FINAL REPORT

GMS 0664: Livestock Guardian Dog/Wild Dog Interaction Study

AIM

To understand how guardian dogs work; provide information on how best to use guardian dogs as a wild dog management tool, and; give confidence to livestock producers considering the acquisition of guardian animals.

OBJECTIVES

1. Investigate the spatial and temporal movements of maremma guardian dogs in relation to sheep and adjacent wild dogs and, specifically, the degree to which guardian dogs and wild dogs intermix, whether they coexist or occupy exclusive areas.
2. Evaluate the relative abundance of meso-predators and macropods (potential over-abundant native wildlife) with and without guardian dogs.
3. Assess if there is any interbreeding between guardian dogs and wild dogs.
4. Recommend best practice guardian dog management for inclusion in Guardian Dog Manual, Leading sheep website, and *'Beefy and the beast'* newsletters.

METHODS

Spatial and Temporal Movements

Simultaneously, we monitored the movements of multiple maremma guardian dogs and sympatric wild dogs at 30 and 60 minute intervals (respectively) over several consecutive months using GPS data loggers and Argos-linked GPS collars on two properties in central west and north Queensland; one producing sheep and the other beef cattle.

Dunluce Station, 2009-10

Dunluce, 36km west of Hughenden (20º 52'S; 143º 51'E), is a beef and sheep producing property of 46,500ha. It is located on the northern extremity of the Mitchell grass plains, a vast undulating, mostly treeless, grassland extending from south-west to north-west Queensland (Figure 1). Prior to 1982, the Wild Dog Barrier Fence (WDBF) separated Dunluce's sheep from its northern paddocks on Flinders River floodplain. Beyond the Flinders River lay the northern beef cattle regions of the Gulf of Carpentaria. The WDBF no longer remains in this region. Currently Dunluce runs 14,000 sheep and is the most northern sheep producer in Australia. The property receives a mean annual rainfall of 490 mm three quarters of which falls between November and March during the monsoonal wet season.

In 2002, after suffering annual predation losses of 15%, the owners of Dunluce switched to using guardian dogs. They initially purchased 24 maremmas. Since that time, losses reduced to 3% within three years. 1080 baiting has ceased on Dunluce except for annual aerial baiting programs conducted by the local graziers along Dunluce's northern boundary on the Flinders River.

Only one pair of entire maremmas is kept near the house for breeding, training and security purposes. All working maremmas are neutered. Pups are bonded to lambs and livestock, provisioned with the sheep and when mature, integrated into sheep paddocks according to best practice (described in Van Bommel 2011).

Figure 1. Looking northwest across Dunluce Station towards the low basalt hills on the horizon, the northern boundary. All but one of the wild dogs were trapped on or north (RHS) of this road in cattle paddocks yet almost all foraged at some time in the sheep paddocks south of this road.

Eight of the 24 mixed gender maremmas were collared on Dunluce in May 2009 and monitored until October 2010. In April 2010 four wild dogs, and the following July a further two wild dogs, were trapped, weighed, ear tagged and fitted with satellite tracking collars (Figure 2). Details of these captures are found in Appendix 1; the aging method is illustrated in Appendix 2.

Of these six wild dogs collared, one collar (Dog #85) failed shortly after being deployed and was never relocated. This wild dog probably dispersed. Another (Dog #86), died next to a public road (shown in Figure 1), on the 13 July 2010 ten weeks after she was released. She was most likely shot by a passer-by or involved in a vehicle collision. Of the first four wild dogs collared, two were monitored for seven months, one for ten weeks. Of the two that were collared in July, one (Dog #87) was destroyed in mid-September in a Dunluce sheep paddock exactly two months after release, and the second, Dog #88 was discovered in October 2010 paired with a female that had previously bred. Both were destroyed. Thus a total of 18 wild dog-months of movement data were captured.

Figure 2. Dunluce owner, Anne Stewart-Moore, about to release one of the eight maremmas monitored during the project in 2010.

Stratford Station, 2011

Stratford is a 13,000 ha beef cattle property located 85km east of Barcaldine (23º 52'S; 145º 47'E) in the Desert Uplands bioregion of the Lake Eyre Basin. It receives a mean annual rainfall of 525 mm in a short summer wet season and is noted for its poor, mineral deficient soils, sparse eucalypt vegetation and native Spinifex grasslands. Stratford runs 600-700 *Bos indicus* cross breeder cattle and typically achieves around 70% branding which is considered good for the desert uplands.

Stratford had not been baited for wild dogs for over a decade and practices no wild dog management other than infrequent, opportunistic shooting and the use of maremma guardian dogs. The management of maremmas departs from recommended 'best practice' (Van Bommel, 2010) in that: all 11 working maremmas are entire; they are not bonded to the livestock but believed bonded to the property, and; they are provisioned at the homestead (Figure 3).

Eight wild dogs were trapped and collared with Argos-GPS satellite transmitters in May 2011 (Details in Appendix 1) and were tracked continuously for seven months until the end of the project in November 2011. Traps for wild dogs were not set within two kilometres of the homestead to avoid accidentally capturing a maremma.

General Procedures

Location data from each collar was displayed over satellite imagery using ArcGIS. The paddocks containing maremmas (and sheep) were then delineated so that forays of wild dogs into sheep paddocks or paddocks patrolled by maremmas could be identified and examined closely. Where wild dogs entered sheep paddocks, the location and movements of maremmas in response to their presence during each intrusion was investigated.

Figure 3 Stratford differed from accepted 'best practice' in the way their maremma guardian dogs were managed. They were not bonded to the livestock and were provisioned at the homestead each day.

Interbreeding

Tissue samples or blood for DNA analysis was collected at the time of collaring the maremmas and when free-ranging wild dogs were shot or trapped locally. Samples were preserved in Longmire's Buffer and sent to the University of NSW (2010 Dunluce samples) and ASAP Laboratories in Victoria (2011 Stratford samples) for analysis to determine if there was any evidence of interbreeding between guardian dogs and wild dogs.

At the University of NSW, DNA samples were analysed by Dr Alan Wilton using 5-20 allele tests that compared the submitted samples to reference groups of pure dingoes, domestic dogs and hybrid dingoes. Results produced in this analysis illustrate test samples against reference samples in respect to the percentage of dog-like and dingo-like alleles in their DNA (see Appendix 3 for details).

ASAP Laboratories analysed the submitted samples for breed ancestry using a sophisticated statistical approach that included Principal Component Analysis, Homozygosity observations and Bayesian Statistical Modelling based on 321 alleles (details of methods in Appendix 4). This approach identifies each individual's breed from over 200 domestic dog breed 'signatures' including its past three generations of parents. However, as 'dingo' is not one of the breeds characterised in the analyses, the program identifies the closest related domestic breed.

Biodiversity Impacts

Simultaneously, the activity of wild dogs (a measure of relative abundance), guardian dogs (distinguished from wild dogs by their much larger foot length), sympatric predators and other wildlife was investigated from spoor at tracking stations using the Activity Index (AI) methodology (Allen *et al.* 1996). Forty-two and sixty-two tracking stations, one kilometre apart, were monitored for two to three consecutive days in three surveys at Dunluce and Stratford respectively (Figure 4). The wildlife activity data collected from tracking stations within paddocks patrolled by maremmas were compared to the data collected from tracking stations in paddocks without maremmas to determine whether the presence of maremmas negatively affected the activity of feral cats, foxes or macropods. As no-one knew where Stratford's maremmas got to at night, separating the paddocks with- and without maremmas on Stratford was done post hoc.

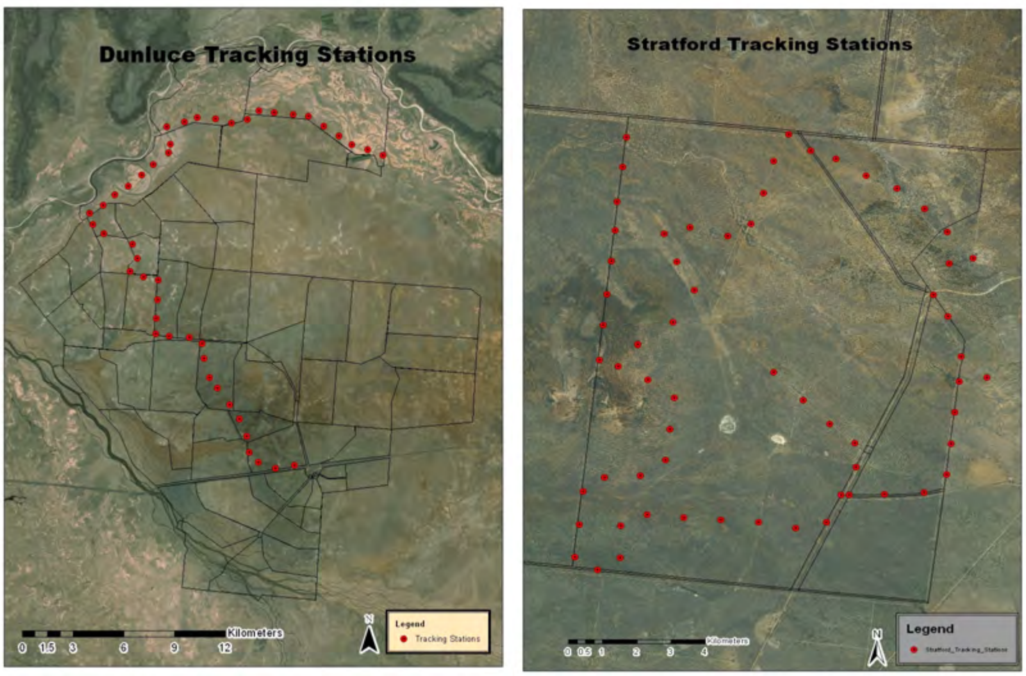


Figure 4. Location of tracking stations for monitoring wildlife abundance trends within the two study sites.

Communication

Progress and results of this study was communicated through numerous seminars and workshops addressed by the Principal Investigator, radio and newspaper interviews, in a Leading Sheep webinar and on Biosecurity Queensland's websites (details in Appendix 5). Progress was reported to the State's QDOG taskforce (Queensland Dog Offensive Group) and National Wild Dog Management Advisory Committee and these reports were further disseminated through member organisations and networks.

RESULTS

Spatial Movements - Dunluce

Figures 5a&b show the movements of the eight maremmas and five wild dogs collared on Dunluce for which location data was obtained. While some maremmas showed strong fidelity to the paddocks or sheep they were assigned to (Nunzio, Eddie,

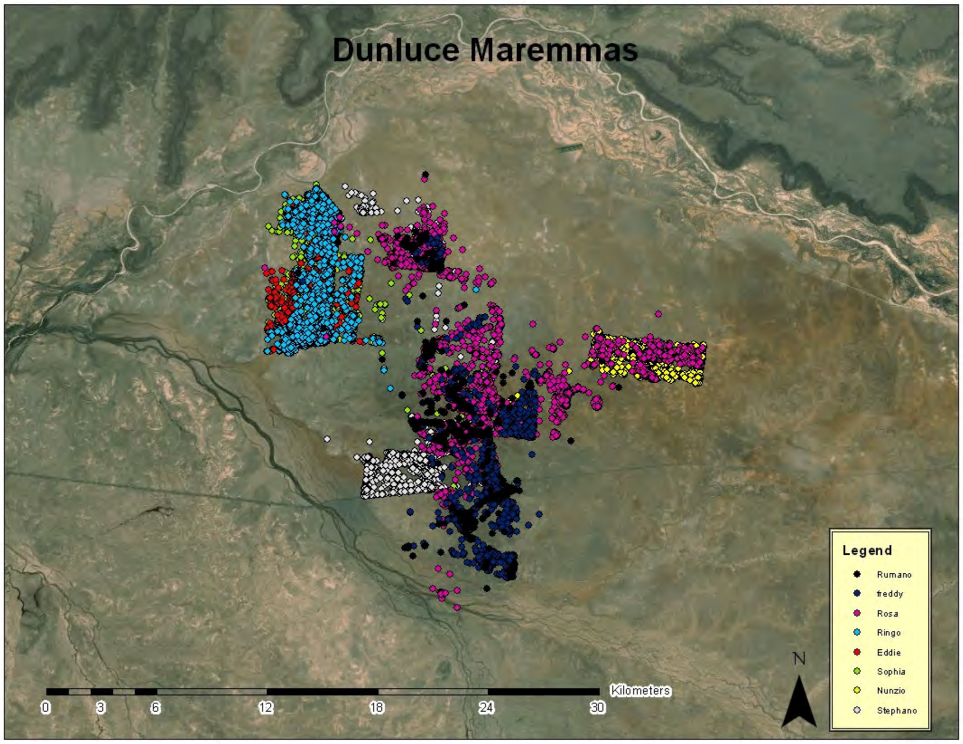
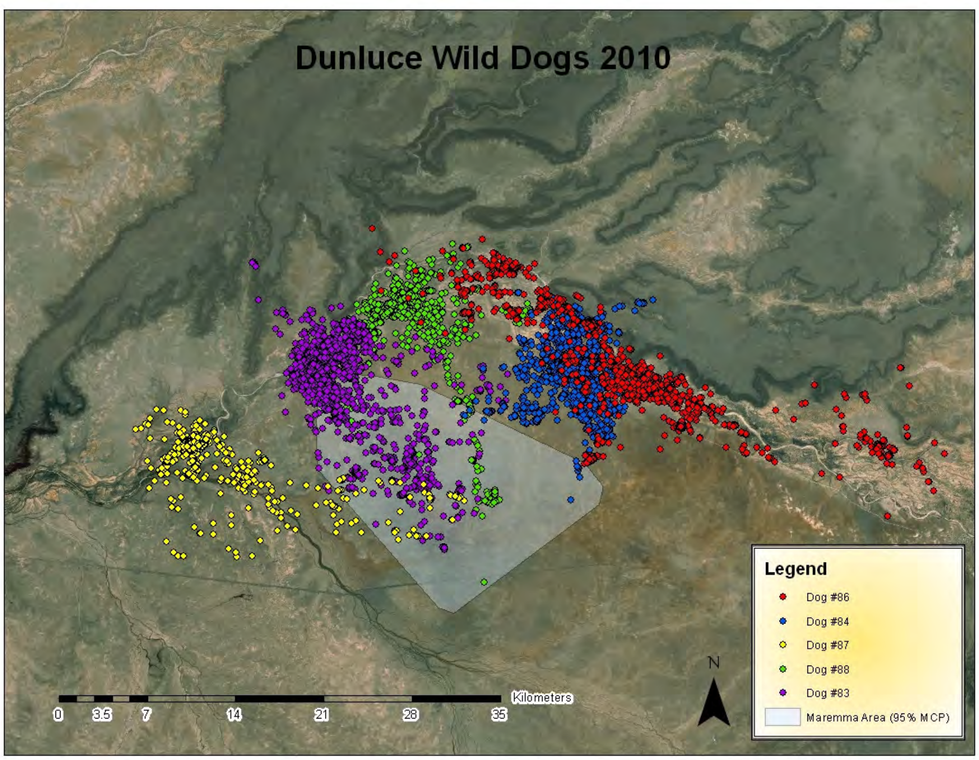


Figure 5a. Half hourly locations of eight of Dunluce's maremmas showing individual differences in their fidelity to the paddocks they are assigned to.



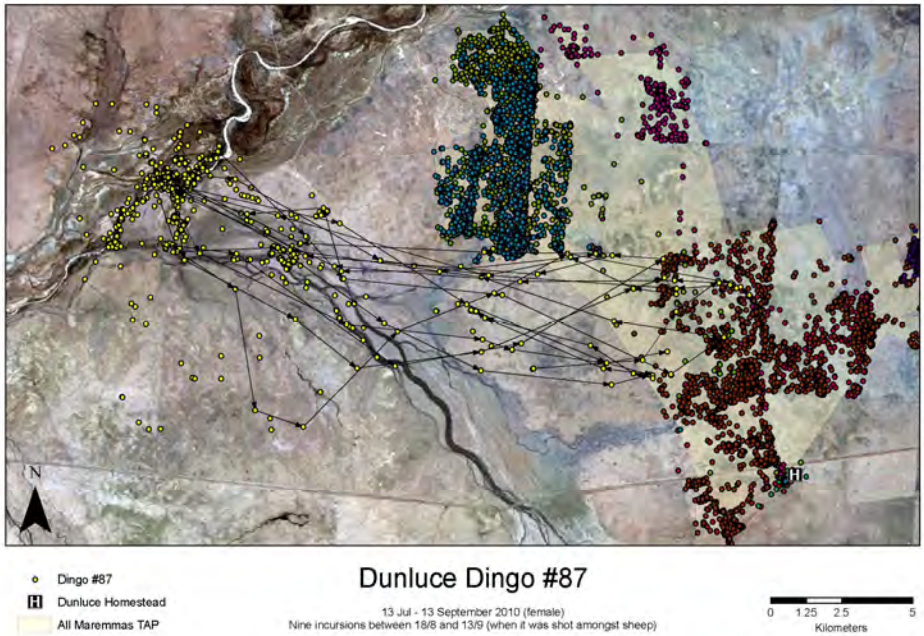
Fig

Figure 5b. Hourly GPS locations of five wild dogs captured and tracked on Dunluce between April and October 2010. Shaded area represents the (95% MCP) maremma-patrolled sheep paddocks shown in Fig 4a.

Sophia and Ringo, Fig 5a) others (Stephano, Romana and Freddie) were found to occasionally move between sheep paddocks ranging up to 20 kilometres away from their 'home' paddock.

Two of the five wild dogs barely intruded into the maremma-patrolled sheep paddocks (Dog # 84 and 86 in Fig 5b) yet 864 hourly GPS locations were recorded inside the maremma-sheep or adjoining paddocks (95% MCP area, Fig 5b) by the other three wild dogs. Sixty-six separate forays of wild dogs venturing into this area were documented in the 18 wild-dog-months of tracking. Twice as many wild dog locations found in sheep paddocks or adjoining paddocks occurred during the night compared to daytime but occasionally wild dog forays extended for up to three days. One wild dog (Dog # 83) had 19.5% of all his hourly locations inside the maremma-patrolled area.

Although movement data show wild dogs bred and mostly confined their activities (territories) to the less accessible, timbered habitats associated with the river and tributaries, they still made regular forays of over twenty kilometres from woodland habitats adjacent to the Flinders River into the open grasslands to forage within and adjacent to sheep paddocks (Figure 6).



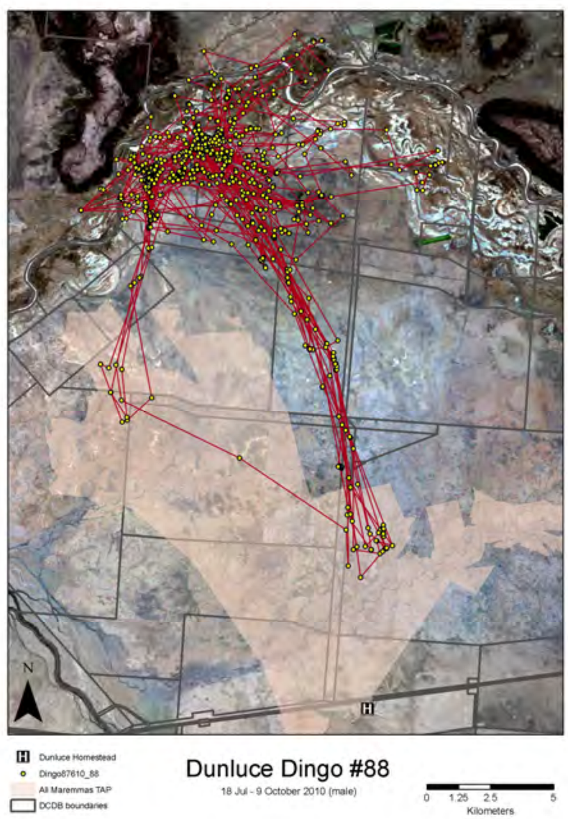


Figure 6. **Two examples of multiple forays by collared wild dogs (yellow dots connected chronologically) 15 - 20 kilometres into the maremma-patrolled sheep paddocks (shaded area) at Dunluce. Coloured dots in upper image show maremma GPS locations in paddocks containing sheep.**

Temporal Movements - Dunluce

Maremmas moved little through the night even when wild dogs foraged in the same paddock (Figure 7a&b). Conversely, wild dogs were most active at dawn and dusk and through the night. Consistent with this, spotlight observations suggest that maremmas camp with the sheep in large single flocks through the night. When disturbed (by spotlights) the sheep reacted by milling around and tightly packing together, so tight in fact that sheep towards the centre of the flock were forced to stand upright. Meanwhile the maremmas barked noisily and circled the perimeter of the flock.

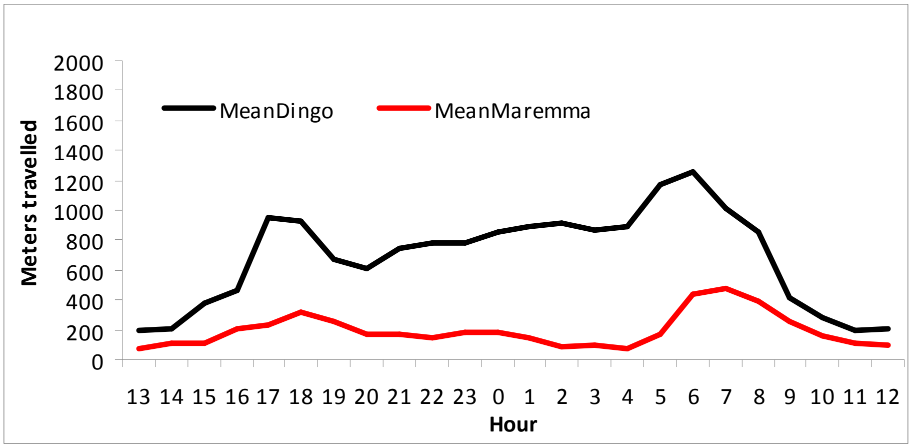


Figure 7a Mean distance maremmas and wild dogs travelled for each hour of the day (midday to midday) on Dunluce between April and October 2010.

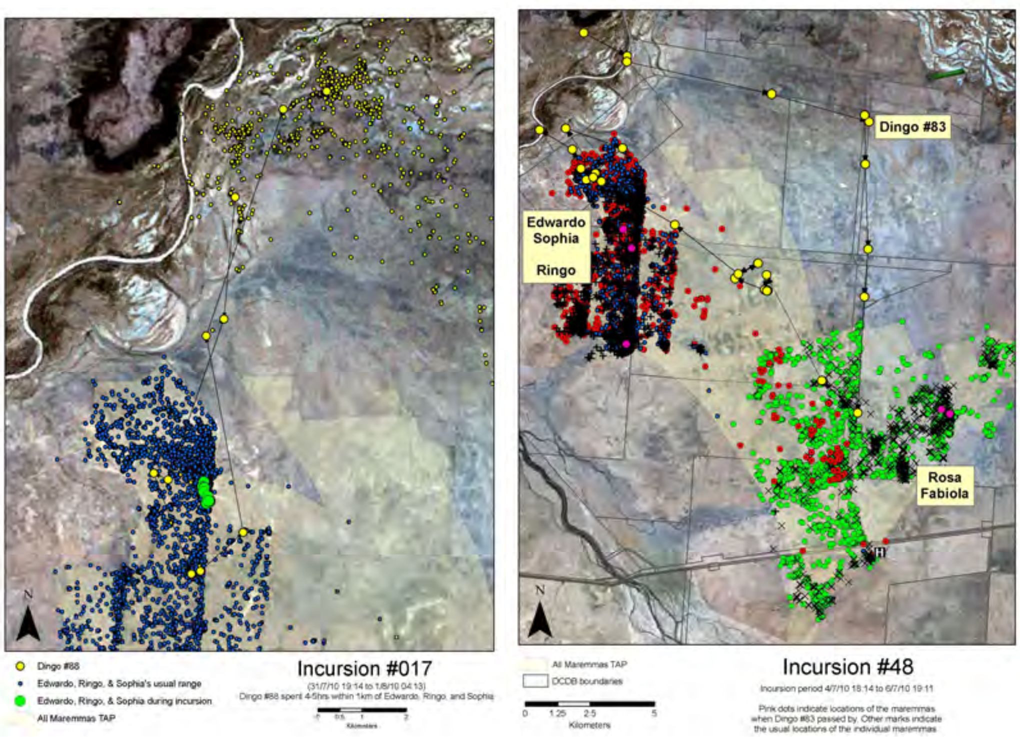


Figure 7b Two of 66 incursions made by collared wild dogs into paddocks containing sheep and maremmas. The concurrent location and movements of the maremmas during the incursion are highlighted in green (left image) and pink (right image) showing that guardian dogs move very little at night in response to wild dogs being in the paddock.

While no conclusive evidence was found showing maremmas chased-off wild dogs, physical encounters between maremmas and wild dogs are believed to occur. In fact, a collared maremma probably died from injuries following an altercation with several wild dogs. Figure 8 shows the concurrent movements of a lone maremma named “Nunzio” and adult female wild dog (Dog #84) hours before Nunzio died. When wild dog #84’s collar was retrieved in October, she was accompanied by a juvenile and on autopsy she had seven placental scars. The interaction took place on the night of 21st July at the peak of whelping season. Therefore, female #84 would have been either heavily pregnant or heavily lactating and likely to be accompanied by un-collared wild dog companions. Nunzio only moved a few hundred yards and died six hours later.

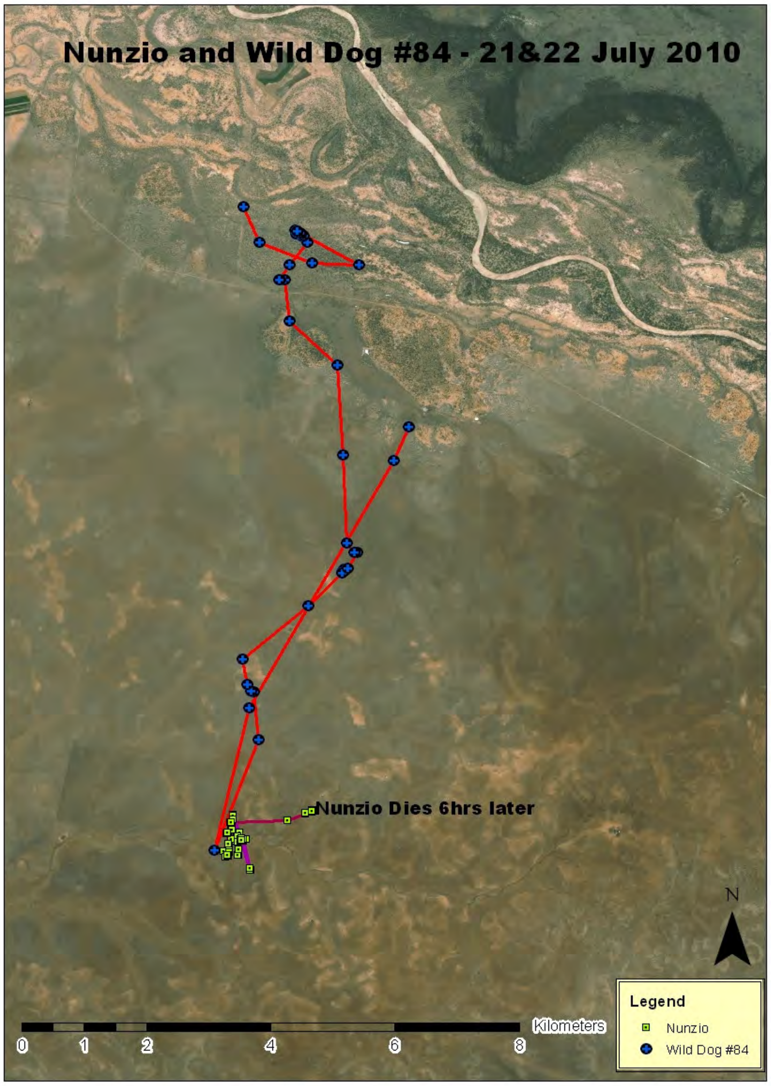


Figure 8 Concurrent movements of adult female wild dog #84 and maremma Nunzio hours before Nunzio died suggesting that aggressive confrontations do occur.

Spatial Movements – Stratford

With little difference between individuals, the maremmas at Stratford were found to rarely venture more than three kilometres from the homestead (Figure 9), three individuals occasionally making it the five kilometres to the mailbox. Most of Stratford's paddocks that contained calves and weaners which were thought to be protected by maremmas were rarely visited. Collared wild dogs ranged widely throughout Stratford and neighbouring properties. However, there was barely any overlap between maremma and wild dog locations.

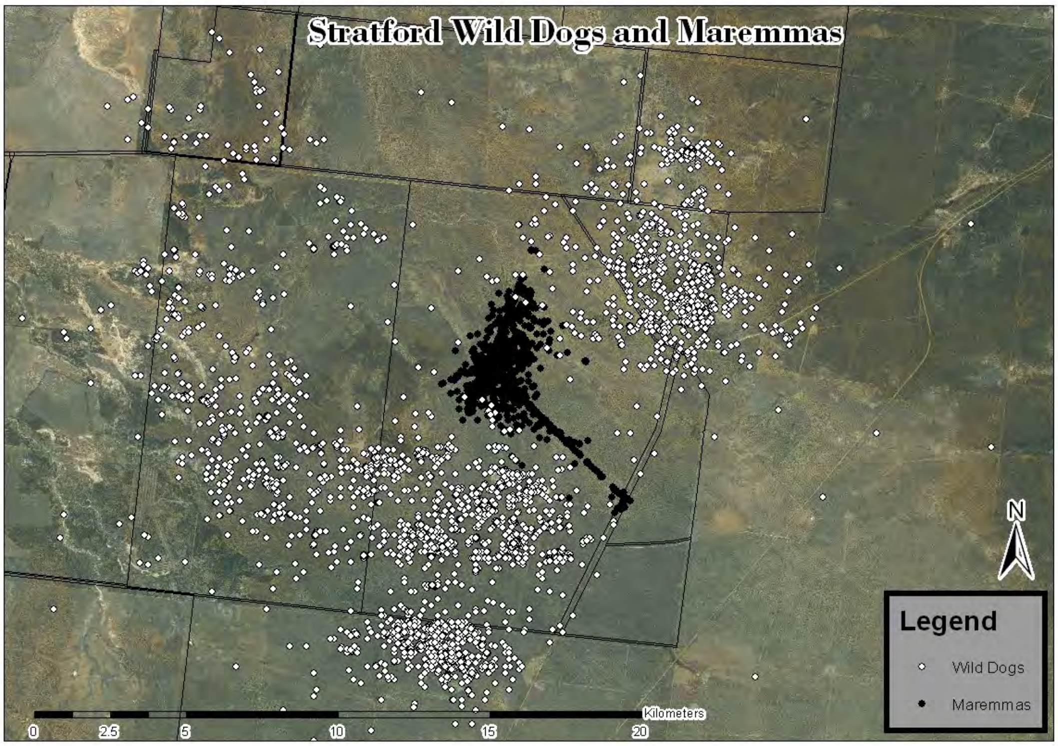


Figure 9. Half hourly locations of seven maremmas (black symbols) and hourly locations of eight wild dogs (white symbols) over seven months at Stratford.

Interbreeding between Maremmas and wild dogs

DNA samples from thirteen wild dogs (five from Dunluce and eight from Stratford) and four, entire male maremmas (one from Dunluce and three from Stratford) were compared. While the samples from Dunluce and Stratford were analysed differently by separate laboratories in Sydney and Melbourne, no evidence of interbreeding between maremmas and wild dogs was found. Results show the wild dogs at Dunluce were all dingo hybrids of varying degrees of purity yet were mostly (>75%) dingo genetics with no evidence of recent domestic dog (maremma) interbreeding (Appendix 3).

The analysis done by ASAP Laboratories investigated breed ancestry (details in Appendix 4). This analysis indicated that Stratford maremmas were slightly inbred but of pure maremma origins with no wild dog or other domestic dog interbreeding in recent generations. The Wisdom Panel Professional program identified a mix of ancient south-east Asian dog breeds (Korean Jindo, Chinese/Mongolian Chow Chow, and Japanese Akita and Shiba Inu) as the nearest domestic dog breed to Stratford wild dogs. However, three of the eight wild dogs (wild dog #89, 93 & 94) had hybrid, (dingo-domestic dog cross) forebears in one or more of the past three generations. However, none of the hybrid-domestic dog forebears were maremmas; they were Australian cattle dog and other domestic breed crosses. Considering the management history of entire maremmas on Stratford and the potential for them to have interbred with local wild dogs over the past decade, these data provide no evidence that interbreeding between maremmas and wild dogs has occurred.

Biodiversity Impacts

Three wildlife surveys were conducted on Dunluce and Stratford during each year of the study. On Dunluce it was possible to divide the tracking stations almost equally between maremma/sheep paddocks and cattle paddocks where maremmas are absent and calculate wildlife abundance indices for each area with- and without maremmas (see Figures 4 and 5a). While there were differences in relative abundance between species and surveys, foxes were only identified on tracking stations in maremma/sheep paddocks (Figure 10). Wild dogs and feral cats were also more active in maremma/sheep paddocks on occasions. However, macropods were more active at times in the cattle paddocks closer to the river and associated trees.

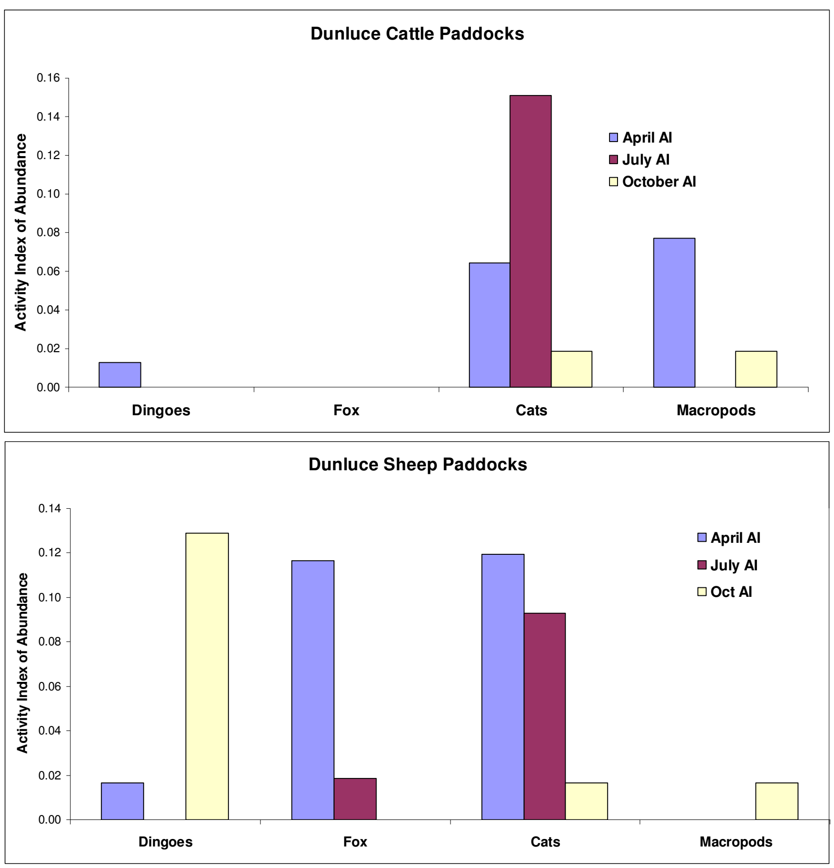


Figure 10 The relative abundance of predators and macropods between the maremma /sheep paddocks and the cattle paddocks.

Overall other species of wildlife showed similar abundance trends between cattle and sheep paddocks except that frog/toad activity, high in sheep paddocks, was not detected in cattle paddocks (Figure 12).

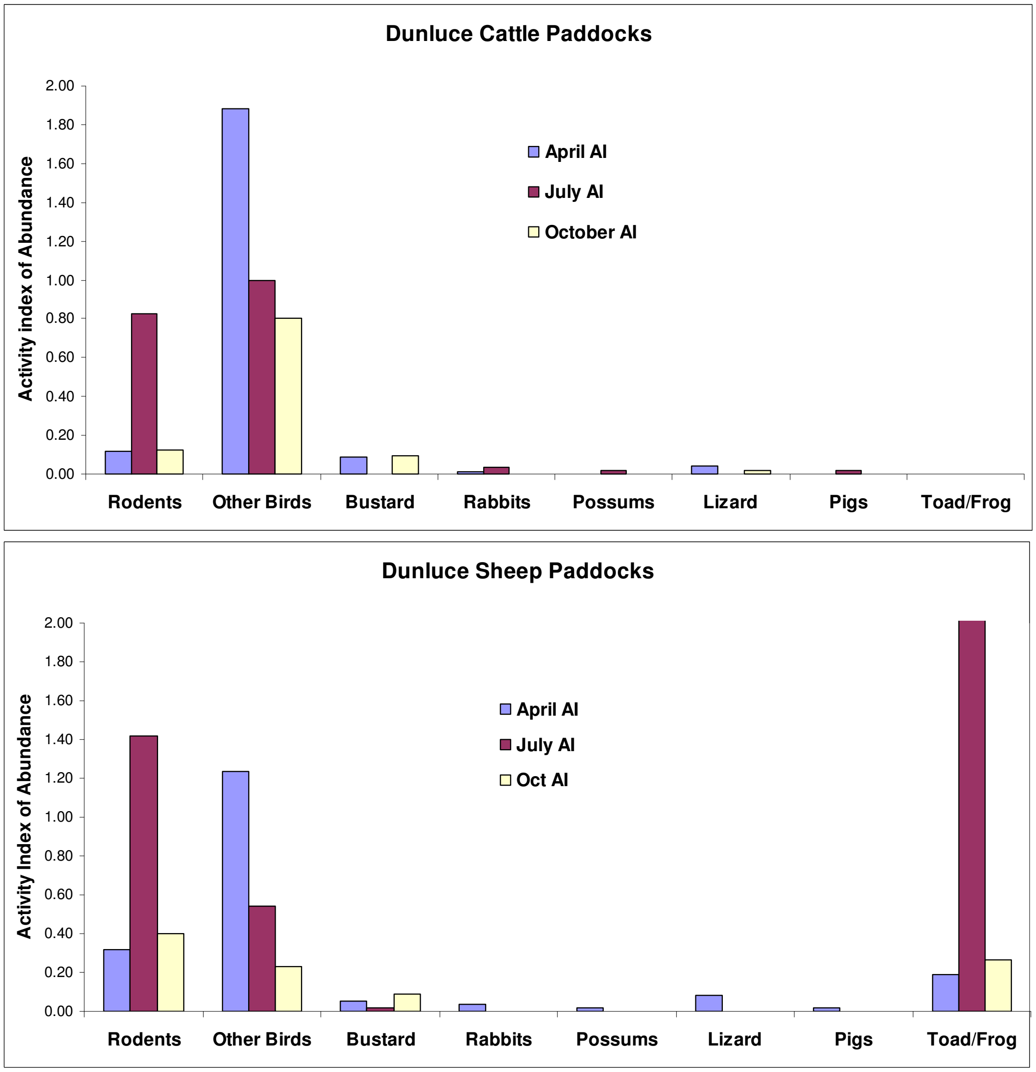


Figure 11 Other species detected on tracking stations on Dunluce during 2010 surveys.

Because the maremmas at Stratford did not venture far from the homestead there were insufficient data to compare wildlife activity in paddocks with maremmas (6 of 62 tracking stations) to those without maremmas (see Figures 4 and 9). Foxes were not detected on Stratford and are infrequently found in the area (unpublished data) but feral cat activity was very high relative to wild dog activity on Stratford (Figure 13) and seldom reaches this level of activity anywhere in the State (unpublished data).

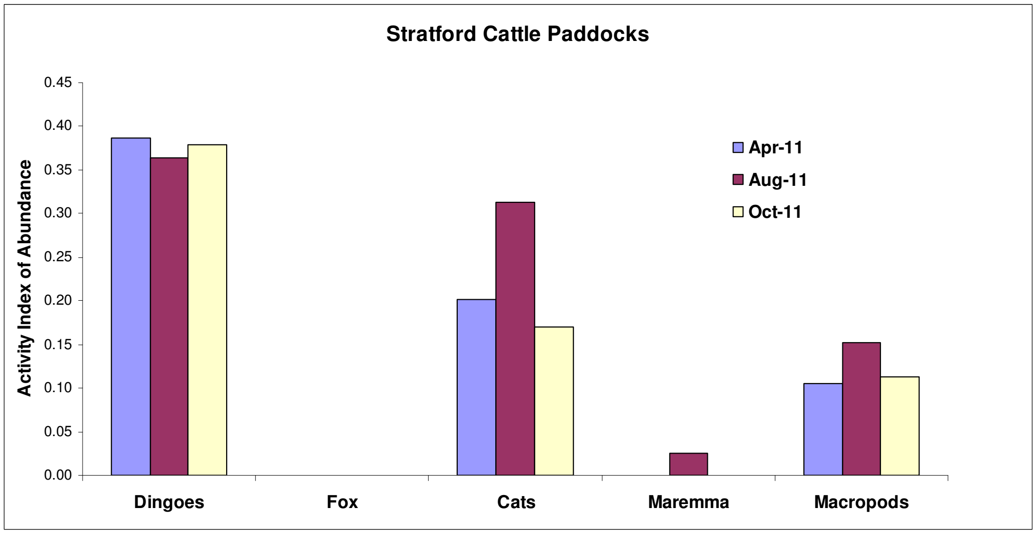


Figure 13 The relative abundance of predators and macropods at Stratford during surveys conducted in 2011.

DISCUSSION

The aim of this project was to discover how guardian dogs work, investigate the risk of maremmas interbreeding with wild dogs and, assess any additional biodiversity benefits maremmas may produce. While Dunluce provides a compelling example where maremmas have practically eliminated sheep predation when these data show wild dogs foraged in and around the sheep paddocks, Stratford's results show maremmas provided little protection to their cattle. When managed according to best practice recommendations (i.e. when maremmas are properly bonded to the livestock and fed in the same paddock, Van Bommel 2011), maremmas can work. However, they may not work if recommended management procedures are not followed.

How maremmas work

Maremmas do not work by marking and defending territories whose boundaries are respected by adjacent wild dogs as initially hypothesised. These movement data show wild dogs regularly foraged in and around the maremma/sheep paddocks. Superficially, Stratford's movement data suggests the maremmas and wild dogs were mutually exclusive; however, wild dog tracks were detected during Activity Index surveys on the six tracking stations located within Stratfords's maremma area (activity index of 0.21). None of Stratford's wild dogs were found to have territories that overlapped the movements of maremmas but this was probably because traps were not set and wild dogs were not collared within two kilometres of the Stratford homestead.

Our data and observations suggest maremmas might work in several other ways:

1. By facilitating and even encouraging sheep to camp together in large flocks at night,
2. By reducing sheep's normal impulse to flee from predators when threatened (as fleeing elicits predators to attack),
3. By aggressively barking at intruding wild dogs,

4. By cooperatively coming to the assistance of neighbouring maremmas in order to repulse wild dogs, and
5. By physically attacking wild dogs if necessary.

Our own observations, confirmed through personal communications with other maremma owners, suggest that sheep generally camp in large, single aggregations at night in the company of attending maremmas. When wild dogs are within the sheep paddock (at night) our data shows that maremmas generally remain stationary (Figure 7b), presumably close to the sheep. In addition, maremmas bark continuously through the night and seem to 'communicate' with maremmas in more distant paddocks; and some individuals will move up to 20 kilometres away from their 'home' paddock (Figure 5a). During this study a freshly-killed wild dog was discovered in a sheep paddock that contained additional maremmas to those that should have been there (Personal communication, Ninian Stewart-Moore) suggesting that some individuals (i.e. the more aggressive, more dominant or more maternalistic maremmas) respond to the vocalisations of others to cooperatively deter wild dog attacks.

The innate behavioural interactions that have evolved between predators and prey are well documented (Mech 1988). Fleeing behaviour by prey animals elicits the chase and attack behaviours of their predators. While sheep, stressed by wild dog predation, are characteristically anxious and easily frightened (Allen and Fleming 2004), they were notably relaxed and unhurried when accompanied by maremmas. Synergistically, the changes in sheep behaviour brought about by the presence and actions of maremmas are probably fundamental to the maremma's ability to eliminate or reduce sheep attacks.

In contrast, several aspects of how maremmas work seem in conflict with cattle behaviour. Cattle, particularly rangeland beef cattle, are likely to resist being shepherded together by maremmas at night preferring instead to stay in smaller, isolated, matrilineal social groups (Lazo 1995, Sewell *et al.* 1999). In addition, the anti-predator behaviours and large body size of cattle makes them inherently less prone to wild dog attack (see Rankine and Donalson's 1968 observations of dingo predation on cows and calves). Establishing and maintaining the bonding between maremmas and cattle may be more challenging in extensive grazing systems compared to smaller, more gregarious and vulnerable species of livestock like sheep, goats and poultry. This may be part of the reason why Stratford's maremmas were not bonded with the cattle and failed to patrol most of the cattle paddocks on the property.

Interbreeding

No evidence was found suggesting that maremmas had interbred with wild dogs (and vice versa) on these two properties when considerable opportunity for interbreeding has existed. I conclude that while interbreeding is an inherent risk when using guardian dog breeds, their disposition to react aggressively towards wild dogs appears to avert copulations.

Biodiversity Impacts and Benefits

Observed differences in macropod numbers between paddocks with- and without maremmas led to earlier speculation that maremmas keep macropod numbers down. In the belief that maremmas could also reduce fox and feral cat activity, it was hypothesised that maremmas might have biodiversity benefits in addition to reducing livestock predation losses. However, these data are unsupportive in this regard.

Maremmas did not exclude wild dogs from foraging in sheep paddocks and certainly did not reduce meso-predators more than wild dogs. The data from Dunluce shows that foxes were only found in the sheep/maremma paddocks and feral cats were just as numerous in paddocks with maremmas as they were in paddocks without maremmas (Figure 10). Their impact on macropods is inconclusive. There were less macropods in the maremma/sheep paddocks at Dunluce at times but this could be an artefact of them harbouring in the more heavily timbered cattle paddocks closer to the Flinders River. Stratford also had macropods but the data provided no evidence that maremmas reduced their numbers.

RECOMMENDATIONS

This project reinforces the utility of using maremmas to guard against wild dogs and to prevent attacks on sheep. Other breeds of guardian dog may work the same way, perhaps not. Other types of guardian animals, donkeys and lamas for example, almost certainly will work differently. **I recommend** future research investigate other guardian dog breeds and alternative guard animal species, particularly, to understand how they all 'work' and how best to use them. Further **research is also recommended** on how best to use guardian dogs to protect rangeland cattle or at least their calves.

ACKNOWLEDGEMENTS

I acknowledge the assistance of Damian Byrne, Ben Allen and James Speed in manipulating and preparing the spatial information; Damian Byrne, Andrew Granzotto and Mark Goullet for assistance in trapping and collaring the wild dogs (even if it was only for cooking some of the meals), and; Matt Gentle for helpful comments on this report. I also acknowledge the support and hospitality of Ninian and Ann Stewart-Moore at Dunluce and Terry Brennan and Robyn Adams of Stratford and for the use of their property and access to their 'workers': Nunzio, Fredrico (Freddie), Ringo, Edwardo (Eddie), Sophia, Rosa, Stephano, Romana, Miranda, Lear, Razor, Richard, Brutus and Ollie. Nunzio, whom paid the ultimate price while defending the sheep, deserves special mention.

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Appendix 1 Details of the Wild Dogs Captured and Collared

Date	Dog #	Gender Male/Fem	Location/Description	Weight Kgs	Est Age Mths*	Trap Description	Comments
20-Apr-10	83	M	Dunluce/Ginger	18.5	**15**	#3 Jake	Shot 9 Oct 2010
22-Apr-10	84	F	Dunluce/Black/Tan	13	**23**	#3 Victor SC	Shot 9/10/10, 7 placental scars
24-Apr-10	85	M	Dunluce/Red	20.5	10	#3 Bridger SC	Collar failed, never recovered
26-Apr-10	86	F	Dunluce/Ginger	14	**8**	#3 Jake	Found dead 8 Oct 2010, had died 13 July 2010.
13-Jul-10	87	M	Dunluce/Ginger	15.5	**17**	#3 Victor SC	Killed in sheep paddock, 13 Sept 2010
18-Jul-10	88	M	Dunluce/Ginger	17	12	#3 Bridger SC	Shot with likely sibling 10 Oct 2010
10-May-11	89	M	Stratford, Red	19	12	#3 Victor SC	
13-May-11	90	M	Stratford, Sable-Black	23	12	#3 Victor SC	
15-May-11	91	M	Stratford, Red	23	12	#3 Bridger SC	Shot 2 Nov 2011
15-May-11	92	F	Stratford, Ginger	17	12	#3 Victor SC	
16-May-11	93	F	Stratford, Ginger	16	9	#3 Victor SC	
17-May-11	94	F	Stratford, Ginger	18	12	#3 Jake	
17-May-11	95	F	Stratford, Ginger	18.5	24	#3 Victor SC	
23-May-11	96	M	Stratford, Ginger	20	24	#3 Bridger SC	Shot 1 Nov 2011

* Age estimated from Pulp Cavity: Tooth Width Ratio (bolded values, Kershaw *et al.* (2005)) or from visual assessment based on O'Brien et al (2011).

Appendix 2

Department of Employment, Economic Development and Innovation

GUIDE TO FIELD ESTIMATING THE AGE OF WILD DOGS

Lee Allen & Lauren O'Bryan

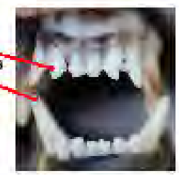
<6 Months Milk teeth, adult canines not yet emerged

Partly emerged canine with juvenile canine not yet pushed out

6 – 9 Months Canines emerged and open rooted

12 Months Canines fully in place with rounded tips.
Pointed cusps and notches on incisors.
Large pulp cavity.

Cusps & notches on incisors. Rounded tips on canines



2 years Canines get slightly flattened tips.
Cusps flatten and notches on incisors begin to wear off.
Pulp cavity slowly closes

17 mth and 23 mth old canines

4 Years Canines get flattened tips, discolorations and show evidence of wear marks from opposing teeth. Notch on incisors worn off

10 Years Some incisors worn to gum-line. Teeth flattened to pegs.
Erosions and discoloration common. Pulp cavity no longer visible or just a thin line

Wear marks on opposing teeth

More information

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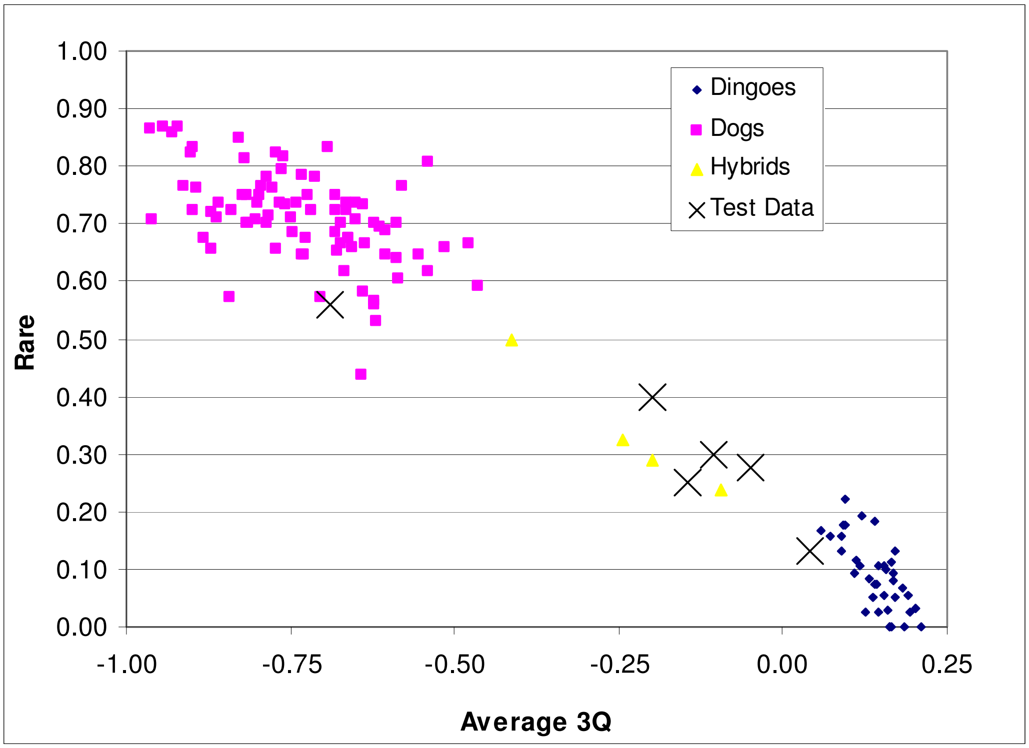
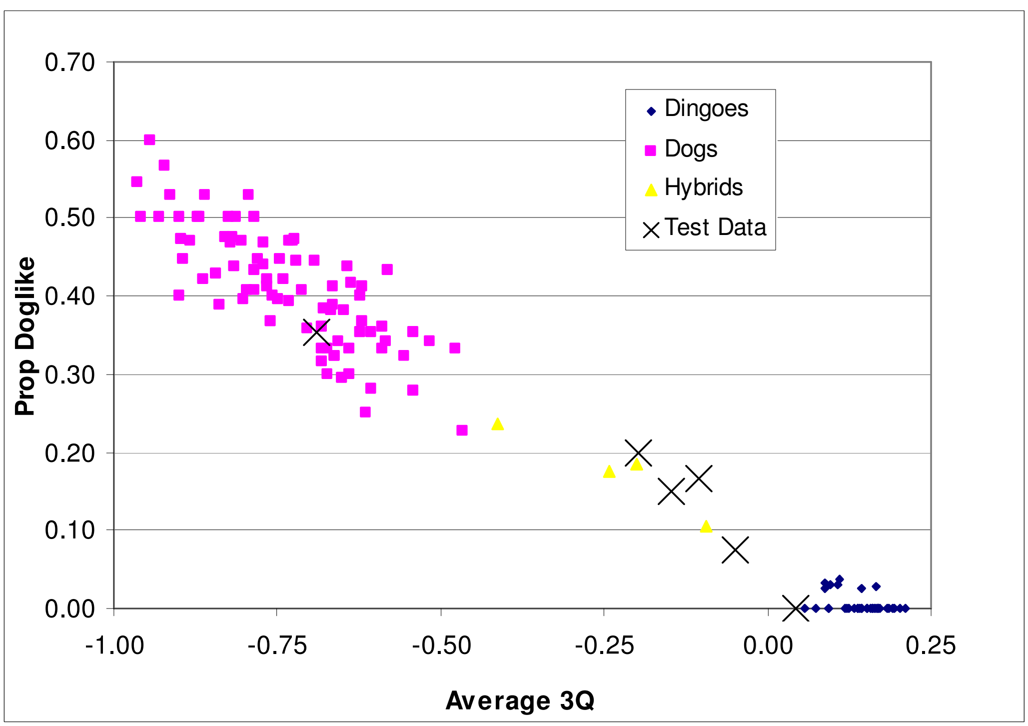
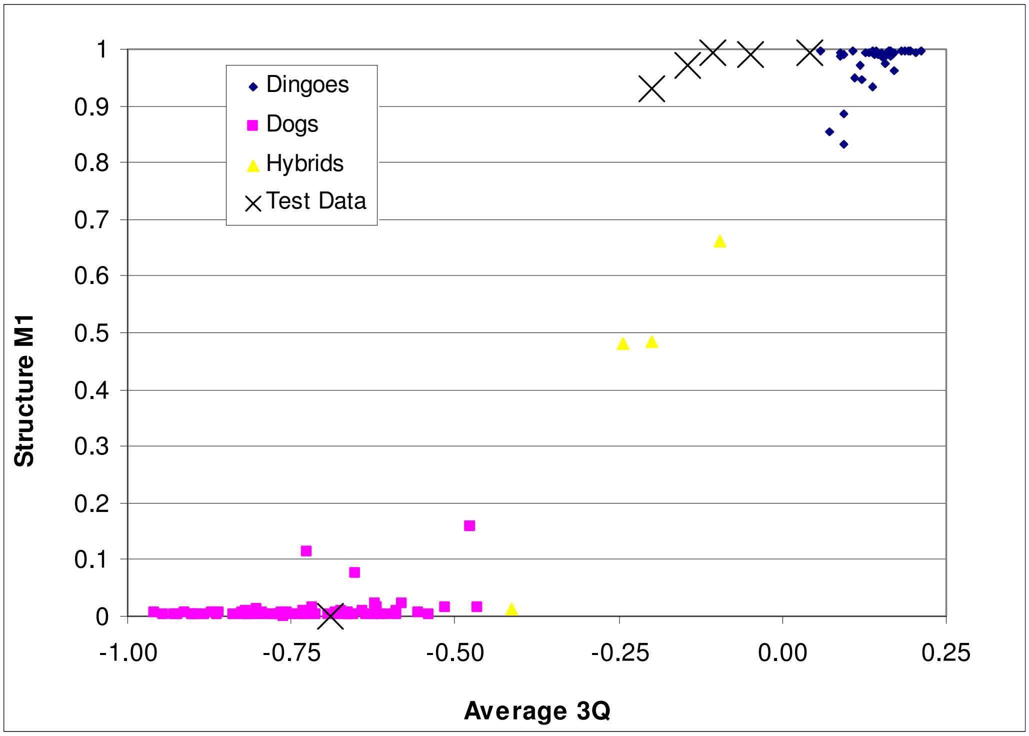
Queensland Government

Appendix 3

Results of DNA testing Dunluce samples (from Dr Alan Wilton)
(The Figures show the maremma Casper falls in a cluster with domestic dogs distinct and separate from the five dingo hybrids captured on the same property)







Dingoes
Dogs
Hybrids
Test Data
Structure M1
Average 3Q
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-1.00
-0.75
-0.50
-0.25
0.00
0.25

Appendix 4. Report on Stratford DNA Samples

Wild Dog Report for Lee Allen

Introduction

Mars Veterinary was provided with a number of wild dog samples from Lee Allen in Queensland as well as a few purebred Maremma Sheepdogs. The intention is to review the DNA marker data to try to ascertain whether there is any evidence for admixture from the Maremma Sheepdog or any other breed population with the wild dog population. The report contains a number of data that consider this problem including Principal Component Analysis (PCA) and Bayesian Statistical ancestry modelling.

Materials

11 canine blood samples were received as EDTA blood samples from ASAP Laboratories from Lee Allen. DNA was extracted using a high throughput commercial kit and processed with the 321 DNA single nucleotide polymorphism (SNP) markers of the Wisdom Panel Professional. 8 multiplex reactions were used to genotype each sample, and the SNP genotype data for each locus was scored automatically using the latest version of the Sequenom Mass Array analysis platform software. Data for each sample was provided to Mars Veterinary in a .csv file format.

The sample identities and markers successfully typed were:

Sample ID	Description	Markers successfully typed	Marker panel Homozygosity
8000000838	Maremma Lear 148	258	72.87%
8000000236	Maremma Richard 149	289	83.74%
8000000268	Maremma Brutus 150	293	70.99%
8000000757	Wild Dog #96 145	302	82.45%
8000000753	Wild Dog #95 147	317	86.44%
8000000786	Wild Dog #94 144	312	83.01%
8000000209	Wild Dog #93 143	317	82.02%
8000000856	Wild Dog #92 140	317	86.12%
8000000055	Wild Dog #91 143	315	83.49%
8000000303	Wild Dog #90 146	317	79.18%
8000000378	Wild Dog #89 142	314	86.62%

Samples were statistically analysed using a number of approaches that included:

Principal Component Analysis (PCA)

Homozygosity observations

Purebred breed likelihood

Bayesian Statistical modelling

Results of these analyses can be found in the following pages of this report with associated commentary.

Results

Homozygosity observations

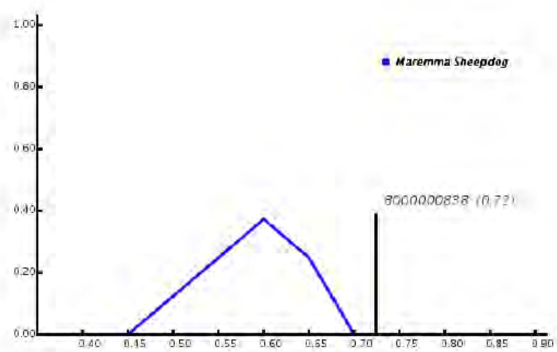
Homozygosity is a measure of how many genetic markers are identical by descent, such that both the sire and the dam passed down the same marker variant (called an allele) – typically each dog inherits two copies of every DNA marker, one from the paternal and the other from the maternal line. Purebred dogs (as small isolated breeding populations) are commonly observed to have a higher average homozygosity across the Wisdom Panel DNA markers than mixed breed dogs, and for each breed there is usually a particular range of values observed that usually form a normal distribution. We calculate the average heterozygosity of a dog based on the percentage of the 321 markers tested that are observed to have inherited the same marker variant. Dogs with a mixed ancestry generally tend to have a lower homozygosity level across the markers of the Wisdom Panel®. Breeds with very small population sizes and limited population diversity (e.g. Basenji) often have a higher average homozygosity than larger more diverse breeds (e.g. The Labrador Retriever).

Initial observations of the raw DNA marker data show that the Maremma Sheepdogs were observed with panel average homozygosity either equal to, or higher than the range previously established for the breed (figure 1). This suggests that these dogs are towards the more inbred end of the breed distribution maybe due to local line breeding, or limited diversity in the local genepool.

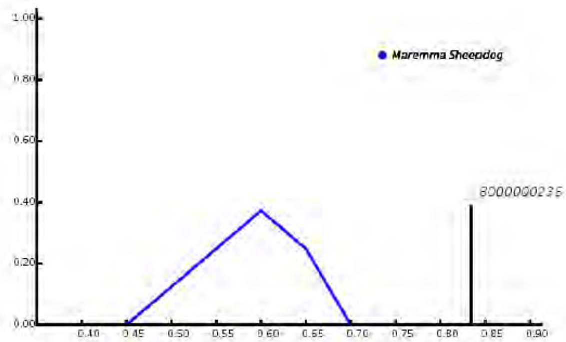
The wild dogs also had very high observed average panel homozygosity figures across the 8 samples – ranging from 79.2% to 86.4%. These values are higher than those seen in all but the most inbred dog breeds suggesting that the local wild dog population has limited diversity across the DNA markers sampled and is likely highly inbred. This also suggests that significant admixture from another canine breed is potentially unlikely as mixing different breed backgrounds would be predicted to lead to much lower panel homozygosity scores – in our experience most mixed breed dogs, even first generation (F1) crosses, have a panel homozygosity below 60% unless line bred specifically as an inbred designer dog population.

Figure 1: Maremma Sheepdog panel homozygosity compared to range seen in previously tested dogs from the breed.

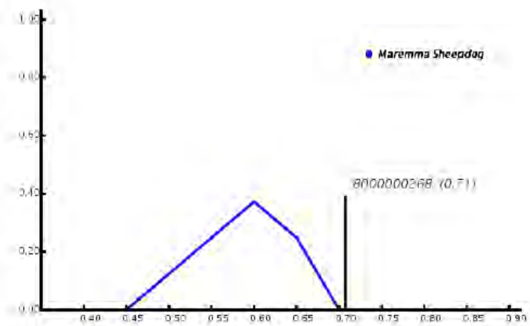
a) 8000000838



b) 8000000236



c) 8000000268

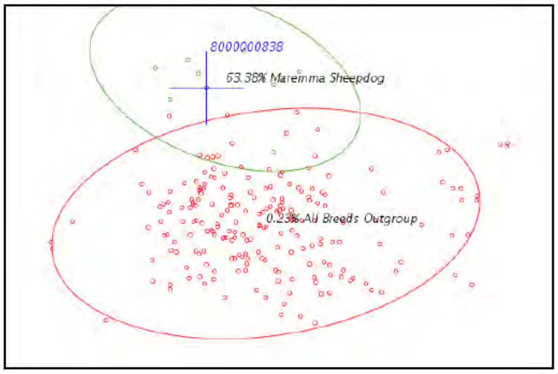


Maremma Breed PCA analysis

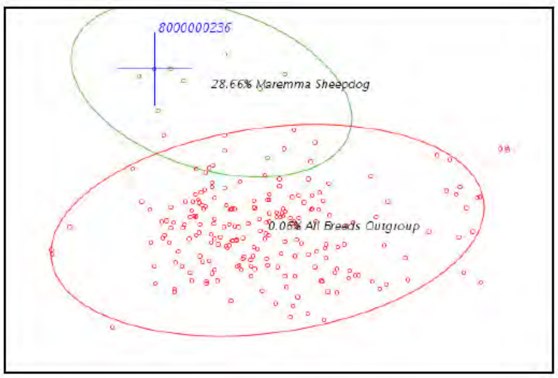
The pure Maremma Sheepdog samples provided for testing by Lee Allen clustered with the known purebred from that breed with high statistical support as expected when examined using an unsupervised clustering software package utilising a technique called Principal Component analysis (PCA - figure 2). PCA summarizes the data by the greatest contributing factors to variance in the data. The effect is to cluster together the most similar samples in the dataset and to separate the more distantly related samples. PCA is a method of simplifying the data in order to focus on the most important factors (principle components) that cause differences between dogs. When plotted in three dimensions, the different axis represent the most important principle components with the x axis being PCA 1 (most different factor), y axis PCA 2 (2nd most different factor) and the z axis being PCA 3 (third most important factor). Often, its operation can be thought of as revealing the internal structure of the data in a way which best explains the variance in the data.

Figure 2: Two dimensional Principal Component analysis showing individual provided Maremma Sheepdog samples, known purebred Maremma Sheepdogs and an all breed outgroup (containing a single sample from each of the 200+ breeds detected by the Wisdom Panel).

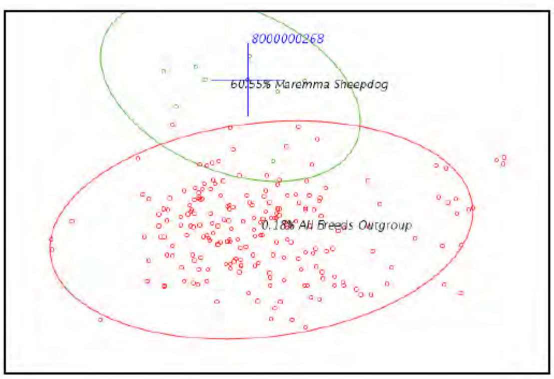
a) 8000000838



b) 8000000236



c) 8000000268



Purebred Likelihood Analysis – Maremma Sheepdogs

Bayesian Statistical analysis with the proprietary Wisdom Panel Professional algorithm suggested that these dogs were indeed purebred Maremma Sheepdogs with a high degree of statistical support using three different maximum statistical likelihood models to analyse the DNA data from each individual dog and compare to Wisdom Panel database reference breed samples (figure 3).

Figure 3: Results of 3 different Bayesian likelihood calculations that query the best breed as a statistical match to the tested sample. Each shows the Maremma Sheepdog to be the clear best statistical breed match for all 3 analyses supporting each as a purebred Maremma Sheepdog.

a) 8000000838

Likelihoods			
Breed	**Likelihood**	**Mix Likelihood**	**IBD Likelihood**
Maremma Sheepdog	113.1	85.8	104.3
Hedge	122.5	93.0	110.8
Treeing Walker Coonhound	125.5	92.5	113.3
Pointing Griffon (Wire)	126.4	91.1	112.3
Guard	126.5	93.1	113.1
Ancient	126.7	93.5	113.8
Munsterlander (Small)	127.2	92.5	114.3
Blue Tick Coonhound	127.6	94.3	115.3
Catahoula Leopard Dog	128.6	93.8	114.4
German Wirehaired Pointer	129.2	92.7	118.1

b) 8000000236

Likelihoods			
Breed	**Likelihood**	**Mix Likelihood**	**IBD Likelihood**
Maremma Sheepdog	130.0	103.9	102.5
Hedge	148.7	114.8	115.5
Guard	153.2	115.9	117.9
Catahoula Leopard Dog	153.8	116.2	119.8
Rat Terrier	154.4	116.9	119.1
Belgian Malinois	154.8	116.5	120.7
Presa Canario	155.4	118.5	119.7
Ancient	155.8	117.0	120.0
Greyhound	155.8	116.8	121.1
Canaan Dog	156.3	116.8	120.1

b) 8000000268

Likelihoods			
Breed	**Likelihood**	**Mix Likelihood**	**IBD Likelihood**
Maremma Sheepdog	124.9	96.1	114.6
Hedge	137.5	105.8	123.5
Ancient	141.1	105.6	125.8
Treeing Walker Coonhound	142.3	106.4	127.2
Guard	143.8	108.4	127.9
Presa Canario	144.8	109.4	129.8
German Wirehaired Pointer	145.1	105.1	129.5
Blue Tick Coonhound	145.3	108.5	129.1
Munsterlander (Small)	145.7	106.6	130.9
Canaan Dog	146.5	107.3	130.0

Bayesian Statistical Analysis of Wild dogs

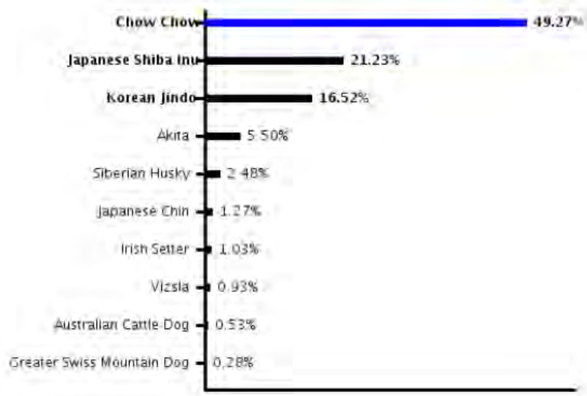
An individual Bayesian statistical breed composition analysis was performed on each Wild Dog using a version of the Wisdom Panel Professional database that lacked a dingo breed signature. This analysis was designed to find the statistically most likely breed matches found in each dog surveyed and results are expressed as the percentage of tree slots filled in each of 7 million Bayesian best fit models considered after a burn-in phase of 10,000 iterations of the MCMC method. Individual breed match results varied as would be expected from such a diverse group, however breeds from the ancient dog cluster comprised the majority of the closest breed matches found in the surveyed dogs which is consistent with the wild dogs having a distinct and ancient heritage (in common with other domestic canid populations such as the Coyote and the Gray Wolf (see individual dog results below)). This finding also reflects the observed close genetic similarity between each of the wild dogs found using all analysis methods in this study applied to the DNA breed signatures.

No evidence of recent admixture was found from the Maremma sheepdog in this survey of 8 wild dogs. We would expect the Bayesian statistical analysis to provide a level of approximately 12.5% or more should a single purebred Maremma Sheepdog grandparent be present in the ancestry of any of the dogs tested. Any breed matches below this level likely reflect noise in the Bayesian analysis rather than genuine evidence for true ancestry from these breeds. Matches to the Maremma Sheepdog breed signature did not exceed 0.85% in any of the 8 dogs studied.

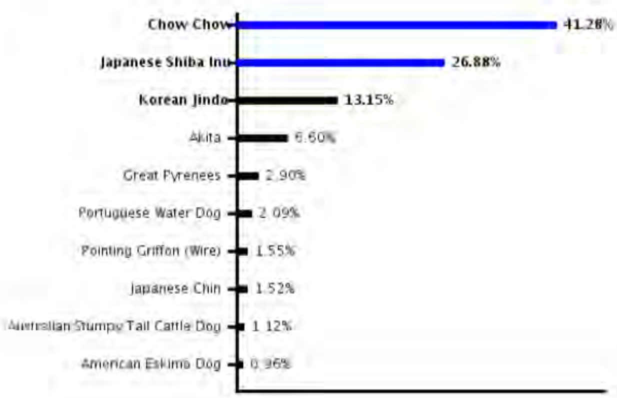
Only one dog 80000000209 showed potential evidence of any significant ancestry from a non ancient breed. There was perhaps suggestive evidence that this dog may have had some ancestry from the Australian Stumpy tailed cattle dog, perhaps three or four generations back. This was not highly supported by PCA analysis (figure 5) and may merely reflect noise in the MCMC data.

Figure 4: Top 10 breed matches found in a Bayesian Statistical Analysis using 7 million 3 generation MCMC tree model iterations on each of the wild dog DNA samples

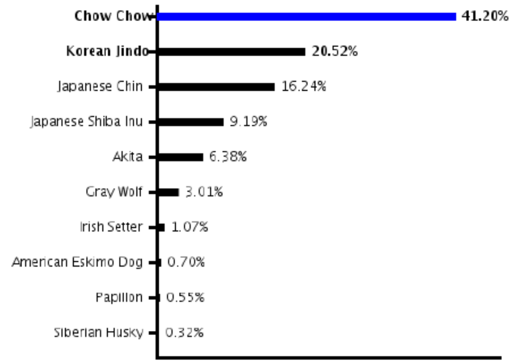
a) 8000000757



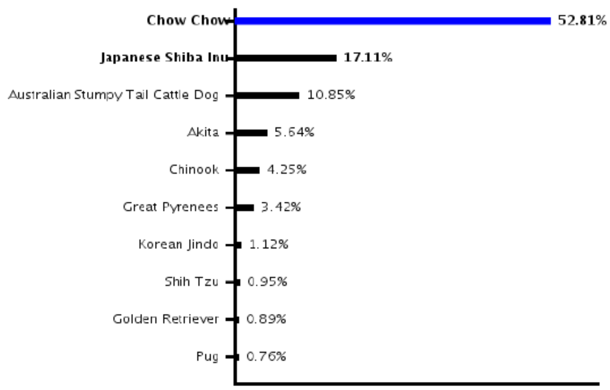
b) 8000000757



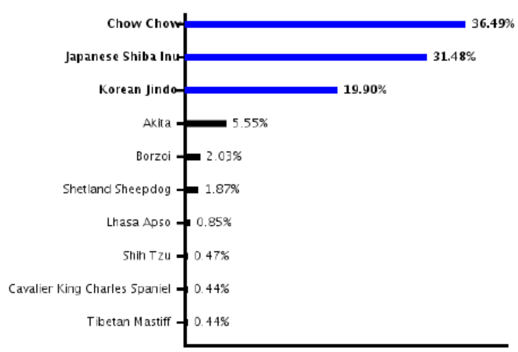
c) 8000000786



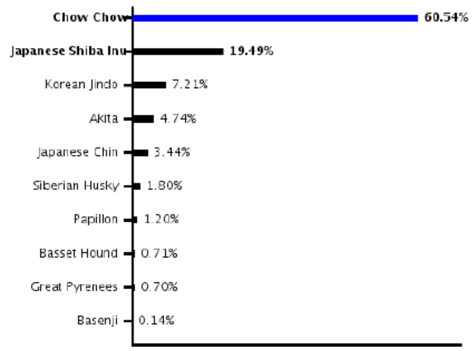
d) 8000000209



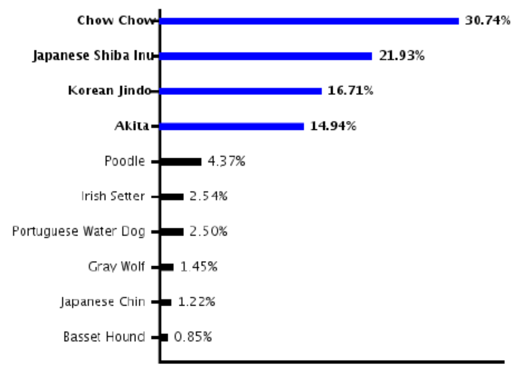
e) 8000000856



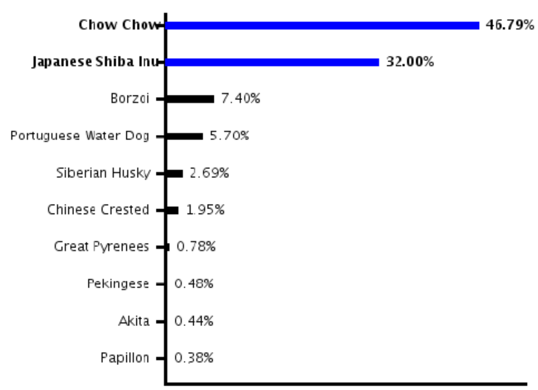
f) 8000000055



g) 8000000303



h) 8000000378



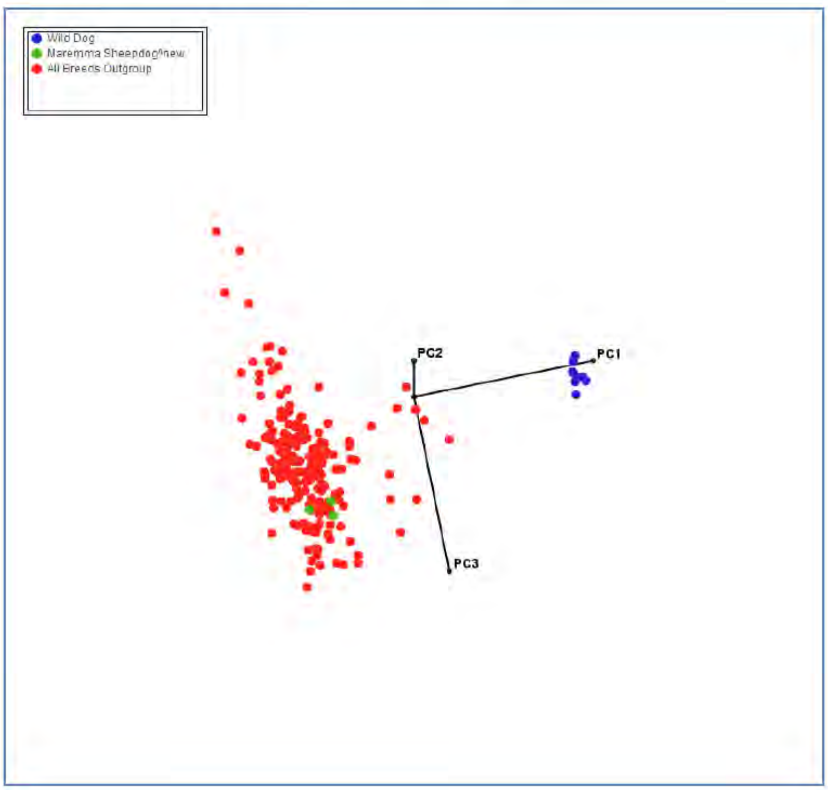
Wild dog PCA analysis

In order to ascertain whether we could find any evidence for admixture from other breeds in the Wisdom Panel breed database we performed a number of additional PCA analyses. In all these different analyses performed, the wild dog samples provided for testing by Lee Allen all clustered very closely together with the other wild dogs with high statistical support when examined using the unsupervised clustering software package utilising a technique called Principal Component analysis (PCA – figures 5a, 5b and 5c). This data supports the observation that the wild dogs surveyed are a homogeneous group that show little evidence of admixture from modern purebred dog breed populations. The Maremma Sheepdogs provided clustered with the existing Maremma Sheepdogs in the Mars Veterinary database as expected.

Figure 5: Three dimensional Principal Component analysis showing clustering of the 8 tested wild dog samples compared with various other breed samples.

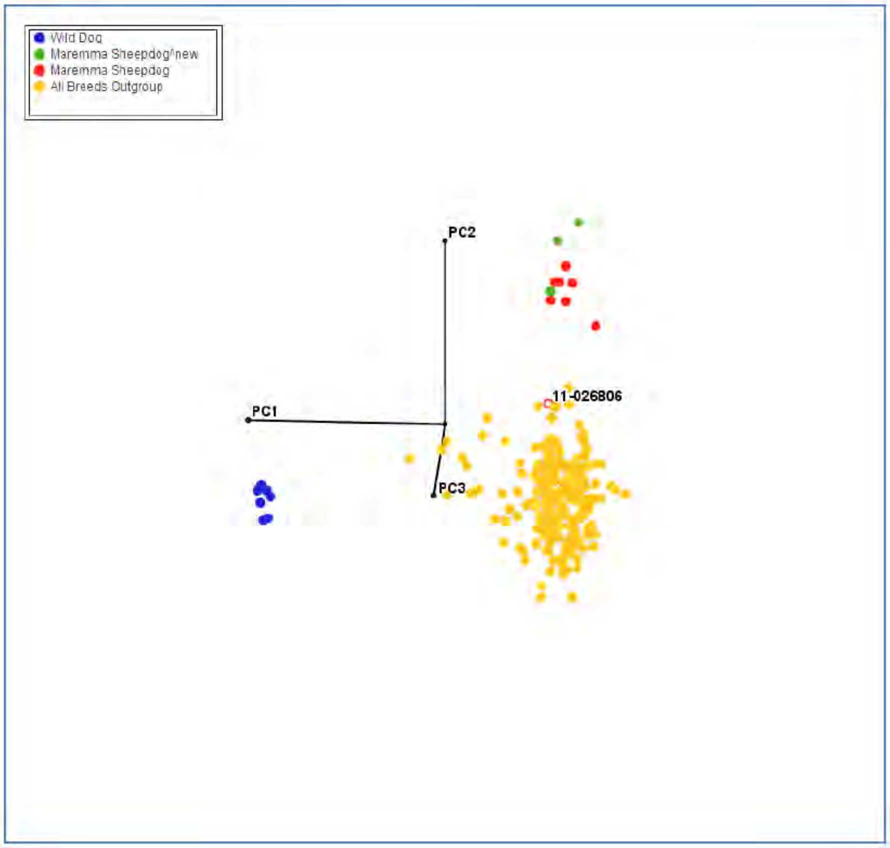
5a: Eight Wild dog samples and three new Maremma sheepdog samples compared to an all other breed outgroup.

The eight wild dogs are clearly separated from all the other dog samples by the first principal component of the analysis and cluster very closely together.



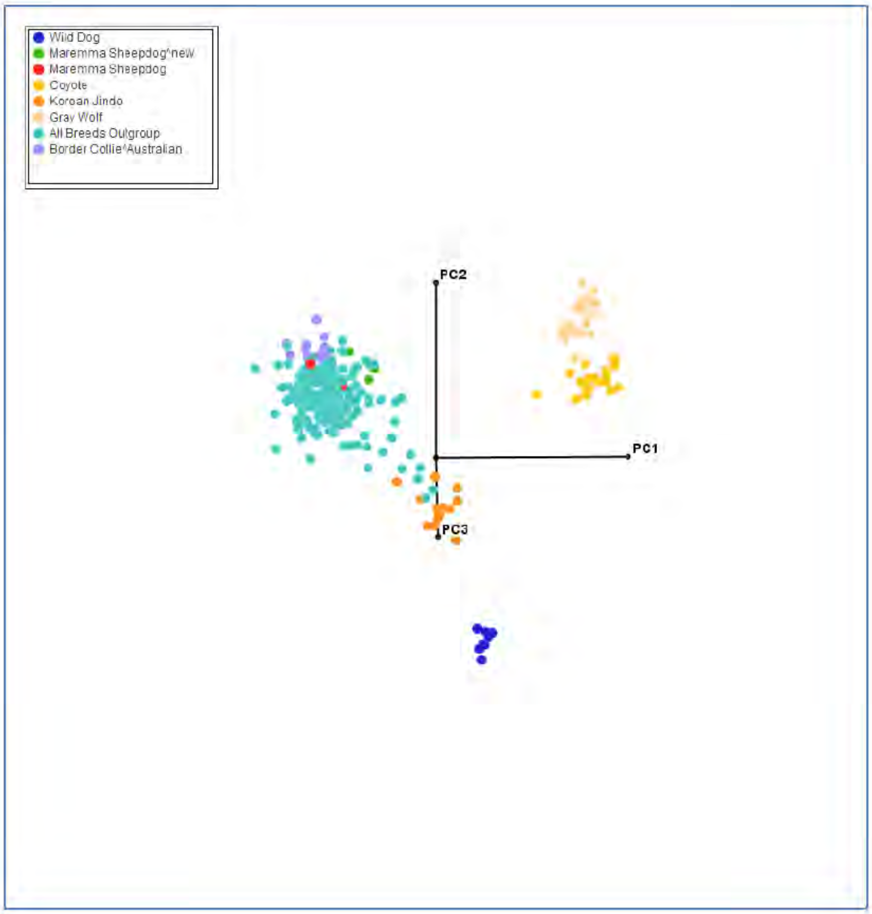
5b: Eight Wild dog samples and all Maremma sheepdog samples compared to an all other breed outgroup.

The eight wild dogs are clearly separated from all the other dog samples by the first principal component of the analysis and cluster very closely together as do the Maremma Sheepdogs with the exception of database sample 11-026806 which may potentially have recent admixture from another on this evidence.



5c: Eight Wild dog samples and all Maremma sheepdog samples compared to an all other breed outgroup, other canid species (Gray wolf and coyote) and other selected dog breeds from ancient or herding ancestry.

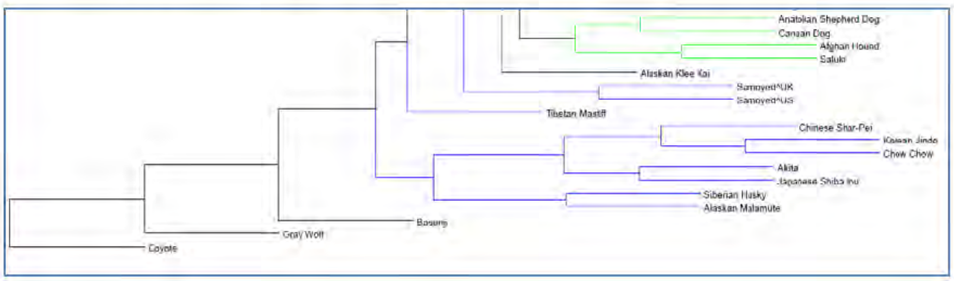
The eight wild dogs and the other canids are clearly separated from all the other dog samples by the first two principal components of the analysis. The nearest dog breed in the distribution is the ancient breed the Korean Jindo as would be predicted from the ancestry analysis. The wild dogs are highly distinguished from the other canid species signifying that they have little in common with either the Gray Wolf or Coyote DNA signatures, which cluster close together.



Summary

Based on the observed results of the various analysis methods performed using 321 DNA markers, there is no evidence in the data to suggest that the wild dogs have significant recent admixture from any modern purebred dog breed, in particular the Maremma sheepdog breed. The eight dogs tested all have a very similar genetic profile that appears distinct from all other canine breeds as well as two other canid groups (notably Gray Wolf and Coyote sourced from the US & Canada). It is thought likely by the authors based on the observed evidence that these wild dogs are from an isolated population that does not interbreed frequently with the local dog population. Based on Bayesian DNA analysis, this population shares closest genetic similarity to dog breeds from the ancient group including the Jindo, Chow Chow, Akita and Shiba Inu which are kown to be closely related breeds based on DNA evidence (see figure 6 below).

Figure 6: Relationship of ancient dog breeds and other canids to each other based upon neighbour joining phylogenetic analysis using Wisdom Panel database breeds and 321 DNA markers



Appendix 5. Media reports and opportunities from this project

	Date	Event	Location	Topic
Presentations	22-Mar-11	SEQ Pest Advisory Forum	Toowoomba	Research Update
	6-8 Apr 11	NWDMAG mtg	Brisbane	Research Update
	6-May-11	Field Day	Charters Towers	Research Update
	7-May-11	Field Day	Townsville	Research Update
	27-May-11	Field Day	Proserpine	Research Update & trap demo
	17-Jun-11	QDOG Mtg	Brisbane	Guardian dog update
	21-23 Jun 11	VPC Conference paper	Sydney	How do guardian dogs work?
	17-Aug-11	Field Day	Nindigully	Guardian dogs
	18-Aug-11	Field Day	Boolba	Guardian dogs
	14-Sep-11	Westek Ag Show	Barcaldine	How do guardian dogs work?
	14-18 Nov 11	Pest Plant & Animal Forum	Goombungee	How do guardian dogs work?
	14-18 Nov 12	Pest Plant & Animal Forum	Dalby	How do guardian dogs work?
	14-18 Nov 13	Pest Plant & Animal Forum	Warwick	How do guardian dogs work?
	14-18 Nov 14	Pest Plant & Animal Forum	Clifton	How do guardian dogs work?
	14-18 Nov 15	Pest Plant & Animal Forum	Bell	How do guardian dogs work?
	14-18 Nov 16	Pest Plant & Animal Forum	Millmerran	How do guardian dogs work?
	14-18 Nov 17	Pest Plant & Animal Forum	Pittsworth	How do guardian dogs work?
	14-18 Nov 18	Pest Plant & Animal Forum	Chinchilla	How do guardian dogs work?
	14-18 Nov 19	Pest Plant & Animal Forum	Condamine	How do guardian dogs work?
	12-Dec-11	Leading Sheep Webinar	State-wide	How do guardian dogs work?

Media	Zinc 666 (Mt Isa)	06:30 News - 09/12/2011 - 06:34 AM
Radio	ABC Southern Queensland (Toowoomba)	06:30 News - 08/11/2011
Print	Stanthorpe Border Post 08-Nov-2011 Page: 9	
	Warwick Daily News 08-Nov-2011 Page: 11	Weeds and pests are in their sights
	Pittsworth Sentinel 9-Nov-2011 Page: 7	Pest and Weed Series
	Queensland Country Life 10-Nov-2011 Page: 14	
	Warwick Rural Weekly insert 11 Nov 2011 p6	
	Barcoo Independent Nov 11	
	November 14-18 Pest plant a& Animal Forum's	
	Pittsworth Sentinel 16-Nov-2011 Page: 3	
	Warwick Daily News 15 Nov 2011 p11	
	Stanthorpe Border Post 15 Nov 2011 p9	Pests, face wrath of agri-allies
	Queensland Country Life 17-Nov-2011 Page: 16	
	Daily News Warwick 22 Nov 2011 Pg 9 New Data Aids Wild dog Control	
	Clifton Courier 23 Nov 2011 p7	Pests and weeds under spotlight at Clifton
	Warwick Daily News 06-Dec-2011 Page: 20	
	Crows Nest Advertiser 06-Dec-2011 Page: 3	Webinars on guardian animals
	Highfields Herald 06-Dec-2011 Page: 3	Webinars on guardian animals
	Rural Weekly insert	'Targeting Pest and Weed Series'
	Chinchilla News & Murilla Advertiser	