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RESEARCH ARTICLE

Feral pig population structuring in the rangelands of eastern Australia: applications for designing adaptive management units

Brendan D. Cowled · Jaclyn Aldenhoven · Inakwu O. A. Odeh · Tom Garrett · Chris Moran · Steven J. Lapidge

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Abstract Feral pigs (*Sus scrofa*) are an invasive species in Australia. Their negative impact on conservation values has been demonstrated, and they are controlled in many areas in the rangelands of Australia. However, they are usually controlled over small, often ad hoc management units (MUs), and previous research has revealed that these MUs can be inadequate. Understanding feral pig population structuring can aid in the design of appropriate MUs. This study documents an approach to improving MUs for feral pig control in the rangelands of Australia. Feral pigs from a 500,000 km^2 region were genotyped with 13 polymorphic markers. Genetic analyses were used to identify population structure. Identified sub-populations were then related to geographical and environmental gradients with geographical information systems, regression analysis and with canonical correspondence analysis. Five sub-populations were identified. These were moderately differentiated, with relatively high-migration rates. Two sub-populations in drier, lower elevation areas overlapped, due to extensive migration, probably along the large, inland rivers and flood plains. Sub-populations in higher rainfall environments appeared less likely to migrate. Sub-population differentiation was also dependant on distance, indicating isolation by distance was present. A case study applying an adaptive MU to a previously controlled area is presented. Generally, however, MUs for feral pig control for natural resource protection and endemic disease eradication in the rangelands should take into account geographical size, but also geographic features, especially major rivers in low-rainfall areas.

Keywords Feral pigs · Population structure · Optimal control · Management units · Wildlife endemic disease management

B. D. Cowled · S. J. Lapidge
Invasive Animals Cooperative Research Centre, University of Canberra, Canberra, ACT 2601, Australia

J. Aldenhoven · C. Moran
Centre for Advanced Technologies in Animal Genetics and Reproduction (Reprogen), Faculty of Veterinary Science, University of Sydney, Sydney, NSW 2006, Australia

I. O. A. Odeh
Faculty of Agriculture, Food and Natural Resources, University of Sydney, Sydney, NSW 2006, Australia

T. Garrett
Queensland Macropod & Wild Game Harvesters Assoc Inc, 1 Creek St, Amby, QLD 4462, Australia

Present Address:
B. D. Cowled (✉)
Department of Agriculture, Fisheries and Forestry, Office of the Chief Veterinary Officer, GPO Box 858, Canberra, ACT 2601, Australia
e-mail: brendan.cowled@daff.gov.au

Introduction

Feral pigs (*Sus scrofa*) are an introduced, invasive pest in Australia. They damage natural resources and conservation values in Australia and in many other areas of the world (Choquenot et al. 1996; Deh 2004, unpublished data). For example, in Australia they have been demonstrated to predate endangered species, modify native habitat and compete for food with endangered species (Miller and Mullette 1985; Laurence and Harrington 1997; Choquenot et al. 1996; Mitchell 2000; Hone 2002; Deh 2004, unpublished data). They also spread endemic diseases and they have the potential be involved in the epidemiology of serious exotic diseases in Australia (e.g. Choquenot et al. 1996; Pech and Hone 1988).

In the western rangelands of New South Wales and Queensland, their distribution and spread is linked with water-courses and associated floodplains (Choquenot et al. 1996). Spread away from these areas is generally limited by availability of water, such as that provided by pastoral watering points (McKnight 1976).

Eradication of established, mainland vertebrate pests is generally considered to be extremely difficult or impossible (Bomford and O'Brien 1995). Control efforts should therefore be focused on efficiently reducing the damage to natural resources to acceptable levels (Braysher 1993). However, disease eradication is often still possible since it does not require the eradication of an entire host (Pech and Hone 1988). In any endemic disease eradication the focus would be likely on progressive elimination of the disease from contiguous regions and preventing re-introduction of disease into these regions. Contingency plans for wildlife exotic disease epizootics in Australia call for the establishment of a control zone based upon the known incidence of disease in wild animals, with a sufficient buffer zone to prevent emigration and immigration (Australian Veterinary Emergency Plan 2000, unpublished data). Generally, whatever the aim of a control program, the size of the land area to be controlled is important to reduce immigration of the species into the controlled area.

Management units (MU) refer to distinct populations of animals that should be treated as one population (Manel et al. 2003). Braysher (1993) used this term for feral animal control for the purpose of natural resource protection. MU for feral pig control in Australia are often based on a variety of practical factors (as opposed to biological factors, such as distinct feral pig populations or natural boundaries) and include the strategic aims of a control program, resources, government jurisdictions, landholder involvement and the budget of the control operation (Choquenot et al. 1996). However, it is commonly acknowledged that utilising a sufficiently large area of land will reduce the speed at which uncontrolled feral pigs surrounding the MU recolonise (Hone et al. 1980; Choquenot et al. 1996; Spencer and Woolnough 2004; Cowled et al. 2006a). Therefore, geographic features can be used in structuring MUs (Saunders and Bryant 1988; Spencer and Woolnough 2004). For example, Hampton et al. (2004) and Spencer and Woolnough (2004) used molecular ecology to investigate population structuring of feral pigs in southwest Western Australia (WA). They found that distinct genetic sub-populations were located over entire contiguous mountainous water catchments, and suggested that MU's should be formed on water catchments (Spencer and Woolnough 2004).

This study was prompted by the research of Spencer et al. (2005) and Cowled et al. (2006a) that revealed a genetically contiguous population of feral pigs over 4,000 km^2 in the centre of the present study area. That studied population showed no decline associated with control, due most likely to an inappropriately structured MU, leading to rapid re-immigration of feral pigs and re-colonisation of the controlled area. The aim of the present study was therefore to investigate the population structure of feral pigs over a larger area of rangelands in eastern Australia, and to assess the predictability of the structure with reference to geographic or environmental gradients. The current study sought to recommend an adaptive MU for feral pigs for natural resource protection and endemic disease eradication in the previously studied site from Cowled et al. (2006a). Additionally, it sought to highlight important considerations for establishing MUs for the Australian rangelands generally.

Methods

Study area

Rangelands occupy 70% or 6 million km^2 of the Australian continent (Smyth and James 2004). The study area extended over 0.5 million km^2 of the Australian rangelands (8%) in eastern Australia, covering portions of south-western Queensland and north western New South Wales (Fig. 1).

Sampling

The majority of feral pig samples were collected from carcasses harvested by commercial feral pig hunters throughout the study area. Each hunter operated a commercial chiller box (a refrigerated site where feral pigs are briefly stored prior to processing) within the centre of a harvesting region. Further samples were also collected opportunistically during other feral pig research (e.g. Cowled et al. 2006b). Attempts were made to collect samples in a grid pattern across the entire study area. Samples were almost invariably small sections of ear tissue and these were stored in ethanol. The sex and weights of feral pigs were recorded. DNA samples from 15 domestic animals from QAF Meat Industries Corowa, NSW, were added to provide an outgroup for analyses.

Molecular analysis

DNA was extracted from portion of the 1 cm^2 section of ear tissue provided by hunters. Thirteen polymorphic loci were selected for this study based on prior research (Alexander et al. 1996; Hampton et al. 2004). These loci have been extensively characterised in feral pigs (e.g. Vernesi et al. 2003; Hampton et al. 2004), were designed

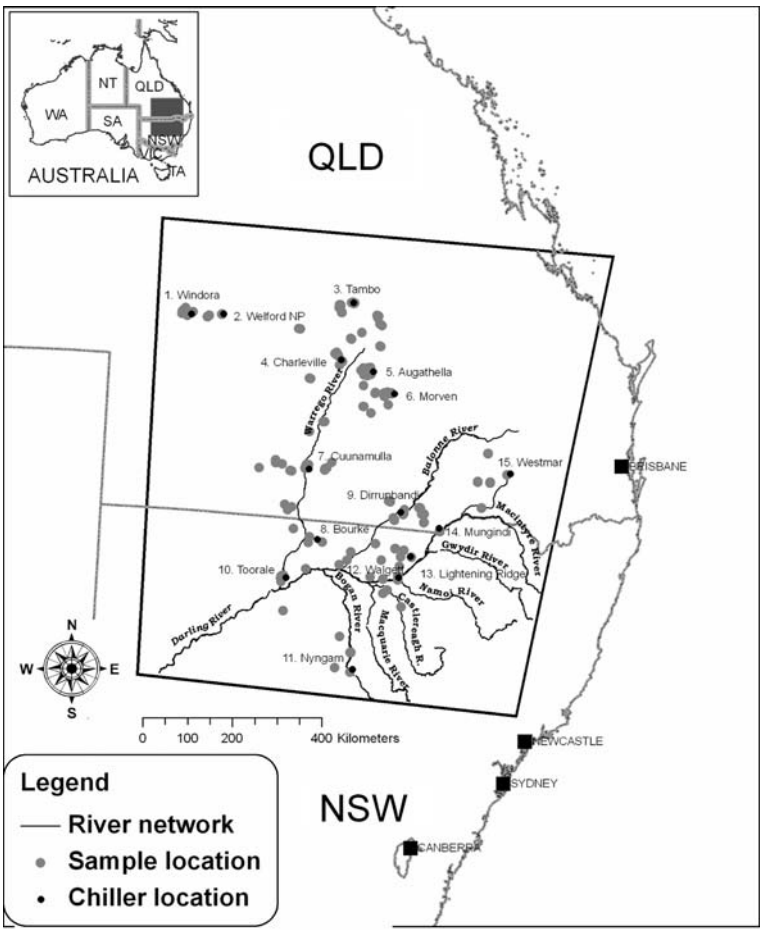

Fig. 1 The study area showing individual sample locations in relation to the Australian continent. *Grey circles* represent either single sample or multiple sample locations. The chiller box (the storage area for harvested feral pig carcasses where much of the sampling occurred) or sample site locations are shown as *dark circles* labelled with their numbered names. The major rivers are overlaid on the map

as part of the Pig Genome Mapping Project, and give no indication of null alleles (Alexander et al. 1996).

PCR reactions for each animal and marker were performed in 15 µl volume containing 40 ng of porcine genomic DNA, 10 × *Taq* DNA polymerase buffer, 80 nM of each primer, 1.5 mM $MgCl_2$ and 100 µM dNTPs covered with two drops of paraffin oil. After denaturation at 95°C for 10 min and then holding at 80°C, 1 U of *Taq* polymerase was added. A touchdown program was used (44 cycles 95°C 40 s, 63–55°C 60 s, 72°C 60 s, 1 cycle 72°C 20 min) for all markers. Amplified products were analysed by capillary electrophoresis and fluorescent detection in an ABI PRISM 3700 DNA analyser (Applied Biosystems, Warrington, UK). GeneScan500 (LIZ) (PN4322682, Applied Biosystems) was used to recognise microsatellite genotypes. Genotypes were called manually using GeneMapper V 3.5.

Population genetic analyses

Descriptive statistics

Descriptive statistics of the microsatellite data, such as number of alleles, observed and expected heterozygosities were determined using POPGENE (Yeh et al. 1999; Version 1.31). FSTAT 2.9.3.2 (Goudet 2002) was used to calculate F_{is} values and probabilities. MICRO-CHECKER, a free software (van Oosterhout et al. 2004) was used to check for genotyping errors. The software determines whether a significant excess of observed homozygotes relative to expected homozygotes is present. A significant deviation can be caused by null alleles, stuttering, large allele drop out, Wahlund effects or non-panmictic populations. If the excess is across most homozygote classes at one locus, then a null allele is probable. However, if all loci show an excess in multilocus genotypes, then the excess of homozygotes is likely due to the population not being in Hardy–Weinberg equilibrium (HWE).

Population structure

The program, STRUCTURE-Version 2.0 (Pritchard et al. 2000), was used to infer the genetic structure and assign individuals to their likely population of origin, which we termed here as an inferred population. The program uses a full Bayesian assignment approach to account for departure from HWE and linkage disequilibrium by applying population structure to minimise these departures. The number

of inferred populations (K) comprising the sampled population, and the proportion of each individual feral pigs genotype attributable to each inferred population was derived, based on the smallest K-value for which the simulation produces a plateau for the probability of K (see Pritchard and Wen 2004). The results generated were based on simulations from one to ten inferred populations (K = 1–10). A burnin period of 50,000 iterations, with 10^6 iterations of a Markov chain Monte Carlo simulation were used in an admixture model (after Hampton et al. 2004). The mean contribution of each inferred population to pooled individuals from a predefined sampling area was determined. The predefined sampling area was composed of individuals from a single sampling area (research site, GPS locations, written directions or chiller box location). The inferred population contributions to a predefined sampling area allowed sampling areas to be assigned to a geographic/inferred sub-population. Thus, sub-populations were composed of geographically contiguous sample sites that had the majority of the genomes of individuals at each sample site composed of one, common inferred population.

Genetic differentiation within sub-populations

An average number of alleles per locus and observed and expected heterozygosities in each of the inferred population clusters were determined using the program POPGENE. All loci were tested for HWE for each inferred sub-population. Exact tests for HWE were performed using the GENEPOP (Raymond and Rousset 1995) program.

To detect evidence for recent population bottlenecks in the inferred clusters a test for excess of heterozygosity was employed using the program BOTTLENECK (Piry et al. 1999). The two-phase model (TPM) was used to analyse the data, as it has been shown to be more appropriate for microsatellite data. With the TPM, single-step mutations were assumed to account for 90% of all mutation events (therefore multiple-step mutation accounted for the remaining 10%) with a variance among multiple step of 12 (Piry et al. 1999). The Wilcoxon sign-rank test, which is most powerful when used with fewer than 20 polymorphic loci, was used.

The effective number of breeding animals, or the effective population size for each population (N_e) was estimated from effective homozygosity values, according to Ohta and Kimura (1973), for populations under mutation drift equilibrium. Mutation rate (u) was estimated to be 1.0×10^{-3} (Weber and Wong, 1993), which is the generally accepted value for pure dinucleotide repeat microsatellites.

Genetic differentiation between sub-populations

Two separate methods were used to determine genetic differentiation between sub-populations using GENEPOP (Raymond and Rousset 1995). First, a Fisher's exact test for genetic differentiation amongst the sub-populations was conducted. Pairwise comparisons of sub-populations at each locus were tested for the same allelic distribution across both sub-populations. Global and pairwise fixation index statistics (F_{st}) were calculated after Cockerham (1973) and Weir and Cockerham (1984). Migration rates were calculated using the private alleles method (Barton and Slatkin 1986).

Environmental explanations for population structuring

GIS analysis

A geographic information system (GIS) program, ArcGIS/ArcInfo (ESRI 2006), was used to identify broad relationships between environmental and geographic factors, and the sub-populations. Specifically, maps were generated with layers of environmental data overlaid on feral pig sub-populations to loosely identify potential relationships before more objective analysis occurred.

The various geographic and environmental layers were obtained from the Australian Bureau of Meteorology, Geoscience Australia and Queensland Department of Natural Resources and Mines for the exploratory investigation and subsequent detailed analyses. The geospatial environmental data layers used are land cover types (1. native forests and woodlands; 2. native shrub lands and heath lands; 3. native grasslands and minimally modified pastures; 4. annual crops and highly modified pastures; 5. permanent and ephemeral water features), average annual rainfall, average maximum annual temperature, elevation above sea level and course cultural topography. Also included in the analysis is the derived distance of feral pigs to major river systems. All of the geospatial layers were converted to a common coordinate system and projection. For such a large region requiring spatial analysis and therefore distance and area consideration, we formulated and adopted Albers Area Conic Projection specific to the Eastern Australian region. Each layer data was extracted at each feral pig location by re-sampling based on nearest neighbour method using ArcInfo version 9.1 (ESRI 2006).

The major rivers within the study area were extracted from the perennial river network included in the database of the drainage network layer obtained from Geoscience Australia (https://www.ga.gov.au). By overlaying the river network layer on the sub-population spatial distribution, we were able to visually identify any possible association with the major rivers.

The contribution of each of the inferred populations to each of the sub-populations was examined to infer gene flow within the study area. More quantifiable migration rates were also examined between sub-populations. A

broad picture of gene flow within the study area was then grossly related to rivers (feral pigs reliant on daily water in Australia- see Choquenot et al. 1996) to determine whether major rivers act as migration paths for feral pigs. The relationship between the major rivers and their tributaries and the contribution of their resident inferred population was investigated using regression analysis. The inferred population contribution (percentage contribution due to the major resident inferred population) at a predefined sample site was plotted against the distance up river from the confluence of the two rivers. In this manner, changes in the contribution of an inferred population to a sample at various points along a river length were examined.

Canonical correspondence analysis (CCA)

The contribution of each of the inferred population (STRUCTURE) to each individual feral pig was related to the extracted geospatial environmental data using canonical correspondence analysis (CCA) using the program, CANOCO (Canonical Community Ordination, Version 4.5; see ter Braak and Smilauer 2002). CCA is a robust ordination technique, useful to decipher relationships between species (in this case inferred population) and environments (Palmer 1993; McCune 1997). This allowed relationships between feral pigs of different inferred populations (''species'') and environmental or geographic variables to be identified spatially.

Isolation by distance

Isolation by distance (null hypothesis was for independence between pairwise R_{st} and distance) was tested using the ISOLDE program (Genepop Version 1.2; Raymond and Rousset 1995). The program conducts a regression of pairwise R_{st}-values against geographic distance, and computes a modified ''Mantel test'' (Mantel 1967). The Mantel test is modified to uses a Spearman rank correlation coefficient, instead of approximations for the distribution of Z, and returns a P-value (Raymond and Rousset 1995). The pairwise R_{st}-values were plotted against distance apart in a scatter-plot with a line of best fit.

Additionally, spatial genetic structuring was assessed using a ''global'' spatial autocorrelation method, since analyses of this nature are more powerful than Mantel tests (Peakall et al. 1995). This method uses a multivariate approach to simultaneously assess the spatial signal generated by multiple genetic loci (Smouse and Peakall 1999) and provides information on contemporaneous patterns of dispersion (Peakall et al. 2003). This test was applied using the program GENALEX 6 (Peakall and Smouse 2005) in a similar fashion to others (Peakall et al. 1995; Smouse and Peakall 1999; Peakall et al. 2003). Specifically, all individual feral pigs underwent pairwise matching across the study site. The squared genetic distance between feral pigs was autocorrelated. This provided a coefficient, r, which gives a measure of the genetic similarity between pairs of individuals whose geographic separation falls within a specified distance range (the linear Euclidean distance between x and y coordinates). The results are summarised by a correlogram. When significant positive spatial genetic structure is present, r will decrease with increasing size of distance classes, and can be best demonstrated with two correlograms at increasing distance classes (Peakall et al. 2003). Genetic correlation across the study site for varying distance size classes, spanning the sampling distances (10–900 km) was conducted, since this can reveal the physical extent of genetic structuring (Peakall et al. 2003). Autocorrelation between age and sex classes can indicate possible reasons for spatial genetic structuring (Peakall et al. 2003). However, further analyses between juvenile offspring, and between sexes was not possible since sampling was strongly biased towards large economically valuable males.

Results

Sampling

In total 306, individual feral pig genetic samples were collected. Returns from some areas were poor, due to low feral pig abundance, or lack of commercial chiller boxes (presumably also partially due to low feral pig abundance). Feral pig samples were collected from 13 chiller boxes and two research sites throughout the region, as shown in Fig. 1. The sample locations were loosely associated with major river systems. Commercial hunters returned 248 samples. Accuracy of recorded locations for individual feral pigs by commercial hunters varied and consisted of: 80 located to property name; 75 with written directions from the nearest town; 58 with an exact GPS location; and, 35 which were assigned to the town containing the chiller box due to no or poor records. The mean distance of feral pigs of known location from a commercial chiller box was 59 ± 40 (s.d.) km. The mean weight of feral pigs sampled by commercial hunters was 40 ± 14 kg, and these were predominantly male (84%). Fifty-eight samples were collected in associated research and had a GPS position recorded. Samples collected during research were 51% female and 49% male, with the mean weight 32 ± 18 kg.

Population genetic analyses

Descriptive statistics

All thirteen loci used showed moderate to high-allele numbers having between 7 and 23 alleles (11.38 ± 4.48).

As shown in Table 1, the heterozygosity ranged between from 0.572 to 0.879 (0.754 ± 0.09). MICRO-CHECKER revealed no false alleles or allele dropout. However, every locus showed a significant excess of homozygotes, indicating that the sample was probably not in Hardy–Weinberg equilibrium (van Oosterhout et al. 2004), rather than molecular analyses being compromised by true null alleles. This is not surprising given the population structuring revealed below.

Population structure

Six inferred populations ($K = 6$) were identified using the STRUCTURE analysis of the sample genotypes from the sampling sites and the domestic pigs (see Table 2). All sampling sites with a proportion >0.1 of a particular inferred population were geographically contiguous in a given region. Overall, five populations (hereafter known as sub-populations) were identified (Table 2 and Fig. 2) with the sixth being the domestic out-group. These five sub-populations comprise of multiple sampling sites across a geographically contiguous area, and consist predominantly of one common inferred population. However, some sampling sites from within the Cunnamulla and Walgett sub-populations, close to the confluence of the Warrego and Darling Rivers are highly admixed populations with contributions from the adjacent sub-populations.

Table 1 Summary statistics from POPGENE for 306 individuals for the 13 microsatellite marker loci from feral pigs in the area

Marker	N_A	H_O	H_E	F_{is}
SW240	14	0.75	0.807	0.092**
S0026	9	0.535	0.691	0.230**
SW857	10	0.590	0.802	0.237**
SW911	9	0.462	0.617	0.248**
SW632	14	0.698	0.796	0.113**
S0005	23	0.712	0.878	0.181**
SW122	11	0.653	0.8288	0.214**
SW951	7	0.531	0.664	0.195**
S0155	8	0.648	0.780	0.189**
S0226	10	0.430	0.732	0.419**
S0002	10	0.614	0.802	0.224**
S0068	16	0.773	0.811	0.055**
S0090	7	0.452	0.5701	0.283**
Mean	11.4	0.604	0.752	0.202
SD	4.4	0.115	0.091	NA

Data includes the observed number of alleles (N_A) observed (H_O), expected (H_E) heterozygosities and F_{is}-values

**Denotes significantly positive F_{is} ($P < 0.02$) indicating a defecit of heterozygotes

Genetic differentiation within sub-populations

The average observed heterozygosity for each of the inferred sub-populations in the sample area was similar (Table 3) with the highest in the Cunnamulla sub-population ($H_o = 0.637 \pm 0.112$) and the lowest in the Windorah sub-population ($H_o = 0.536 \pm 0.180$). The observed heterozygosity was lower than the expected in the inferred populations.

Hardy–Weinberg Equilibrium was tested for each locus for each inferred population. HWE tests on the inferred populations revealed that all populations except for the domestic animals (6) showed a departure from HWE at most loci. Each population was then tested to see whether there was either a heterozygote deficiency or excess. The loci from the populations in the sample area almost invariably exhibited a heterozygote deficiency.

Tests for recent bottlenecks revealed no evidence of any recent ($P > 0.05$) or any long-term bottlenecks. The effective population sizes were determined to range between 960 and 1,477, with the Windorah sub-population having the lowest effective population size and the Cunnamulla sub-population the highest (see Table 3).

Genetic differentiation between sub-populations

Pairwise comparisons of sub-populations at each locus revealed that 124/130 (96%) allele frequencies were significantly different ($P < 0.05$). Of the six comparisons where there was no significant difference, four were between geographically adjacent populations. Global F_{st}-values indicated moderate differentiation (Wright 1978) between sub-populations (0.070). Pairwise calculations of F_{st} ranged from 0.02, indicating little genetic differentiation between sub-populations, to 0.11 indicating moderate genetic differentiation. Contiguous sub-populations had the lowest genetic differentiation, in contrast to geographically disparate sub-populations, where moderate to high-genetic differentiation was recorded (Table 4). Mean migration rates 1.88 individuals per generation. The largest migration rates were between the Cunnamulla and Walgett sub-populations.

Environmental explanations for population structuring

GIS analysis and migration along major rivers

The Warrego River, which runs from north to south (Fig. 1), is one of two major rivers in the study area (~600 km in length). This river's upper tributaries (not shown here) start within the Tambo and upper Cunnamulla sub-populations (Fig. 2), and flow south/south west through the Cunnamulla sub-population till it joins with the

Table 2 Inferred population contribution to predefined sample areas and sub-populations after assignment testing

Sample areas	Sub-population	Contribution of inferred population					
		A	B	C	D	E	F
1. Windorah	Windorah (n = 35)	0.07	0.03	0.02	0.03	0.07	0.77
2. Welford N.P.		0.02	0.01	0.01	0.01	0.01	0.89
Mean		0.04	0.02	0.04	0.02	0.04	0.84
3. Tambo	Tambo (n = 57)	0.72	0.01	0.02	0.09	0.02	0.13
4. Charleville		0.71	0.05	0.08	0.04	0.04	0.09
5. Augathella		0.76	0.01	0.07	0.07	0.03	0.06
Mean		0.73	0.02	0.06	0.07	0.03	0.09
6. Morven	Cunnamulla (n = 65)	0.1	0.1	0.64	0.04	0.09	0.04
7. Cunnamulla		0.05	0.04	0.58	0.06	0.21	0.06
8. Bourke		0.01	0.04	0.45	0.12	0.37	0.01
9. Dirranbandi		0.03	0.04	0.66	0.11	0.13	0.04
Mean		0.05	0.06	0.60	0.07	0.18	0.04
10. Toorale	Walgett (n = 100)	0.02	0.1	0.33	0.02	0.51	0.01
11. Nyngan		0.07	0.07	0.34	0.02	0.46	0.04
12. Walgett		0.06	0.04	0.12	0.04	0.69	0.06
13. Lightning ridge		0.03	0.03	0.19	0.07	0.67	0.01
Mean		0.04	0.07	0.26	0.03	0.57	0.03
14. Mungindi	Westmar (n = 49)	0.03	0.04	0.03	0.84	0.04	0.01
15. Westmar		0.05	0.02	0.1	0.8	0.02	0.02
Mean		0.04	0.03	0.07	0.82	0.03	0.02
Domestic	Domestic (n = 15)	0.01	0.96	0.01	0.01	0.01	0.01

Fifteen predefined sampling sites showing proportion of genotype attributable to an inferred population cluster (rounded to two decimal places). The numbers are listed on Fig. 1 to allow identification of sub-populations

Darling River. The Darling River (and its tributaries) is the major river system draining from the east in a south westerly direction through the Westmar sub-population, and then the Walget sub-population.

The northern, southern and eastern extent of the Cunnamulla sub-population was identified and covered at least 85,000 km^2 in the Cunnamulla area (see Fig. 2). This population was located in semi-arid plains, characterised by a relatively undulating landscape with low relief (120–250 m above sea level) and a dry climate (annual average rainfall of between 300 and 500 mm). The Warrego River was contained within its distribution. The influence of the Cunnamulla sub-population increased as it extended from the confluence of the Warrego and Darling Rivers upstream along the Warrego, but not to the upper reaches of the Warrego (Tambo sub-population).

The Walget sub-population area is within the upper Darling River and the lower catchments of its tributaries. This population's area was also semi-arid with 300–400 mm average annual and was also characterised with lower elevation. This populations influence increased as it extended from the confluence of the rivers, upstream along the Darling, but not to the higher tributaries of the Darling (Westmar sub-population). Migration rates between the Cunnamulla and Walget sub-populations were the highest recorded throughout the study area, despite many other contiguous populations occurring in the study. The only common geographical features identified between the two sub-populations was the Warrego and Darling Rivers. In contrast, the Tambo and Westmar sub-populations on the upper tributaries of the same rivers had no dispersal along stream lines. These 2 sub-populations are located in the higher rainfall landscapes of south Central Queensland (400–700 mm average annual rainfall), at relatively higher elevation, especially the Tambo sub-population.

This relationship between the Walgett and Cunnamulla sub-populations and their respective rivers is demonstrated by regression analysis (see Fig. 3). As the distance of the locations of individual feral pig samples in the Cunnamulla and Walgett sub-populations increases from the confluences of the various tributaries of the Darling and Warrego, the mean proportion of Walgett genetics (i.e. inferred population contribution) in the samples declined linearly whilst the mean proportion of Cunnamulla genetics increased linearly. An inverse relationship is therefore evident along the Darling River and along the lower parts of its tributaries. In contrast, the Charleville and Augathella samples (Tambo sub-population) experienced little influence

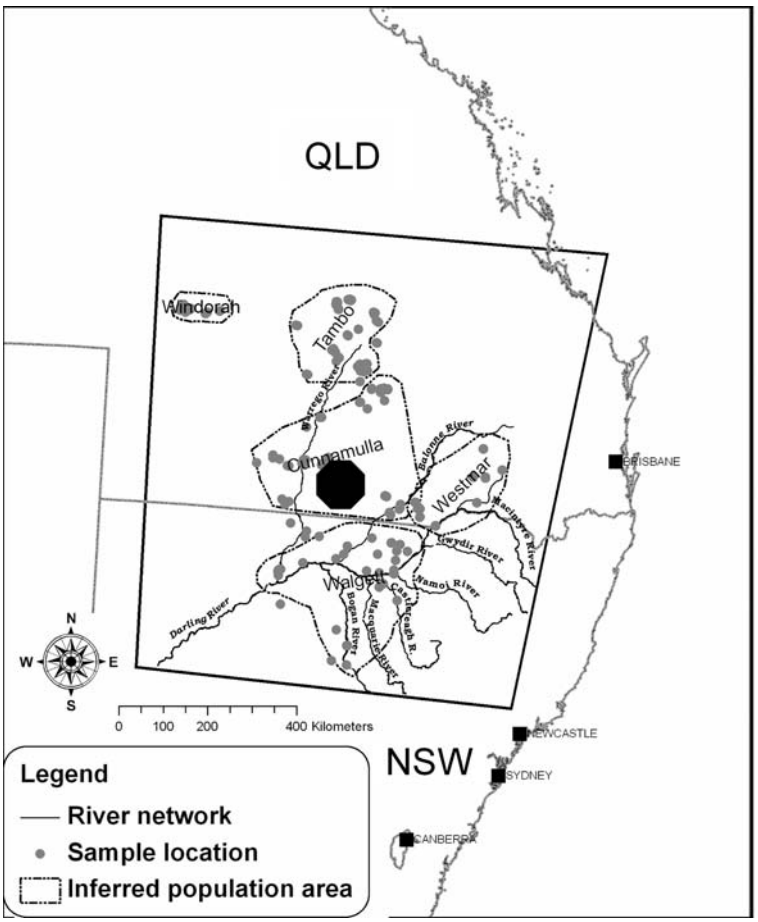

Fig. 2 The study area showing the sub-population areas. *Grey circles* represent either single sample or multiple sample locations. The major rivers are overlaid on the map. The *dark octagon* represents the previous ineffective MU for feral pig control, which was compromised by immigrating feral pigs (Cowled et al. 2006a). The *dashed lines* represent the approximate location of sub-populations following assignment testing and geographic assessment. Note, all sub-populations may be larger than shown, since sampling may not have reached the edge of the population

Table 3 Genetic diversity: mean observed number of alleles (N_A), mean observed (H_O), mean expected heterozygosity (H_E), effective population size (Ne) for the populations

Sub-population	*n*	N_A	H_O	H_E	(Ne)
Tambo	57	7.69 ± 2.72	0.601 ± 0.136	0.699 ± 0.127**	1,257
Cunnamulla	65	7.92 ± 2.36	0.637 ± 0.112	0.712 ± 0.082**	1,377
Walgett	100	8.69 ± 2.69	0.603 ± 0.131	0.701 ± 0.091**	1,269
Westmar	49	8.08 ± 3.12	0.606 ± 0.159	0.721 ± 0.115**	1,477
Windorah	35	5.62 ± 2.75	0.536 ± 0.180	0.661 ± 0.102**	960
Domestic	15	3.69 ± 1.89	0.611 ± 0.268	0.568 ± 0.206	NA

** Adherence to HWE tested as null hypothesis (Rousset and Raymond 1995), stars indicate $P < 0.00$

Table 4 Pairwise F_{st}, and migration rates between sub-populations

Sub-population	Cunnamulla	Tambo	Westmar	Windorah	Walgett
Cunnamulla	–	2.2	2.2	1.5	2.9
Tambo	0.08**	–	2.3	1.4	2.2
Westmar	0.05**	0.05**	–	1.0	1.5
Windorah	0.11**	0.07**	0.10**	–	1.6
Walgett	0.02**	0.08**	0.07**	0.11**	–

The table displays pairwise F_{st} below the diagonal, and migration rate calculated from the private alleles method above the diagonal

** Significance of pairwise F_{st} assessed after Simard et al. (1999). **Indicates significant ($P < 0.05$)

(<8%) from the Cunnamulla sub-population. despite being located on the same river system and containing many individuals a similar distance from the confluence. Additionally, the Mungindi samples (part of the Westmar sub-population), experienced little influence (<4%) from the Walgett sub-population. despite being located on the same river system with individuals being a maximum of 100 km further upstream along the catchment.

Canonical correspondence analysis (CCA)

In the CCA the total amount of variation explained by the geographic or environmental variables is 1.22 (sum of constrained eigenvalues). Specifically, the sum of all inertia (variance) in inferred population dispersion was 2.72 (sum of unconstrained eigenvalues). After variance partitioning, geographic variables (land use and elevation) uniquely described ~40% of the explainable inertia. Climatic data (rainfall and temperature) explains a similar amount of inertia, and the remainder of the variance is jointly accounted for by the geographic and climatic variables (or 20% of the explainable inertia was redundant). The first two axes (canonical roots) account for 34.8% of the overall variance of inferred population dispersion, or 78% of the variance explainable by the geospatial environmental variables. Climatic variables had the highest weighted correlation with the first canonical axis (temperature = 0.73, Rain = 0.46, native woodland and forest = 0.30, native shrubland = 0.27). On the other hand, elevation and rainfall have high-weighted correlations with axis 2 (~0.52) native shrub lands and heath lands was 0.36 and water features was 0.31.

The results of the CCA are illustrated in canonical biplot in Fig. 4. The environmental variables are represented by the arrows. The sub-population scores of CCA are represented by points. According to ter Braak (1986), the feral pig sub-population centroids, as represented by points on the ordination biplot, characterise the community association as they are explained by the environmental variables. The sub-population centroid points and arrows of the environmental variables ''reflect'' the sub-population distribution along the environmental variables (the arrows). Thus, in Fig. 4, the Walgett sub-population centroids is closest to the end of the ''Water features'' arrow. It thus indicates that the Walgett sub-population is affected by the proximity of the sample sites to water features. To a lesser extent the same can be said of the Cunnamulla sub-population. The Tambo sub-population is thus associated strongly with higher elevation and higher rainfall environments. The Westmar population is associated with native scrublands in higher rainfall environments.

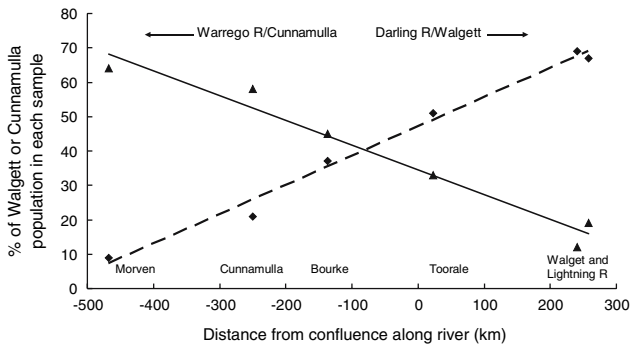

Fig. 3 The high degree of admixture between parental sub-populations at the confluence of the Warrego and Darling Rivers. The *solid regression line* ($R^2 = 0.96$) represents the contribution of the Cunnamulla inferred sub-population to samples, and declines with sampling distance down the Warrego River. The contribution continues to decline at increasing distances up the Darling River System. The dashed regression line ($R^2 = 0.99$) is similar, but demonstrates the contribution of the Walgett inferred population to samples which declines with distance down the Darling River System and continues to decline with increasing distances up the Warrego River. The names along the horizontal axis represent the sample sites, and their distance from the river confluence (0 km) where admixture is high

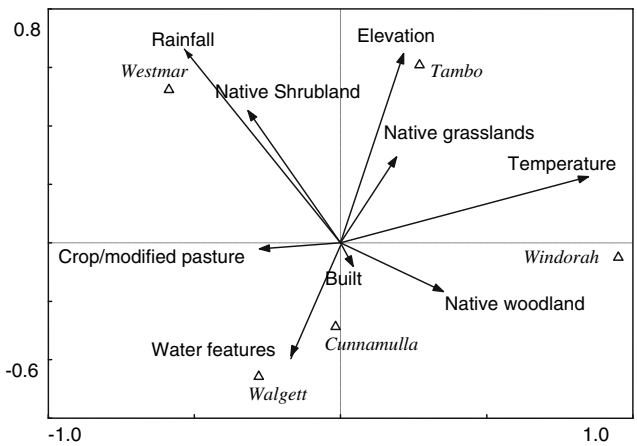

Fig. 4 Ordination plot of inferred populations against geographic and environmental gradients (after ter Braak and Verdonschot 1995). ''Population'' conditional biplot based on canonical correspondence analysis of the distribution of inferred population across varying geographical and environmental factors. The plot displays 34.8% of the inferred population abundance inertia (weighted variance) and 77.5% of variance in weighted averages and class totals with respect to the variables. The first axis (*horizontal*) had an eigenvalue of 0.514 and the second axis had an eigenvalue of 0.434. Sub-populations (*open triangular*) are weighted averages of regional scores. Quantitative environmental variables are indicated by *arrows*. The plot indicates that some sub-populations are strongly associated with various environmental or geographic gradients. For example, Walgett and Cunnamulla sub-popualtions are associated with water features, and were in lower elevation landscapes. The Tambo sub-population is associated with higher elevation landscapes, with relatively high rainfall. The Westmar subpopulation is associated with native shrub and heath lands in higher rainfall areas

Isolation by distance

A strong correlation between pairwise R_{st} and the distance between sub-populations was obvious on simple linear regression ($R = 0.74$). The Mantel test revealed a significant positive correlation between pairwise R_{st} counts and the distance between sub-populations (One tailed test; $\rho = 0.746$; $P = 0.026$) indicating that isolation by distance (IBD) was present. Greater than half ($R^2 = 0.55$) of the genetic differentiation between sub-populations was explained by the distance between them (Fig. 5).

Following autocorrelation at increasing size of distance classes, r decreased, revealing a significant non-random (positive) genetic structure (figures not shown for brevity). The autocorrelation values leave little doubt as to the strength of genetic correlation (Peakall et al. 2003). A comparison of combined r for different class sizes demonstrates that the majority of genetic structuring is evident for distances between feral pigs of <400 km (Fig. 6). Only a small amount of genetic structure is still present for distances >400 km.

Discussion

The findings of this study have applications to establishing an adaptive MU for feral pig management in the previously controlled area in Cowled et al. (2006a), with more general applications to the rangelands. Cowled et al. (2006a) showed that an extensive, repeated aerial shooting program across a 4,000 km^2 MU (see the octagon in Fig. 2 for an approximate area), within the Cunnamulla population's geographic distribution, produced no demographic or genetic changes. The distribution of an identical population surrounding the previously shot population, as revealed in this paper, and the extensive movements of feral pigs in the area (Spencer et al. 2005) supports the conjecture of

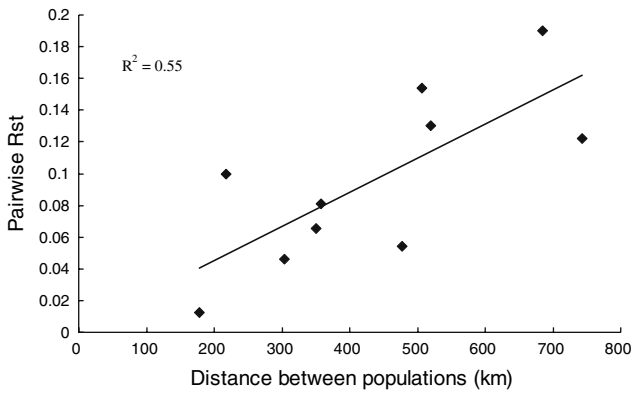

Fig. 5 Relationship between distance and genetic differentiation between sub-populations

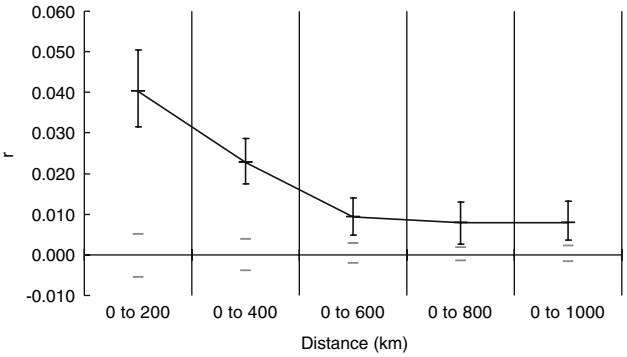

Fig. 6 Correlogram showing combined r-values at multiple distance class sizes [1].The *grey dashes* represent upper and lower 95% CI about the null hypothesis, of a random distribution of feral pigs (Peakall et al. 2003). The *error bars* are 95% CI based on bootstrapping (Peakall et al. 2003). The *solid line* represents combined r-values

Cowled et al. (2006a), that inward migration of similar feral pigs from the same sub-population were responsible for the unchanged genetic and demographic parameters in their study site. This implies generally, that MUs for feral pigs in the semi-arid rangelands would need to be much larger than currently, or perhaps incorporate the entire sub-population into the MU, to produce long-lasting or permanent changes in localised feral pig populations.

To determine the complete extent of the Cunnamulla sub-population in order to establish a MU would require further research, since the western and north eastern boundaries of the population were not isolated (sampling did not extend far enough). However, based on the known structuring of the Cunnamulla population, and genetic contributions from surrounding populations in the region, some recommendations can be made for a better MU for the study site of Cowled et al. (2006a). A MU should be much larger, with the MU extending across large portions of the identified Cunnamulla population. This may markedly reduce immigration into the controlled area. Additionally, the MU should extend down to the confluence of the rivers to prevent immigration of the Walgett population. Ideally it should also have depopulation buffers into the geographically contiguous sub-populations where populations showed a small degree of admixture with the Cunnamulla population, indicating gene flow (prior migration) between these populations. The establishment of such a MU would produce the most efficient and greatest natural resource protection and have the greatest positive impact on conservation values.

The resources, coordination and will to conduct such large control programs (>85,000 km^2 to cover the known extent of the Cunnamulla population) may be impossible, and unnecessary, if simple natural resource or agricultural protection is the aim of the program. For example, at

Toorale Station at the confluence of the two rivers, no bottleneck was evident in sampled individuals (B. Cowled, unpublished data) following nearly a decade of intensive annual shooting. However, a significant increase in the number of lambs raised per ewe was evident over this time, indicating reduced predation of lambs by feral pigs (Tony McManus, Personal Communication September 2005). Nevertheless, application of control programs across entire sub-populations and sub-populations contributing immigrants should lead to the most efficient and long lasting reductions in feral pig densities (Spencer and Woolnough 2004).

It is interesting to compare the establishment of MUs for conservation of species and for the control of invasive species. Palsbøll et al. (2007) recommended that MUs for conservation of species should be based on divergence measures between populations rather than departure from panmixia. One approach suggested by them was to establish MUs based on divergent clusters identified with assignment testing [e.g. Pritchard et al. (2000)]. Our study took a similar approach but with a different emphasis. We have recommended a MU based on clusters and the degree of *similarity* with neighbouring clusters. For example, to optimally reduce the abundance and distribution of feral pigs in the controlled area of Cowled et al. (2006a) would require that immigrating feral pigs be prevented or reduced. Thus, it is necessary to concurrently control all the sources of immigrating feral pigs (the larger Cunnamulla population *and* other populations that contribute dispersing individuals). We took the historical gene flow evident from assignment testing and more traditional descriptive genetic analyses to recommend which neighbouring populations were contributing dispersing feral pigs, at least historically.

The sampled population in the study area contained five sub-populations based on an analysis of geographic locations and inferred populations. These sub-populations were moderately to highly differentiated based on fixation indices, yet, migration rates between the sub-populations were relatively high (>1). The explanation for this is indicated by the likely reason that each of these populations was in HW disequilibrium. Some admixed or immigrant individuals were assigned to a sub-population based on geographic location, even though they may have been suitably placed in an alternate, neighbouring population. Thus, departure from HWE was evident because each sub-population was composed of individuals from the main inferred population for that sub-population, but with some geographically concordant but genetically discordant individuals with contributions from nearby sub-populations. This lead to a Wahlund effect, whereby a significant deficit of heterozygosity was recorded for each population, because, more than one genetic population was considered together in each of our main sub-populations (e.g. see Freeland 2005). This statement is supported since most groups (5/6) of pooled ''pure'' individuals from each population (with greater than 95% contribution from the inferred population for that geographic population) were in HWE (B. D. Cowled, unpublished data). This compromised the calculation of fixation indices, which use the expected heterozygosity of subpopulations based on assumptions of HWE. Thus, migration rates based on the private alleles method may not correspond intuitively with values of fixation indices. However, this has little effect on establishing MU for invasive species control, since the important consideration is the admixture, and gene flow of individuals (indicating historical dispersal) of individuals in order to guide MU to best reduce immigration into controlled areas.

Interestingly, analysis revealed that some IBD was evident (Wright 1943; Slatkin 1993), although this has not been detected in other studied wild pig populations (Vernesi et al. 2003; Hampton et al. 2004). Slightly more than half of the variability in genetic differentiation between sub-populations was due to their distance apart. Autocorrelation supported this conclusion with a strong positive spatial genetic structure for sub-populations less than 400 km apart. However, geographic features also appeared to contribute heavily to gene flow and subsequent genetic variability between sub-populations.

The two major rivers in the region appeared to have a major affect on the population sub-structure. Highly admixed samples (Cunnamulla and Walget sub-populations) were present at the river confluence with the admixture declining linearly upstream. Pairwise parameters reflecting genetic differentiation between these two sub-populations were the lowest in the study, with migration rates being the highest, despite the large distances between the confluence and farthest extent of the two sub-populations (500 and 300 km). This suggested that the rivers and associated floodplains acted as a major migration route for feral pigs.

Other geographical factors appeared to be contributing to population sub-structuring. Some sub-populations were geographically contiguous, but had a sudden genetic demarcation between them. The Tambo sub-population contributed little to the inferred population structure of the geographically contiguous Cunnamulla sub-population (and vice versa). The major difference appeared to be rainfall. The Tambo sub-population was located at significantly higher elevations, with a correspondingly higher average annual rainfall (500–700 mm compared with <300–500 mm across the Cunnamulla sub-population). With more locally available water across the landscape, and a relatively small upper tributary river of the Warrego, the Tambo sub-population would be less likely to use the river as an important migration route southwards to the lower, drier Cunnamulla region. In contrast, the larger Warrego River in the lower elevations to the south was in a

drier landscape and appeared to be an important migration route between the Cunnamulla and Walgett sub-populations. In this landscape, the river, with its extensive flood plains would provide a more favourable habitat compared with the relatively harsh surrounding low rainfall, semi-arid rangelands. A similar situation was apparent between the more easterly, higher rainfall (500–700 mm) Westmar sub-population compared with the contiguous, lower rainfall (<300–400 mm) Walgett sub-population (which also shared less than 7% of each others inferred populations). The CCA analysis supported this conclusion, with the Tambo sub-population being strongly associated with higher elevation, higher rainfall areas, and the Westmar sub-population being associated with easterly, high-rainfall areas in native scrublands.

It is important to note that the Cunnamulla sub-population distribution was not always associated with the Warrego River. For example, the Dirranbandi sample was composed predominantly of the same inferred population as the greater Cunnamulla population, yet was located a significant distance from the Warrego River. A likely explanation is the extensive series of bore drains that provide artificial watering points for sheep throughout the Cunnamulla area. These would allow free migrations of feral pigs away from the Warrego River towards Dirranbandi during good seasons when food was also plentiful. Management of bore drains in the eastern Australia rangelands may therefore be crucial to preventing re-establishment of feral pig populations following control campaigns.

This approach also has utility for endemic disease eradication in wildlife. The targeting of entire sub-populations and establishment of buffer zones into surrounding sub-populations that contribute genetics (and therefore potentially infected individuals) would seem the most efficient way to eradicate endemic disease from a region. This approach has little applicability to exotic disease outbreaks in wild animals, since the extent of infection would be established with surveillance (Australian Veterinary Emergency Plan 2000, unpublished). However, an exotic disease may remain undetected for an extensive period of time in feral pigs with subsequent widespread distribution (Hone and Pech 1990). In these situations, disease would more rapidly spread within a sub-population, and initial disease surveillance should be focused in areas within a sub-population, or along migration pathways (major rivers in drier regions).

Although IBD and autocorrelation analyses indicate that the size of MUs is important, geographical features could also guide decisions in other similar areas within the rangelands. When feral pigs are in drier areas (<300–500 mm annual rainfall), migration, disease spread and re-colonisation will be likely along major rivers and associated floodplains. Thus, MUs in drier inland areas need to take account of major rivers. Buffer zones to account for migration in these areas, should extend extensive distance up-river and along flood plains, rather than in simple arbitrary concentric circles from an area. The Cunnamulla population extended ~500 km along the river, where it was located in lower rainfall area, suggesting length of buffers should be large in some instances. In areas of higher rainfall (e.g. 400–600 mm), for example in higher elevation areas, or areas closer to the eastern mountain ranges, feral pigs will be less likely to migrate along rivers, presumably due to less reliance on rivers and flood plains for water, food or shelter.

It is informative to compare these findings with those of other studies. Migration rates calculated in this study were higher than in similar studies in Australia in mountainous regions closer to the coast (Hampton et al. 2004). This is supported by the much higher effective population sizes in the current study compared with Hampton et al. (2004). Other comparisons between the two studies reveal that genetic differentiation between the sub-populations was higher, and that each sub-population was located entirely in a single catchment in Hampton et al. (2004). The difference between the two studies was likely due to habitat differences. In Hampton et al. (2004), the steep catchments and lack of comparable, periodic flooding across broad flat landscapes may have prevented spread of feral pigs away from the sub-population core area.

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