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Quantifying the population dynamics of camels in the arid and semiarid rangelands of Australia

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EXECUTIVE SUMMARY

The effective control and abatement of invasive species is an emerging wildlife management problem that is expected to increase in coming decades with the rise in globalisation, urban encroachment, climate change and spread of zoonotic diseases. A common thread with many of the pest species is that the ecological information pertaining to their ecology, such as understanding the movements and population structure remains undetermined. The other problem with invasive species is they share a number of common traits, namely that they are generalists, highly fecund, with expanding ranges and have high evolutionary potential. One such species is the dromedary, or one-humped camel in Australia.

Thirty seven percent of the Australian continent is now occupied by feral camels with recent population estimates of 1,000,000 individuals. At the current rate of increase, the population is estimated to double every six to eight years. There is growing evidence that at these densities, feral camels are adversely impacting on environmental and cultural values and infrastructure in the arid zone. Control of feral animal populations is best achieved when population (and social) structuring has been delineated, and as such the aim of this study was to generate the genetic data on feral camels in Australia. This report describes the development of nuclear and mitochondrial molecular markers for *Camelus dromedaries* with the aim of providing a greater understanding of feral camel dispersal and structuring.

Conserved mitochondrial DNA (mtDNA) sequences were used to characterise the control region of *C. dromedarius*. Alignments generated from sequencing a variable segment of the control region (n=104 camels) from around Australia were used to construct a phylogeny that revealed the population has only little control region variation with only three haplotypes detected. The limited mtDNA variation observed is likely due to a combination of both founder effects and domestication processes.

We also used STR markers to profile 390 camels. Results suggested the presence of a single panmictic population. Consistent with the mtDNA analysis low levels of genetic diversity were observed. We conclude that camels in Australia have a high potential for long-distance dispersal. Thus, localised control operations may be too narrow in their capacity to contain the rapid re-invasion of camels from surrounding areas.

If, as this study suggests, the feral camel consists of a single population, the localised control strategies need to be reassessed. As such, new and undoubtedly costly approaches may be critical to the long-term goal of eliminating the camel from inland regions.. Management plans should be adopted on a national basis rather than by state boundaries – moreover, innovative approaches will be required to manage an invasive species with perhaps the single largest population of any vertebrate yet studied – encompassing a size of ≥2.8 million km^2.

Name of Project

Quantifying the population dynamics of camels in the arid and semiarid rangelands of Australia

Project aims and objectives

We proposed to examine the population genetics of feral camel populations. With a better understanding of camel populations, strategic decisions and clear guidelines on how and where camels should be managed can be made to minimise impacts on pastoral enterprises, maximise conservation values and integrate these into management in remote indigenous communities. The project utilised technology (DNA fingerprinting) used in a similar way for feral pigs to address two major aspects of camel ecology to define: (i) how large are camel populations and (ii) what are the dispersal dynamics that maintain these populations (where do camels move to)? More specifically we aimed to;

Optimise the methodology for DNA profiling (DNA fingerprinting) camels in Australia.

1. Determine the population boundaries (size) of camel populations in the arid and semi-arid rangelands of the NT, SA and WA. And to determine the genetic effective population size of each of those populations
2. Quantify the rates of immigration and emigration and to identify key source populations of reinvasion.
3. Define exactly which populations are likely to respond best to control and use this to identify adaptive management and cost effective strategies for the management of camels, for both the agricultural and conservation stakeholders
4. Determine the historical links between multiple introduction sites and modern-day impacts to assist with management strategies.

Project location

The project was based in Perth, WA but utilised resources from across the entire distribution of the feral camel in Australia. The project collected data from all states with major camel populations (WA, SA & NT), with many regional areas represented.

Background information

Feral camels in Australia

The Australian feral dromedary camel (*Camelus dromedarius*), often referred to as the single humped camel, belongs to the family Camelidae. This group is made up of the 'New World Camelids' from South America (llamas, alpacas, vicunas and guanaco) together with the larger 'Old World Camelids' of Asia and Africa. This latter group consists of only two species, the bactrian camel (*Camelus bactrianus*) and the dromedary camel.

The natural range of *C. dromedarius* is concentrated around Northern Africa and middle-east states (Fig 1). Attempts have been made at introducing the dromedary camel to many parts of the world, however most have been unsuccessful, with the exception of Australia (Long 2003) which contains the only and largest known feral camel population in the world (Mason 1979).

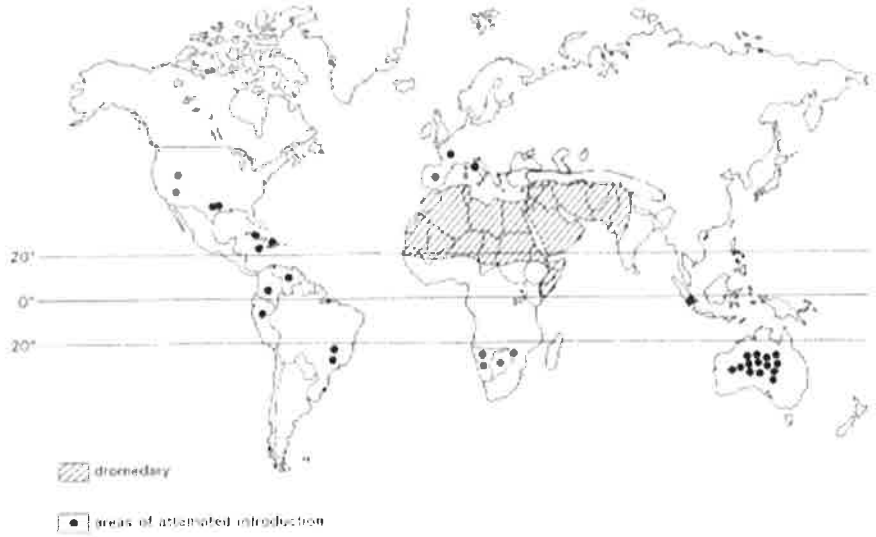

Figure 1
Global representation of the attempted introduction sites of dromedary camels (from Köhler-Rollefson 1991).

Introduction of camels in Australia

The dromedary camel was first introduced into Australia in 1840 (McKnight 1969). The first importation of camels into Australia occurred in October of 1840 into South Australia; only one camel survived the shipment. A second importation occurred in December of 1840 into Tasmania, where two camels were imported (and bred). The third importation of camels occurred in 1860, where 24 camels were imported into Victoria for the Burke and Wills expedition, followed by importations by Sir Thomas Elder and Samuel Struckey to establish a breeding property, Beltana station in South Australia. This importation was the initial nucleus of camel source population. Camels were beginning to prove their usefulness in commercial freight hauling and were dispersed from Beltana station to NSW, QLD and WA. Further importation of camels from India into Port Augusta (SA) and Fremantle port (WA) also occurred (McKnight 1969; Rolls 1969).

An expansion of camel breeding occurred across Australia, with the focus being on natural increase rather than continued importation. This was enhanced by the establishment of camel depots that served as centres for breeding and training, servicing Ghan towns which housed the skilled Afghans, who were originally used to train and handle the camels. Camels were commonly used for riding, as draught and pack animals for exploration, as commercial carriers, and in major infrastructure projects; for example in the construction of the overland telegraph line, the Canning Stock Route in WA, the Rabbit Proof Fence and the Oodnadatta-Alice Springs railway (McKnight 1969).

Estimation of camel numbers

In total, an estimated 10 000 to 20 000 camels were imported into Australia between 1880 and 1907 (the origin of which is unknown due to the many importations and poor records held; Edwards *et al.* 2004; McKnight 1969). Registered records show that the number of domesticated camels held in Australia peaked in 1920 at 12,649 but then declined steadily - with the advent of modern transport. With mechanisation, camels were superseded and many were released (McKnight 1969; Dörges & Heucke 2003). Their suitability to the Australian climate led to the feral camels breeding prolifically and spreading across Australia. The current estimation of feral camel numbers is approximately 1,000,000 (Glen Edwards, *pers. comm.*) (Table 1) with populations estimated at doubling every six to eight years (Edwards et al. 2004; Dörges & Heucke 2003), inhabiting 2.8million km^2 or 37% of Australian mainland (McLeod 2004). Recent aerial surveys in the Great Victoria Desert region of WA indicate that the Australian population may be considerably less than 1,000,000 (Ward *pers. comm.*).

Table 1. Estimated camel population sizes for Australia from 1966 to 2007 (adapted from Ward *et al.* 2005 to include information from Edwards *et al.* 2004 & B.Ward, *pers. comm.)*

Year	State	Population estimate	Source
1966	NT	4 500-6 000	McKnight 1969
		15 000-20 000	McKnight 1969
1979	NT	3 000-6 000	Letts *et al.* 1979
1986	NT	31 570 - 100 000	Graham *et al.* 1986
1994	NT	60 000 - 200 000	NT Conservation Commission 1994
2001	NT	80 533 - 300 000	Edwards *et al.* 2004
2005	WA	238 000 - 476 000	Ward & Burrows 2005
2007	WA	364 000 - 728 000	B.Ward & N. Burrows *pers. comm.*

Decline in the domestic requirement and establishment of the feral population

The decline and phasing out of the dromedary was due to a number of reasons including prolonged drought, proliferation of motor cars and trucks, the development of four wheel drives, the increased rate of roadway construction and reduced remoteness of the outback (McKnight 1969).

The decline in demand for camel use also led to a decrease in the demand for camel depots. Camel depots were still in operation in the early 1920's with large inventories of stock. The last camel depot was closed in 1938. Camels were either sold at a reduced cost (as little as three pounds) or slaughtered. Many camels escaped, or released by their owners, two noted cases inlude Marble Bar where 50 camels were released and Wiluna

where 200 camels were released by the last Afghan carter working there (Ameer Bux). It is likely that a considerable number of camels were released intentionally, or escaped, but the number remains unquantifiable.

Impacts of feral camels

Feral camels are estimated to contribute $200K worth of economic loss (approximately 25% is sheep and 75% cattle production loss). However, they inhabit mostly non-agricultural areas at present and important environmental and social impacts are yet to be quantified (McLeod 2004). Despite the small agricultural impact of camels (in comparison to other pests) they are still a major vertebrate pest to Australia and this estimate is presumably a gross underestimate of the true costs.

In terms of environmental costs, when camel numbers remain at low population densities they do not appear to have a major impact on the environment. Their padded leathery-feet do less damage than the hard-hoofed ungulates and their browsing patterns (on the move) means that they do not generally feed intensively in one area. Camels feed on more than 80% of the plant species in arid Australia (Dörges & Heucke 2003). While the impact of camel browsing does not contribute to a loss of diversity, their continual browsing can have a serious impact on some shrub and tree species including the curly pod wattle and bean tree (Dörges & Heucke 2003). Interestingly, they may in fact have replaced the role of the large mega-herbivours that have long vanished from mainland Australia.

The impact of feral camels can change dramatically and be very pronounced when at high population densities (>2 camels per km^2) and during periods of drought (where they remain around waterholes). Feral camels reduce shelter for small desert mammals and also compete with food sources traditionally harvested by indigenous Australians. They can also spoil natural water sources and artificial water points. Agriculturally feral camels can damage fences and infrastructure at cattle watering points (Edwards *et al.* 2004). Camels also compete with cattle forage (McLeod 2004) and have the potential of spreading livestock diseases when densities are high.

Biology adaptation and population dynamics of camels

The morphological adaptations of feral camels have made them important and successful invaders in arid zones of Australia. Adaptations to the desert environment include their dual purpose coat, insulating in winter and reflecting the heat in summer. Camels are highly mobile, foraging up to 70 km per day (facilitated by long legs which enable rapid movement over long distances) even during food abundance. Their feet are padded, adapted for movement on sand, and providing insulation from the heat of ground surfaces. They can conserve water by reducing sweat and concentrating their urine. Camels also have the ability of increasing body temperature up to 3 degrees Celsius, storing heat in the body: further conserving water instead of being dissipated by its evaporation. They have the ability to withstand prolonged dehydration due to their tolerance of a high degree of desiccation of their bodies. Their hump is a store of fat, which is a source of energy when food is scarce. Camels also have a high tolerance to salt, and therefore have the ability to consume plants and water with a high salt concentration (McKnight 1968; Ward *et al. 2005 &;* Siebert & Newman, available online). All of these physiological adaptations make camelids well suited to life in arid Australian conditions.

Sexual maturity is reached at approximately 4 years of age. Females come into oestrus several times a year; males come into rut only when food is abundant (usually in winter), when the pituitary glands secrete gonad stimulating hormones. Gestation lasts between 360 and 380 days. The young is suckled for more than a year (Siebert & Newman, available online).

Group size appears to be dependent on the amount of rainfall, with a high amount of rainfall being directly proportional to larger group sizes. Camels appear to live in non territorial groups of three kinds; year round groups of bulls (bachelor groups), summer groups of cows and calves and winter breeding groups (mature bull with several cows and their calves). During the breeding season (May to October) the females group together to defend against advances from other males. Seasonal range patterns were also recorded on a study conducted by Heucke *et al.* (1992) who found that during summer camel groups stayed in small areas not wandering far. Alternatively in winter breeding groups meet frequently with bull groups, wandering great distances restricted only by access to areas (Heucke *et al.* 1992).

Control strategies

The primary aim in controlling any pest species is to remove or reduce the animal's adverse effects (Cowan & Tyndale-Biscoe 1997). For eradication to be successful mortality rates must exceed birth rates and no immigration can occur to prevent reinvasion of pest free areas (Bomford & O'Brien 1995).

There have not been any limiting factors to the growth of feral camel populations in Australia. Camels do not have natural predators, disease has not been recorded as having an impact on numbers and food supply is always sufficient (Dörges & Heucke 2003; Edwards *et al.* 2004). Camels also travel great distances (up to 70km a day) (Siebert & Newman, available online), have extensive home ranges and are distributed over large uninhabited areas (Norris & Low 2005) making control strategies difficult to implement.

Dörges and Heucke (2003) suggest that approximately 10% of the camel population numbers need to be removed annually to maintain the stability of the population (as populations double every 6-8 years). The most commonly used techniques to control feral camel numbers are aerial and ground shooting. These methods are most effective; however are often opportunistic and therefore ineffective as management strategies. Other methods also used are fencing off key areas and live wild harvesting for commercial sale (Edwards *et al.* 2004). At present population numbers (estimated at 730 000 (B. Ward *pers. comm.*) control strategies will require substantial efforts to remove the approximate 80 000 individuals needed to halt the natural rate of increase per year.

A better understanding of the demographic structure of camels is therefore needed for more effective control efforts, this is particularly important in highly mobile large herbivores (Edwards *et al.* 2004). The application of molecular ecological data to feral animal management has huge potential by establishing the size and rate of dispersal patterns. An effective control programme requires the knowledge of immigration rate and source/sink dynamics. Genetic techniques which are able to identify the stage of population growth or change are useful in management so that a population can be targeted when eradication will be most successful. The detection of population contractions (bottlenecks) can be

measured by detection of excess heterozygosity and through identification of shifts in allele frequency distributions (Rollins *et al.* 2006).

Application of molecular ecology to feral animal management
Molecular ecology is the use of genetic information to assist demographic studies of populations (Hampton et al. 2004). It provides an ideal complementary technology for field based studies by providing additional information on population parameters of animal species that field based techniques would not provide (Sunnucks 2000). Information from molecular ecology studies can be generated for all levels of population structure; from a landscape scale involving relationships between separate populations, to a management scale; concerning relatedness of social groups within a population, as well as fine scale social analysis, involving relatedness of individual animals within a group (Milligan *et al.* 1994; Sunnucks 2000). Current management of feral camels includes fencing off key areas, live wild harvesting for commercial sale and culling (ground and aerial based) (Edwards *et al.* 2004). Management techniques require a different and innovative approach due to the population size and difficulty in managing the Australian feral camel population. Molecular techniques can provide the underlying framework to assist in designing a more effective population control *strategy*. Molecular markers such as microsatellites and mitochondrial DNA allow a large amount of information from an individual in a population to be determined. This information can be used to compare to other populations of the same species and allow the inference of gene flow, species boundaries, parentage and molecular evolution.

Aims and scope of report

The remote and arid habitat of the Australian feral camel makes it a difficult species to study. The research in this report forms a pilot study that aimed to identify and develop molecular markers that will ultimately lead to informed decision making regarding the control strategies for feral camels.

Development of mitochondrial markers:

- For the first time sequence the control region of the Australian dromedary camel.
- Develop and optimise a PCR assay to amplify the control region of *C.dromedarius*.
- Determine the phylogenetic relationship among a subset of *C.dromedarius* sampled from across Australia, and to determine the relationship with other camelid species.
- Identify if any phylogeographic signals exists within the Australian population.

Development of microsatellite markers:

- Develop efficient, high throughput STR markers for profiling feral camels based on existing camelid STR markers.
- Determine what population boundaries and social structure, if any, exist in the arid and semi arid rangelands of Western Australia.
- Compare the Western Australian, South Australian and Northern Territory camel populations to determine genetic differences between populations in the aid of

targeting control efforts.

- Quantify the rates of immigration and emigration to identify key source populations of reinvasion.
- Define specific populations which are likely to respond best to control and use this to identify adaptive management and cost effective strategies for the management of camels for both agricultural and conservation stakeholders.

Methodology

Sampling and study sites

A total of 558 feral camel samples (ear, skeletal muscle and liver) were collected between September 2005 to October 2007. A subsample of 390 camels were used in this study from localities in Western Australia (n=332), Northern Territory (n=47) and from South Australia (n=11) (Fig 2 & Table 2). All available samples were used from sampling points from the Northern Territory and South Australia. Samples were collected with records/details of demographic data including sex, weight, age class (juvenile or adult) and social grouping as well as a GPS coordinate location.

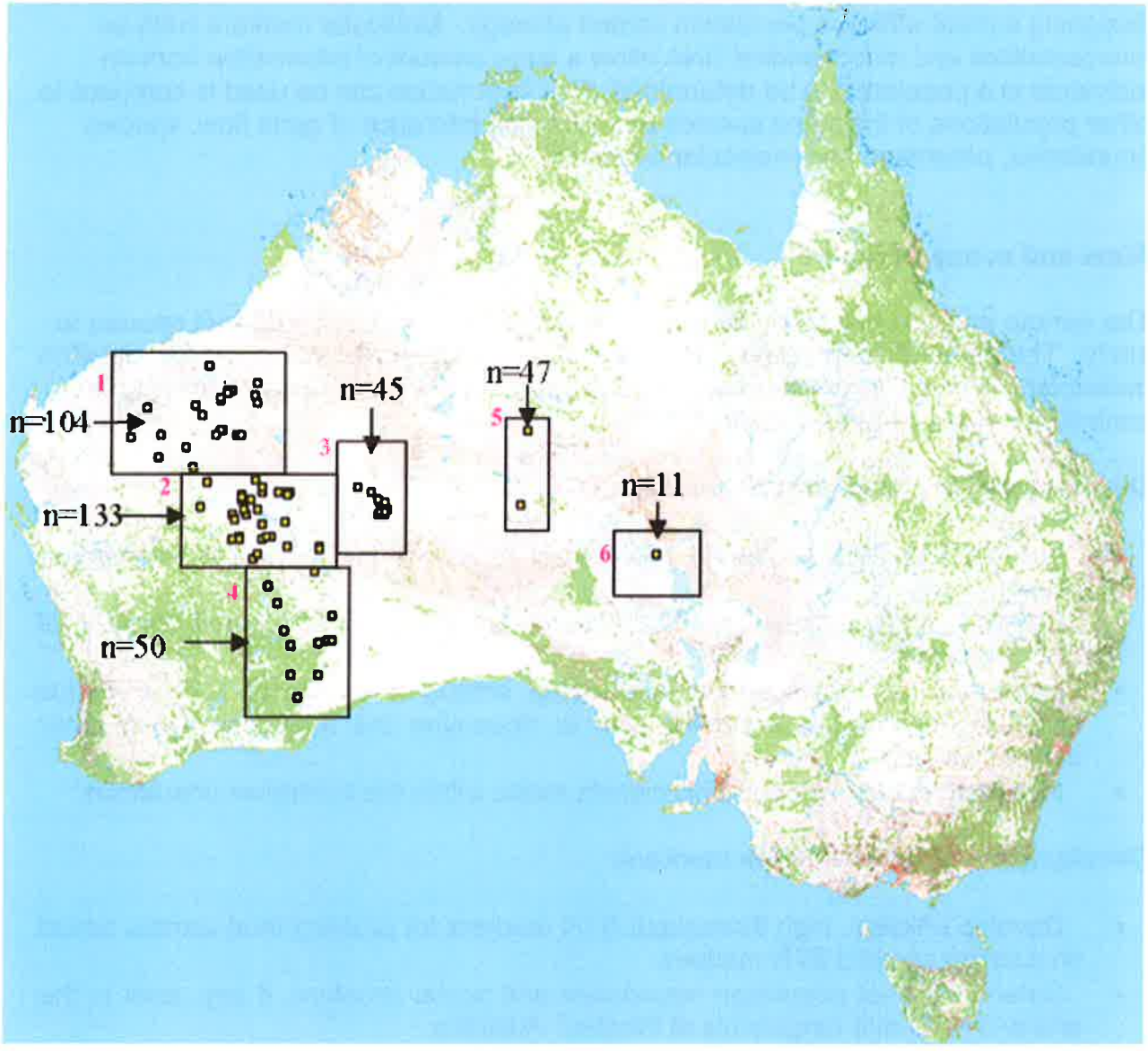

Figure 2
Map of feral camel tissue sample collection sites & sample groups. Numbers are representative of population groupings based on distribution given in Table 2.

Table 2 The number of camels sampled across Australia grouped based on distribution, and the number of these which are adults.

Populations groupings based on distribution	Location	Number of camels samples (*Adults & Juveniles*)	Adults Only
1	WA (North)	104	77
2	WA (Middle West)	133	106
3	WA (Middle East)	45	28
4	WA (South)	50	37
5	NT	47	41
6	SA	11	11
TOTAL		390	300

DNA extraction
Tissue (ear, muscle and liver) samples were preserved in a solution of 20% dimethylsulphoxide (DMSO) saturated with NaCl. DNA was extracted from a small piece of tissue (approximately $2mm^3$) which was suspended in tissue digestion buffer (20mM EDTA; 50mM Tris; 120mM NaCl; 1.5% SDS) containing 0.1mg of Proteinase K solution following standard procedures described in Hampton *et al.(* 2004).

Mitochondrial amplification
The lack of any previous characterisation of the *C. dromedarius* control region meant that the first stage of this project involved designing new generic camelid primers. Initially primers designed for the amplification of Llama subspecies (*L. guanicoe*, *L. glama* and *L.pacos*) were employed. These primers were targeted to sequences in tRNAPro and tRNAPhe genes (Table 3; Maté *et* al 2004) and should have amplified a 1000bp fragment. However, after numerous sequencing attempts we failed to obtain the *C. dromedarius* control region.

The lack of success with the tRNAPro and tRNAPhe primers necessitated a second attempt using degenerate primers designed for camelid species (our data). Primers LthrArtio (Forward) and H362 (Reverse) (Table 3) were used. Amplification produced a fragment with an approximate size of 380bp, which conferred with the expected size (Maté *et al.* 2004). The resulting amplicon was sequenced (data in Appendix 1) and a more specific primer set was designed to include the region with the most variation, based on an alignment of all camelids taxa.

Table 3 Control region primers for *C. Dromedarius*

Primer	Sequence (5`→3`)	Initially designed for
tRNAPro	CTGATAAATCCCATAGAGC	*Lama* [1]

tRNAPhe	TTTCAGCGCCTTGCTTTAAG	*Lama*[1]
LthrArtio (F)	GGTCCTGTAAGCCGAAAAAGGA	*C. bactrianus*[1]
H362 (R)	GGTTTCACGCGGCATGGTGATT	*C. bactrianus*[1]
CR_71F	CCAGCACCCAAAGCTGGA	*C. dromedarius* [2]
CR_29F	CACCCTCCCTAAGACTCAGGG	*C. dromedarius*[2]
CR_305R	TTGACTGGAAATGATTTGACATAATG	*C. dromedarius*[2]
CR_302R	GGAAATGATTTGACATAATGCGC	*C. dromedarius*[2]

[1]: Maté *et al.* (2004); [2]: *this study*

Four primers were designed: CR_71F, CR_29F, CR_305R and CR_302R (Table 3), and optimised under various conditions. The primer combination that produced the most efficient PCR was found to be CR_29F and CR_305R. Using this PCR assay a sample of camels from a geographic spread across WA, and a subset of NT and SA camels was chosen to conduct a pilot study into the levels of mtDNA diversity in the population.

PCR reactions were performed in a total reaction volume of 25µL with approximately 50-100ng DNA. Final concentrations were 1x Reaction Buffer (Fisher Biotech), 2.5mM $MgCl_2$ (Fisher Biotech), 0.1mM bovine serum albumen (Biotech International), 0.25mM of each deoxynucleotide (Astral), 10 pmole of each primer and 0.25U of *Taq* polymerase. PCR reactions consisted of an initial denaturation of 95°C for 3 minutes, followed by 40 cycles of 95°C 45 seconds, 55°C 45 seconds and 72°C 45 seconds. This was followed by an extension step of 72°C for 10 minutes.

Amplicons were cleaned up and sequenced using BigDye 3.1 chemistries and 3730 capillary sequencer. DNA sequences were checked for fidelity by eye and deposited into an alignment in Geneous (BioMatters) together with other camelid species.

Microsatellite amplification

Eighteen microsatellite primers previously used in the Camelidae family (*Camelus bactrianus*, *C. dromedarius*, *Llama glama* and *L. pacos*) were chosen for optimisation in Australian camels. Of these, six were omitted due to poor amplification or difficulty with allele scoring. Of the remaining, 12 microsatellite loci were shown to be highly informative for *C. dromedarius* (Table 4).

PCR reactions were performed in a total reaction volume of 40µL with approximately 50-100ng of DNA. Final concentrations were 1x Reaction Buffer (Fisher Biotech), 2.0mM $MgCl_2$ (Fisher Biotech), 0.1mM bovine serum albumen (Biotech International), 0.4mM of each deoxynucleotide (Astral), 8 pmole of each primer and 0.15U of *Taq* polymerase (Fisher Biotech). Primers were fluorescently labelled with either FAM, VIC, NED or PET (Table 4). Temperature optimisation between 50 and 65°C was performed for all primers. All primers perform optimally at 55°C. As such, PCR conditions for all reactions entailed an initial denaturation of 95°C for 5 minutes, followed by 35 cycles of 94°C 1 minute, 55°C 1 minute and 72°C 1 minute. This was followed by an extension step of 72°C for 10 minutes.

Fragment analysis

Fragments were combined with loading mix, containing Hi-Di Formamide (Applied Biosystems) and Genescan Liz-600 size standard (Applied Biosystems). Fluorescently-labelled DNA fragments were run on an Applied Biosystems 3730 40 capillary sequencer.

DNA fragments were scored with the aid of GeneMarker software (v1.5, Soft Genetics), and recorded in an electronic spreadsheet.

Table 4 The characteristics of the 11 microsatellite loci amplified in feral camels (adapted from Penedo et al. 1998 Penedo et al. 1999; Mburu et al. 2003; Obreque et al. 1998; Lang et al. 1996 and Mariasegaram et al. 2002). N/A= information was not available; *represents fluorescent dye on reverse primer

Primer	GenBank	Allele size range	Fluorescent Dye*	Forward Primer (5`→3`)	Reverse Primer (5`→3`)
VOLP03	AF305228	144-176	**FAM**	AGACGGTTGGGAAGGTGGTA	CGACAGCAAGGCACAGGA
VOLP32	AF305234	256-262	**VIC**	GTGATCGGAATGGCTTGAAA	CAGCGAGCACCTGAAAGAA
YWLL08	N/A	134-172	**NED**	ATCAAGTTTGAGGTGCTTTCC	CCATGGCATTGTGTTGAAGAC
YWLL38	N/A	182-188	**FAM***	GGCCTAAATCCTACTAGAC	CCTCTCACTCTTGTTCTCCTC
YWLL44	N/A	90-114	**FAM**	CTCAACAATGCTAGACCTTGG	GAGAACACAGGCTGGTGAATA
LCA33	AF060103	136-164	**PET**	GAGCACAGGGAAGGATATTCA	ACAGCAAAGTGATTCCATAATA
LCA37	AF060105	132-133	**VIC**	AAACCTAATTACCTCCCCCA	CCATGTAGTTGCAGGACACG
LCA56	AF091122	133-169	**VIC**	ATGGTGTTTACAGGGCGTTG	GCATTACTGAAAAGCCCAGG
LCA66	AF091125	240-244	**VIC**	GTGCAGCGTCCAAATAGTCA	CCAGCATCGTCCAGTATTCA
CMS50	AF329149	170-190	**PET**	TTTATAGTCAGAGAGAGTGCTG	TGTAGGGTTCATTGTAACA
CVRL01	AF217601	196-242	**FAM***	GAAGAGGTTGGGGCACTAC	CAGGCAGATATCCATTGAA

Data analysis and statistical calculations

Population structure

The programme Structure (Pritchard et al. 2000) was used to determine population structure from the total sample before any further statistical calculations were made. This is an increasingly utilised approach when individuals are not arranged in a clustered distribution and also for detecting cryptic structure that is not obvious from sampling locations. Structure uses the Bayesian clustering method to identify the inferred population based on the likelihood of a given genotype estimated by allele frequencies. K populations are characterised by a set of allele frequencies at each locus, individuals are then assigned to populations on the basis of genotype. The assumptions of Structure are that populations are within Hardy Weinberg equilibrium (HWE) and the marker loci are unlinked and at linkage equilibrium within the population. Thus each allele at each locus for each individual is an independent representative of frequency distribution (Pritchard et al. 2000). Structure therefore models the data to minimise linkage and increase conformance to HWE. Using this approach the relative contribution of each inferred population into each predefined sample group and each individual animal was determined. Individuals were organized into predefined sample groups, based on the geographic area samples were collected from. Inferred populations were calculated based on the groups

that clustered together from assignment results, determined by implementation of Markov Chain Monte Carlo (MCMC) algorithm. This approach requires no prior knowledge or assumptions of the true population structure (Pritchard et al. 2000).

The results generated were based on simulations from one to ten (K=1-10) inferred populations, using a burn-in period of 50,000 iterations with 10^6 iterations of MCMC simulation (repeated 10 times). The number of inferred populations (K) most compatible with the data site was estimated following Evanno et al. (2006). The sampled population was run in a variety of combinations: including the entire group (as one population, and also on the assigned groupings based on the distribution), all juveniles excluded and juveniles excluded and male and females treated as separate groups. Various combinations were run to ensure populations were assigned correctly.

The programme GeneClass2 (Piry et al. 2004) was also used to determine population structure, as an alternative method. This method assigns individuals based on the locus data for a reference population. A Monte Carlo re-sampling method is used to identify a statistical threshold beyond which individuals are likely to be excluded from the reference population. This is based on the distribution of genotype likelihoods in a reference population sample, and is also compared to the likelihood for the individual to be assigned to that distribution. The camel genotypic information was again run multiple times, with the possibility of belonging to one population, and again based on the assigned group numbers based on distribution. The results generated were based on the assigning of individuals to the reference population, using the Rannala & Mountain (1997) Bayesian method, with the simulation algorithm of Paetkau et al. (2004), simulated 10000 times with an α of 0.01. Genepop (Raymond & Rousset 1995) was used to test the assumptions of HWE and marker linkage.

Genetic variation within populations

Measures of genetic diversity within populations: mean observed (H_o) and mean expected heterozygosity (H_e) (Nei 1987) and mean observed (NA) and mean expected number of alleles (NE) (Kimura & Ohta 1964) were calculated using PopGene2 (Yeh et al. 1999). Bottleneck (Piry et al. 1999) was also used to test if the populations have undergone recent bottlenecks using a Wilcoxon sign-rank test with parameters of 1000 iterations, using a transitional phase mutation model (TPM) (consisting of 90% SMM and 10% IAM) (see Section 1.7).

Estimates of effective population size (N_e)

Effective population size is the size of an ideal population that looses genetic variation at the same rate as the real population. Effective population size is approximately equal to or more likely less than the number of breeding individuals in a population (Caughley 1994; Frankham et al. 2002). Ne is a relative measure of population size and may differ widely from census population size based on observational studies. The effective size of a population is correlated with the rate of change of genetic variation and the level of genetic diversity already existing within that population (Crow & Denniston 1988). Estimates of effective population size was calculated based on (i) the population groups assigned by Structure and (ii) as a one single population (juveniles excluded) and (iii) Adult Male and Females treated as separate population; using the programme NeEstimator (version 1.3, Peel et al. 2004) which estimates effective population size using a point estimation method based on allele frequency data using linkage disequilibrium (Hill 1981).

Results

Mitochondrial DNA analysis in Australian camelids

MtDNA can provide valuable information on evolutionary relationships among the camelid species (Ballard & Whitlock 2004). In the context of this study it was suggested that the control region sequence might provide information on the effect of introduction on species diversity allowing comparison between the camelid lineages. Mitochondrial DNA is maternally inherited, and as such mutations do not arise from recombination during sexual reproduction. The variation in the mitochondrial DNA shows the differences in maternal lineages. The mitochondrial genome has a higher rate of mutation than nuclear DNA, which accumulates genetic variation within and between populations. This high rate of mutation allows the use of mtDNA to distinguish variation between closely related species, which forms the basis of the construction of phylogenetic trees (Avise *et al.* 1987; Baker 2000). During the course of this study, the entire mtDNA genome of the two humped *C. bactrianus* was sequenced by Cui *et al.* (2007) (GenBank accession number: EF212037, EF507798, EF507799). This is fortunate as it allowed a direct alignment of *C. dromedarius* closest relative. This study is the first to characterise the mtDNA control region in the dromedary camel.

Two generic primer combinations were used in this study; LthrArtio and H362 (Marín *et al.* 2007) which provided the expected amplicon size. Specific dromedary camel control region primers were developed for the most heterogeneous section of the control region (Table 2.2); two forward (CR_71F and CR_29F) and two reverse (CR_305R and CR_392R) primers were designed (Fig 3). Various primer combinations were tried, and CR_29F and CR_305R were found to be the most specific and robust (Fig 3).

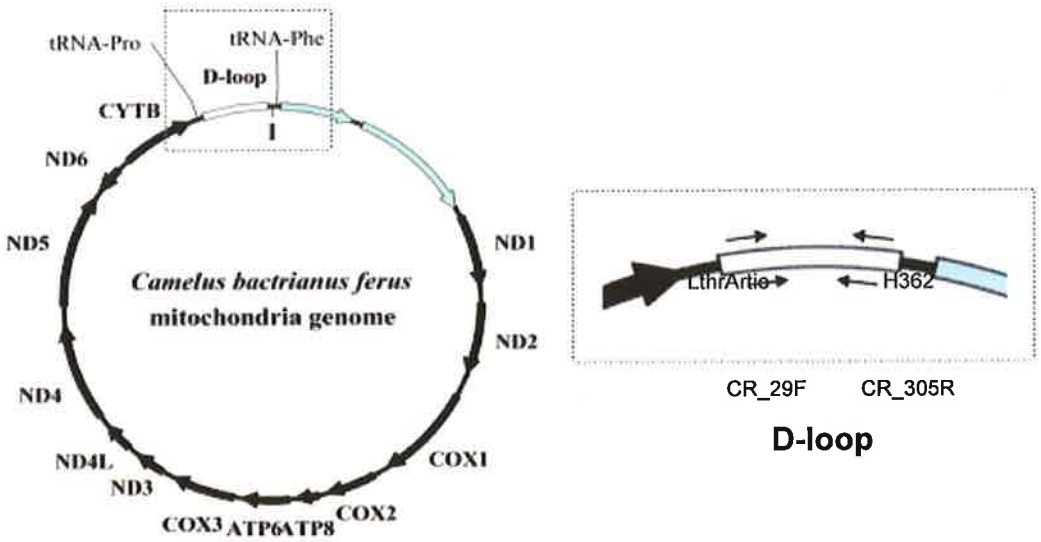

Figure 3

A schematic map of the mitochondrial genome of *C.bactrianus ferus* illustrating the position of the control region and primer binding sites. The camelid CR is situated between tRNA proline and phenylalanine. Locations of LthrArtio and H362_R primers (generic camelid primers) are shown as well as the location of the designed dromedary specific primers 29_F and 305_R, the main primers employed in this study (adapted from Cui *et al.* 2007).

Sequence variation in the control region of the bactrian and dromedary camel can be visualised in the alignment below (Figure 4). There are a total of 15 base differences between the two species, of which 3 substitutions are transversions, the rest being transitions.

```
                                1         11         21         31         41         51
                                |         |          |          |          |          |
C_bactrianus_D1_EF212037 GAGCCACACT CTCCCTAAGA CTCAGGGAAG AGGCCAAAGC CCCACCACCA GCACCCAAAG
camel_C0102              .G.......C .......... .......G.. .......... .......... ..........
camelCR_29F              -------CACC CTCCCTAAGA CTCAGGG--- ---------- ---------- ----------
camelCR_305R             ---------- ---------- ---------- ---------- ---------- ----------

                                61         71         81         91         101        111
                                |          |          |          |          |          |
C_bactrianus_D1_EF212037 CTGGAATTCT CATTAAACTA CCCCCTGACC CCCGCCAAAA CGGCGGTAGC CCTTGAGTAT
camel_C0102              .......... T......... .......... T......... ....AA.... ..........
camelCR_29F              ---------- ---------- ---------- ---------- ---------- ----------
camelCR_305R             ---------- ---------- ---------- ---------- ---------- ----------

                                121        131        141        151        161        171
                                |          |          |          |          |          |
C_bactrianus_D1_EF212037 TATTACAGTA CTAAAAACCA GATAACATGC CCAACGTGCA TGAAACTTCA ATACTGACAT
camel_C0102              ....T..... .......... C..GT..... .TGG...... ......C... ..........
camelCR_29F              ---------- ---------- ---------- ---------- ---------- ----------
camelCR_305R             ---------- ---------- ---------- ---------- ---------- ----------

                                181        191        201        211        221        231
                                |          |          |          |          |          |
C_bactrianus_D1_EF212037 GTCACAGCAC GCGTTGCGTG CTATATGTAC ATCGTGCATA AATTTGTTTG CCCCATGCAT
camel_C0102              .......... .......... .......... .......... .......... ..........
camelCR_29F              ---------- ---------- ---------- ---------- ---------- ----------
camelCR_305R             ---------- ---------- ---------- ---------- ---------- ----------

                                241        251        261        271        281        291
                                |          |          |          |          |          |
C_bactrianus_D1_EF212037 ATAAGCATGT ACATCTTATC CTTGTTCGTG CATAGCACAT TATGTCAAAT CATTTCCAGT
camel_C0102              .......... .........T .......... ......G... .......... ..........
camelCR_29F              ---------- ---------- ---------- ---------- ---------- ----------
camelCR_305R             ---------- ---------- ---------- -------CAT TATGTCAAAT CATTTCCAGT

                               301        311
                               |          |
C_bactrianus_D1_EF212037 CAATACGCAT ATCATAACC
camel_C0102              .......... .........
camelCR_29F              ---------- ---------
camelCR_305R             CAA------- ---------
```

Figure 4

Alignment of the control region of *C. bactrianus* and *C. dromedarius* (ID number C0102). Forward and reverse primer binding sites as indicated. The sites of variation between the dromedary and bactrian camels can be seen as indicated by the change in bases. Dots are indicative of identity with the *C. bactrianus* sequence.

Characterisation of the dromedary control region

A representative spread of camels across a wide geographical distribution from Australia were chosen for the control region analysis using the CR_29F and CR_305R PCR assay. This was to ensure all areas were adequately represented with the aim of maximising the chance of seeing phylogeographic differences across the Australian population.

The raw sequence chromatographs obtained from the CR_29F and CR_305R assay of *C.dromedarius* was aligned by eye using Geneious software (Biomatters). The phylogenetic tree shows three maternal haplotypes within the Australian dromedary camel population. The phylogenetic signal of *C. dromedarius* is a result of nucleotide substitutions at three sites. The variable sites in the alignment can be seen in Appendix 3 (found on the attached CD). These occur at position number 54 (A → G), position number 96 (G → A) and position number 230 (G →A). These mutations are all purine transversions. Sequence heterogeneity can occur for one; two or all three variable sites, and as such result in the different tree topology seen in Figure 3.3. The alignment (Appendix 3) generated for the different camelid subspecies also shows that within the Camelidae family there are many nucleotide substitutions; highlighting the separation of species and hence different branching on the phylogenetic tree (Purdue *et al.* 2006).

Furthermore, the mtDNA haplotypes were mapped on the basis of their distribution within Australia (Figs 5 and 6). The three haplotypes are each represented by a different colour (as indicated by the blue, yellow and pink shading on the phylogenetic tree) which correspond to the branching on the tree which have been mapped based on distribution below and show that there is poor phylogeographic signal based on haplotype (Figures 5 and 6). Geographic distributions such as found above correspond with the absence of geographical barriers constrictive to movement, which is seen in the Australian dromedary population who do not have any factors limiting their movement and range (see Avise *et al.* 1987)

Phylogenetic reconstructions

A phylogenetic analysis was conducted using Geneious software (Biomatters) using a neighbour joining tree method under a Hasegawa Kishino Yano (HKY) nucleotide substitution model. The HKY model allows for the asymmetric base frequencies (transitions and transversions). The resulting phylogeny of camels is very shallow, and give three clear maternal haplotypes within the Australian dromedary camel (Fig 5). The haplotypes based on the distribution within Australia (given in different shades in Fig 5 and 6) clearly demonstrate the poor phylogeographical structure.

Bootstrap values were calculated with 1000 bootstrap replicates showing support values greater than 80%. Bootstrapping measures how consistently the data supports the topology by re-sampling data. A high bootstrap value of 100% for the dromedary and bactrian branches indicated that there is uniform support for the monophyly of the bactrian and dromedary camel, excluding them from other South American camelid species

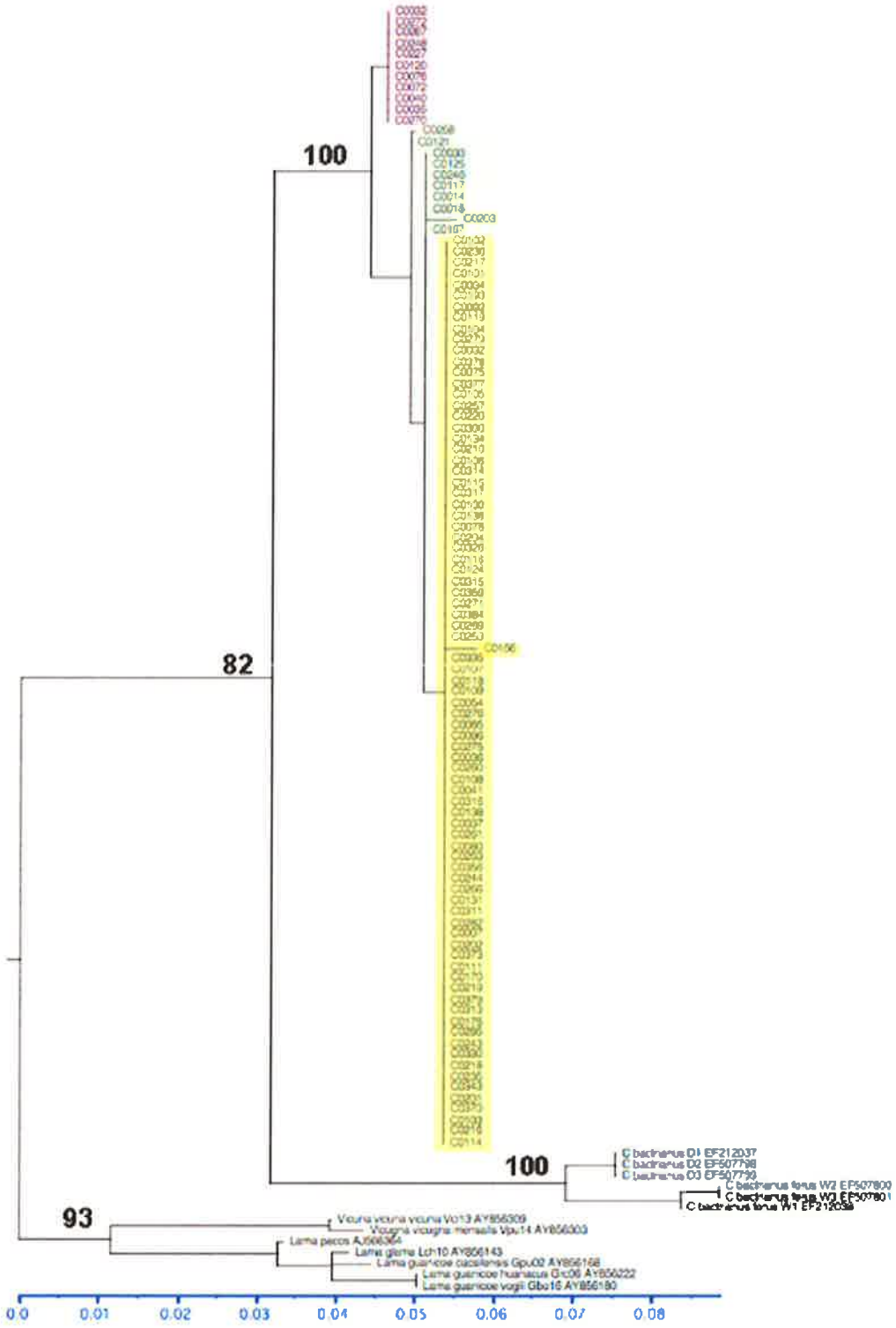

Figure 5

Phylogeny of the mtDNA haplotypes found in Australian camels. The NJ tree was constructed under a HKY model using 1000 bootstrap replicates. The haplotypes shaded correspond to the lineages colouring in the following figure (Fig 6).

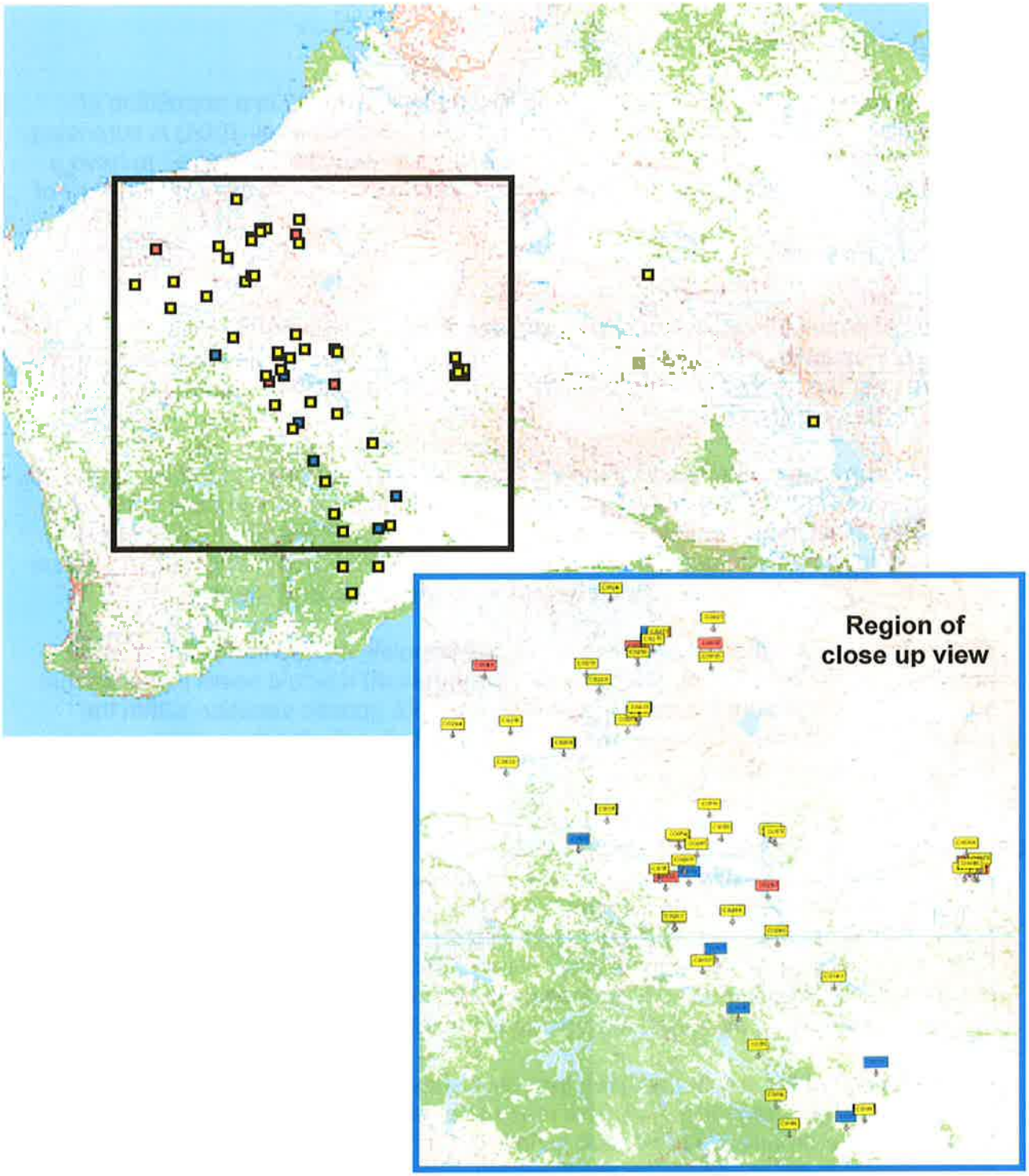

Figure 6
The geographic distribution of the different mtDNA haplotypes found in the *C.dromedarius* represented by different colours indicating the haplotypes given in the phylogenetic tree, Figure 3.3. The close-up view of the WA camels demonstrate that these haplotypes are mixed.

(Muyzer *et al.* 1995).

Causes of reduced genetic variability

Finding only three mtDNA haplotypes with limited genetic diversity in a population of camels estimated to be approximately 700 000 (*sensu* Edwards et al. 2004) is surprising. There are many examples where a population of this size would be expected to have a much greater amount of genetic diversity. There are two possible causes for this lack of diversity:

Founder effect

The camel population in Australia was founded from a limited number of maternal lineages. Populations founded by a small number of individuals will carry only a small proportion of the total genetic variation of the parental population (Carson & Templeton 1984; Halliburton 2004).

Based on the known number of camels introduced into Australia it is plausible that this is the case, a limited number of camels were initially introduced and bred. The most successful initial importation being that of Sir Thomas Elder and Samuel Strucke. In 1866 they imported 124 camels and established the Beltana breeding station. Focus was placed on breeding rather than the continual importation of camels.

Due to the small number of founders within the population (who carry only a small proportion of the parental population's genetic variation) it would seem plausible that the founder effect is the cause of the reduced mtDNA genetic variation within the population. However a selective sweep of the population (in the time since release) also cannot be discounted.

Bottleneck prior to introduction in Australia

The dromedary camel population may have bottlenecked prior to introduction into Australia. Bottlenecks occur as a result of a reduction in population size. Population bottlenecks reduce genetic variation and increase the level of inbreeding (Hedrick & Miller 1992; Maruyama & Fuerst 1985; Leberg 1992). Domestication, a process of reorganisation of wild species to domesticated forms according to human requirements, can be attributed to population bottlenecks (Hummer 1990).

Domestication of the dromedary was thought to have occurred around 3000 BC (Mburu *et al.* 2003; Mason 1979). The reasons behind the capture, taming and breeding of the dromedary may have initially been non-utilitarian however would have eventuated the use of camels for work related purposes (Mikesell 1955). Bulliet (1975) suggests that domestication was effected by an unknown pre-Semitic hunting and fishing people who tamed the camel for milk during this period. Circumstantial evidence shows the domestication of the dromedary occurred in Arabia; rock art found with camels represented as riding animals dates back to this time (Mason 1979). Arabic texts detail the use of the dromedary for trade of spice, salt and incense around 1000 BC, indicating domestication had occurred prior to this (Mikesell 1955). No archaeological records and evidence exists to support this; based on purely circumstantial evidence Arabia is thought to be the probable origin of domestication.

It is plausible that bottlenecks are the cause of the reduced genetic variation within the dromedary camel, causing the three maternal haplotypes. The genetic consequences of a bottleneck are identical to the founder effect. If the camel population had bottlenecked prior to being introduced into Australia then those which were introduced already had a reduced amount of allelic diversity and genetic variation, and as such upon introduction and mating would produce further individuals with reduced genetic variation, as seen in the results.

The lack of genetic diversity found within the Australian dromedary population is likely to be due to a combination of bottlenecking and the founder effect. If populations bottlenecked before they were introduced into Australia, the population would already have reduced allelic diversity. Adding to this, removing a small subset of these bottlenecked individuals and introducing them into Australia would further reduce the genetic variation as the founders carry a smaller proportion of the total genetic variation of the parental population, and the parental population would have already undergone a bottleneck and have a pre-existing reduction of genetic variation (Carson & Templeton 1984; Halliburton 2004; Hedrick & Miller 1992; Maruyama & Fuerst 1985; Leberg 1992).

A more in-depth phylogenetic analysis using this dataset is beyond the scope of this thesis. The data clearly indicates minimal signal from the mtDNA analysis; the limited variation within the most variable region of the mtDNA as indicated by the small number of variable sites shows that there is little mtDNA diversity within the Australian dromedary population. For this reason analysis of more variable loci is required, the only markers with this resolution are microsatellite markers. The direction is then shifted away from a species evolutionary level towards a population level. This will be done with the use of microsatellite markers to look at population structuring and group dynamics within *C. dromedarius* as mtDNA alone is unable to provide this information.

Summary of the findings from the mtDNA analysis

- Characterisation of the dromedary control region is never previously been done.
- Primers were specifically designed for the analysis of the control region in the Australian dromedary camels.
- Phylogeographic reconstruction (Fig. 5) showed that three main haplotypes existed for the Australian *C. Dromedaries*.
- High bootstrap values show uniform support for the monophyly of the bactrian and dromedary camel excluding them from other South American camelid species.
- There is no phylogeographic signal based on mitochondrial haplotypes
- It can be inferred that the Australian camel population has limited genetic variation as a result of the founder effect, population bottleneck (prior to or upon introduction into Australia), severe limitation on breeding individuals or a combination of these.

Nuclear DNA analysis in Australian camelids
Inferred population structure

This study is the first to use highly variable microsatellite markers to examine the population structure of the Australian dromedary camels. Analysis with the programme *Structure* (Pritchard *et al.* 2000) under various groupings (detailed below) showed the presence of two populations had the highest likelihood. The number of inferred populations (K) most compatible with the data site was assumed to be the smallest K value for which the simulation produces a plateau for the probability of ΔK. This value was found to be K = 2. Inferred populations are referred to as Population A and Population B in future discussions.

The sampled population was run in a variety of combinations: including on (i) the assigned groupings based on the geographic location (Table 5), (ii) all juveniles excluded and male and females treated as separate groups (Table 6) and (iii) the entire group as one population (Table 7; Fig. 7). For individuals where age information was not available (not recorded upon sample collection) it was assumed that these individuals were adults. Of the 390 camels sampled, 300 of these were adults. Juvenile individuals were excluded from the analyses to avoid overrepresentation of genotypes from related individuals (see Table 2). Various combinations were run independently to ensure populations were assigned correctly.

As camel population structuring is unknown and previously untested, all possibilities needed to be tested to ensure an accurate representation of the data. Camels are said to occur in three non territorial groups, bachelor groups, summer groups of cows and calves and winter breeding groups (Heucke *et al.* 1992). Analysis of males and females treated as separate populations(Table 6; to determine if groups are structured as mentioned above) found that inferred population structure is still found to be two, therefore male and female populations are still grouped into two inferred populations which are unrelated to the sex of the individual.

Analysis of adult individuals based on geographic location also produced an inferred population of two (Table 7). Inferred population structure is determined from model and independent of geographic sampling location. The inferred populations A and B are not correlated with geographic distribution. All combinations resulted in inferred population of two.

Table 5 The proportion of camels assigned based on *Structure*'s inferred population groupings, Population A and B relative to the assigned population based on geographic sampling location.

Assigned population number based on geographic location	Proportion of camels in inferred Population A	Proportion of camels in inferred Population B
1 WA North (n=104)	0.258	0.742
2 WA Middle (n=133)	0.414	0.586
3 WA Middle east (n=45)	0.564	0.436
4 WA South (n=50)	0.374	0.626
5 NT (n=47)	0.325	0.675
6 SA (n=11)	0.629	0.371

Table 6 The proportion of camels found in inferred populations A and B assigned on the separation of males and females

Sex	Proportion of camels in inferred Population A	Proportion of camels in inferred Population B
Male (n=141)	0.511	0.489
Female (n=105)	0.413	0.587

Table 7 The total camel population (excluding juveniles) and the proportion found in inferred populations A and B.

	Proportion of camels in inferred Population A	Proportion of camels in inferred Population B
The total camel population (n=300)	0.386	0.614

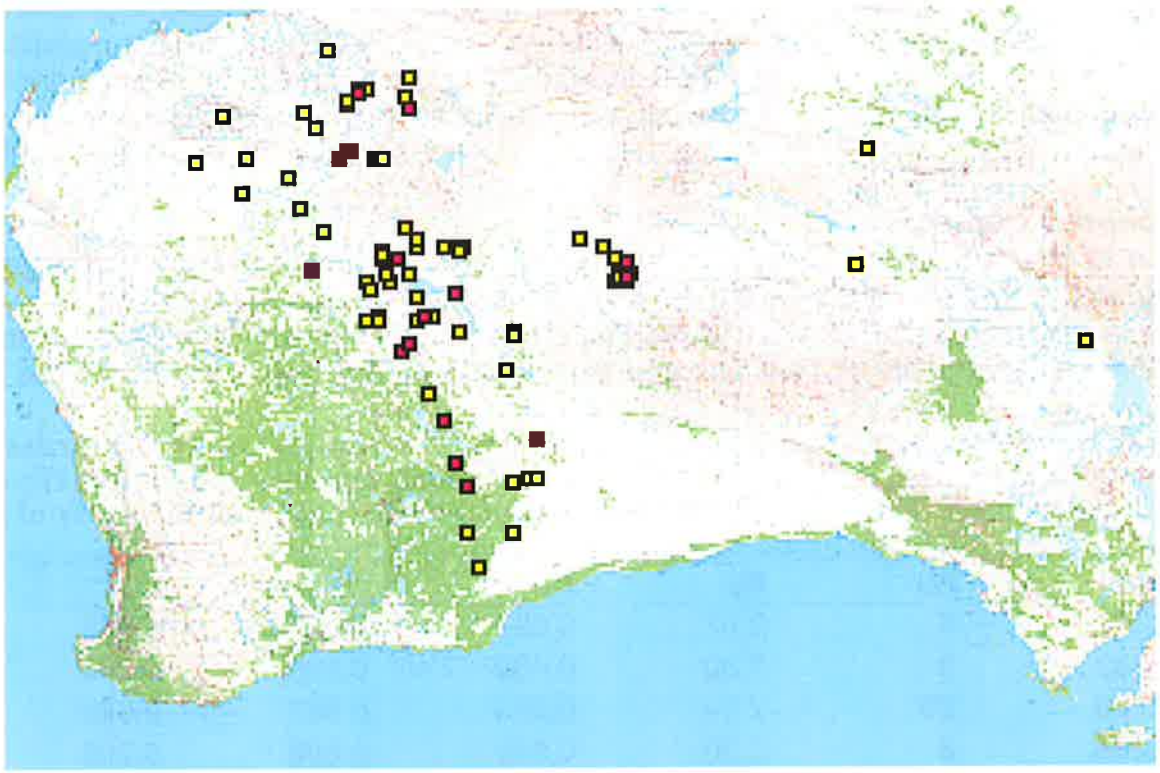

Figure 7
Geographic distribution of inferred populations A (yellow) and B (Pink) as designated by *Structure.*

The assumptions of *Structure* are that populations are within Hardy Weinberg Equilibrium (HWE) and the marker loci are unlinked and at linkage equilibrium within the population (Pritchard *et al.* 2000). Testing for HWE and marker linkage using the programme *Genepop* (Raymond & Rousset 1995) confirms these assumptions, finding all loci are at HWE within the population, and not linked. The *Genepop* data provides validation that results obtained from *Structure* analysis are not biased by linkage variation from HWE.

The programme *GeneClass2* (Piry *et* al. 2004) was also used to determine population structure, as an alternative method. *GeneClass2* differs from *Structure* in that *Structure* assigns individuals to populations requiring no prior knowledge of population structure (using the Markov Chain Monte Carlo algorithm) (Pritchard *et al.* 2000), whereas *GeneClass2* assigns individuals based on the locus data for a reference population. Therefore an individual needs to be assigned to a reference population so that other individuals can then be assigned to an inferred population. Results of analysis of *GeneClass2* imply that populations are structured loosely, as no definitive population grouping is inferred by the output.

The data generated from the 11 microsatellite loci suggest upon analysis by the programme *Structure* that there are two likely populations of camels. However this structuring is not related to location, sex and age, which are most frequent morphological characteristics to cause population structuring. As the cause of population structuring is unable to be identified, the Australian dromedary camel population can be assumed to be panmictic, with some unknown form of weak cryptic population structuring. Analysis with *GeneClass2* confirms this conclusion, as results also implicated that loose population structuring among Australian dromedary camels exists. Based on the 12 microsatellite markers used the Australian *C. dromedarius* can therefore be said to be a single panmictic population, with some cryptic structuring unrelated to location, sex and age of the population.

Descriptive statistics

Of the 12 microsatellite loci characterised in feral camel population analyses, 11 were moderately polymorphic, containing between 2 and 20 alleles per locus (with the exception of marker LCA77 which is monomorphic) with the percent of polymorphic loci at 91.67%. Heterozygosity (H_e) values range from 0.062 to 0.862 (0.536 ± 0.299; Table 8).

Table 8. Measures of genetic variability; mean observed (N_A) and mean expected number of alleles (N_E), mean observed (H_o) and mean expected (H_e) heterozygosity and fixation index (F_{IS}) for the 12 microsatellite markers chosen for the study of feral camels.

Marker	N_A	N_E	H_o	H_e	F_{IS}
VOLP03	5	2.07	0.027	0.518	0.949
VOLP32	3	2.30	0.400	0.566	0.290
YWLL08	20	7.14	0.823	0.862	0.043
YWLL38	8	3.30	0.552	0.698	0.208
YWLL44	4	1.23	0.084	0.186	0.549
LCA33	13	2.75	0.437	0.637	0.313
LCA37	2	1.07	0.037	0.062	0.402
LCA56	4	2.03	0.620	0.509	-0.221
LCA66	6	4.15	0.571	0.760	0.248
LCA77	1	1	0.000	0.000	-
CMS50	11	5.63	0.591	0.8240	0.282
CVRL01	20	5.13	0.705	0.8063	0.125
Mean (± s.d)	**8.1 ± 6.6**	**3.15 ± 1.98**	**0.40 ± 0.30**	**0.54 ± 0.30**	**0.245**

Genetic diversity within feral camel populations

Measures of genetic variability based on the inferred population structure (Table 9) assigned by *Structure* and based on geographic location (Table 8) are included below. This information is included for comparative purposes only, as population structure is not actually grouped as such. Population B (as assigned by *Structure*; Table 10) had a marginally higher level of expected heterozygosity than Population A.

Table 9. Measures of genetic variability; mean observed (N_A) and mean expected number of alleles (N_E), mean observed (H_o) and mean expected (H_e) heterozygosity for the assigned camel populations based on geographic location.

Population	n	N_A (± s.d)	N_E (± s.d)	H_o (± s.d)	H_e (± s.d)
1 WA North	77	6.667±5.483	3.024±1.781	0.401±0.291	0.531±0.301
2 WA Middle	106	7.167±6.235	3.271±2.318	0.399±0.293	0.538±0.300
3 WA Middle East	28	4.917±4.231	2.872±1.850	0.386±0.291	0.516±0.301
4 WA South	37	5.750±4.181	2.781±1.585	0.422±0.297	0.508±0.302
5 NT	41	5.750±4.093	2.641±1.309	0.417±0.326	0.512±0.283
6 SA	11	3.750±2.527	2.501±1.306	0.405±0.335	0.512±0.286

Table 10. Measures of genetic variability; mean observed (N_A) and mean expected number of alleles (N_E), mean observed (H_o) and mean expected (H_e) heterozygosity for the inferred population groupings based on analysis by *Structure*.

Population	n	N_A (± s.d)	N_E (± s.d)	H_o (± s.d)	H_e (± s.d)
Population A	107	6.833±5.750	3.165±2.454	0.3823±0.283	0.492±0.339
Population B	193	7.750±6.269	2.998±1.878	0.416±0.300	0.505±0.315

Effective population size and Population Bottleneck

Estimates of effective population size (N_e) were calculated for three different situations: (1) as one single adult population, (2) the population groups assigned by *Structure*; populations A and B and (3) Adult populations of male and female individuals treated as separate populations based on sex (Table 11).

Table11. Estimates of effective population size and bottlenecking from 12 microsatellite loci for various situations.

Population	N_e	Probability of recent genetic bottleneck
(1) Pooled samples		
Entire Adult Population	429	0.949
(2) Inferred Populations		
Population A	232	0.897
Population B	375	0.966
(3) Sex		
Male Adults	1005	0.913
Female Adults	201	0.966

Effective population size is an estimate of the size of an ideal population that loses genetic variation at the same rate as the real population. Simulations under various models revealed that effective population size was similar for all populations (with the exclusion of males as its own subgroup), ranging from 232 to 429 breeding adult individuals. Males however showed a marked increase in the effective population size, with an estimated value of 1005, in comparison with 201 in females. Males contribute approximately a five-fold increase in the populations when compared to females. Despite this being an abstract measure, effective population size is influenced by a population's geographic structure, dispersal, presence or absence of overlapping generations, breeding sex ratio, distribution of family sizes and the degree of differentiation of populations (Barrowclough 1980). It is a relative measure of population size and may differ widely from census population size based on observational studies. The effective size of a population is correlated with the rate of change of genetic variation and the level of genetic diversity already existing within that population (Crow & Denniston 1988). The effective population size estimated in camels is significantly less than the actual population size; this correlates with what is expected as the N_e is related to the level of genetic variation within the dromedary population. Microsatellite and mitochondrial studies both show limited genetic variation within the Australian dromedary population, resulting in the smaller effective population size than census population size as observed in the dromedary camels.

The significance of a recent genetic bottleneck on feral camel populations was also estimated, using the demarcation of the sampling units listed in Table 11, corresponding to (i) a single camel population, (ii) inferred populations A and B and (iii) male and females treated as separate sampling units. Calculations were based on a transitional phase mutation model with a Wilcoxon sign-rank test with 1000 iterations. Findings for all situations previously mentioned show the probability of a heterozygosity excess (one tail for H excess) was always greater than 0.05. No population structure tested showed evidence of a recent genetic population bottleneck, but with limited heterozygosity (Table 9), suggest that they have had substantial long-term demographic bottlenecking.

Comparison of the genetic findings with previous camel studies

Previous studies using the same camelid markers allow the comparison of Australian dromedary camel to other camelid species. The split between the New World (llamas, alpacas, vicunas and guanacos) and Old World camelid species (bactrian and dromedary camels) occurred approximately 11 million years ago (Cui *et al.* 2007). This section will look at comparison of the genetic variability of the 12 microsatellite markers used for Australian *C. dromedarius* (Table 8) to those used in previous experiments (Table 12). Many of these experiments did not publish the associated information in the journal articles, and as such has been left blank where the information could not be obtained.

The majority of the markers used in previous experiments were used in a different camelid species, for example all primers except CMS50 and CVRL01 were performed on various species including *L. glama* and *L. pacos*. Primers CMS50 and CVRL01 in previous experiments were performed on *C. dromedarius* species from different origins (not Australian dromedaries). Comparison of LCA primers (LCA33, 37, 56, 66 and 77), where information for the mean observed number of alleles (N_A) and expected heterozygosity (H_e) was available showed a significant difference in the results obtained for the Australian dromedary camel and the *L.glama* results as found by Penedo and co-workers (1998,

1999). For example Penedo found for *L.glama* with marker LCA33 N_A to be 3 and H_e to be 0.23, whereas in comparison to results of this experiment with 12 and 0.637 respectively, there is a substantial difference between the old and new world camelids. There is also a substantial difference between species with many of the other primers used.

On comparison of primers which were used on the dromedary species CMS50 (Kenyan origin) and CVRL01 (unknown origin) results for N_A and H_e (Table 12) were similar to those found in the experiment (Table 9). CMS50 results from Edvotchneko *et al.* (2003) found N_A to be 8 and H_e to be 0.82, results for Australian dromedary show N_A to be 11 and H_e to be 0.83. CVRL01 shows a H_e of 0.73 in previous studies and in the Australian dromedary is found to be 0.806. This indicates that primer results are species specific, as Australian dromedary camels produced similar values to other dromedary camels. This also demonstrates that primers which have only previously been used in New World camelids show cross species amplification. For example primer YWLL08, which has previously been used in llama and alpaca species (Lang *et al.* 1996; Table 12) shows high levels of allelic variation in dromedary camels, with a mean observed number of alleles of 20 (Table 12). Primer LCA33 shows a mean observed number of alleles in llamas of 3 (Penedo *et al.* 1999; Table 12) compared with 13 for the dromedary (Table 11). This information is useful for future studies as primers with high levels of allelic diversity which amplify cross species (even though camelid species are estimated to have split 11 million years ago) can be used in comparative studies of species

Table 12. Measures of genetic variability; mean observed (N_A) and mean expected number of alleles (N_E), mean observed (H_o) and mean expected (H_e) heterozygosity and fixation index (F_{IS}) for the 12 microsatellite markers chosen for the study of feral camels as found in previous studies. Blanks in table and N/A represent information unavailability.

Marker	N_A	N_E	H_o	H_e	Camelid species	Reference
VOLP03				0.80	*Lama pacos*	Obreque *et al.* 1998
VOLP32				0.80	*Lama pacos*	Obreque *et al.* 1998
YWLL08					llamas and alpacas	Lang *et al.* 1996
YWLL38					llamas and alpacas	Lang *et al.* 1996
YWLL44					llamas and alpacas	Lang *et al.* 1996
LCA33	3			0.23	*Lama glama*	Lang *et al.* 1996
LCA37	9			0.82	*Lama glama*	Penedo *et al.* 1998
LCA56	12			0.78	*Lama glama*	Penedo *et al.* 1999
LCA66	18			0.83	*Lama glama*	Penedo *et al.* 1999
LCA77	10			0.79	*Lama glama*	Penedo *et al.* 1999
CMS50	8	4.97	0.70	0.82	*C. dromedarius* (Kenya)	Edvotchenko *et al.* 2003
CVRL01			0.60	0.73	*C. dromedarius*	Ariasegaram *et al.* 2002

The genetic characterisation of *Camelus dromedarius* in Southern Africa (Nolte 2003) also used some of the STR markers described in this study, finding that the dromedary have less genetic variation than the alpaca. On comparing the dromedary in different populations they also found that there is little genetic variation within them (Nolte 2003). The average expected heterozygosity found for the South African population is 0.505 (± 0.241), in comparison with findings in Australia of 0.404 (± 0.299) (Table 9) indicating that the Australian population has lower genetic variation than the South African population.

This corresponds with what is expected; Australia's population is the introduced population, and the site of the source of introducees (the founder of the population, which is potentially Africa and India based on McKnight 1969) would be expected to have a higher level of genetic variation than the introduced population.

The above findings need to be explored in further detail to allow comparison of findings to those of the Australian dromedary. Problems in comparing specific values obtained from the South African study arise when details are looked at: minor variations in experimental conditions, allele scoring and statistical programmes used from that of the Australian study. To be able to better compare genetic variability of the dromedary camel from Australia to other populations, specific experimental details should be replicated to maintain the accuracy of the report.

By comparing the potential founders of the Australian dromedary population to the results of this experiment, the potential for discovering unknown information arises, for example the source of the cryptic structuring of the dromedary population in Australia.

Summary of findings from the STR analysis

- Inferred population structure found two populations (K=2). Population structuring with *GeneClass2* showed weak population structuring, with a definitive inferred populations unable to be determined.
- The Australian dromedary camel population can therefore be said to be a single panmictic population, with weak cryptic structuring unrelated to geographic location, sex and age of species.
- 11 of the 12 microsatellite loci used are moderately polymorphic, with the percent of polymorphic loci at 91.67%.
- Males showed a marked increase in the effective population size, approximately five times higher proportion of males than females contributing genes to the next generation.
- The Australian dromedary camel showed no evidence of population bottlenecking.
- Comparison of Australian findings with those from previous studies showed that results vary considerably with primers based on species. South African dromedary populations had a higher expected heterozygosity than Australian populations, which would be expected with founder and introduced populations.
- Focus can now be placed on the application of findings of STR and CR of the Australian dromedary camel for control and management purposes.

General discussion and future directions

Overview

Molecular techniques have been used in invasive species management with the aim of focusing control efforts on the pest species where success will be maximised, preventing re-invasion of eradicated areas and wasting resources. This thesis has applied molecular genetic techniques to characterise the Australian dromedary camel population, more specifically microsatellite and mitochondrial markers to gain an insight into the Australian population's structure for use in control applications. MtDNA analysis found that only three maternal haplotypes existed for the Australian dromedary camel population. These haplotypes are differentiated on the basis of three nucleotide substitutions (all purine transversions), indicating limited genetic variability within the Australian dromedary population, possibly due to bottlenecking prior to being introduced into Australia as a result of the domestication process and or due to the founder effect. The haplotypes showed no phylogeographic correlation. Analysis of more variable microsatellite loci concur with this, population structuring is not related to location, sex or age of camels. The Australian camel population showed weak population structuring; given this finding our current hypothesis is that the dromedary camel population within Australia is a single panmictic population with weak cryptic structuring due to some undetectable cause.

Overall the findings suggest that using the current technology, Australian dromedary camels display a lack of genetic differentiation. Panmixia, is usually seen in species which are capable of dispersing over large distances and in the absence of barriers to dispersal (Feldheim *et al.* 2001) for example panmixia is frequent in birds and is attributed to their dispersal ability (Miller 1947). Australian dromedary camels are capable of travelling long distances of up to 70km a day, they also do not have barriers to movement within the arid and semi arid zones of Australia are limited, with the occasional fence as a barrier, which is easily destroyed by camels if not built adequately, making camels ideally suited to being a panmictic population.

The findings of the presence of a single panmictic population within the Australian dromedary camel population will have implication for the management of this invasive pest, making control even more difficult.

Implications for control programmes and management

Current management of feral camel populations

Current management of feral camels focuses on three main strategies; culling, harvesting and exclusion fencing. Culling occurs via ground and aerial based shooting predominantly through agencies such as Department of Environment and Conservation (DEC) and also by private landholders. Aerial shooting is highly effective, and allows broad scale control over remote and difficult to access areas. Ground based shooting is somewhat less effective, and is confined to accessible locations, however does allow target specific control for landholders (Norris & Low 2005).

Commercial harvesting includes live wild harvesting and use in pet meat. Live wild harvesting is rare, infrequent and occurs in low numbers. Commercial harvesting has no prospect at reducing camel numbers in the near future, as demand for camel products is low, and lack of

a purpose built abattoir makes camel meat trade difficult. The major restrictions to meat and live animal trade of the Australian camels are the infrastructure requirements. Even with the presence of a purpose built abattoir the success of camel meat industry would be dependent on a number of factors including the net return of camel, which is reliant on the method of capture and distance required for transport (Norris & Low 2005; Ellard 2000).

Fencing of camels excludes them from an area, but does not do anything to control population numbers. Fencing is useful for conserving areas of high importance, as a fence provides a barrier which shifts the point of impact away from the area inside the fence. Exclusion fences needs to be of at least a height of 1.6metres and reinforced, fences which are inadequately built are easily destroyed by camels (Norris & Low 2005).

Aerial and ground based shooting are the most effective way at controlling camel population numbers. A substantial amount of camels need to be removed annually to maintain current population numbers. As such, a means is needed to optimise the success of culling, focusing efforts on individuals which will have the most effect.

Management implications and recommendations

Management of feral camels poses a problem for Australia as camels are an invasive pest, however they are also viewed as a resource by many people (especially the aborigines). The results generated from this study suggest that the management of the Australian feral camel population will be a difficult problem. The larger the area invaded by an invasive species the harder the task of controlling them (Hulme 2006). The Australian camel population has invaded approximately 37% of its mainland (McLeod 2004), indicating they have dispersed large distances, making control efforts difficult. The amount of camels needed to be culled to maintain the current population size is significantly large (approximately 80 000 p.a.). Adding to this the finding that the Australian feral camel population is one single panmictic population (with some form of weak structuring which cannot clearly be identified), poses further difficulty for the control and management of camel populations as the lack of discrete groups means that the entire camel population needs to be eradicated for effective control. Culling in one locality, removing only a fraction of the population would inevitably result in a rapid re-colonisation of the area as migrating camels would simply reinvade the area previously cleared by culling, wasting resources. Eradicating the entire camel population is also improbable. A more innovative means by which to control camels is therefore needed.

The following are suggestions only, on ways to control camels, based on the current knowledge of camel populations. If camels could somehow be separated into distinct populations creating barriers between them, population structure would be altered, allowing specific populations to be targeted at a time and hence preventing the re-invasion of that area. Associated with this are difficulties with creating boundaries and targeting populations. Camel movements and migration routes could perhaps be used to target populations and create boundary fences. For example camels are known to concentrate around areas of salt lakes particularly in winter and within regions with plants containing a high salt content. The migration patterns and population distribution of feral camels in Western Australia differ from that of the Northern Territory due to the regular movement between uninhabited desert regions and pastoral properties. Surveying of land owners report that groups of camels regularly move onto properties along defined migration routes dependent on seasonal conditions (Ellard 2000). Group size appears to

be dependent on the amount of rainfall, with a high amount of rainfall being directly proportional to larger group sizes (Heucke *et al.* 1992). These characteristics could enable the prediction of camel movements to create barriers to movement and isolate populations in different areas. If populations are isolated into discrete groups, culling of these groups could be successful if re-invasion does not occur.

Understanding dynamics and population structure of invasive species is important for the management and control of these species. Further information on population group dynamics of the Australian dromedary camel is needed to be able to effectively control them. Camels appear to live in non territorial groups of three kinds; year round groups of bulls (bachelor groups), summer groups of cows and calves and winter breeding groups (mature bull with several cows and their calves; Heucke *et al.* 1992). The structure of these groups could be used in a fertility control strategy.

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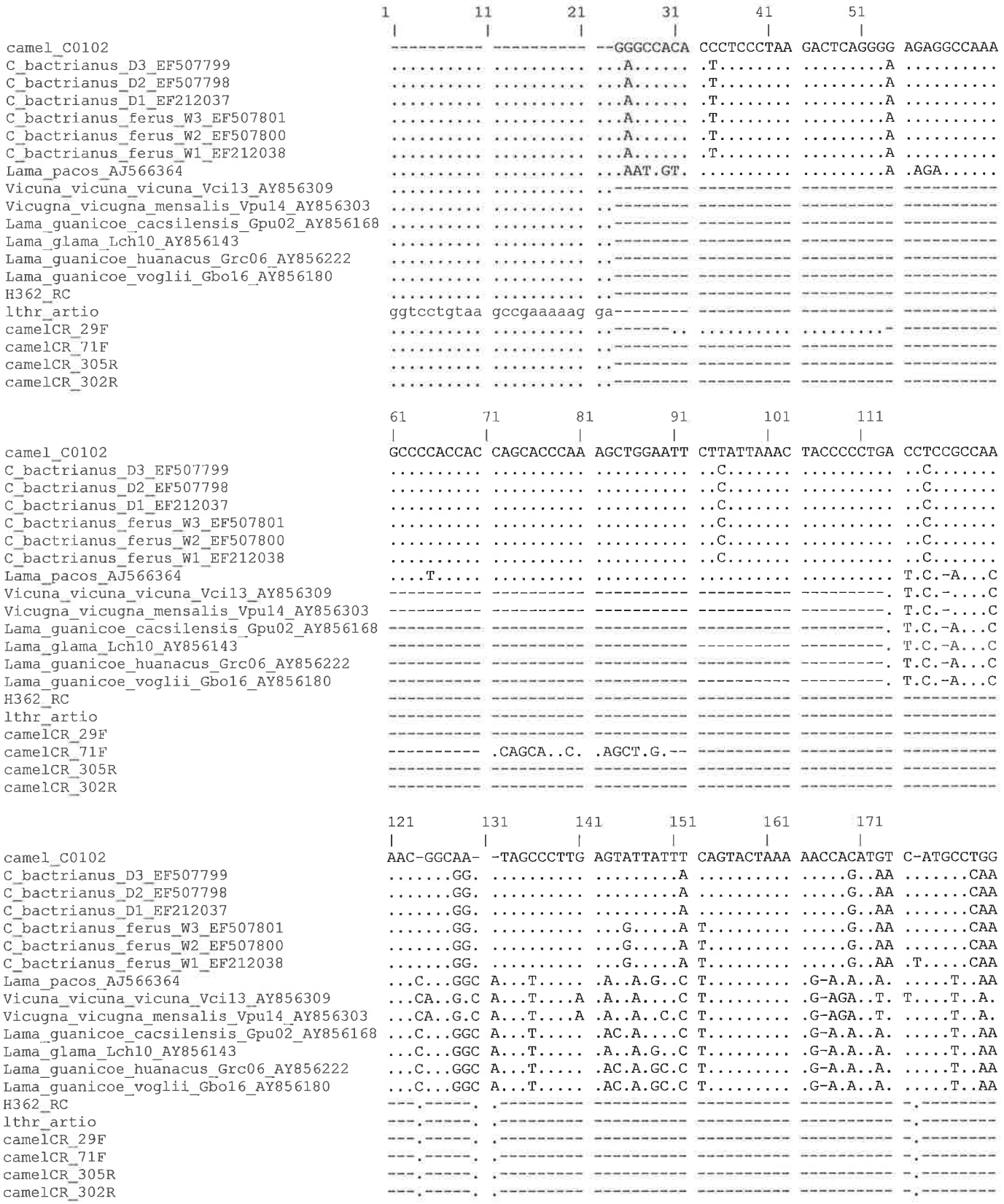
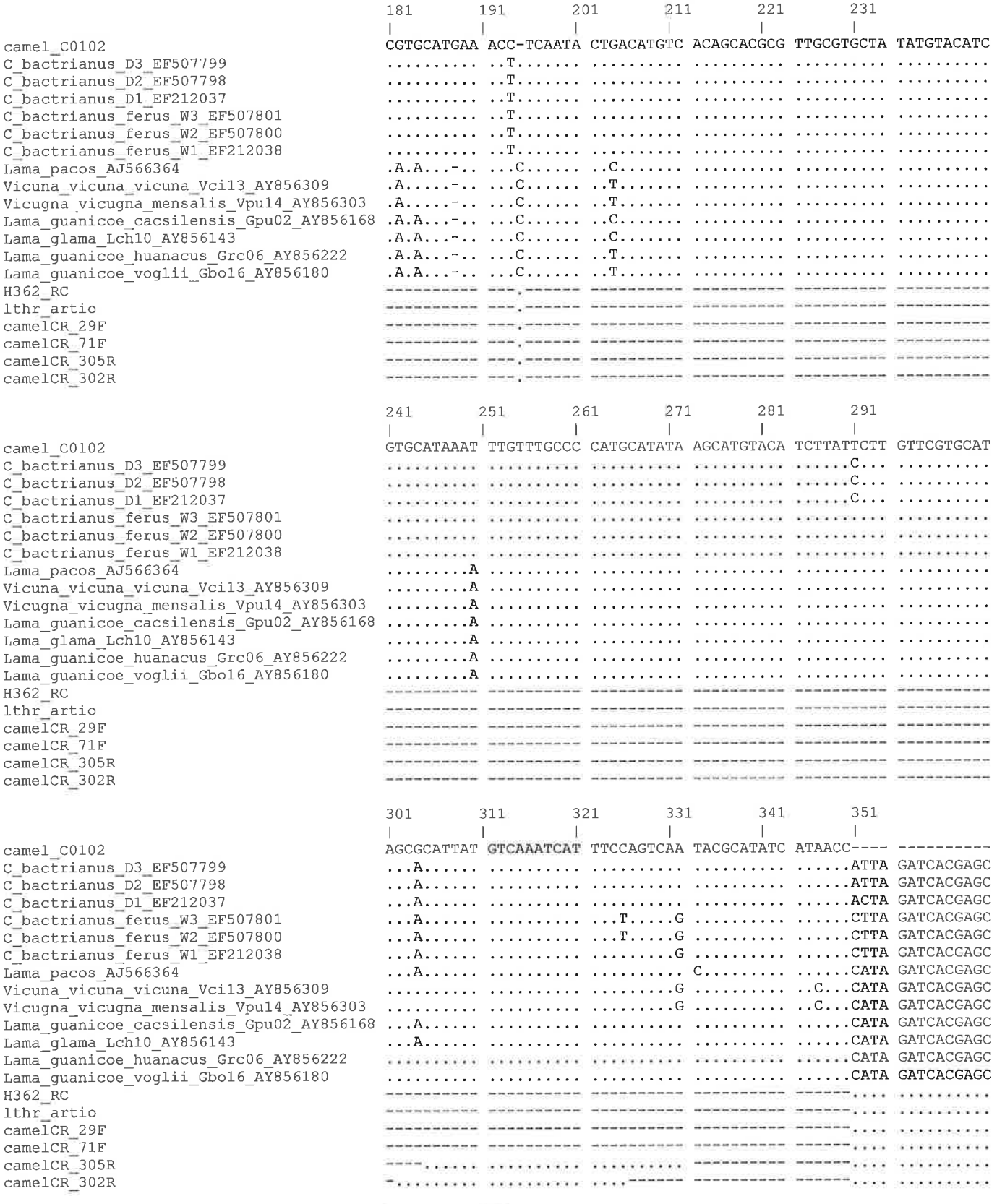
Appendix 1 Sequence alignment from a range of camels and relatives, including representatives from Australian *C. dromedarius* using primers Lthr Artio (F) and H362 (R).




181 191 201 211 221 231
camel_C0102 CGTGCATGAA ACC-TCAATA CTGACATGTC ACAGCACGCG TTGCGTGCTA TATGTACATC
C_bactrianus_D3_EF507799 ....T..... ....
C_bactrianus_D2_EF507798 ....T..... ....
C_bactrianus_D1_EF212037 ....T..... ....
C_bactrianus_ferus_W3_EF507801 ....T..... ....
C_bactrianus_ferus_W2_EF507800 ....T..... ....
C_bactrianus_ferus_W1_EF212038 ....T..... ....
Lama_pacos_AJ566364 .A.A...-.. ...C..... ..C..... ....
Vicuna_vicuna_vicuna_Vci13_AY856309 .A.....-.. ...C..... ..T..... ....
Vicugna_vicugna_mensalis_Vpu14_AY856303 .A.....-.. ...C..... ..T..... ....
Lama_guanicoe_cacsilensis_Gpu02_AY856168 .A.A...-.. ...C..... ..C..... ....
Lama_glama_Lch10_AY856143 .A.A...-.. ...C..... ..C..... ....
Lama_guanicoe_huanacus_Grc06_AY856222 .A.A...-.. ...C..... ..T..... ....
Lama_guanicoe_voglii_Gbo16_AY856180 .A.A...-.. ...C..... ..T..... ....
H362_RC ---------- ---.------ ---------- ---------- ---------- ----------
lthr_artio ---------- ---.------ ---------- ---------- ---------- ----------
camelCR_29F ---------- ---.------ ---------- ---------- ---------- ----------
camelCR_71F ---------- ---.------ ---------- ---------- ---------- ----------
camelCR_305R ---------- ---.------ ---------- ---------- ---------- ----------
camelCR_302R ---------- ---.------ ---------- ---------- ---------- ----------
241 251 261 271 281 291
camel_C0102 GTGCATAAAT TTGTTTGCCC CATGCATATA AGCATGTACA TCTTATTCTT GTTCGTGCAT
C_bactrianus_D3_EF507799 ....C... .....
C_bactrianus_D2_EF507798 ....C... .....
C_bactrianus_D1_EF212037 ....C... .....
C_bactrianus_ferus_W3_EF507801 ....
C_bactrianus_ferus_W2_EF507800 ....
C_bactrianus_ferus_W1_EF212038 ....
Lama_pacos_AJ566364 .....A ....
Vicuna_vicuna_vicuna_Vci13_AY856309 .....A ....
Vicugna_vicugna_mensalis_Vpu14_AY856303 .....A ....
Lama_guanicoe_cacsilensis_Gpu02_AY856168 .....A ....
Lama_glama_Lch10_AY856143 .....A ....
Lama_guanicoe_huanacus_Grc06_AY856222 .....A ....
Lama_guanicoe_voglii_Gbo16_AY856180 .....A ....
H362_RC ---------- ---------- ---------- ---------- ---------- ----------
lthr_artio ---------- ---------- ---------- ---------- ---------- ----------
camelCR_29F ---------- ---------- ---------- ---------- ---------- ----------
camelCR_71F ---------- ---------- ---------- ---------- ---------- ----------
camelCR_305R ---------- ---------- ---------- ---------- ---------- ----------
camelCR_302R ---------- ---------- ---------- ---------- ---------- ----------
301 311 321 331 341 351
camel_C0102 AGCGCATTAT GTCAAATCAT TTCCAGTCAA TACGCATATC ATAACC---- ----------
C_bactrianus_D3_EF507799 ...A..... ....ATTA GATCACGAGC
C_bactrianus_D2_EF507798 ...A..... ....ATTA GATCACGAGC
C_bactrianus_D1_EF212037 ...A..... ....ACTA GATCACGAGC
C_bactrianus_ferus_W3_EF507801 ...A..... ....T.....G ....CTTA GATCACGAGC
C_bactrianus_ferus_W2_EF507800 ...A..... ....T.....G ....CTTA GATCACGAGC
C_bactrianus_ferus_W1_EF212038 ...A..... ....G ....CTTA GATCACGAGC
Lama_pacos_AJ566364 ...A..... .... C..... ......CATA GATCACGAGC
Vicuna_vicuna_vicuna_Vci13_AY856309 ....G ....C...CATA GATCACGAGC
Vicugna_vicugna_mensalis_Vpu14_AY856303 ....G ....C...CATA GATCACGAGC
Lama_guanicoe_cacsilensis_Gpu02_AY856168 ...A..... ....CATA GATCACGAGC
Lama_glama_Lch10_AY856143 ...A..... ....CATA GATCACGAGC
Lama_guanicoe_huanacus_Grc06_AY856222 ....CATA GATCACGAGC
Lama_guanicoe_voglii_Gbo16_AY856180 ....CATA GATCACGAGC
H362_RC ---------- ---------- ---------- ---------- ------.... .....
lthr_artio ---------- ---------- ---------- ---------- ------.... .....
camelCR_29F ---------- ---------- ---------- ---------- ------.... .....
camelCR_71F ---------- ---------- ---------- ---------- ------.... .....
camelCR_305R ----..... .... ---------- ------.... .....
camelCR_302R -..... ....------ ---------- ------.... .....
361 371 381

```
camel_C0102                                 ---------- ---------- ----
C_bactrianus_D3_EF507799                    TT........ .......... ....
C_bactrianus_D2_EF507798                    TT........ .......... ....
C_bactrianus_D1_EF212037                    TT........ .......... ....
C_bactrianus_ferus_W3_EF507801              TT........ .......... ....
C_bactrianus_ferus_W2_EF507800              TT........ .......... ....
C_bactrianus_ferus_W1_EF212038              TT........ .......... ....
Lama_pacos_AJ566364                         TT........ .......... ....
Vicuna_vicuna_vicuna_Vci13_AY856309         TT........ .......... ....
Vicugna_vicugna_mensalis_Vpu14_AY856303     TT........ .......... ....
Lama_guanicoe_cacsilensis_Gpu02_AY856168    TT........ .......... ....
Lama_glama_Lch10_AY856143                   TT........ .......... ....
Lama_guanicoe_huanacus_Grc06_AY856222       TT........ .......... ....
Lama_guanicoe_voglii_Gbo16_AY856180         TT........ .......... ....
H362_RC                                     ..AATCACCA TGCCGCGTGA AACC
lthr_artio                                  .......... .......... ....
camelCR_29F                                 .......... .......... ....
camelCR_71F                                 .......... .......... ....
camelCR_305R                                .......... .......... ....
camelCR_302R                                .......... .......... ....
```

ANNEX A – NATURAL HERITAGE TRUST - Administrative outputs

Communication

Project participants were kept up-to-date with project progress, new innovations and knowledge in camel control through regular presentations to Agriculture boards (ZCA, VPS), the web (http://wwwstaff.murdoch.edu.au/~pspencer/what_we_do/the_ecology_of_large_feral_/camel_research.html) , interactive maps (see CD Appendix) and reports. Field days and workshops were held and attended throughout the project.

See appendix Y for sample collection protocol.
See attachment CD for the Camel.KZM file for use with Google Earth that illustrates the map and sampling locations

Discussion of the results and the implications for future management of pest animal damage

This study represents the first study to develop nuclear and mitochondrial molecular markers for *Camelus dromedaries*. It has provided a greater understanding of feral camel population structuring across Australian. Namely that,

- the control region of *C. dromedarius* was characterised with specifically designed control region primers. Alignments generated a phylogeny revealing limited genetic variation, with three haplotypes detected.
- there is no phylogeographic signal based on the detected haplotypes.
- The limited mtDNA variation is likely due to a combination of the founder effect and or population bottleneck.
- 12 microsatellite loci were used to genotype 390 individuals within Australia.
- Bayesian statistical programs were used to elucidate population structure from the genetic data generated.
- based on current knowledge analyses suggest that the Australian feral camels are a single panmictic population, with evidence of some weak cryptic population structuring.
- findings of this thesis pose difficulty for the management and control of feral camel populations.
- alternative methods for population management are needed. Information from mob dynamics, mating systems and migration routes could provide a means by which to target camel populations.
- a national based approach is needed for control of the Australian dromedary population; control on a regional basis will be unsuccessful.

General

Progress towards project aims & objectives, milestones, outcomes & outputs and performance indicators are summarised in appendix V. More detailed information can be found in attachment 1.

Media coverage

ABC Rural report	DNA helps in hunt for wild camels
ABC regional – southern goldfields	Disease spread by illegal dumping of pigs
ABC Regional - Karratha	Camels to cop-it
ABC North West WA rural report	Crime scene camels

Training & information workshops

John Curtin College of the Arts. STAR tutoring, visit to Murdoch on 26 March 2008

Managing vertebrate pest species – the DNA way!
NRM (Natural Resource Management) Board, Kalgoorlie September 2007.

Large vertebrate pest control: Pigs, camels and dogs.
Goldfields ZCA (Zone Council Authority, Kalgoorlie). September 2007

Audited Statement of expenditure

The last payment for this project was received in February 2001. This amount was acquitted in previous progress reports. For information, a copy of the Natural Heritage Trust acquittal form for period ending 30th June 2008 appears on the following page.

Appendix I: Summary of project dates, extensions and amendments

- Funding started 01/07and ended on 06/08

- Project started 01/07/06. One investigator had a major health scare in early 2007, which has delayed outputs/completion. The outputs are still in preparation, but may be delayed. Details relayed to Bureau of Rural Sciences (BRS).

Appendix Y: Group and participant statistics

Number of participants	?	
Regions represented	?	groups
Key enterprises represented	?	

We have also had numerous meetings (Murdoch, DEC, AgWA etc) in order to obtain samples.

Contact with NRM, Cons Dept(s),

Ag Depts and other interested parties

DEC Dr Mark Cowan (Regional Ecologist, Rangelands), Kevin Marshall (Wildlife Officer, Geraldton)

NRM Groups (e.g. Alinytjara Wilurara Natural Resources Management Board), State Governm Departments (e.g. NT Department of Natural Resources, Environment and Arts, SA Department Environment and Heritage), Indigenous Organisations (e.g. Central Land Council, NT)

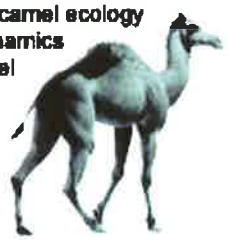
The Pastoral Industry (e.g. Warrawagine Cattle Co, WA) etc

Appendix Y: Training material (brochures, newsletters, workbooks)

Data collection forms

CAMEL GENETICS STUDY – SOME EXPLANATORY NOTES

Camels have a major impact on the environment, agriculture and are potential disease carriers in Australia. Despite this, little is known of their population ecology and genetics. The integration of camel ecology together with the modern tools of molecular biology will allow robust analyses of the dynamics occurring within and between populations. The scope of these disciplines to aid in camel control is potentially enormous. This project, will investigate the genetic structure and relatedness within and between camel populations to determine the structure, gene flow and genetic 'health' of existing populations of the camels. This will be done using the latest and most powerful molecular markers available - exactly the same approached that is used in the TV 'CSI' shows. More specifically, this study will:



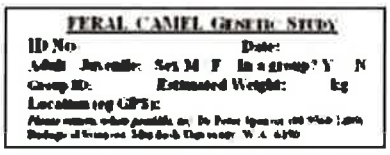
- compare the levels of genetic 'health' within and among populations
- determine the genetic mating system (do big males always father all the young?)
- determine the population size, and how big they are (pigs live in populations >100,000 km^2)
- estimate where, and how often individual camels move among populations
- help to determine better, more effective control strategy(s);

Outcomes of this research

The 'DNA fingerprinting' techniques required to undertake the study are well established - we have now completed a large project using the same techniques with feral pigs, mice and feral cats. This project provides an exciting opportunity to evaluate the relative importance of dispersal and population dynamics of camels. One of the outstanding features of using this molecular approach is that it is very difficult to achieve comparable findings using field-based methods, such as mark and recapture studies, alone as very few camels are allowed the opportunity to be recaptured for future study! However, they all have genotypes and these genotypes contain a population history that we can 'read'. **The vials are for tissue from each animal that you can get a sample for. We are particularly interested in getting as many individuals from a group as possible.**

Preservation of tissue for DNA analysis. We have tried to make the collection process as simple as possible. Keep in mind that only ONE (1) individual can go in each tube, but as many pieces of that individual can be included in the tube (for example the ear, liver and kidney bit, and anything else that might look strange –eg, 'growths', worms, ticks, lesions). All that is needed is a small piece of tissue (~ 2 cm^2) from the ear (or kidney or liver) to be put into the preservative supplied (20% DMSO saturated with table-salt). The size of the piece of tissue should be about as big as that shown in the picture of a tube.

Place the tissues into a small tube that contains the amount of preservative. Rotate a few times to mix in the preservative. The preservative is a 20% solution of DMSO (Dimethyl sulfoxide), saturated with Sodium Chloride. This is the latest whiz-bang molecular preservative and we can get DNA from these samples for years afterwards (even if they were stored on your desk). The most important step is to properly label the container (hopefully the label will stay on the tube!). To do this we need a reasonable amount of data to be recorded for each sample. It may sound like a lot of info, but what appears obvious to you in the field can be difficult for us to work out much later once it arrives back in our laboratory.


FERAL CAMEL GENETIC STUDY

ID No Date:

Adult Juvenile Sex M F In a group? Y N

Group ID: Estimated Weight: kg

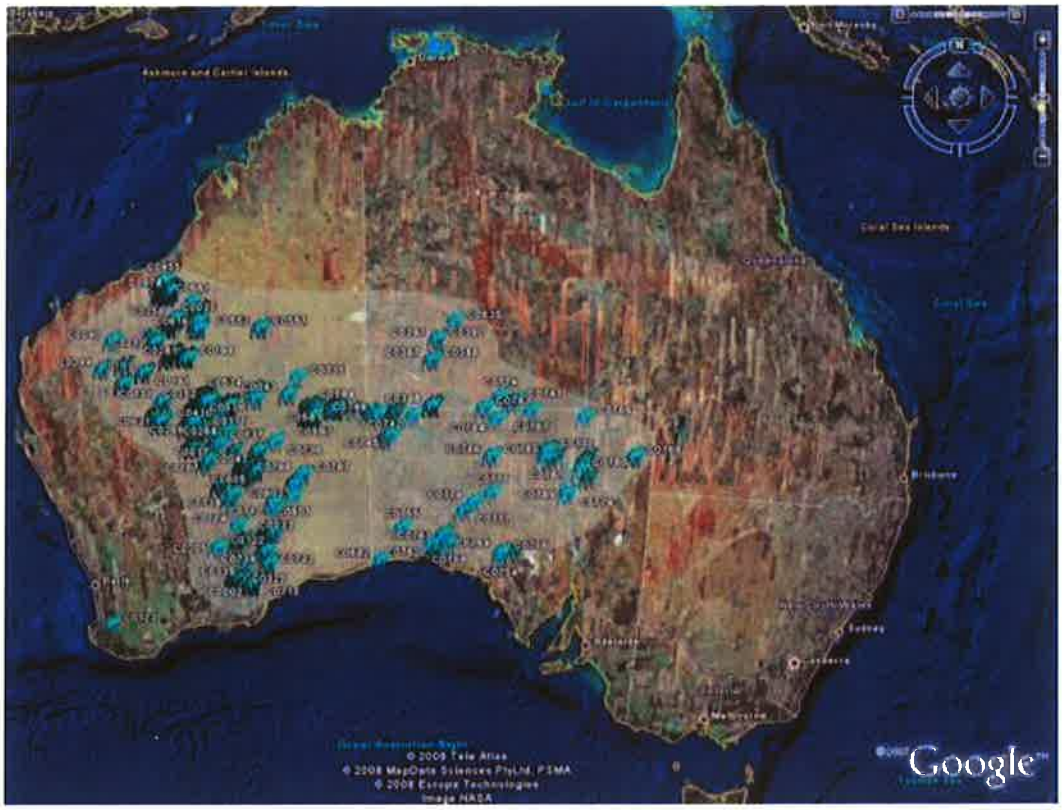
Location (eg GPS):

The information that is needed is on the labels supplied with the tubes. This information is generally easy to record, with many options simply requiring to be circled, for example whether it was an Adult or Juvenile - to give a general idea of age; Male or Female; Was the animal in a group? Other information on the label should include; ID No. (A number that you designate to the specimen (so we can track it later); Date (the date the sample was collected (D/M/Y); Location (an accurate description or possibly a GIS-fix of its location); Estimated Weight: in kg (an *estimate* of its weight – to identify its social status).

Thank you for your interest, input and effort in helping this project, and if there is anything that you need more information on, please do not hesitate to contact me. My contact details are;

Peter Spencer
School of Biological Sciences;
Murdoch University, Perth, W.A. 6150.
Phone (08) 9360 2489; Fax: (08) 9310 4144
Mobile: 0406993293; Email: P.Spencer@murdoch.edu.au;
WGL homepage: http://www.staff.murdoch.edu.au/~pspencer/

Snapshot of the (i) sample locations and (ii) detail available from the .kmz file for use with Google Earth



Appendix Y: Progress towards project aims & objectives, milestones, outcomes & outputs and performance indicators

Aims & Objectives	Comments
A. Optimise the methodology for DNA profiling (DNA fingerprinting) camels in Australia	A set of 11 informative STR markers for identifying individual camels. Publication (#1) describing novel MS loci and levels of diversity within Australian camels in preparation
B. Determine the population boundaries (size) of camel populations in the arid and semi-arid rangelands of the NT, SA and WA. And to determine the genetic effective population size of each of those populations	We describe a single continuous population across Australia. This is the largest vertebrate population ever recorded.
C. Quantify the rates of immigration and emigration and to identify key source populations of reinvasion	We are unable to quantify absolute rates of movement, as the data suggests single large panmictic population.
D. Define exactly which populations are likely to respond best to control and use this to identify adaptive management and cost effective strategies for the management of camels, for both the agricultural and conservation stakeholders	This will now be difficult and expensive, as the population appea4rs to be a single population encompassing >2.8 million km^2.
E. Determine the historical links between multiple introduction sites and modern-day impacts to assist with management strategies	Unable to determine multiple introductions of camels. Data suggests that they are highly inbred and are a genetically 'simple' population.
F. identifying better pest animal management strategies and encouraging adoption of 'best practice' pest animal management	A number of strategies will be offered once a larger set of data has been completed.

Milestones	**Outcomes/comments**
1. To optimise the methodology for DNA fingerprinting camels in Australia.	A set of highly variable and informative STR markers for identifying individual camels. This is being completed as a publication (#1) describing novel MS loci and levels of diversity within Australian camels
2. Raise public awareness and attendance to Zone Control Authority (ZCA) meeting	Media release joint release with CALM and DAFWA. Also disseminate via articles to DAFWA northern and southern pastoral memo. Public feedback (radio and community news) & Attendance
3. Determine the population boundaries (size) of camel populations in the arid and semi-arid rangelands of the NT, SA and WA. and to determine the genetic effective population size of each of those populations	Publication (#2 near submission): describing landscape and structural complexity within and between Australian camel populations, and a publication (3; in preparation): describing social organisation and effective population size within Australian camels
4. Quantify the rates of immigration and emigration and to identify key source populations of reinvasion.	Publication describing local, regional and landscape level migration and gene flow patterns within Australian camels. Unlikely to be done due to the discovery of a single genetic population.
5. Define exactly which populations are likely to respond best to control and use this to identify adaptive management and cost effective strategies for the management of camels, for both the agricultural and conservation stakeholders	Publication (5): Applied Management of Australian camels. A concept paper investigating the 'local neighbourhood' in camels. Community information booklet and material suitable for outcomes desired by agricultural and conservation stakeholders
6. Determine the historical links between multiple introduction sites and modern-day impacts to assist with management strategies.	Publication (6): Linking all previous work into an encompassing publication on the social and genetic structure of camels within Australia (in preparation)
7. Final report	Sent to funding body July 2008.

ANNEX

The table below identifies the NHT outputs that the project has complete over its funded period. Actual project performance against this table was reported six-monthly in accordance with Schedule 2. Provided ion the table are the actual versus anticipated performance indicators against the milestone dates outlined in your original application (i.e. Annex B of this Contract); and, in accordance with Annex A of this Contract.

Output unit of measure #1			Output unit of measure #2	
CB1.1 Number of awareness-raising events such as demonstrations, field days or study tours ***(if any, please also respond to next column)***	?	▶	**CB1.1** Number of awareness-raising event participants in person-days ***(e.g. 20 participants at a 2-day workshop equates to 40 person-days)*** Biosecurity meeting WA (July 2006) 80 DAFWA staff Presentation - APB Pastoral conference 80 participants (pastoralists) Camel workshop (desert knowledge CRC) 15 APB annual meeting, Perth, 40 participants WA Pest animal workshop (IACRC sponsored) at UWA - 30 attendees	
CB1.2 Number of written products such as brochures, newsletters, posters or factsheets ***(if any, please also respond to next column)***		▶	**CB1.2** Estimated number of recipients of written products News flyer: "Studying the DNA of camels?" Newsletter for the Sporting Shooters community, Conservation Branch (500 members) Camel sampling protocol – 140 (estimated) Paper presentation, APB Pastoral conference 80	**1000**
CB1.4 Number of media opportunities resulting in articles in newspapers or on radio or television	6	▶	ABC Region - Karratha (PS) ABC Region - Pilbara (PS) American Broadcasting (AW) Stateline (ABC TV) ABC WA	
CB1.5 Number of websites developed or significantly enhanced	1		Inclusion of updated information within Academic websites (e.g. http://wwwstaff.murdoch.edu.au/~pspencer/what_we_do/the_ecology_of	

CB2.1 Number of training sessions, workshops, seminars or other skills and training events conducted ***(if any, please also respond to next column)***	**3**
CB2.2 Number of workbooks, course notes or other key materials developed ***(if any, please also respond to next column)***	**?**
CB4.4 Number of new databases developed	**1**
CB5.1 Number of community groups OR projects assisted	**1**
OG8.3 Area (ha) of pest animal control measures	**20000**
P1.1 Number of best management practice codes or guidelines completed	**?**
RA3.1 Number of models developed	**?**
RA3.2 Number of information management systems developed	**?**
RA3.3 Number of other decision support tools developed	**?**
RA4.1 Number of research and development studies completed	**2**
Other [your description]	**?**

_large_feral_/camel_research.html	
CB2.1 Number of training participants in person-days Undergraduate research training - Honours student Undergraduate course in Conservation Biology - 60 students	**20**
CB2.2 Quantity of workbooks etc. distributed	**?**
?	**?**