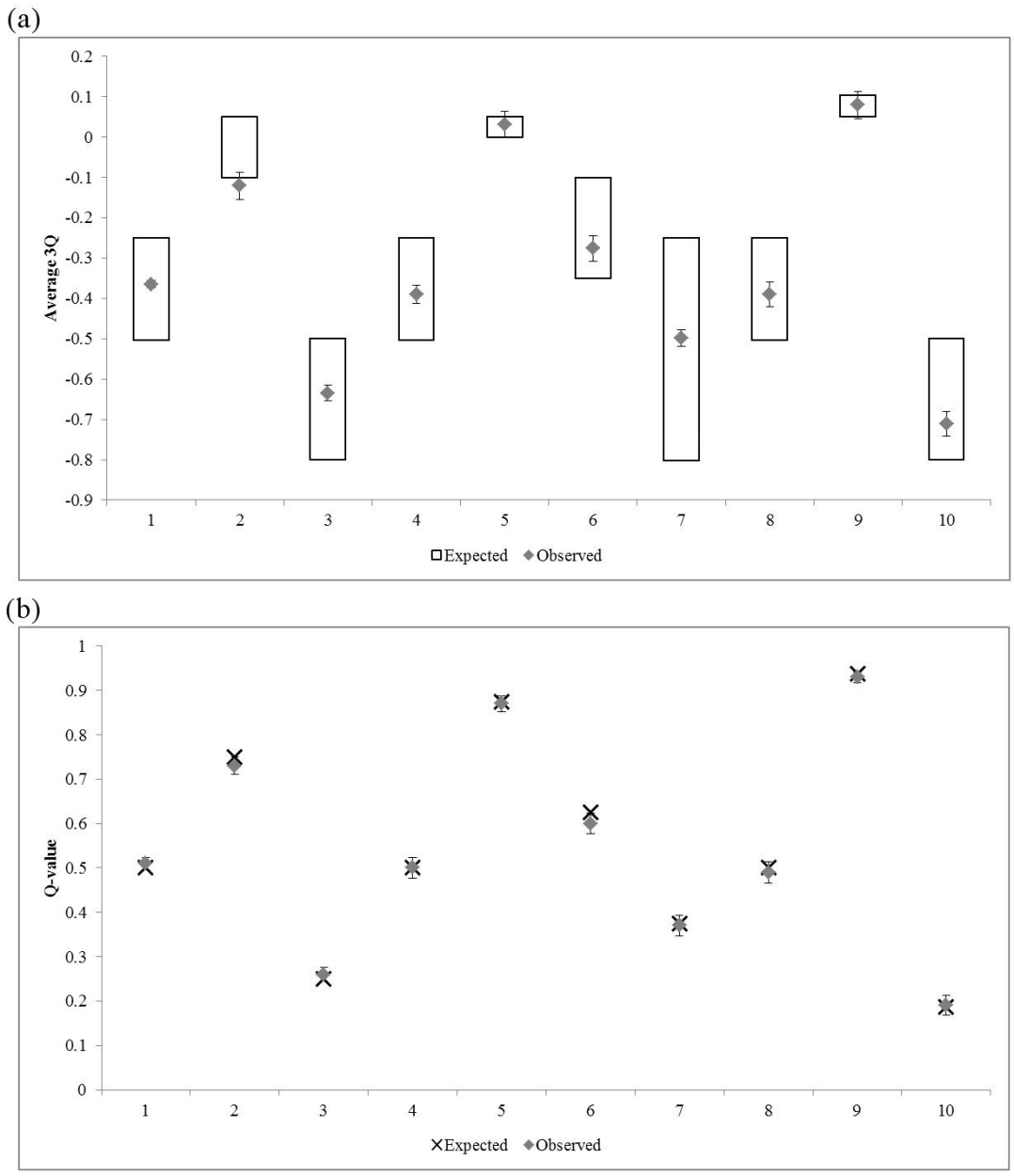

Fig. 3.4. Comparison of expected component of dingo ancestry from simulated data and the results from both the reference and clustering methods. The x-axis values are the simulated hybrid types 1-10 as classified in Table 3.2. Error bars show 95% confidence intervals. (a) Results from the average 3Q method categories. Each box for the 'expected' values shows the range of the average 3Q score for the appropriate reference category. The expected values for crosses 2 and 7 cover two categories as the expected value is in between them. (b) Clustering results showing the percentage of dingo ancestry for each cross.

The pattern of hybridisation found in this study is in agreement with previous morphometrics studies (e.g. Newsome & Corbett 1985; Woodall *et al.* 1996; Corbett 2001), but provides much more detail. This is particularly the case

in Western Australia, where only one study has been reported, which found 74% pure dingoes in the Pilbara region (Corbett 2001), and in South Australia, where no studies have been published. The trend to hybridisation in coastal areas is shown on the west coast, but to a less extreme degree to that found in the southeast. Hybridisation around the town of Kununurra in the northwest (Fig. 3.3) was influenced by the collection of free-roaming domestic dogs in this area. The areas north of Geraldton, around Broome, around Townsville in Qld and, for the average 3Q method, inland of Kalgoorlie were also associated with a localised increase in hybridisation. Although the north-central areas in the Northern Territory contained mostly pure dingoes, the specimens were mostly sourced near a gold mine and associated accommodation, so the actual purity level may be higher in the more remote areas.

Southeastern Australia has a much higher proportion of hybrids than the rest of the continent, which probably reflects the progression of dense European settlement (Fleming *et al.* 2001). A high incidence of hybrids in this area has been reported previously (ranging from 23% pure dingoes on the south coast of New South Wales to 65% dingoes in the Victorian highlands using the morphometric method; Newsome & Corbett 1985), but not at the level found in this study (1-4% pure dingoes; Fig. 3.3). Some of the discrepancy between the findings would be caused by differences in methodology or sampling intensity, but given the magnitude of the difference, the proportion of hybrids has certainly increased since Newsome & Corbett (1985) collected skulls from this area in the 1960s and 1970s. The question of dingo purity is particularly relevant to the state of Victoria, as the dingo has recently been declared a threatened species, with requirements for its protection (Anonymous 2007; Glen 2010). Efforts for dingo conservation in Victoria would therefore require a comprehensive program to address the process of hybridisation wherever pure dingoes are found. Because of the ubiquity of hybrids, such a program would require isolation of extant dingo populations with exclusion fencing and a trap-hold-test -and cull/release process for all wild dogs in a designated dingo reserve. Reserve size would be dependent on information on group dog home range size (e.g. Robley *et al.* 2010), shape and resource distribution (e.g. Claridge et al. 2009).

Relative to studies of hybridisation between wolves and dogs, the high prevalence of introgression found in dingoes may be due to their semi-domesticated history causing a decreased wariness of human settlements, their concentration around livestock or artificial watering points on grazing land containing working dogs, or due to a higher population density of dingoes than wolves (Koblmüller *et al.* 2009; Muñoz-Fuentes *et al.* 2010). The disruption of dingo social groups and decrease in population densities caused by culling may also increase the likelihood that a dingo will encounter, and mate with, a domestic dog before they encounter another dingo (Corbett 2001; also discussed with respect to wolves in Vilà *et al.* 2003).

3.5.2 Distribution of feral domestic dogs

The low incidence of purely domestic dogs in wild populations (<1% of all specimens) suggests that they have poor survivorship in the wild. The most likely mating between dingoes and dogs is generally considered to be between a female dingo and a male dog, due to the difficulty of a domestic bitch raising a litter outside of captivity (Corbett 2001). The hybrid offspring of captive dogs and dingoes may be better able to survive and integrate into dingo societies than domestic dogs raised in captivity, which would have little experience hunting or socialising with dingoes (Vilà & Wayne 1999; Daniels & Corbett 2003).

As populations of feral domestic dogs have not been reported in Australia (Jones 2009; this study), the extent of hybridisation is caused either by historical introgression or an ongoing influx of genes from roaming domestic pets or wild bitches visiting domestic dogs and subsequent interbreeding of hybrids. The presence of mostly hybrids with few representatives from either of the parent taxa suggests that wild dogs in some areas may be approaching the status of a stable hybrid swarm, where the majority of individuals are the product of multiple generations of hybrid crosses and backcrosses (Rhymer & Simberloff 1996; Allendorf *et al.* 2001). Ongoing monitoring of the proportion of hybrids in these areas, including much of southeast Australia, could confirm whether this is the case.

3.5.3 Performance of the methods against simulated data

Both of the purity testing methods identified a variety of simulated hybrid crosses with high accuracy (Fig. 3.4). The minimal bias exhibited by the clustering method provides encouragement it can measure dingo purity within a few per cent of the true value. For both methods the simulation provide a conservative measure of accuracy, as in the clustering method 100 of the specimens were removed from the *a posteriori* reference groups to create the simulated hybrids, and in the average 3Q method only the 3Q score was considered, not the inclusion of doglike alleles. This also affects the calculation of misclassification rates, which are an underestimate of the true likelihood of misclassification, but provide a useful comparison between the methods. Although the clustering method still relies on reference samples, as criticised by Daniels and Corbett (2003), the samples used were selected due to their distinctness by 3 sequentially applied tests (the average 3Q method, PCoA and Structure Q-assignment), rather than prior assumptions of purity.

The *a posteriori* dingo reference group used in this study is the most geographically diverse for testing purity to date (Fig 3.2); the references used to develop canonical skull scores were selected from central Australia (Newsome *et al.*1980; Newsome & Corbett 1982), and the *a priori* dingo reference group is mostly from eastern Australia. It should be noted, however, that despite a western continent bias in the *a posteriori* reference groups, and an eastern continent bias in the *a priori* groups, the patterns of hybridisation and total number of pure dingoes found are similar, possibly due to the low genetic diversity within dingoes across Australia, as reported by Savolainen *et al.* (2004). Despite this, the impact of founder effects and genetic drift on isolated areas such as Fraser Island, where entry of domestic dogs is prohibited to maintain the purity of its dingoes, should be considered when using any reference method, as the effects of regional allelic variation and hybridisation may otherwise be confounded. Although Woodall *et al.* (1996) found that 17% of Fraser Island wild dogs were hybrids using skull morphometrics, this method also relies on comparison to a reference group and could therefore show confounding between regional variation and proportion of admixture.

The DNA-based methods presented here provide a considerable advance in precision and versatility relative to morphometric methods, due to their ability to test juveniles and live animals, detect backcrossed hybrids and for the clustering method to give a precise percentage of dingo ancestry. This study also demonstrates the ability to apply the DNA testing of hybrid individuals on a large scale; it would have been unfeasible to measure the number of skull samples equal to the number of tissue specimens used in this study.

3.5.4 Implications for the management of wild dogs

Like wolves in Europe and North America, the dingo retains a unique status as both an icon of the natural world and an agricultural pest. There are cultural considerations surrounding the loss of pure dingoes, including their value to indigenous Australian cultures, tourism, and the perception by many Australians that they are part of the native fauna (Fleming *et al.* 2001). Conflict between cultural and community attitudes, and management targets (e.g. culling for livestock protection), can be more productively addressed when the true status of wild dog populations is known.

The impact of dingo hybridisation on the dynamics of ecosystems could be informed by this study and the application of purity testing. The suppression of fox and cat activity by dingoes has been the focus of particular attention (e.g. Johnson *et al.* 2007; Dickman *et al.* 2009; Letnic & Koch 2010), as these mesopredators are a key threat to the survival of many native fauna species. As a result of this mesopredator release research, it has been suggested that dingoes be reintroduced into the wild to protect small native mammal species, and farmers compensated for any losses to stock that occur as a result (Glen *et al.* 2007; Dickman *et al.* 2009). An important aspect to predicting the success of these reintroductions is the effect that hybridisation will have in changing the ecology of wild dogs, if any.

A decision on whether some degree of mixing between dogs and dingoes will affect conservation goals, and whether it is possible or necessary to attempt to retain historical patterns of genetic diversity, should be made prior to the development of management strategies (Daniels & Corbett 2003; Claridge & Hunt 2008; Rutledge *et al.* 2010). This question has also been raised for the preservation of wolves in the Great Lakes Region of North America, as pre-European admixture

between grey wolves and Great Lakes wolves has been found (Schwartz & Vucetich 2009; Wheeldon & White 2009). The answer to this question for dingoes is somewhat dependent on whether admixture has affected their fitness or adaptive potential. Savolainen *et al.* (2004) detected evidence that a severe bottleneck occurred in dingoes upon their arrival in Australia, to the extent that perhaps only one family had arrived. Significantly lower genetic diversity in dingoes than dogs was also found in this study, on all measures tested, and by Wilton (1999). Breeding with dogs, and the associated increase in genetic diversity, may be beneficial to dingoes' ability to adapt, but whether this is the case requires careful testing (Barton 2001; Yuri *et al.* 2009). However, the high number of hybrids, including backcrosses, found in this study supports the capacity of hybrids to survive and breed successfully in the wild. Maladaptation or wasted reproductive opportunities therefore do not appear to be major issues for dingo-dog hybridisation, but whether traits beneficial to wild dogs but undesirable for humans and livestock will emerge remains unknown.

The rapid hybridisation between dingoes and domestic dogs at a continental scale illustrates the importance of establishing baseline genetic data for taxa in the early stages of admixture, if genetic swamping is likely to become a problem in the future. Once hybridisation has become widespread it becomes more difficult to reconstruct what constitutes a 'pure' wild animal, although this study shows that methods can be developed if a sufficient sample size is available. Additionally, some genetic diversity within the 'pure' population may be lost by the almost complete admixture in certain regions of the animal's range, such as appears to be the case in south-eastern Australia. Because the extent of hybridisation in dingoes is far greater than that of wolves and dogs in Europe (Randi & Lucchini 2002) or North America (Muñoz-Fuentes *et al.* 2010) the study of dingoes provides a unique opportunity to test the impact of large-scale hybridisation on an ecologically significant top-order predator.

3.5.5 Conclusions and applications

Both of the purity testing methods revealed very similar patterns of the distribution of dingoes (Fig. 3.3), showing hybridisation mainly concentrated around the more densely inhabited coastal areas and settlements. This supports

previous assertions that duration and the density of settlement since 1788 are strong predictors of dingo hybridisation levels (Newsome & Corbett 1985; Woodall *et al.* 1996). The low genetic diversity found in dingoes may indicate that conservation efforts would be better targeted at the more remote regions or islands where successful isolation of dingoes and dogs is more probable, as minimal genetic diversity would be lost overall. A study of whether dingoes exhibit population structure between regions would assist in establishing if this is an appropriate strategy.

The extent and pace of hybridisation found in this study means that preventing gene flow between dogs and dingoes requires concerted efforts (Fleming *et al.* 2001). There are three main areas where a better understanding of the pattern of hybridisation has advantages in wildlife management: establishing the roles of dingoes and hybrids within ecosystems; understanding the effect that hybridisation may have on livestock predation; and providing evidence for the formulation of dingo conservation policy encompassing social attitudes and values. Changes in body size, prey preference and pack structure through the hybridisation process could all have great ramifications for the status quo of ecosystems across the Australian continent and require investigation before the dingo is lost.

3.6 Acknowledgements

Over 280 people contributed DNA samples for this study. A. Woolnough, B. Davies, T. Thompson and Y. Hitchen coordinated and encouraged sampling in Western Australia, as well as providing support and feedback throughout the project. Y. Hitchen also provided valuable laboratory assistance. P. Thomson and K. Rose provided feedback on project design and shared their extensive knowledge of dingo ecology. T. Newsome and G. Ballard provided the Yuendumu dog samples used in the dog reference population and assisted with discussions on project design. Phil Goulding and Mike Nunweek provided advice on GIS analysis.

Chapter 4

Human developments impact upon the ecology of dingoes in the Tanami Desert, Australia

4.1 Abstract

Dingoes (*Canis lupus dingo*) are a naturalised component of Australian ecosystems and the top predators since the extinction of the Tasmanian tiger (*Thylacinus cynocephalus*). Provision of extra water and food resources since European settlement of Australia has probably allowed overall dingo numbers to increase, but the ubiquity of domestic dogs with human settlements has caused extensive hybridisation, to the degree that the survival of pure dingoes is threatened. Central Australia, owing to its isolation, has retained a high proportion of dingoes, but human activities within the region may compromise these populations. Here I analyse microsatellite DNA variation to examine the differences in spatial pattern of dingo-dog hybridisation and gene flow within and adjacent to an active gold mining operation in the Tanami Desert, central Australia. Wild dogs were more abundant around the mine than surrounding undeveloped areas, and particularly around a rubbish disposal facility. Analysis of relatedness among the local wild dog populations indicated that many of the individuals around the mine were closely related, suggesting an increase in breeding rather than immigration was the main cause of differences in abundance. Tests of dingo purity suggested a higher proportion of hybrids at the mine sites (14% hybrids) than at undeveloped sites (3%), although this difference was not statistically significant. Sterilisation and increased supervision of domestic dogs, more careful management of waste, and ongoing monitoring of the wild populations are recommended to conserve dingoes and manage overpopulation.

4.2 Introduction

Dingoes (*Canis lupus dingo*) are generalist predators with adaptable foraging tactics. They are present in diverse habitats across Australia, and were able to colonise arid and monsoon-arid central Australia soon after their arrival from Asia approximately 5,000 ya (Corbett 2001; Fleming *et al.* 2001). The current

population of dingoes is intermixed with hybrids from dingo-domestic dog crosses (collectively 'wild dogs'), although higher proportions of pure dingoes are found in central Australia (Chapter 3). The central desert regions are therefore of particular interest in understanding wild dog ecology, because pure dingoes living in a relatively undisturbed state are juxtaposed with isolated pockets of human settlements, which affect dingoes through the introduction of domestic dogs (*C. l. familiaris*) and by increasing availability of food and water. Understanding the magnitude and extent of these effects is important for effective management of these unique populations.

Studies of foxes (*Vulpes vulpes*; Bino *et al.* 2010) and coyotes (*C. latrans*; Fedriani *et al.* 2001) have demonstrated that anthropogenic food sources support higher densities of canids than nearby unmodified landscapes. Experimental removal of anthropogenic resources resulted in a rapid decline in fox survivorship and a shift in home range away from the resources, providing strong evidence for a direct effect of human activity on canid behaviour (Bino *et al.* 2010). Smaller home ranges are also associated with higher resource availability in wild dogs (Thomson 1992c), although this correlation was found in naturally flush seasons and areas rather than as by-products of human settlements. Jackals (*C. aureus*) utilising resources from villages with poor sanitation and accessible fruit crops in Israel also showed a decrease in home range size when compared with nearby 'pristine' land (Rotem *et al.* 2011).

The Tanami Desert is a sandy, arid region in the central-north of Australia. Europeans have been active at various stages in the Tanami since the early 1900s (Gibson 1986; Baume 1994), and this area currently contains sparse and highly localized occurrences of human-associated resources around mines sites. Rubbish mounds in particular can provide an abundance of food for wild dogs. The Tanami mining sites provide an opportunity to investigate whether access to supplementary resources could also cause a shift in wild dog breeding behaviour, thus leading to abnormal increases in the population.

Alteration of social structure is a potential mechanism for affecting gene flow and population demographics of wild dogs, specifically population size and interbreeding between dingoes and domestic dogs (Allen & Gonzalez 1998; Elledge *et al.* 2006; Glen *et al.* 2007; Wallach *et al.* 2009). In regions with scarce or

unpredictable resource availability, such as unmodified areas of central Australia, wild dogs are generally found in stable social groups ('packs') to improve the success of hunting (Thomson 1992c; Corbett 2001). Packs of dogs have been observed limiting the number of offspring born within the group by aggressive prevention of mating between socially subordinate individuals, or infanticide if pups are born to dogs other than the dominant breeding pair (Corbett 1988; Corbett 2001). The pack structure is also hypothesised to limit interbreeding between dingoes and domestic dogs because the packs are hostile towards strangers and defend territories against them (Elledge *et al.* 2006). The absence of domestic dogs found in the wild supports the hypothesis that domestic dogs are unlikely to integrate into dingo societies, and interbreeding may occur most commonly between a lone dingo bitch and a free-roaming pet or working dog (Chapter 3). The provision of an unnaturally high volume and stability of food, such as the Tanami mine sites, may result in the disintegration of wild dog packs if individual foraging is more beneficial. Such a response can remove these limitations to reproduction and cause increases in fecundity, with more individuals producing pups for the same amount of adults.

As hybridisation with domestic dogs threatens the survival of the Australian dingo, identifying areas where pure dingoes remain becomes increasingly important. Surveys of dingo purity have revealed that central and northern Australia contains a high proportion of pure dingoes (Chapter 3; Newsome *et al.* 1980; Newsome & Corbett 1982; 1985). Analysis of skull morphology in remote and settled areas of inland central Australia has found 97.5% pure dingoes (Newsome *et al.* 1980; Newsome & Corbett 1982; 1985), and genetic analysis in Chapter 3 designated 88% of individuals in the Northern Territory as pure dingoes. Because many of the wild dogs sampled for the genetic analysis in Chapter 3 were collected near a mining site with considerably greater human activity than the surrounding regions, it is important to examine the impact that the mine may be having on the population of wild dogs in the Tanami Desert, to provide context for the results.

In this study I use molecular methods to investigate whether wild dogs sampled around mine sites have different population characteristics than those in surrounding areas within the Tanami Desert. Wild dogs were sampled at adjacent

pastoral and mining operations to compare the population characteristics at and away from the mines. I investigate levels of hybridization across the region, and provide management recommendations to reduce breeding between domestic dogs and dingoes in the area. I also investigate whether there are any distinct population clusters to infer movement and breeding patterns within the study area. The high dispersal capacity of wild dogs and the potential disruption since the establishment of the mine sites may have precluded the establishment of stable population structure; therefore, I also examined the data for family groups to test for patterns of gene flow on a smaller temporal scale. The results provide an insight into how access to human-provided resources might alter typical wild dog breeding and social behaviour.

4.3 Methods

4.3.1 DNA collection

Sampling of wild dogs focused around two mine sites, The Granites and DBS, and along an east to west transect approximately 100 km either side of the mines (Fig. 4.1b). Wild dogs were trapped using Victor #3 soft jaw steel traps (Oneida Victor Ltd, Ohio, USA) in April, August and November 2008, April and August 2009, and April 2010. A relatively even trap effort was applied across all sites with the exception of Windy Hill, which was sampled on only one trip. A 2 mm biopsy punch was used to take tissue from the dogs' inner ears, and tissue samples were stored in lysis buffer (Longmire *et al.* 1997) at room temperature until DNA extraction. If a dead wild dog was encountered in the field ear tissue was collected, following the same procedure as for a trapped wild dog. A handheld GPS was used to record the collection location of DNA samples. Twenty-one samples of ear tissue were also collected at The Granites in 2006 by Northern Territory Government Parks and Wildlife Service, and stored in lysis buffer at room temperature until DNA extraction.

4.3.4 DNA extraction and amplification

DNA extractions and amplifications were performed as described in Chapter 3. All loci used in Chapter 3 were included in purity analyses (23 for the clustering method and 22 for the reference method), but loci m13tt and m13c19 were

excluded from population structure and kin analyses because of their low number of alleles and specific purpose as dingo diagnostic alleles.

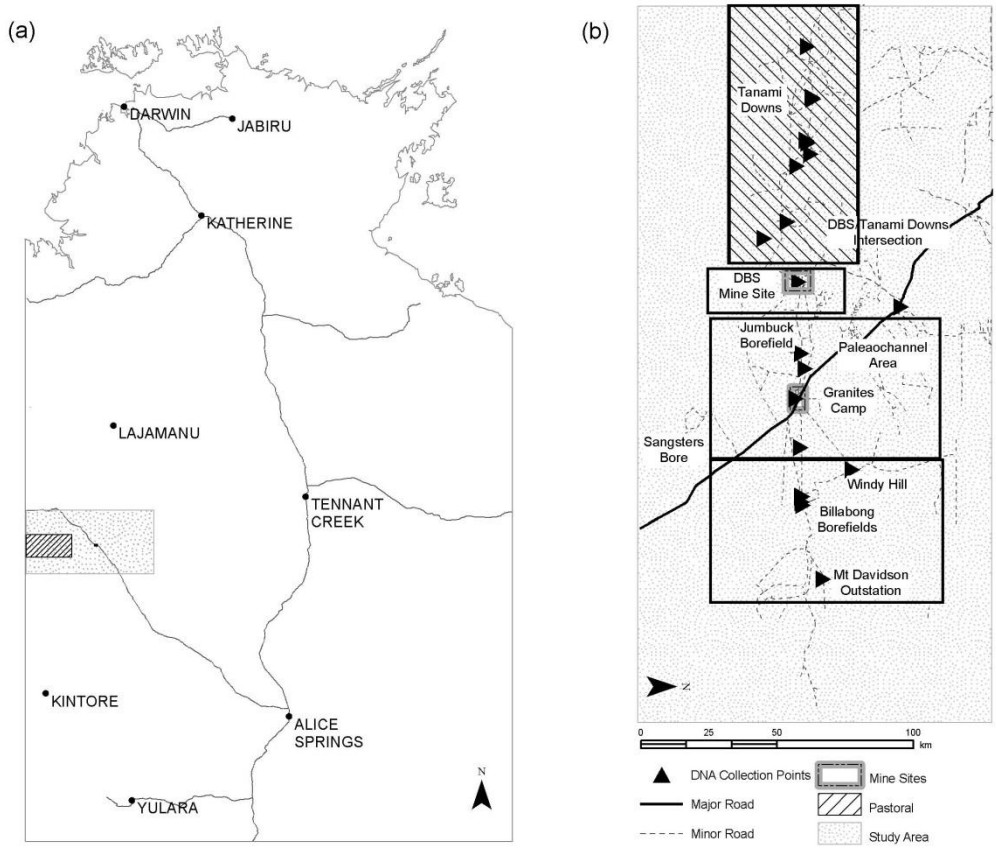

Fig. 4.1. (a) Map of the Northern Territory, showing location of the study region (box) in the relation to major towns and roads. (b) Detail of the study region, oriented at 90° to the map in (a), showing locations where DNA samples were collected from wild dogs. Each triangle represents a trapping location. Individuals from the trapping sites within rectangle outlines were pooled for analysis of genetic structure.

4.3.5 Hybridization and population assignment

Hybridisation scores using both the clustering and reference methods were taken from Chapter 3, and mapped using ArcGIS v.9.3 (ESRI Inc., Redlands, CA, USA).

I used the program Structure (Pritchard *et al.* 2000) on all samples to determine whether there were distinct population clusters in the northern region. I incorporated spatial data into the model to increase the sensitivity of the clustering method, by using the sampling site of each individual to inform the prior distribution of the model (Hubisz *et al.* 2009). Because only two wild dogs were sampled at the Jumbuck site, these individuals were pooled with individuals from

The Granites to increase sample size. Dogs caught at Windy Hill (n = l), Billabong (n = 9) and Mt Davidson (n = 5) were also pooled to increase the samples size of this group (Fig. 4.1b).

For all Structure analyses I used the admixture model, and correlated allele frequencies with a 20,000 chain burn-in and 200,000 replicates, which was sufficient to reach convergence. All other parameters were at the default values. To determine the probable number of clusters (K), I used the 'ΔK' method (described in Evanno *et al.* 2005). Each value of K from 1 to 10 was run for 10 replicates, and the results collated using Structure Harvester (Earl 2011). Once the optimum K value was selected, I averaged each individual population assignment ('Q-value') across the 10 replicates, using the program CLUMPP (Jakobsson & Rosenberg 2007). Samples with a Q-value assignment above 0.90 to a cluster were considered to belong fully to that cluster; samples with Q-values below 0.90 were considered 'admixed', having ancestry from more than one cluster.

4.3.6 Analysis of kin relationships

I used the program Kingroup v2 (Konovalov *et al.* 2004) to test for the presence of first-order (parent-offspring or full sibling) relationships among wild dogs. To do this I ran a full sibship reconstruction, using the descending ratio algorithm (Konovalov *et al.* 2004). The primary hypothesis I used was that individuals were full siblings or parent-offspring, and the complex null hypothesis was that individuals were half-siblings or unrelated. I also attempted to evaluate kin relationships using the program Colony 2.0 (Jones & Wang 2010), but the results are not included because testing against data with known ages and sexes resulted in erroneous assignment of parents and offspring.

4.4 Results

4.4.1 DNA sample collection

Tissue samples were obtained from 152 wild dogs (63 Female, 86 Males, 3 unknown). The total included the 21 specimens collected in 2006, 16 found carcasses and 115 from wild dogs that were trapped. The majority of these specimens were obtained from The Granites/Jumbuck (65%).

4.4.2 Distribution of dingoes and hybrids

I obtained a successful genotype (≥14 loci amplified) from 142 of the 152 tissue samples collected. Using the Average 3Q method, I identified 16 of these as dog-dingo hybrids (Category 3–4; Fig. 4.2): 14 at The Granites (including four directly around the waste facility), one at the DBS mine and one on the eastern edge of Tanami Downs (Fig. 4.2). No individuals in categories 5–7 (less than 65% dingo) were found. A one-tailed Fisher's Exact Test for difference in the number of hybrids at the mine sites (Granites and DBS; 15 hybrids, n = 109) and the remaining sites (1 hybrid, n=33) was not significant (P= 0.073).

The clustering method to determine purity identified 10 hybrid individuals with q < 0.90. The individuals with the three lowest Q-assignment values were the same individuals identified as Category 4 using the average 3Q method. Six of the hybrid individuals identified by this method were from The Granites, one each from the Billabong Borefield and DBS sites, and two from Tanami Downs. One of the Tanami Downs hybrids was the same as that identified by the Average 3Q method, and the additional hybrid at Tanami Downs had a Q-assignment value of 0.89, close to the arbitrary threshold for assuming purity. A one-sided Student's t-test showed no significant difference between the mean q-value between the mine sites and at the other sites (P = 0.17).

4.4.3 Population assignment and relatedness

Results from the Bayesian clustering analysis, the ΔK values and the consistency of results among replicate runs indicated that the probable number of clusters in the northern region was K = 2 (Fig. 4.3). No geographically discrete population clusters were apparent, but one cluster constituted the majority of the samples around The Granites mine site, and representatives were not found in the outlying areas. Admixed individuals were found at all sites except for Tanami Downs (Fig. 4.4a).

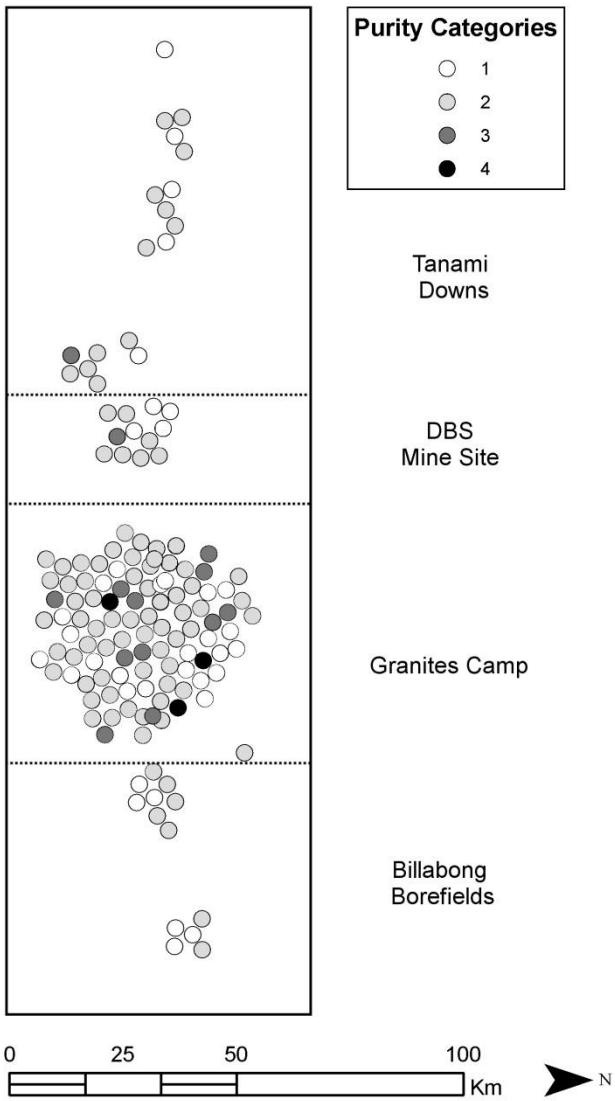

Fig. 4.2. Distribution of dingoes and hybrids as indicated by the reference method (Chapter 3; Wilton 2001). Samples have been randomly dispersed around a central point when multiple individuals were collected at the same location, to display all individuals. The inset box shows the purity categories, as described in Chapter 3 (with category 1 being the most likely to be a pure dingo). No individuals from categories 5–7 were found. The study sites are listed at right.

Kinship analysis found 14 family groups, defined as cases where two or more animals were related at the full sibling or parent offspring level (Fig. 4.4b), the largest containing 55 individuals (mean group size = 9.34 ± 14 SD). Groups found at Tanami Downs were all restricted to that area. All other sites contained kin that were also found in other sites, demonstrating recent gene flow in the area between DBS and Mt Davidson (Fig. 4.4b).

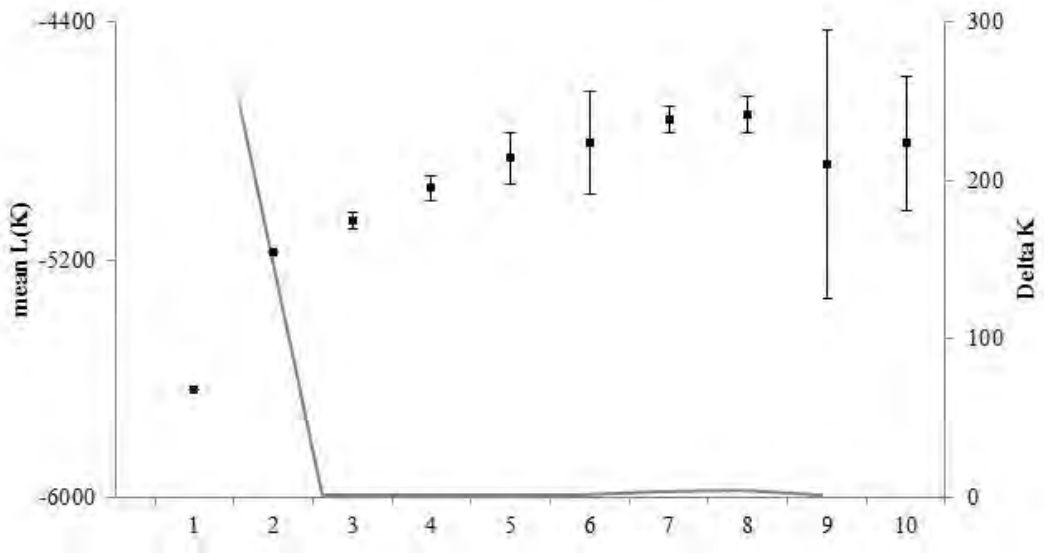

Fig. 4.3. Estimators of the 'true' number of populations (K). Mean L(K) (filled squares) show the mean estimate of the log probability of each K described in Pritchard *et al.* (2000). Bars show standard deviation. The most likely K is at the point where gains in likelihood begin to diminish (K=2). Delta K (solid line) shows a peak at the probable value of K (Evanno *et al.* 2005). K=1 cannot be evaluated by this method as the calculations require the difference in the rate of change from the previous value.

4.5 Discussion

There is a clear increase in numbers of wild dogs at the mine sites compared to surrounding areas, with two closely related groups containing the majority of individuals at the mine. Formation of packs is correlated with the type of food available (Newsome *et al.* 1983; Thomson *et al* 1992b), and the stable and constant food source provided by the mine refuse may provide enough food for the dogs to forage independently, without the need for cooperative hunting. The absence of a stable pack structure in wild dogs is hypothesised to have two main effects: an increase in population size, and increased potential for hybridisation (Elledge *et al.* 2006; Wallach *et al.* 2009). These potential increases in population size and hybridisation are thought to be caused by the removal of breeding restrictions enforced in a pack hierarchy, where only the 'alpha pair' is permitted to produce offspring. Destruction of subordinate females' pups by the alpha bitch is a major regulator of population size when pack structure is present (Corbett 1988), but the reduction of intraspecific competition or pack cohesiveness caused by the high availability of resources could have removed this constraint at The Granites and DBS.

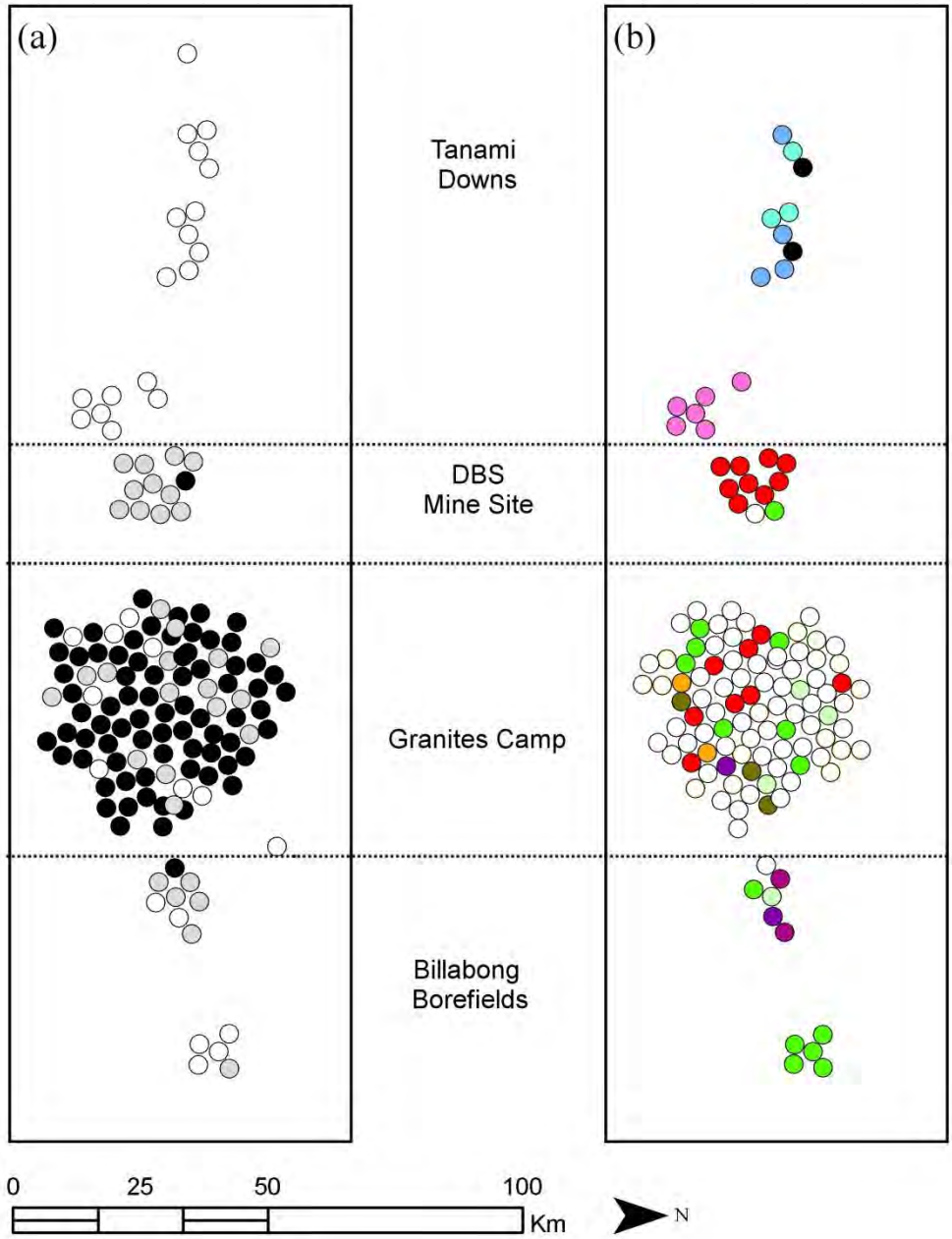

Fig. 4.4. Genetic segregation of wild dogs by (a) population clusters and (b) family groups. (a) Results of Structure analysis for K=2. Black and white circles represent each of the two clusters found, grey circles are admixed individuals (q-value < 0.90). (b) Family groups as determined by full sibship reconstruction. Each circle colour represents a unique family group. Individuals that were not assigned any kin are not shown.

4.5.1 Hybridisation and the conservation of dingoes

This study shows that the hybrid individuals in the region are centred on the mine site, with only two outside the Granites site and none in the outermost areas using the Average 3Q method (Fig. 4.2). This difference in hybrid proportions was not

statistically significant, however, and the study area contained mostly pure dingoes at all trapping sites. The few hybrids that were found could indicate the early stages of introgression in this area, and monitoring is recommended.

The majority of hybrids were identified at The Granites mine, so this site could become a source of hybrids to the surrounding region. Baume (1994) stated that both dogs and dingoes were kept as pets at The Granites during the early years of exploration (pre-1940s). More recently, however, there may have been domestic dogs brought to the outstation near The Granites, where there is frequent human habitation.

The overall proportion of "pure" dingoes identified is lower than reported by Newsome & Corbett (1985) (97.5%) for inland central Australia; however, the latter samples were from more remote regions, and the difference between skull morphology and genetic tests may affect the results (Elledge *et al.* 2008). Even with a potential sampling bias towards hybrids due to sampling near human settlements in this study, the central Australian region still has the highest proportion of pure dingoes found, making it an important region for conservation efforts.

4.5.2 Population structure and distribution of kin

Our primary objective in investigating population and kin structure was to determine whether there was any evidence that access to human-provided food alters typical wild dog breeding behaviour. Although we did not have observational data to determine whether the dogs belonged to socially stable packs, our results revealed important trends. Because a low number of individuals were caught outside the mine, conclusions from genetic data are more difficult, but the asymmetry itself indicates the effect of the mine. The high number of wild dogs captured at The Granites for an approximately equal trap effort across the study region suggests that the mine site is supporting a higher density of wild dogs than the surrounding sites. In addition, the large family group found by kin analysis around the mine (Fig 4.4b; white dots) suggests a rapidly breeding family or inbred group resides in the area. This is supported by the Structure analysis, with 91% of the 'black' population (Fig. 4.4a) consisting of individuals from the two largest family groups.

The high level of relatedness around the mine indicates that the higher numbers of wild dogs is mainly caused by higher reproductive output, possibly in combination with increased immigration (P. Fleming, pers. obs.). The high proportion of related individuals suggests that wild dogs can increase their numbers rapidly through breeding alone when enough resources are available. This finding may be significant in debates on whether dispersal or *in situ* reproduction has the greatest effect on increases in wild dog numbers after lethal control programs; i.e. whether dogs are entering pastoral territory from other regions, or whether wild dog problems stem from incomplete eradication of the local dog population (Chapter 6). Relatedness testing on populations in pastoral areas could resolve this question, and determine whether the increased availability in resources from removing competing individuals (through baiting, trapping and shooting) is sufficient to show the same increase in wild dog numbers as found from the provision of anthropogenic food and water sources.

Samples from Tanami Downs were not directly related to those sampled elsewhere (Fig. 4.4b), and contained no admixed individuals in the Structure analysis (Fig. 4.4a). We also did not re-capture or sight any wild dogs caught from another site on Tanami Downs or vice-versa, and no wild dogs fitted with GPS collars in the surrounding region during the study period visited Tanami Downs (T. Newsome, unpublished data). These results in combination suggest that the Tanami Downs region has the lowest level of gene flow with surrounding sites. Given that there was evidence of gene flow among all other sites, including across 102 km between Mt Davidson and DBS, this suggests that a feature other than distance is causing the isolation of Tanami Downs. The movement of dogs toward Tanami Downs through the path of the study area could be interrupted by the presence of the mines, if dogs find sufficient resources and mating opportunities there and have no reason to continue west to the pastoral region.

4.5.4 Management recommendations

The proportion of pure dingoes throughout Australia is thought to be declining, with estimates that only hybrids and feral dogs will exist by the end of the 21st century (Daniels & Corbett 2003). The present study identified a high proportion of pure dingoes in the Tanami Desert, particularly in areas away from human

activity. Previous studies also indicate low hybridisation rates in inland Australia (Newsome & Corbett 1985). These remote regions therefore offer the best hope of preserving dingoes on mainland Australia. To maintain these dingo populations, domestic dog numbers in settlements areas should be reduced, and programs to encourage sterilisation and restrictions on allowing dogs to roam freely be expanded. Sterilisation programs are already underway at Yuendumu, a settlement approximately 250 km southeast of the study site, to limit the population of free-roaming dogs, improve animal welfare, and reduce transmission of zoonotic diseases. Removing hybrids from populations in the wild might be less feasible due to the inability to identify hybrids easily in the field (Elledge *et al.* 2008).

The incidence of hybrids and abnormal abundance of closely related wild dogs around The Granites mine site provide compelling evidence that supplementary resources can cause a shift in typical breeding and social behaviour. A decrease in resource availability has the potential to reduce densities rapidly to more usual levels (Bino *et al.* 2010), so more careful handling of waste should be considered if the high number of wild dogs at the site becomes problematic for either dingo conservation or human health through the spreading of waste or disease.

The genetic separation of Tanami Downs shows that, in general, genetic subdivision as well as distance should be considered when attempting to determine the spatial extent at which anthropogenic activities will affect wild dog populations. For this region, however, an indication of the minimum area of impact to the west of the mine is provided. Extension of sampling to the east to find the extent of gene flow and ongoing monitoring to determine the response of the dogs to management are recommended. The importance of central Australian dingoes to the conservation of the dingo lineage as a whole and the rapidity with which hybridisation is progressing in other areas makes management of these populations an urgent concern.

4.6 Acknowledgements

Samples were collected by T Newsome, G Ballard and P Fleming.

Chapter 5

Variable population structure despite high dispersal capability in Australian wild dogs

5.1 Abstract

Effective management of mobile and adaptable predators, such as Australian wild dogs, requires an understanding of their movement patterns and population structure. The impact of dingoes, feral dogs and their hybrids on both native wildlife and commercial livestock are significant, but wild dogs are difficult to study due to the cryptic and wary nature. Wild dogs are controlled in pastoral regions to protect livestock, but reinvasion of the controlled areas is a persistent problem. Information about the movement patterns of wild dogs can indicate the scale of control needed to protect livestock from predation, because culling efforts within the area of wild dog populations will result in more rapid reinvasion than those performed at the scale of the population. Although individual movements of wild dogs have been well studied by radio and satellite telemetry in some areas, the average movement of dogs through generations and across a broad geographic scale are better addressed by genetic methods. To determine the extent and pattern of gene flow in wild dogs, I analysed genetic structure and spatial autocorrelation from 300 individuals in eastern Australia. DNA specimens were collected in south Queensland across an area of sheep and cattle grazing land approximately 533,000 km^2. Bayesian clustering analysis revealed four genetically distinct clusters of dogs. The geographic extent of these groups differed significantly: two groups in the south of the study area showed a sharp geographic separation and restricted distribution, whereas the other populations exhibited considerable overlap. This pattern indicates a barrier to gene flow is present between the two south populations, which is not affecting dogs in other areas. The variable extent and strength of spatial genetic structuring illustrates the value in using data from local populations to inform management plans, because the variability in the genetic structure of wild dogs found here implies that the scale of spatial management cannot be readily transferred, even between adjacent regions.

5.2 Introduction

Wild canids are present on all continents except Antarctica, and play an important role in most ecosystems through their impact on prey and mesopredator populations (Berger *et al.* 2008; Beschta & Ripple 2009; Ritchie & Johnson 2009; Vanak *et al.* 2009). Understanding the ecological role of canids requires information on their density, predation habits, reproduction, and movement, the latter two of which can be informed by their genetic structure. Despite the widespread use of population genetics to study wild canids (e.g. Gottelli *et al.* 2004; Dalen *et al.* 2005; Pilot *et al.* 2006; Carmichael *et al.* 2007), this approach has not yet been applied to wild dogs in Australia (including dingoes, *Canis lupus dingo*, feral domestic dogs, *C. l. familiaris*, and their hybrids).

Australian wild dogs are highly adaptable apex predators, and thrive in diverse habitats, from deserts to alpine forests (Corbett 2001). They have extensive dispersal capacity, but frequently live within defined territories either in socially stable groups ('packs') or as solitary hunters (Corbett 1988; Thomson 1992c; Thomson *et al.* 1992a). Dingoes have a unique evolutionary history within the wolf-like canids, because they were semi-domesticated as companion and protection dogs for indigenous Asians and Australians (Corbett 2001), but in most regions now live with little human interaction. Wild dogs have ecological significance as apex predators and economic significance as predators of livestock and as tourist attractions, and dingoes have cultural significance as both totemic animals in Aboriginal culture and iconic Australian animals (Fleming *et al.* 2001). Management objectives for wild dogs therefore vary among regions, including lethal control in pastoral regions and conservation of dingoes in national parks.

Successful management can be aided by information about the genetic structure of wild dogs, which can indicate their typical movement and reproductive behaviours. Landscape and population genetic studies have provided substantial advances in our understanding of the ecology of many wild canids. Studies of coyotes (*C. latrans*) have found distinct genetic structure, despite their potential for long-range dispersal, putatively due to roads (Riley *et al.* 2006) or habitat bioregions (Sacks *et al.* 2004) acting as barriers to gene flow. Landscape features, social structure and prey specialisation, are also important determinants of the pattern of population structure for wolves (*C. l. lupus*; Carmichael *et al.* 2001;

Weckworth *et al.* 2005; Randall *et al.* 2010). These studies have shown that multiple factors can influence canid behaviour, and there is likely to be an interaction between habitat and inter- and intra-specific interactions, which make it difficult to generalise ecological patterns across canid species or ecosystems.

The elusive nature and sometimes low densities of wild dogs can make it difficult to study their ecology, but genetic approaches can overcome some of these problems (Chapter 2). The relatively recent arrival of dingoes in Australia (~5,000 ya), and the bottleneck effect caused by the few individuals in the founder population (Savolainen *et al.* 2004) may affect the likelihood of finding genetic structure in dingoes. One extra complication to the study of gene flow in dingoes, compared to other canids, is the high proportion of hybrids between dingoes and feral domestic dogs. The impact of hybridisation on the social structure, predation behaviour, and other characteristics in wild populations is unknown, yet introgression is widespread across Australia (Chapter 3).

Wild dogs have variable behavioural characteristics across their range and through time, which is likely to affect their pattern of genetic structure. Most data on wild dog ecology have been collected using radio tracking collars, or more recently, GPS collars (Thomson *et al.* 1992b; Claridge *et al.* 2009; Robley *et al.* 2010). The distances reported for movements of wild dogs differ widely, both between studies and for individual dogs. Studies in the southeast of Australia have found home ranges of 27 km^2 (Harden 1985) to 100 km^2 (Claridge *et al.* 2009), although Robley *et al.* (2010) found individual dogs that moved up to 230 km before returning to their usual home range.

Collaring studies, along with observations of captive animals, have also shown that wild dogs display flexible social structure, particularly in response to availability of prey and water (Corbett 2001). If easily captured prey are available, dogs tend to hunt alone, but to capture more difficult prey, such as kangaroos, or to survive in otherwise harsh environmental conditions wild dogs may form packs of up to 12 animals (Corbett & Newsome 1987; Thomson 1992c; Thomson *et al.* 1992a). The formation of packs has important consequences for gene flow, particularly as it affects the range and likelihood of dispersal, the incidence of sex-biased dispersal, the exclusion of other animals from the territory area, and the pattern of breeding (Corbett 2001; Fleming *et al.* 2001). Thomson (1992c) found

that site fidelity of Western Australian dingoes in packs was high, but dingoes with no obvious pack affiliation had higher average daily movements (5.6 ± 0.39 SE km/day cf. 3.3 ± 0.6 km/day for individuals in packs), although the sample size for lone individuals was low (n = 3), as few solitary individuals were found in the study. Persecution of wild dogs to protect livestock may also affect pack stability, and therefore dispersal. Specifically, it is hypothesised that control of wild dogs may increase livestock losses, either through the disruption of pack structure, leading to an increase in population size (Chapter 4; Wallach *et al.* 2009), or through the increased targeting of easy prey, such as sheep by inexperienced lone juvenile hunters (Fleming *et al.* 2006). Pack fragmentation may also cause an increased rate of reinvasion where control has successfully reduced the number of wild dogs, due to a decrease in site fidelity and territoriality, and inexperienced young dogs seeking easy prey (Glen *et al.* 2007).

The adaptability displayed by wild dogs has been established at the individual level, but it is not yet known whether this extends to variability in genetic structure at the population and landscape scales. In this study I use microsatellite DNA to examine whether wild dogs exhibit genetic structure in south Queensland, Australia, and to infer the extent of gene flow within the study area. Using genetic data to examine the ecology of wild dogs offers potential benefits over collaring approaches: more specimens over a wider area can be obtained more easily and at lower cost; patterns of gene flow can be tracked through time without ongoing monitoring; and it allows inclusion of animals that are too small for collars. The study area contains several potential barriers to gene flow and contrasting histories of human influence on the population, making this a suitable area in which to examine whether genetic structure is detectable. The primary aims of this study are to determine whether genetic structure is present within the scale of the study site and, given the variability in dingo ecology found by non-genetic studies, to compare patterns of gene flow across the study area. I also examine whether population structure is related to physical barriers, such as a dingo barrier fence or rivers, or the degree of dingo-dog hybridisation.

5.3 Methods

5.3.1 Study area

The study area covers approximately 533,000 km^2 in south Queensland, Australia (Fig. 5.1). The area is used primarily for livestock grazing, and high levels of predation on stock by wild dogs are reported. The costs to the Queensland agricultural industry of wild dog impacts have been estimated at AU$33million/year (Anonymous 2004). For this reason regular culling is undertaken by land managers and government, both southeast of a dingo barrier fence to protect sheep enterprises, and along a buffer zone north of the fence to reduce or slow the migration of wild dogs to the south. The dingo barrier fence is approximately 5,600 km long, and runs through the study area, extending through South Australia and Queensland, and following part of the NSW border. The fence began as a series of local individual barrier fences, which were consolidated into a single structure in 1945 (Bauer 1964). Due to the short time the fence has been present in its current form and the opportunity for dogs to cross through gates or areas damaged by storms, the fence itself may not present a detectable barrier to gene flow. The difference in stocking regimes and levels of persecution on either side of the fence may, however, have an impact on the social structure and behaviour of wild dogs (Allen & Gonzalez 1998; Boyko *et al.* 2009). The study area also contains a series of river systems south of the barrier fence, which may impact wild dog movement.

5.3.2 Sample collection

Samples of dog ear tissue were collected in south Queensland between December 2003 and June 2009 by officers from Queensland Parks and Wildlife, Queensland Department of Environment and Resource Management and Queensland Department of Primary Industries, during regular dog-control or research operations. GPS coordinates, or the property where the dogs were culled, were recorded at the time of collection. Specimens were dried or stored in lysis buffer (Longmire *et al.* 1997) at room temperature until DNA extraction.

5.3.3 DNA Extraction and Amplification

I extracted tissue DNA using a manual glass-fibre method (Ivanova *et al.* 2006) on 96-well plates, with a 1/3 dilution of extracts used for amplification. I amplified 33 microsatellite loci in seven multiplex PCR reactions. Loci and multiplex combinations are listed in Chapter 3 (Table 3.1) and Table 5.1. I conducted PCRs in 10 µl volumes consisting of: 5 µl Qiagen Multiplex PCR solution (Qiagen Inc. Valencia, CA, USA), 1 µl Qiagen Q-Solution, 1 µl DNA, 0.2 µM of each primer and DNAase/RNAase-free water. I ran PCRs with 15 minutes at 95 ^{o}C for polymerase activation, followed by 35 cycles of 30 s at 94 ^{o}C, 90 s at 60 ^{o}C and 60 s at 72 ^{o}C, and 30 minutes final extension at 60 ^{o}C. Fragments were run on an ABI 3730 capillary sequencer and analysed using GeneMarker® software (SoftGenetics, LLC.). For capillary sequencing analysis, I combined the multiplexes 2/3, 4/5 (Chapter 3) and 6/7 (Table 5.1) into panels. Loci m13tt and m13c19 were used only for tests of dingo purity, as they displayed just two and three alleles, respectively. They are also closely associated with dingo ancestry (Elledge *et al.* 2008), making them less suitable for reconstructing spatial and family histories.

Table 5.1. Microsatellite loci used in this study in addition to those detailed in Chapter 3. Size range and the number of alleles (NA) were calculated from the 300 specimens used for analysis

Locus	Multiplex	Reference	Size Range (base pairs)	NA
FH2168	6	Francisco *et al.* 1996	201-239	27
Wan142	6	Ostrander *et al.* 1993	129-140	6
FH3413	6	Guyon *et al.* 2003	341-389	13
Ren195	6	Ostrander *et al.* 1993	131-140	5
FH2537	6	Guyon *et al.* 2003	149-176	12
FH3591	7	Guyon *et al.* 2003	298-334	8
FH3278	7	Lingaas *et al.* 2003	300-393	9
Ren47D17	7	Jouquand *et al.* 2000	332-348	7
Ren229	7	Breen *et al.* 2001	307-321	8

5.3.4 Analysis of population structure

I examined genetic clustering with the Bayesian clustering software Structure v.2.3.1 (Pritchard *et al.* 2000), using the admixture model, correlated allele frequencies and no prior population information. Excluding population information was preferred in this study because samples were not collected in discrete locations, so there was no basis for *a priori* groupings. Each run had a 20,000 chain burn-in and 200,000 replicates, which was sufficient to reach convergence. I estimated the number of populations present (K) using the 'ΔK' method of Evanno *et al.* (2005), with K evaluated from one to 12 with 10 replicate runs at each K. This method provides the second order rate of change of the log probability between values of K, as generated by Structure. The 'true' value of K at the highest level of structure (i.e. the fewest number of populations with identifiable structure) is then assumed to be the value with the highest ΔK value. Results of the clustering analysis in Structure were displayed using ArcMap 9.3 (ESRI Inc.).

To measure genetic differentiation and subdivision between genetic clusters identified in the clustering analysis I calculated D_{EST} (Jost 2008) in the program SMOGD v1.2.5 (Crawford 2010), and F_{ST} (Weir & Cockerham 1984) using Genepop v.4 (Raymond & Rousset 1995; Rousset 2008). For the latter I used comparison of the observed value to 999 random permutations to determine significance.

The extent of dog-dingo hybridisation may affect the consistency of clusters, if it is uneven across the study area, due to either a skew in behaviour or - if extreme - confounding signal from the dingo and domestic dog lineages. To test whether there were significant differences in purity between the genetic clusters I performed a one-way analysis of variance on the q-values of purity (Chapter 3) within each cluster, because the distribution of purity values was approximately normal. This was followed by pairwise Tukey-Kramer tests using PASW statistics 18 for Windows (SPSS Inc., IL, USA).

5.3.5 Spatial autocorrelation and relatedness

I employed spatial autocorrelation analysis to compare the extent of gene flow within genetic clusters. This was done using the 'multiple distance class' function

in Genalex v6.41 (Peakall & Smouse 2006), which pools all specimens below a given distance for increasing distance classes, e.g. 0-10 km and 0-20 km groupings (Peakall *et al.* 2003). This method provides a more sensitive test for non-random gene flow than discrete distance class autocorrelations because the samples sizes are larger, particularly at higher distance classes, and should be more sensitive to infrequent long-distance dispersal events (Lachish *et al.* 2011). For spatial autocorrelation tests, I used 999 permutations of the data, and 999 bootstrap resampling replicates to generate 95% confidence intervals.

At the time of sampling, potential siblings were identified when groups of pups were collected together. To confirm the distribution of such family groups and determine the effect that they may have on clustering analysis, I identified relatives with the program Kingroup v2 (Konovalov *et al.* 2004). I analysed the data with the full sibship reconstruction, using the descending ratio algorithm. I used a primary hypothesis of full siblings or parent-offspring, and complex null hypotheses of half-siblings or unrelated. To test for the impact of kin groups on the population clusters, I re-ran Structure with all but one of the members from each kin group with >3 members removed.

5.4 Results

Of the 332 specimens collected, I removed 17 from the data set due to suspicion of DNA cross-contamination (>4 loci showing evidence of more than two alleles at a locus), nine for having <20 loci amplified, and six for having <50% dingo ancestry (possible stray/feral domestic dogs), based on the 'reference method' dingo purity testing protocols (Chapter 3; Wilton 2001; Elledge *et al.* 2008), leaving 300 specimens for analysis.

5.4.1 Population structure

I removed locus FH2175 from analysis due to an inconsistent repeat motif, leaving 30 loci for spatial analyses. The ΔK output for estimating the number of populations from Structure showed the highest peak at K=4 (ΔK=10.8). All other ΔK values were ≤ 4.6. K=4 was therefore assumed to be the 'true' K for further analysis.

F_{ST} values calculated between clusters showed significant subdivision between all groups (Table 5.2). The highest values for both F_{ST} and D_{EST} were between populations Southwest (SW) and Southeast (SE), which were geographically closest (Fig. 5.1). The least difference was found between populations North (N) and Coastal (C), which also showed considerable geographic overlap.

One-way ANOVA of the q-value of purity in each cluster found an overall significant difference in the degree of dog-dingo hybridisation among clusters ($P<0.001$). Pairwise Tukey-Kramer tests showed significant differences only between the Coastal cluster and the others ($P < 0.001$ between Coastal and North/Southeast, and $P = 0.02$ between Coastal and Southwest). Mean purity and standard deviation values (in brackets) for each cluster were: SW, 0.83 (0.08); C, 0.78 (0.10); N, 0.86 (0.06); and SE, 0.86 (0.07).

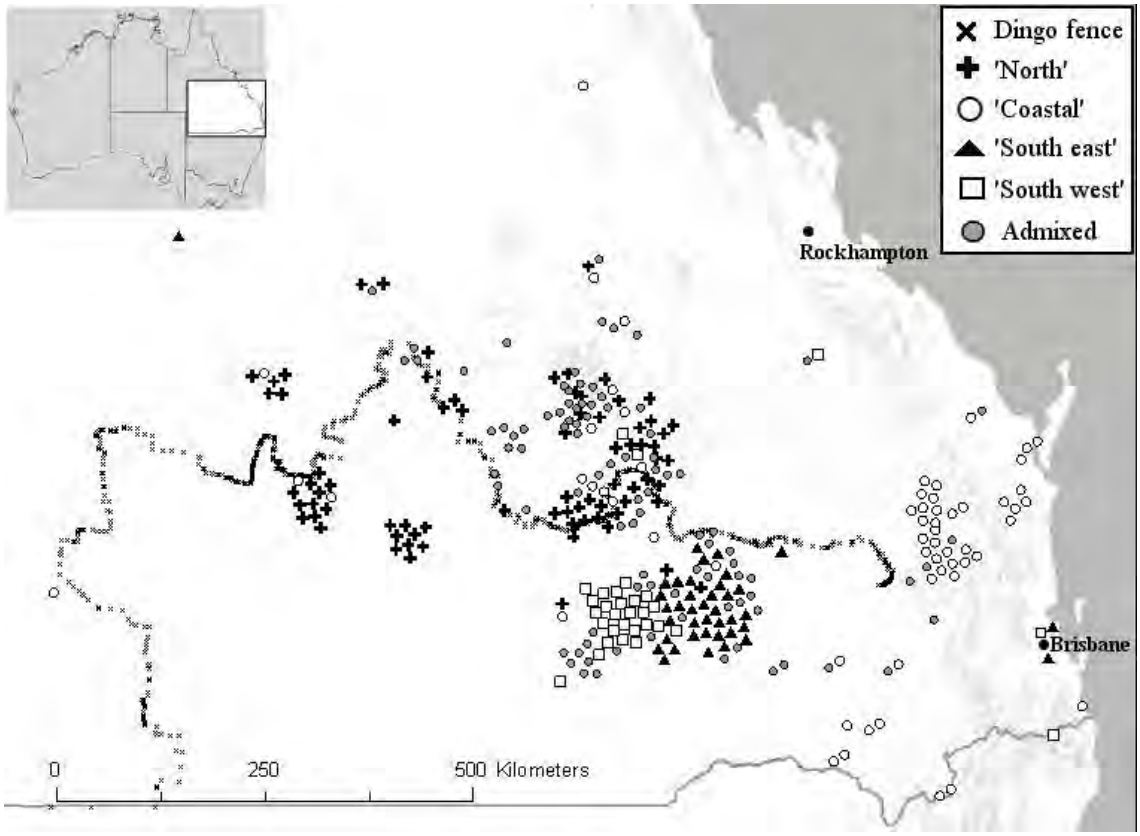

Fig. 5.1. Genetic clusters of wild dogs identified in south Queensland. The inset map shows the location of the study area within Australia (white rectangle). Specimens caught at the same location have been randomly dispersed around the location so that all specimen results are visible. Individuals with a q-value (the probability of belonging to a cluster) of <0.90 are shown as 'admixed'. From the 300 specimens, 69% had Q ≥0.90.

Table 5.2. F_{ST} and D_{EST} values between structure populations. Pairwise F_{ST} values are reported below the diagonal, D_{EST} are reported above. Values include all individuals assigned to the population cluster with their highest proportion of membership (q). All values of F_{ST} were significant ($P < 0.001$). Groups are as identified in Fig. 5.1, abbreviations are: SW = Southwest; C= Coastal; N = North; SE = Southeast.

	SW	**C**	**N**	**SE**
SW	-	0.073	0.072	0.097
C	0.061	-	0.026	0.084
N	0.069	0.018	-	0.084
SE	0.103	0.065	0.069	-

5.4.2 Spatial Autocorrelation

Spatial autocorrelation results are displayed using increasing distance classes in Fig. 5.2. The maximum distance between specimens was 1,236 km, but the maximum distance shown in Fig. 5.2 is 500 km because there was no detectable correlation between geographic and genetic distance beyond this distance. The extent of significant relatedness varied considerably among the clusters. Using the conservative measure of classifying the x–intercept as point where the bootstrap error bar first intersects zero, the maximum extents of significantly positive spatial autocorrelation using the multiple distance class method were: all data=400 km; SE=30 km; SW=90 km; N=300 km; and C=425 km.

5.4.3 Relatedness

Eight groups of > 3 individual animals significantly more likely to represent first-order relatives than second order or unrelated were identified with descending ratio analysis. Groups of four or more individuals were used, because I considered groups of two or three related individuals unlikely to have as much effect on the results as collection of full-sib litters. Four of these groups were identified in cluster Southeast (containing four, four, six and seven individuals), two from cluster Southwest (five and 11 individuals), one from cluster North (five individuals) and one split between clusters North and Coastal (four individuals, two adults and two pups, which could represent a mating between an individual from each of these clusters).

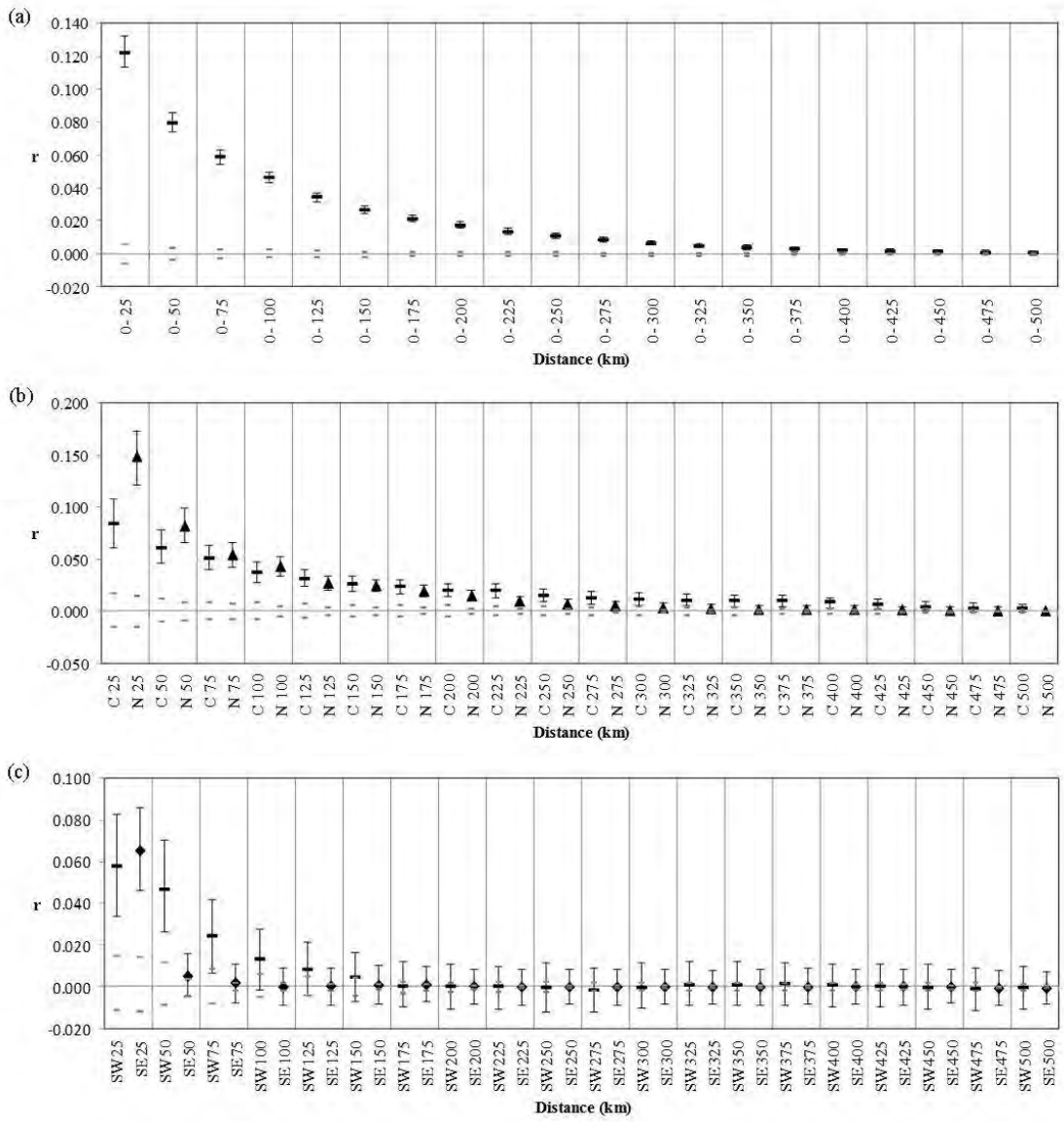

Fig. 5.2. Genetic spatial autocorrelation of wild dogs in southern Queensland. For all graphs whiskers show the 95% limits of 999 bootstrap replicates, the grey lines show the upper and lower 95% bounds from 999 permutations with randomly shuffled data. 'r' (y-axis) is the correlation coefficient of genetic similarity (Peakall & Smouse 2006). (a) Multiple distance class spatial autocorrelation for all data up to 500 km. (b) Multiple distance class display for the Coastal (C, ▬) and North (N, ▲) clusters. (c) Multiple distance class display for clusters Southwest (SW, ▬) and Southeast (SE, ♦).

Structure clustering analysis with all but one sib removed from each group showed 94% of individuals were assigned the majority of their ancestry to the same cluster as the first analysis (n=262), with a similar spatial pattern displayed (data not shown), indicating that the presence of kin groups alone did not greatly affect the clustering output. The clustering therefore displays a pattern of genetic

discontinuity at a deeper temporal scale, rather than being an artefact of sampling litters of pups.

5.5 Discussion

Analysis of microsatellite DNA genotypes showed clear evidence of genetic structure, despite the capacity of wild dogs for long-distance movement (Thomson *et al.* 1992b; Robley *et al.* 2010). Significantly, the differing extent of gene flow found between the Southwest/Southeast clusters and the North/Coastal clusters in both the Bayesian clustering and spatial autocorrelation results suggest marked behavioural differences between the dogs in these areas despite their close proximity.

The most striking result of this study is the abrupt genetic discontinuity between the Southeast and Southwest clusters (Fig 5.1; Table 5.2). The appearance of limited genetic structure is consistent with collaring studies in northwestern Australia (Thomson 1992c; Thomson *et al.* 1992b), which found wild dogs generally remained within a limited territory relative to their capacity for dispersal.

5.5.1 Landscape barriers in the study area

The highest F_{ST} and D_{EST} values were found between the Southwest and Southeast clusters, despite their boundaries being immediately adjacent (Table 5.2). The most obvious landscape feature separating the two populations is the Condamine River, which runs partway between the two clusters. The main river, however, veers east through the Southeast cluster without having a noticeable effect on genetic subdivision within this cluster. A tributary of the Condamine does, however, continue north along the division between the Southeast and Southwest populations, along with the Carnarvon Highway. The highway continues through the North cluster without detectable impact, so is unlikely to be a cause of population structure on its own. There is therefore no obvious, consistent physical barrier to movement between the Southeast and Southwest populations. The rivers and roads may, however, represent a 'marker' to delineate pack territories, implying a behavioural separation of clusters which could be confirmed through observation or tracking studies.

The dingo fence did not have a detectable effect on gene flow, based on the clustering results, shown by the extension of the North cluster across both sides of the fence. In addition, preliminary studies comparing F_{ST} of comparable populations across and along the fence, and simulation studies on the number of generations of separation required to create detectable subdivision, support the absence of a significant effect on gene flow (data not shown). Standards of maintenance for the fence have increased in recent decades, however, so an effect may become apparent in the future. Ongoing monitoring of relationships among individuals around the fence would provide an indicator of the effectiveness of the fence in containing wild dogs.

5.5.2 Hybridisation and genetic structure

It is not known whether pure dingoes have greater pack cohesion, and hence philopatry, than hybrids; yet pack stability is an important consideration when attempting to establish the ecological role of hybrid wild dogs (Claridge & Hunt 2008). The populations with the most geographically contained population structure (Southwest and Southeast) and the lowest inferred distance of gene flow from spatial autocorrelations have approximately equal numbers of dingoes and hybrids (data not shown), and the purity levels are not significantly different to North cluster, which has a very different distribution pattern. The lower average purity in the Coastal cluster did not create any noticeable differences between the pattern of genetic structure between the North and Coastal clusters (Figs. 5.1; 5.2b), although the difference in purity is only 8%. It therefore seems unlikely that the purity of dingoes and any associated social and behavioural differences are the most important factor in creating patterns of population structuring. Comparison of genetic structure in pure and hybrid populations in similar habitats would address this question more explicitly, if such populations can be found.

5.5.3 The effect of persecution on social cohesion and dispersal

The size and stability of wild dog packs can be affected by persecution and competition with neighbouring dogs (Thomson *et al.* 1992a; Glen *et al.* 2007). The area covered in this study has regular wild dog control, but the cattle regions to the north of the study area typically have less control than the sheep regions to the

south. If wild dog packs were being fractured by lethal control the expected pattern would be large, wide ranging clusters south of the dingo barrier fence, and more restricted, established clusters in the north, that are being prevented from moving further north by established packs above the area sampled. In this study, the reverse was true. The implementation of control along a 'buffer zone' to the north of the fence in this instance may be attracting dogs from further north (outside the study area) to regularly recolonise the region around the barrier fence, which is consistent with the distribution of the North cluster. It is, however, possible that the frequency and intensity of control is not tightly correlated with pack fragmentation, and that occasional control will have the same impact as regular control. Comparison of the genetics and behaviour of strongly persecuted, weakly persecuted and unpersecuted areas would be valuable to determine the effect that lethal control has on wild dog demography.

The sampling in this study does not cover the outside limits of all the clusters (Fig. 5.1), so extending the sampling may provide valuable information about the extent of the clusters. The flexibility in the formation and size of wild dog packs has been linked to prey availability and stability of resources (Newsome *et al.* 1983; Corbett 2001), therefore further study of the diet or direct observation of these animals may reveal differences in hunting behaviour that could also affect pack cohesion and site fidelity. Integration of genetic data with information about local environmental and landscape factors, and preferably also observation of dogs, would also assist in determining social dynamics and the reasons for genetic structure (Chapter 6 and references therein).

5.5.4 Management Implications

The pattern of dispersal inferred from this study may assist planning for management of wild dogs. The size of interbreeding groups shows the spatial scale at which gene flow is more likely to occur within rather than between populations. Direction of control measures at this scale rather than within populations may slow the reinvasion of dogs to sheep-producing areas (Hampton *et al.* 2004; Cowled *et al.* 2006). The geographic extent of gene flow, particularly in the North and Coastal clusters, highlights the advantage of coordinated 'nil-tenure' management plans, which observe the areas covered by wild dogs rather than

isolating management to property boundaries. The uneven distribution of genetic clusters and the differences in the strengths of boundaries separating the clusters also highlights the problems with applying assumptions about wild dog ecology across a broad scale and the value of replication in landscape genetic studies (Short Bull *et al.* 2011).

The multigenerational inferences from DNA-based analysis in this study provide a valuable addition to the detailed, short-term data gained from collaring studies, to provide a more complete picture of the demographic and ecological patterns of wild dogs. The underlying reason for the patterns of population structure and gene flow found in this study may be a combination of social factors, landscape features, and anthropogenic impacts via persecution and altering the availability of resources. Further examination of the behaviour of dingoes and their relationships with these variables may provide great insight into the demography of wild dogs as well as their responses to the many changes that have affected them, and continue to affect them, since European settlement of Australia. Understanding these responses will help all management relevant to wild dogs, whether it is related to reducing impacts on livestock or to conserving dingoes.

5.6 Acknowledgements

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Chapter 6

The dingo, an adaptable and mobile apex predator, displays distinct genetic clusters in a low relief landscape

6.1 Abstract

The effective management of trophically important species, such as apex predators, should be underpinned by an understanding of their population structure. In this study I provide a broad-scale assessment of genetic structure for the dingo (*Canis lupus dingo*), Australia's largest terrestrial predator, across approximately 2.5 million km^2 in Western Australia. Wild dogs (including dingoes) are of considerable management interest due to their predation on livestock and status as a threatened species in some regions. Through the analysis of 34 microsatellite DNA loci with Bayesian clustering and spatial autocorrelation analyses I identified four genetically distinct and adjacent groups of wild dogs that are not separated by topographic barriers. The association of climate and vegetation with genetic structure was assessed using discriminant analysis. Mean and mid-summer temperature were the most strongly associated with the observed differentiation overall, but the mechanisms by which this could dictate population structure are unclear. I therefore evaluate the possibilities that colonisation history or current ecological specialisation have influenced the pattern of population structure, although further research into predator-prey interactions is recommended to resolve these hypotheses. The presence of pronounced population structure in Western Australian wild dogs in the absence of sharp habitat changes or landscape barriers demonstrates the potential for cryptic discontinuities in gene flow to exist in large and mobile organisms.

6.2 Introduction

Wolf-like canids – such as coyotes (*Canis latrans*), jackals (*Canis* spp.), wolves (*C. lupus* ssp.), and Australian dingoes (*C. l. dingo*) – occur on all continents except Antarctica, and are often apex or high order predators (Berger *et al.* 2008; Beschta & Ripple 2009; Ritchie & Johnson 2009; Vanak *et al.* 2009). Understanding the ecology of large predators is important because their roles in trophic cascades

cause effects on ecosystem processes that are disproportionate to their abundance (Pace *et al.* 1999). Changes in canid abundance and behaviour can therefore affect mesopredators, herbivores, and other species in the ecosystem. Understanding the genetic structure within predator species provides an important perspective on their ecology as it illuminates their movement and reproductive patterns, and can also provide clues to how they interact with other ecosystem components.

Canids in general have impressive capacity for dispersal (Linnell *et al.* 2005), and occupy a great variety of habitats. These traits suggest that wild canids should exhibit extensive gene flow over vast distances that are little influenced by topographic relief. Nevertheless, genetic structure has been consistently detected in wolves and coyotes (Geffen *et al.* 2004; Sacks *et al.* 2004; Pilot *et al.* 2006; Carmichael *et al.* 2007; Muñoz-Fuentes *et al.* 2009). Providing an explanation for these observations in canids, and in other apex predators, has been the subject of concerted research. One prominent hypothesis is that behavioural differences reproductively isolate clusters of individuals from each other (Muñoz-Fuentes *et al.* 2009; Geffen *et al.* 2004; Sacks *et al.* 2005; Sacks *et al.* 2008; Carmichael *et al.* 2007). Preference for a particular prey (Carmichael *et al.* 2001) or habitat type (Pilot *et al*. 2006) may be learned during the juvenile stage; the individuals with this knowledge are then predisposed to move within habitat similar to the familiar natal area, decreasing dispersal, and hence gene flow, to surrounding areas (Benard & McCauley 2008). Other large, mobile predators, both aquatic (e.g. bass, Adams *et al.* 1982; and whales, Ford *et al.* 1998; Hoelzel *et al.* 2007) and terrestrial (e.g. lynx, Rueness *et al.* 2003; Stenseth *et al.* 2004), also display genetic differentiation in the absence of physical barriers to gene flow. The study of parapatry in these vagile species can provide insights into the early stages of cryptic speciation (Bush 1994; Shoemaker & Ross 1996; Mallet 2008). Although speciation is generally considered to occur in allopatry, these examples highlight the potential for behaviour to be an important mechanism.

The current apex predator in Australia is the dingo, a medium sized (mean weight 15kg; Corbett 2001) wolf-like canid. Dingoes entered Australia along the northern coastline from southeast Asia approximately 5,000 ya, and have spread across the mainland from a small founder population (Savolainen *et al.* 2004). There is currently no evidence that more than one introduction occurred, although

dingoes appear to have been transported back to Asia, based on the presence of kangaroo-associated lice in Asiatic dingo populations (Hopkins 1949). Dingoes probably had a second population expansion approximately 200 years ago, as towns and agriculture were established across the continent, adding fixed watering holes and livestock, which supported higher dingo densities (Corbett 2001; Fleming *et al.* 2001). Some macropods, a staple part of many dingoes' diet, also display evidence of expansion after European expansion due to an increase in watering points (Caughley *et al.* 1984; Caughley *et al.* 1987), providing further resources for dingoes to support their expansion. Despite their high vagility, wild dogs display population structure that may extend tens to hundreds of kilometres, although the degree of differentiation can be highly variable over even a relatively moderate geographic area (Chapter 5).

Wild dogs are declared pests in agricultural regions because of their predation of sheep and calves, but also have special conservation status in some areas, and are a listed threatened species in the state of Victoria (Anonymous 2007). This combination necessitates explicit policies on dingo management in most areas of Australia. Concerns regarding frequent long-distance movement of wild dogs into pastoral areas have influenced dingo management in Western Australia (WA) to the extent that dingo control was often undertaken far from any sheep areas prior to 1970. This policy was amended after radio-tracking studies showed that wild dogs displayed high site fidelity within areas up to 80 km^2 in the northwest of the state (Hogstrom 1986; Thomson *et al.* 1992a). These studies were undertaken in a single area in the northwest of WA, however, and whether these findings apply to other regions has not been investigated.

Determining the extent of population subdivision and the environmental correlates can assist in mitigating damage to other species and agricultural enterprises (e.g. Hampton *et al*. 2004; Cowled *et al.* 2006) and establishing whether there are genetically unique populations that may have particular conservation value (Allendorf & Luikart 2007). Studies of population structure in other recently introduced, wide-ranging species in Australia have found evidence of population subdivision. Feral pigs (*Sus scrofa*) in the southwest of the Western Australia show structure associated with river catchments (Hampton *et al.* 2004), although pigs in south Queensland are genetically homogenous within areas up to

85,000 km^2. Starlings (*Sturnus vulgaris*) display two distinct clusters at the fringes of their expansion range, associated with their pattern of colonisation (Rollins *et al*. 2009). Conversely, camels (*Camelus dromedaries*) do not display any evidence of structure across western and central Australia (Spencer & Woolnough 2010). Previous analysis of genetic structure of wild dogs in Queensland over 533,000 km^2 did not reveal the full extent of clusters (Chapter 5), so a larger area is examined here.

In this study I present the first large-scale assessment of population structure of wild dogs, in the western third of the Australian continent (approximately 2.5 million km^2). By comparison to similar sized regions on other continents, the landscape in this area is relatively flat (Hopper 1979), without major inland rivers or other abrupt landscape features likely to limit the movement of wild dogs. The northern area of the state has a tropical climate with a summer wet season and mostly low savannah vegetation; the remainder is largely temperate with a gradient between a Mediterranean climate with winter wet season near the coast to arid deserts inland. Here I use individual-based Bayesian clustering of microsatellite genotypes to test for the presence of genetic structure of wild dogs, using both spatially explicit and spatially naïve analyses. To distinguish clustering caused by genuine discontinuities in gene flow from those resulting from isolation by distance, I also perform spatial interpolation of clusters and spatial autocorrelation analysis within and between clusters. Finally, I perform discriminant analysis on climate and landscape variables to determine whether there are habitat features that are consistently associated with population structure.

6.3 Methods

6.3.1 Sampling and genetic analysis

Tissue samples were collected from 2,286 individual wild dogs as described in Chapter 3. I amplified thirty-five microsatellite DNA loci from these samples following the laboratory procedures described in Chapters 3 and 5, with the addition of two loci: ladeC213 in multiplex 6 (Ostrander *et al.* 1993) and FH3295 in multiplex 7 (Guyon *et al.* 2003).

In addition to samples collected through control programs, dedicated sampling was also undertaken to expand the area covered. Live-capture of dogs was performed using Victor #3 soft-catch traps by experienced operators. Traps were checked at dawn each day, and captured animals restrained by a noose pole. DNA samples were then taken from the ear with a sterilised hole punch, and the animal released. Lethal capture of wild dogs was performed in more inaccessible areas, as above but using strychnine on the traps to ensure rapid death of the animal. The inability to check traps daily in remote areas necessitated lethal capture to prevent animals being caught in traps for unacceptable lengths of time. These traps were examined up to two weeks apart. DNA samples were then stored in lysis buffer (Longmire *et al.* 1997) until extraction. The areas not sampled, the extreme southwest and central deserts, have low densities of wild dogs (Fleming *et al* 2001). Access to the central desert regions was not pursued due to cultural considerations, because dingoes are a totemic species to the traditional owners.

6.3.2 Individual-based clustering

I employed two model-based clustering methods to identify genetic subdivision: the Bayesian MCMC methods implemented in Structure (Pritchard *et al*. 2000) and the spatially-explicit Vorronoi tessellation model using TESS v.2.3.1 (Francois *et al* 2006; Chen *et al*. 2007; Durand *et al*. 2009). I used TESS in preference to the conceptually similar Geneland (Guillot *et al*. 2005) because the latter lacks an admixture model; wild dogs are distributed continuously across the landscape, rather than in discrete groups (as reflected by the sampling pattern), so a model that permits admixture is more likely to be appropriate for this study. The algorithms implemented by TESS differ from those in Structure in their incorporation of spatial information for each individual to inform the prior distributions, under the assumption that neighbouring individuals are more likely to share genetic affinity.

The parameters used for Structure analysis were 20,000 burn-in chains, followed by 200,000 iterations, using the admixture model and correlated allele frequencies, with all other parameters at the default values. I evaluated values of K (number of clusters) from 1 to 15, using 10 replicates for each K. Both the ΔK method (Evanno *et al*. 2005) and ln(K) were considered for the selection of the

'true' value of K. Because the ΔK method of selecting K returns only the highest level of population structure (Rosenberg *et al*. 2002) - potentially concealing hierarchical structure within the most differentiated clusters - I repeated the above method for the selection of K within each cluster found by the initial testing. I then averaged the individual q-assignments (the estimated proportion of an individual's genome assigned to each cluster) from the 10 replicates of the optimal K value using the 'greedy' algorithm in the program CLUMPP (Jakobsson & Rosenberg 2007) to minimise stochastic errors.

For spatially explicit Bayesian clustering using TESS I weighted spatial coordinates using a Euclidian geographic distance matrix. I chose this option for better modelling of the pattern of continuous sampling and tight geographic clustering of some individuals (Durand *et al.* 2009). I performed preliminary runs of 1,000 burn-in sweeps, followed by 4,000 iterations to determine the probable value of K, using models with and without admixture. For all comparisons between modelling options the Deviance Information Criterion (DIC) was averaged over 10 runs, and the lowest average value selected as optimal. I monitored the DIC over increasing values of K, starting from K=2, until changes in the DIC had stabilised (ceased at K=10). To obtain robust individual assignment values for the optimal K, I then ran 100 iterations using the BYM admixture model, 10,000 burn-in sweeps and 50,000 iterations. I selected the 20 runs with the lowest DIC values and averaged them in CLUMPP as described above.

To visualise the extent of population structure and the gradient of change in cluster assignment I interpolated the q-value for each cluster using ordinary kriging, following the procedure described in Chapter 3. I also assessed Weir & Cockerham (1984) pairwise F_{ST} between the clusters using the program Genepop v4 (Raymond & Rousset 1995; Rousset 2008), and D_{EST} (Jost 2008) using the program SMOGD v1.2.5 (Crawford 2010).

6.3.3 Spatial autocorrelation and isolation by distance

Simulation studies have demonstrated that Bayesian clustering programs may overestimate the number of clusters or create spurious clusters in the presence of isolation-by-distance (Frantz *et al.* 2009; Ball *et al*. 2010; Pritchard *et al.* 2010). In this scenario, clusters will be identified even if there are no discontinuities in gene

flow, as individuals become less related as the distance between them increases. To evaluate whether this affected the results of this study, I employed spatial autocorrelation analysis to test the relationship between genetic and geographic distance within and between clusters identified in the Structure analysis (see Results). This analysis also enabled me to evaluate the extent of non-random gene flow in Western Australian wild dogs.

Autocorrelation analyses were performed on all samples within clusters and along transects between clusters where sufficient samples were available. Our sampling included four targeted transects between the Structure clusters (see Results). Spatial autocorrelation analyses were performed using a relatedness coefficient *r* (similar to Moran's *I*) at distance intervals of 50 km, implemented in the program Genalex (Peakall & Smouse 2006). This approach was used in favour of the cumulative distance class method employed in Chapter 5, because although the cumulative method has greater statistical power for comparisons of different populations, the absolute value of the x-intercept may be biased by the strength of relationships in earlier distance classes. The use of individual distance classes in this study was therefore preferable to infer the absolute extent of significant relatedness. I tested the significance of deviations from zero by performing 999 permutations of the data, and 999 bootstrap resampling replicates to generate 95% confidence bars.

6.3.4 Landscape correlations with genetic structure

To assess the associations between features of the landscape and population structure, I performed discriminant function analysis using JMP v9 (SAS Institute Inc. Cary, NC). In this analysis I used the maximum q-assignment to a cluster from the Structure analysis as the grouping category because most individuals had low levels of admixture (see results). To provide an overview of environmental differences across the study area, multiple climate and vegetation variables from each individual's sampling location were included as predictive variables to form the classifications (listed in Table 6.1). These variables were chosen for this study because similar variables have been associated with genetic structure in wolves (Geffen *et al.* 2004; Pilot *et al.* 2006). Purity scores from the clustering method (Chapter 3) were also included to assess whether introgression from domestic

dogs affected genetic structure. Vegetation measurements from both 1788 and 1988 were used to represent pre-European and current landscapes, because the extant vegetation in some areas has been changed considerably since European settlement of Australia.

Table 6.1 Landscape variables used as explanatory variables to predict the pattern of population clusters. Footnotes refer to the data source.

Variable Type	Variables	Abbreviation
Climatic[1]	Mean rainfall	MeanRain
	Mean temperature	MeanTemp
	January mean temperature	JanTemp
	July mean temperature	JulyTemp
Vegetation[2]	1788 (Pre-European) tallest vegetation density	PTD
	1788 tallest growth form (e.g. grasses/shrubs/trees)	PTGF
	1788 lowest stratum growth form	PLGF
	1988 tallest vegetation density	CTD
	1988 tallest growth form	CTGF
	1988 lowest stratum growth form	CLGF
Other[3]	Hybridisation (q-value)	Qpurity

[1] Bureau of Meteorology, averaged from 1961-1990 data; [2] Geoscience Australia; [3] Chapter 3

6.4 Results

6.4.1 Distribution of population clusters

Both individual clustering methods revealed an optimal K of 4 ($\Delta K = 150.3$). Repeating the selection of K within the four clusters with Structure found no evidence of hierarchical population structure, as determined by peak of ΔK at K=2, with the majority of individuals assigned jointly to both clusters, and the absence of geographic pattern to the clustering. The assignment of individuals to clusters agreed closely between the two methods, with 97% of individuals assigned the majority of their ancestry to the same cluster by both programs. Therefore, only the Structure results are shown to maintain consistency with previous chapters (Fig. 6.1a-b).

Interpolated clusters exhibited abrupt transitions in q-assignment, with gradual transitions occurring only in areas with sparse sampling (Fig. 6.1c). The measure of admixture (α) determined by Structure for K=4 was 0.065, indicating

low admixture between clusters (α=0 indicates no admixture, and α>1 indicates most individuals are admixed; Pritchard *et al.* 2010).

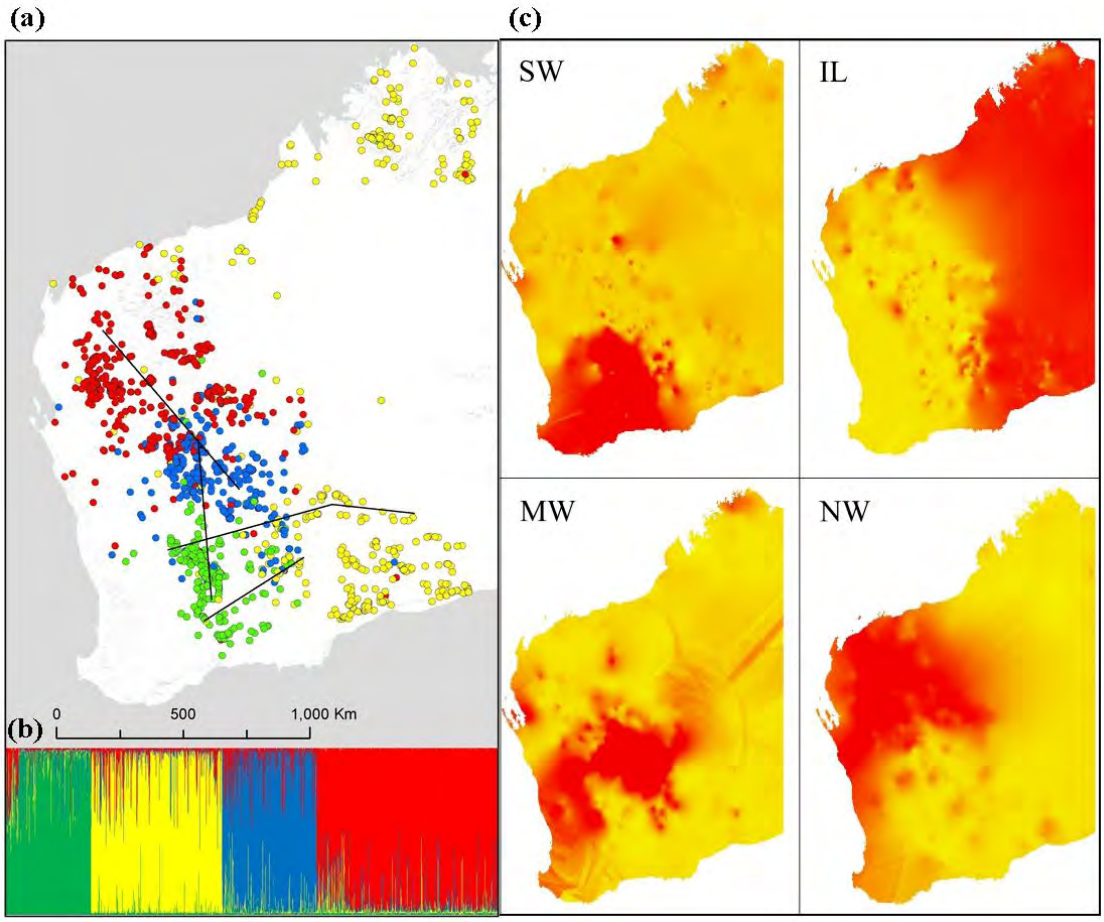

Fig. 6.1. Population assignment of individual wild dogs, as determined by non-spatially explicit clustering in Structure. (a)Assignment of individual wild dogs (represented by circles) to one of four clusters by the maximum q-value, with each cluster represented by a unique colour. The black lines show the locations of the transects analysed for spatial autocorrelation. (b) Barplot of estimates of membership coefficient (q) at K=4. Each individual is represented by a vertical bar broken into K coloured segments, where the length of the segment is proportional to the probability of assignment to each cluster. Colours correspond to the geographical distributions on the upper left map (a). (c) Individual clusters interpolated by kriging using their q-value assignment from zero (yellow) to 100% (red). Cluster names (top-right of each panel) are taken from their location: SW = southwest (n=399); IL = inland (n=608); MW = Midwest (n=438); NW = northwest (n=841).

The clusters showed statistically significant ($p < 0.05$) low to moderate subdivision and differentiation as measured by F_{ST} and D_{EST} respectively (Table 6.2), with the highest level of subdivision between the adjacent MW and SW clusters. F_{ST} and D_{EST} had similar values.

Table 6.2. Pairwise F_{ST} (below diagonal) and D_{EST} (above diagonal) values between the clusters described in Fig. 6.1.

	SW	IL	MW	NW
SW	-	0.050	0.078	0.053
IL	0.054	-	0.064	0.031
MW	0.093	0.070	-	0.040
NW	0.060	0.035	0.055	-

6.4.2 Spatial autocorrelation

Tests performed within clusters MW, IL and NW revealed a pattern consistent with isolation by distance: an initial steep decline in the relationship coefficient followed by slightly negative values or fluctuation around zero or average relatedness (Fig 6.2). The SW cluster was the exception, showing significantly negative values after the x-intercept until the 500km distance class, which may indicate weak subdivision within the cluster that was not detected by Structure. Transects taken between clusters (Fig. 6.3), however, displayed a pattern consistent with a long-distance cline: a transition from significantly positively correlated relationships at distances up to 250km followed by decline to significantly negative relatedness of a similar magnitude to the positive relatedness (Diniz-Filho & Telles 2002). The significantly negative values beyond the x-intercept indicate gene flow is occurring at a lower level than would be expected under the assumption of random mating.

6.4.3 Landscape genetics

Discriminant analysis classified 78.4% of the individuals to their correct Structure cluster based on the climate, vegetation and purity variables (Fig. 6.4). Temperature was the strongest predictor; in particular the annual mean and January temperatures separated the IL and NW clusters. Rainfall contributed most

strongly to the classification of the SW cluster in the southwest. Vegetation and purity showed low contributions to the classifications.

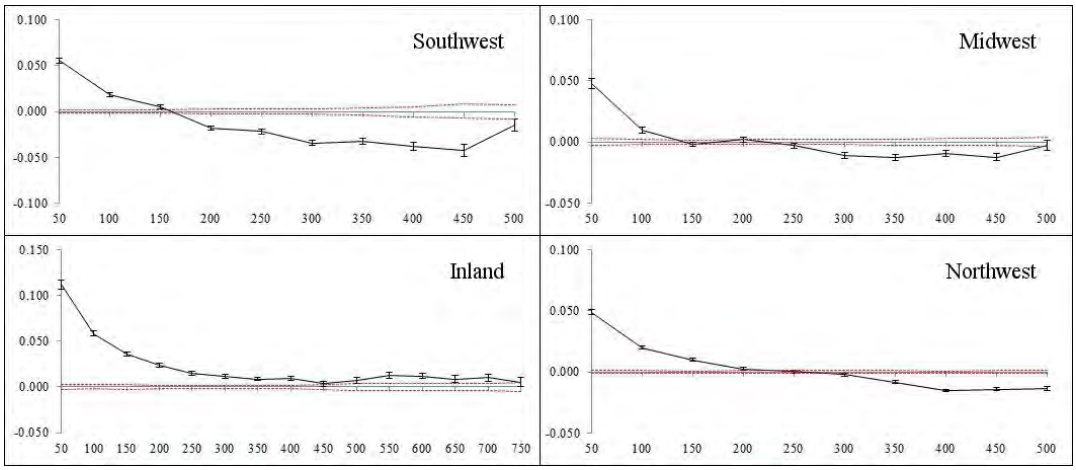

Fig. 6.2 Spatial autocorrelation within Structure population clusters. Distance classes (x-axis) are in kilometres, y-axes show the relatedness coefficient *r*. Error bars display the 95% limits of 999 bootstrap replicates, and the dotted lines show the upper and lower 95% bounds from 999 permutations with randomly shuffled data. Conservatively, points where the error bars are outside the upper and lower permutation bounds show significant deviation from random genetic mixing.

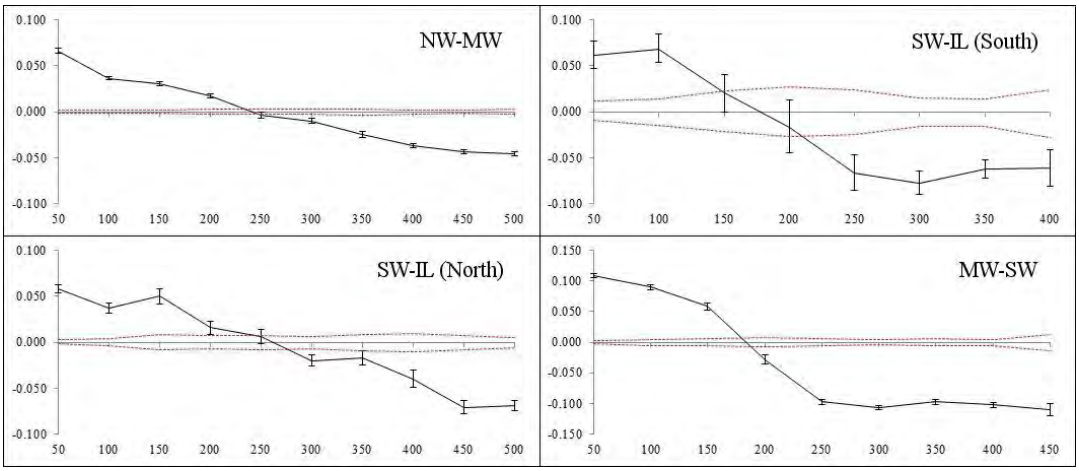

Fig. 6.3 Spatial autocorrelation calculated from samples collected along transects between the population clusters identified in Structure. Axes are as per Fig. 6.2. Transect locations are shown in Fig. 6.1. Transects are labelled according to the clusters they intersect (Fig. 6.1), with the two transects between the SW and IL clusters identified as north and south. Sample sizes for each transect were: SW-IL (south), n = 59; MW-SW, n = 237; NW-MW, n = 431; SW-IL (north), n =196.

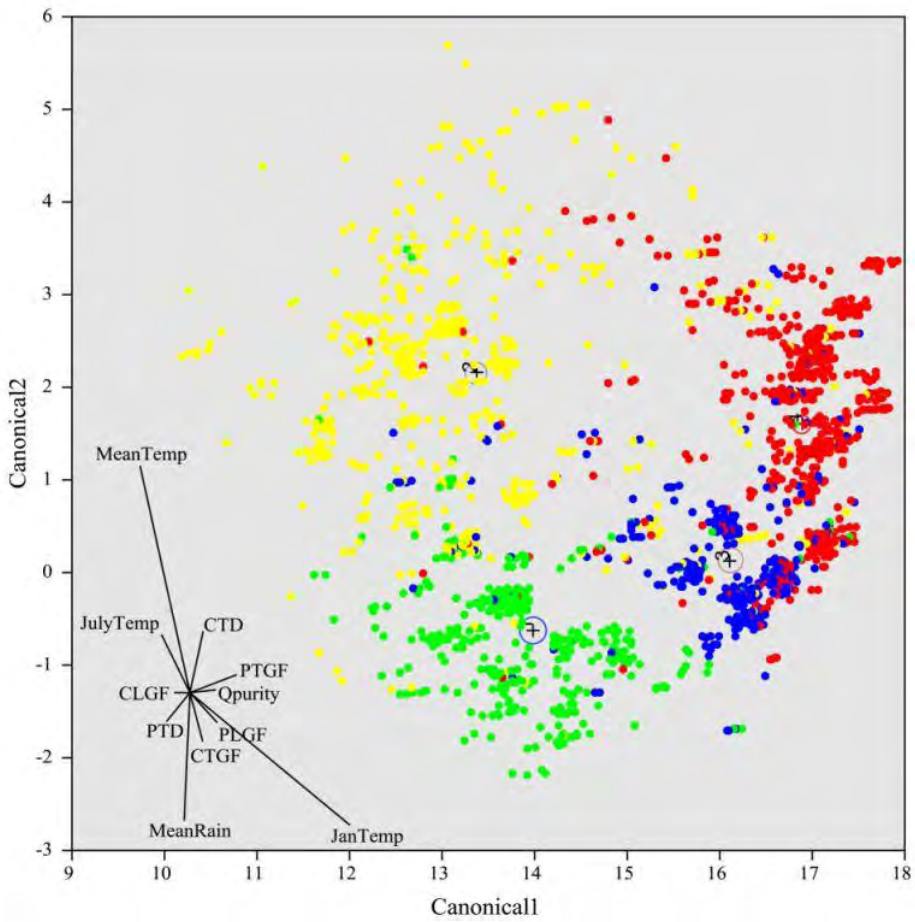

Fig. 6.4. Discriminant analysis of climate and vegetation variables to predict genetic clusters of wild dogs identified in the Structure analysis. The biplot ray (lower left) indicates the direction and magnitude of separation of the clusters by each variable. The colours of each group correspond to the clusters in Fig. 6.1. Circled crosses show the centroid for each cluster.

6.5 Discussion

6.5.1 Population structure and the extent of gene flow

Although wild dogs have become an important component in Australian ecosystems (Johnson *et al.* 2007), their arrival in Australia is recent in evolutionary timescales, having been present for only ~5,000 years (approximately 2,500 generations; Savolainen *et al.* 2004). Despite this, I have demonstrated distinct, large-scale population clustering in Western Australian wild dogs, shown by the abrupt transitions between clusters in both the interpolated clustering maps and the spatial autocorrelations (Figs. 6.1-6.3). The geographic scale of the clusters is an order of magnitude larger than those examined in Chapter 5, although the full geographic extent of clustering in Chapter 5 could not be determined due to the

limited range of sampling. The inclusion of hundreds of samples in each cluster here also precludes the possibility that the genetic structure is driven by recent and close relationships between the dogs, i.e. pack structure. This study is therefore the first to demonstrate the geographic extent of genetic clustering in Australian wild dogs. The sizes of clusters found in this study (250 - 2,000 km across) are comparable to the larger groups of grey wolves in arctic North America (Carmichael *et al.* 2007) and those found in eastern Europe (Pilot *et al.* 2006), despite dingoes having been in Australia for a much shorter time period than these wolves in their native regions.

With the exception of the IL cluster, all the spatial autocorrelations within clusters displayed similar x-intercepts (approximately 150-250 km; Fig. 6.2), which contrasts with the uneven population structure found in south Queensland (Chapter 5). A broader-scale process may affect the individuals in WA than those in south Queensland, the latter of which may operate at the level of inter-pack interactions. This extent of non-random gene flow also indicates that the movement of wild dogs is generally restricted relative to their dispersal capabilities (Corbett 1988; Thomson 1992c; Thomson *et al.* 1992a,b). The extended intercept value in the IL cluster (450 km) may be due to habitat with lower water and food availability in this region, as wild dogs have been shown to increase their home range sizes in areas with fewer resources (Thomson 1992b,c). The gap in sampling in the central area of this cluster would not have affected the spatial autocorrelation, because the gap exceeds 1,000 km from north to south, and distance classes of this extent were not assessed.

The SW and the MW populations occupy similar geographic extents, but the NW and particularly the IL populations cover broader ranges. The extent of the IL cluster was unexpected, because this area covers tropical habitat in the north and arid, scrubby habitat in the south. Increasing the value of K did not separate this cluster into northern and southern groups, nor did the hierarchical analysis within the cluster. Although there are thought to be relatively few wild dogs in the area between the Kimberly and the Goldfields-Esperance regions, dedicated sampling of these individuals and those further to the east would establish the eastern boundary of genetic structure in this area and confirm whether the clustering of the north and south sampling areas is accurate. The results of the discriminant

function analysis, however, do support a level of homogeneity in some of the environmental characteristics across this area, which may be a cohesive force for this wide-ranging group, despite the cluster covering the largest amount of canonical space.

Given the large geographical scales of the genetic clusters, the sharp boundaries between them are especially striking. Although the autocorrelation patterns shown in Fig. 6.3 can be produced by either an absence of genetic structure (i.e., solely due to isolation-by-distance) or the presence of spatially discrete populations (Diniz-Filho & Telles 2002, Fig. 1), the latter interpretation is supported in this study when the results are considered in combination with the interpolations (Fig. 6.1c), and F_{ST}/D_{EST} results (Table 6.2). Narrow regions at the intersection of clusters, however, showed individuals from multiple clusters in spatial overlap (Fig. 6.1a). This is consistent with the absence of physical barriers between clusters, and suggests areas of resistance to gene flow, with some mating and dispersal still occurring between clusters, but at a much lower level than occurs within the clusters. The causes for this pattern of population structure are unclear, but the most likely candidates are: (1) the pattern of colonisation or (2) habitat fidelity, particularly due to dietary specialisation. Below I evaluate the evidence for these causes of population structure, given the results of this study and previous research on the ecology of wild dogs and other vertebrate predators.

6.5.2 Historical explanations for population structure

Simulation and experimental evidence show that range expansion can cause population structure in the absence of selective pressure or geographical isolation (Ibrahim *et al.* 1996; Excoffier & Ray 2008). Occasional long-distance dispersal can allow a few individuals to establish ahead of the main expansion, and these small populations are especially susceptible to genetic drift. Sharp clines in allele frequency can result, and these can remain evident for hundreds or thousands of generations (Ibrahim *et al.* 1996). Dingoes have probably undergone two expansion events since they were transported to Australia: one approximately 5,000 ya upon their arrival and a second approximately 200 ya as European settlement expanded across the country and provided additional water and food resources, increasing the carrying capacity for dingoes in many areas (Corbett

2001; Fleming *et al.* 2001). Both expansions may have been assisted by travel with humans, which would extend the range of occasional dispersal events, and potentially the number of dingoes transported, beyond the extent possible by independent dispersal.

Without human assistance, individual wild dogs have been recorded on forays up to 230 km over 9 days (Robley *et al*. 2010), and more extensive occasional long-distance forays are starting to be revealed by satellite telemetry studies (L. Allen, pers. comm.). Small families of dingoes may have established new colonies across Western Australia, and those that reproduced successfully established in the previously uninhabited locations. Tests of relative genetic diversity measures, dispersal and the presence of private alleles can reveal species' history (Johnson 1988; Allendorf & Luikart 2007; Rollins *et al.* 2009). Decreases in genetic diversity and the number of private alleles are associated with the most extreme edge of the colonisation range, due to small founder populations. These genetic indicators in the dingo were equivocal, as the IL population has significantly more private alleles (3.15 averaged across loci, cf. <0.2 in the other clusters) but not significantly higher heterozygosity (data not shown). It is therefore plausible that the colonisation process has contributed to the pattern of population structure found in this study. Further analysis of phylogeography (incorporating Asiatic dingoes) and comparison with historical samples (e.g. Sacks *et al.* 2010; Rollins *et al.* 2011) within the study area could provide greater insight into the historical movement pattern of these dingoes. If colonisation history has affected genetic structure, however, this does not negate the possibility that current ecological pressures are maintaining population subdivisions. Indeed, the abrupt divisions between the clusters found in this study suggest that current behavioural characteristics may be sustaining geographic separation of wild dogs, regardless of whether the initial cause was historical or ecological.

6.5.3 Behavioural characteristics affecting gene flow

Studies of gene flow associated with physical barriers, at various spatial scales, have shown that significant genetic differentiation of populations can be detected after relatively few generations, even in wide-ranging species (Hampton *et al.* 2004; Epps *et al.* 2005; Riley *et al.* 2006). Differentiation due to permeable barriers

to gene flow, such as social constraints or ecological specialisation, is expected to be weaker, because some gene flow is more likely to occur at the fringes of clusters (Slatkin 1987; Saint-Laurent *et al.* 2003). Examples of population structure attributable to previously unseen ecological processes are increasing, as more detailed genetic and field studies are performed (e.g. Ford *et al.* 1998; Hoelzel *et al.* 2007). The analysis of climate and vegetation in this study showed that temperature variables had the greatest association with population structure (Fig. 6.4). If the correlation between climate and the clusters is due to differences in landscape rather than covariance with latitude and longitude, climatic factors may affect dingo ecology directly or indirectly. Temperature may directly affect survival, particularly in the summer, supported by the much stronger discriminating contribution of the January (summer) temperature over the July (winter) temperature. Climate may also act indirectly at the level of vegetation (such as providing den sites; Thomson 1992a; Muñoz-Fuentes *et al.* 2009), by affecting the type or abundance of prey (which in itself may be determined by vegetation types), or from other complex interactions within these trophic levels. The vegetation structure variables tested in this study did not show consistent or strong influence on the predictive assignment in discriminant analysis, so it is unlikely that vegetation has a straightforward impact on wild dogs, which is consistent with their noted adaptability to a variety of conditions (Corbett 2001).

The availability and type of prey may exert a more direct effect on wild dogs than climate or vegetation, as it has in other vertebrate predators. Arctic foxes (Dalen *et al.* 2009) and wolves (Carmichael *et al.* 2007), for example, have shown population subdivision between specialists on a particular prey and generalists. Dingo dietary studies have reported mixed conclusions about the adaptability of their foraging habits. Whitehouse (1977) found mammals, reptiles and insects in the stomach contents of wild dogs caught across Western Australia over a 5-year period, and concluded that they are opportunistic feeders. Wild dogs in the southeast of Australia, however, were found to continue to predate on wallabies, even when these were no longer the most abundant prey source (Robertshaw & Harden 1985). The complex nature of dingo predation led Corbett (2001) to classify wild dogs as specialist feeders, because few prey types make up the majority of their diet, but generalist hunters, due to the flexibility of their hunting

tactics. In some regions there is evidence that variability in prey availability may be a more important driver of dingo behaviour than the specific prey available (Newsome & Corbett 1985; Brook & Kutt 2011).

Assessing the impact of prey on dingo ecology requires more detailed information on (a) the prey type favoured in different regions and (b) the cycle of prey availability, particularly in prey species prone to plague such as rabbits (*Oryctolagus cuniculus*). The association with prey-related variables and population structure in other canids, particularly wolves, makes this a strong candidate for a driver of population subdivision, and a potential mechanism for maintaining the sharp boundaries between large genetic clusters. Analyses of diet have been undertaken at several sites within the study area (Whitehouse 1977; Thomson 1992b; Williams *et al* 1995), but the combination of genetic data, field observation and dietary studies would provide a broader view of dingo ecology, and help to identify whether prey-associated dingo ecotypes exist.

Hybridisation with domestic dogs may also affect genetic structure over time, but only 71 (3%) of the dogs sampled had less than 80% dingo ancestry, based on the clustering method described in Chapter 3, and the discriminant function analysis showed low association between the clusters and the proportion of hybrids present. Interbreeding with domestic dogs therefore does not appear to have affected genetic structure in this study, but may be a consideration in other areas with higher levels of introgression.

6.5.4 Management implications

Efforts to manage the impacts of wild dogs on stock in Western Australia are often hampered by the widespread belief that wild dogs regularly move large distances into sheep-grazing land, particularly from government-managed land (Hogstrom 1986). The results from this study, particularly the spatial autocorrelation analyses, support the findings of Thomson *et al.* (1992a,b), that wild dogs usually remain within a limited area relative to their dispersal capabilities. The pattern of population structure revealed here is also relevant for establishing the optimal scale for regional control, because wild dogs are more likely to move within clusters than between them. Although occasional long-distance dispersal may occur (Thomson *et al.* 1992b; Robley *et al.* 2010), the limited gene flow displayed

by the spatial autocorrelations indicates that it should not be considered a regular feature of dingo behaviour.

Historically, dingoes have been divided into three ecotypes (tropical, desert and alpine), and priority placed on preserving these entities in captive breeding programs (Corbett 2001). The results of this study and those presented in Chapter 5 suggest that genetic differentiation within dingoes may be influenced by more complicated ecological drivers than just these broad landscape descriptors. The identification of dingo ecotypes should therefore be more useful designations than the current delineation into desert, alpine and tropical races, especially given the extension of cluster IL across both tropical and desert habitats. Further research into the mechanisms underlying population structure would benefit the conservation of dingoes, because maintaining their genetic diversity, and adaptive potential, will be best served by managing ecologically and genetically distinct groups.

6.6 Acknowledgements

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Chapter 7

General Discussion

7.1 Summary and main conclusions

Apex predators can have profound effects on ecosystem structure and function, even when they occur at low densities (Pace *et al.* 1999). When those predators are also introduced taxa, they have the potential to create a unique and sometimes unstable ecosystem dynamics, because of the naivety of the native fauna to the new predator. The dingo is an example of such an apex predator, and its role as an ecosystem engineer as well as a declared pest has generated significant interest and research. One difficulty in understanding the ecological role of the dingo is that the Australian landscape has undergone rapid change due to land clearing, the introduction of invasive species and loss of native fauna (Johnson 2006), so the role of the dingo may also be evolving. Because of the ecological, economic and cultural importance of the dingo and its rapidly changing genetic profile due to introgression, this project sought to provide fundamental knowledge on the biology of Australian dingoes that may be used to manage them more effectively. The main outcomes of this project include a continent-wide study of dingo-domestic dog hybridisation, the first studies of genetic structure in wild dogs, and a demonstration of the variability of that structure in different regions.

The collection of samples from a broad geographic range (Chapters 3 and 6), as well as intensive sampling for fine-scale analysis (Chapters 1 and 4) in this study has established that genetic analysis is a valuable option for gathering data on cryptic wild dogs. This study identified two DNA sources (tissue from culled animals and scats) and multiple analyses that can be applied to assist wild dog management. Tissue samples from culled or collared dogs in particular provide genotypes of high quality that are excellent sources of information about kin relationships, population structure, hybridisation and movements. Collection of these samples can also be performed at low cost, easily integrated into existing control programs, used for multiple analyses, and stored long-term for reanalysis or longitudinal studies. Use of non-invasive scat samples may also be a feasible option in situations where tissue samples cannot be collected, or intensive

sampling of a small area is required, but the costs and time for processing would be higher.

The pattern of hybridisation demonstrated in Chapter 3, with higher levels of hybridisation in the southeast and lower levels in central Australia, is consistent with studies using skull measurements, whilst providing information on many areas that had never been surveyed for purity. The regions with the densest human settlement in southeast Australia displayed the highest proportion of hybrids, and the wild dogs in this area may have reached the state of 'stable hybrid swarm' (Rhymer & Simberloff 1996; Allendorf *et al.* 2001). There are, however, higher proportions of dingoes in the more remote regions of Australia, which may be more viable populations for conservation. The development of a novel Bayesian clustering analysis to determine dingo purity showed accuracy and precision when tested against simulated data, and mitigates some potential biases from using *a priori* reference groups. Both the method developed for purity testing and the broad scale of sampling for purity in this thesis will provide a strong foundation for future research into the pace and effects of hybridisation.

The variable scales and strength of differentiation of genetic structure between the three regions studied in Chapters 4, 5 and 6 indicate that the spatial ecology of wild dogs varies significantly between different Australian landscapes, and that caution should be used when generalising behaviour across the range of wild dogs. The adaptability of the dingo, which allowed it to colonise the continent so rapidly, has probably also allowed it to adapt its behaviour to different habitat types. This variability could be a result of differences in social interactions, densities, quality of habitat, prey type, anthropogenic alteration of the landscape, persecution or other cryptic factors. Although the underlying reasons for structure of the wild populations are not comprehensively addressed in this study, the presence of roads and rivers does not appear to structure dingo populations, contrary to what has been found in some other highly mobile mammals (Epps *et al.* 2005; Riley *et al.* 2006). Despite this, the structure found in Chapter 6 and in particular Chapter 5 is surprisingly abrupt, considering the dispersal capacity and adaptability of dingoes.

The microsatellite DNA genotypes collected here showed great versatility in the information they provided. Through a combination of relatedness, population

structure and individual identification, it is possible to characterise aspects of dingo ecology at multiple temporal and geographical scales. Identification of kin groups can aid in understanding recent and small-scale patterns of gene flow if sampling includes a large portion of the population (Chapter 4) or if combined with observational studies. Individual identification (Chapter 2) can be used to track the movements of individual dogs in a limited area through mark-recapture analysis, or to estimate fundamental population parameters such as abundance and survival. On a larger geographic scale, genetic structure and analyses of gene flow have provided an understanding of the average movement of dogs and the pattern of interbreeding between dingoes and dogs over the past 220 years. The combined results of this study therefore demonstrate the utility of molecular ecology to provide information on dingoes for management, from single transects to continental scale, and information on relationships, from immediate kin to between sub-species.

7.2 Management implications

The impacts of human activity on Australian dingoes have been significant, from their initial transportation to Australia, to the introduction of domestic dogs (Chapter 3), the provision of resources (Chapter 4), and their persecution. Likewise, dingoes have affected the activities of Australian settlers both indigenous and European, from their initial interactions with indigenous inhabitants immediately after their transportation, to the threat they posed to the fledgling livestock industry after European colonisation (Chapter 1). The often emotionally charged interactions between dingoes and humans have spurred the development of explicit management plans for their protection or persecution across most of their range.

For regions where conservation is the priority, this study has provided the first comprehensive predictions of current levels of hybridisation in many areas. Areas of high purity, particularly central and western Australia, have been identified, and these may be appropriate regions for conservation efforts away from pastoral enterprises. Ongoing monitoring of dingo hybridisation can be best undertaken using DNA from carcasses or buccal swabs and the application of clustering methods to determine purity.

The results from this thesis can also be applied to management of wild dogs to reduce stock losses. The extent of non-random gene flow found in Chapter 6 (less than 250 km) supports the results of studies using radio collars, that wild dogs do not routinely move over large distances (Thomson *et al*. 1992a,b). The genetic study found that this is consistent across Western Australia (although not within Queensland, or between Western Australia and Queensland), whereas the collaring studies had looked only at one area in northwest Australia. Management of these wild dogs would be best served by focussing control within areas of a few hundred kilometres, rather than expecting ingress from remote areas. Ongoing monitoring would then assist to determine the extent to which dogs move into territories cleared of resident dogs by control efforts. Although the extent of gene flow was reasonably consistent within Western Australia, the variation found in spatial autocorrelation between clusters in Queensland (Chapter 5) suggests that local estimates of the extent of gene flow are valuable for planning control strategies, rather than attempting to transfer findings across ecosystems that may not represent the local behaviour of wild dogs.

Monitoring the effectiveness of control efforts could be best performed using genotypes from scats and carcasses. This study found that non-invasive samples provided insufficient samples for mark-recapture analyses in the areas examined, although refining laboratory procedures or examining areas with a higher density of wild dogs may improve the usefulness of this approach. In low density areas, however, estimates of the minimum number alive and tracking of survival or movement of wild dogs that are recaptured can also provide insight into the behaviour of these cryptic animals. Combining monitoring of individual dogs (Chapter 2) with local estimates of gene flow (Chapters 4-6) could indicate the best scale and locations to focus control efforts.

7.3 Directions for future research

The genetic information presented here has raised further ecological questions, many of which can be addressed best by integration of molecular and field studies. Because hybridisation has become widespread since the introduction of domestic dogs, a major focus should be research into whether this will affect the ecological role of wild dogs. Social interactions among hybrids, particularly their propensity

to form packs and whether this limits reproduction, are the most likely change that could affect their density and predation ecology. Combining genetic relatedness, estimates of purity and observations of social interactions could provide valuable information on the cohesiveness of packs and whether this varies with levels of purity. Changes in prey preferences, hunting strategies, fear of humans and increased size are also potential implications of hybridisation, and therefore could affect not only wild dogs but also mesopredators and prey species within their ecosystems, through trophic cascades (Clarigde & Hunt 2008; Spencer *et al.* 2008; Glen 2010). This study has provided the first data on the scale of wild dog genetic structure and the locations of several genetic discontinuities, the understanding of which can be developed by incorporation with field data.

Ongoing monitoring of hybridisation and the extension of sampling into more remote areas could give a clearer picture of the extent and rate at which hybridisation is occurring. Due to the nature of community-assisted sampling and the management focus of this thesis, the sampling here was skewed towards inhabited and pastoral areas. Although this approach provided many samples, particularly in areas of conflict between wild dogs and humans, further focus on more remote areas through dedicated sampling would help create an even more comprehensive picture of the purity of dingoes. It may also reveal areas of high purity that are critical for conservation of the dingo's unique evolutionary heritage.

Finding the causes of genetic structure in wild dogs, and whether they are consistent across their range, could provide critical information for understanding and predicting their behaviour. As with wolves on other continents (Geffen *et al.* 2004; Pilot *et al.* 2006; Carmichael *et al.* 2007; Muñoz-Fuentes *et al.* 2009), topographic barriers do not appear to affect wild dog genetic structure noticeably, probably due to canids' adaptability and dispersal capacity. Nonetheless, they still exhibit strong and abrupt genetic structure. Integrating detailed studies of predation ecology of wild dogs with their genetic structure would test the hypothesis that variables related to prey are the most likely cause of genetic discontinuities found in Chapter 6. The growing recognition of the role dingoes play in Australian ecosystems (Johnson *et al.* 2007; Claridge & Hunt 2008; Glen 2010) provides strong impetus for continuing research into this evolutionarily and ecologically unique predator.

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