RESEARCH NOTE

An assessment of the genetic diversity and structure within and among populations of wild pigs (*Sus scrofa*) from Australia and Papua New Guinea

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Introduction

In many regions in the world the reduction in population sizes of native pigs is a conservation concern (Li *et al*. 2000; Martinez *et al*. 2000; Lemus-Flores *et al*. 2001). However, in Australia, feral (or wild) pigs are a significant invasive species, and there are upwards of 10 million feral pigs present, inhabiting over 40% of the continent (Choquenot *et al*. 1996). Coupled with these large numbers and advances made in marker technology, there is an increasing awareness of the value in quantifying (and understanding) the biodiversity retained in noncommercial livestock breeds (e.g. Hall and Bradley 1995). Well-characterized microsatellite markers, such as those recommended by the Food and Agriculture Organization and International Society for Animal Genetics (FAO-ISAG), are ideal for such studies. There is an increasing amount of data being generated from indigenous pigs, including Asian (Li *et al*. 2000; Kaul *et al*. 2001), American (Lemus-Flores *et al*. 2001) and wild European (Laval *et al*. 2000; Martinez *et al*. 2000; Vernesi *et al*. 2003) breeds. However, there is no such information available on the diversity of wild pigs from Australia or Papua New Guinea. Overall, the preliminary findings suggest that Australian feral pigs are genetically diverse, with heterozygosity and allelic diversity at 0.758 and 11.0 alleles per locus on average, respectively.

Methods

In this preliminary study, we collected a total of 320 samples from adult feral pigs from five populations in Australia and Papua New Guinea (PNG), and from 41 commercial pigs (abbreviated as COMM; a mix of Large White and Landrace ancestry). The feral populations were from Muir in the southwest of Western Australia (MUIR; 47 ♂; 54 ♀), Northampton (NORT; 18 ♂; 16 ♀), Noorama (NOOR; 62 ♂; 83 ♀), and smaller samples from Cape York (CY; 7 ♂; 7 ♀) and PNG (16 ♂; 10 ♀; table 1). We generated genotypes for all these individuals using a subset (n = 14; table 1) of microsatellite loci recommended for diversity studies using primers supplied courtesy of Professor M. Rothschild (Pig Genome Coordination Project of the US Department of Agriculture; http://www.genome.iastate.edu/pig). Allele sizes were estimated using an internal size standard (Tamara-350; Applied Biosystems, Melbourne). The sizes were calibrated relative to control animals: F9110010 and F9110012 (courtesy of L. Ollivier, INRA, France).

We calculated descriptive measures of genetic variability (e.g. allelic diversity, heterozygosity) for each locus and at each population using Popgene (Version 1.3.1 available from http://www.ualberta.ca/~fyeh/). Allele frequencies are available from the first author. Departures from Hardy–Weinberg equilibrium were tested using the Markov chain method of exact probability using Genepop (Version 3.3; Raymond and Rousset 1995) with (α = 0.05) table-wide corrections using a Bonferroni test. The level of genetic differentiation among populations was determined by measuring Fisher's exact tests for genetic differentiation using the program Genepop 3.3 (Raymond and Rousset 1995) with Bonferroni correction and by estimates of F_{ST} using the program FSTAT 2.9.3 (see Goudet 1995). The calculation of a standard genetic distance (Nei 1978) and UPGMA dendrogram and bootstrap analysis were performed using the program

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Table 1. Expected heterozygosity, number of alleles and polymorphic information content of 320 adult individuals at each of 14 microsatellite loci in five Australian wild pig populations.

	Expected heterozygosity (H_E)*						Number of alleles						Polymorphic information content					
Marker	MUIR (n = 202)	NOOR (n = 290)	NORT (n = 68)	PNG (n = 52)	CY (n = 28)	COMM (n = 82)	MUIR	NOOR	NORT	PNG	CY	COMM	MUIR	NOOR	NORT	PNG	CY	COMM
SW936	0.689	0.766	0.352	0.827	0.844	0.695	4	5	3	10	8	5	0.626	0.724	0.317	0.788	0.790	0.648
S0026	0.475	0.732	0.442	0.577	0.720	0.686	4	4	3	5	4	4	0.382	0.682	0.353	0.523	0.641	0.616
SW240	0.758	0.716	0.571	0.793	0.815	0.795	7	7	3	8	6	7	0.721	0.664	0.482	0.751	0.754	0.761
SW951	0.502	0.506	0.000	0.621	0.706	0.646	2	3	1	5	4	6	0.375	0.410	0.000	0.570	0.630	0.568
S0155	0.634	0.793	0.549	0.856	0.825	0.671	4	6	3	9	5	5	0.554	0.759	0.435	0.819	0.763	0.602
SW632	0.674	0.765	0.295	0.702	0.817	0.816	4	7	2	8	6	7	0.623	0.728	0.248	0.649	0.755	0.779
S0002	0.559	0.769	0.550	0.723	0.772	0.488	4	9	3	8	7	6	0.517	0.731	0.480	0.675	0.715	0.453
S0068	0.672	0.656	0.664	0.819	0.844	0.849	6	10	7	11	7	9	0.608	0.594	0.623	0.780	0.789	0.821
SW122	0.787	0.761	0.467	0.769	0.905	0.672	5	9	4	8	10	7	0.747	0.730	0.418	0.727	0.859	0.635
SW911	0.577	0.662	0.413	0.754	0.667	0.696	3	6	4	6	4	6	0.490	0.616	0.372	0.697	0.585	0.635
S0005	0.674	0.746	0.647	0.893	0.000	0.887	6	12	4	11	0	13	0.616	0.705	0.571	0.863	0.000	0.864
S0090	0.454	0.574	0.689	0.683	0.643	0.667	4	6	4	4	4	6	0.410	0.500	0.623	0.611	0.543	0.620
SW857	0.660	0.654	0.538	0.933	0.817	0.742	5	9	3	5	7	6	0.591	0.597	0.472	0.744	0.758	0.685
S0226	0.610	0.720	0.450	0.805	0.915	0.742	3	8	3	8	9	6	0.530	0.673	0.384	0.757	0.871	0.685
Average	0.623	0.701	0.473	0.768	0.735	0.718	4.36	7.21	3.36	7.57	5.79	6.64	0.557	0.651	0.413	0.711	0.675	0.669
SE	0.027	0.022	0.048	0.027	0.024	0.027	1.34	2.46	1.34	2.28	2.31	2.17						

*Nei's unbiased estimate (Nei 1978). n = Genic number.
MUIR, Western Australia (32° 02′ S, 116° 09'E); NOOR, Noorama, Queensland (28° 29′S, 146° 16'E); NORT, Northampton, Western Australia (28° 32′S, 114° 37'E); PNG, Papua New Guinea (15° 38′S, 141° 50'E); CY, Cape York, Queensland (14° 29′S, 143° 40'E); COMM, a sample from an Australian commercial piggery.

DISPAN (available from http://iubio.bio.indiana.edu/soft/molbio/ibmpc/dispan.readme). Polymorphic information content (PIC) values were calculated using CERVUS (Marshall *et al*. 1998).

Results

For each locus we generated genotypes and descriptive statistics (direct count, effective number of alleles, observed and expected heterozygosities) for all of these at 14 microsatellite loci (table 1). A total of 154 different alleles (mean = 11.0 ± 3.92 s.d. alleles per locus) were detected at the 14 loci, and all loci were polymorphic in all populations. The expected heterozygosity (H_E) estimates at each locus were between 0.473 and 0.801 with a mean of 0.758 (±0.022 s.d.; table 1). These values are similar to results obtained from different pig breeds in Asia, America and Europe (Laval *et al*. 2000; Li *et al*. 2000; Martinez *et al*. 2000; Lemus-Flores *et al*. 2001; Vernesi *et al*. 2003). Interestingly, one population from Northampton in Western Australia contained about half the variability (H_E = 0.473), compared to the 70–80% contained in the Queensland or other wild populations (table 2).

Table 2. Matrix of Nei's (1978) standard genetic distance (above diagonal) and pairwise population differentiation using estimates of F_{ST} (Weir and Cockerham 1984; below diagonal) between the pig populations sampled in this study.

Population	MUIR	NOOR	NORT	PNG	CY	COMM
MUIR	***	0.189	0.337	0.259	0.215	0.156
NOOR	0.3704	***	0.245	0.108	0.128	0.103
NORT	0.5987	0.6911	***	0.338	0.302	0.270
PNG	0.4664	0.3734	0.7324	***	0.130	0.077
CY	0.3481	0.3701	0.8040	0.2056	***	0.058
COMM	0.4401	0.7556	0.3255	0.2670	0.2939	***

Allele frequencies differed significantly at each locus among the populations sampled ($P < 0.01$ in all but six of 210 pairwise comparisons) and in nearly all comparisons the probability was $P << 0.001$. PNG, NOOR, COMM, CY, MUIR and NORT had 106, 101, 93, 80, 61 and 47 alleles, respectively at the 14 loci. Overall, the populations studied were highly structured (global F_{ST} = 0.159 ± 0.013 s.e.; 95% CI 0.133 – 0.183), and nearly all populations displayed F_{ST} values greater than 0.1. Establishing the usefulness of these loci in breed classification requires further study, although they have been shown to be extremely discriminatory (Hampton *et al*. 2004) in some populations.

In terms of diversity contained in the wild Australian and PNG pigs, the feral pigs we sampled had 63 (41%) alleles that were not represented in the genotypes from the commercial pigs we sampled. Alternatively, the commercial breed had only seven alleles (out of 154) not represented in the wild populations. However, some caution needs to be given, as we utilized only a small number of loci in this study. Additionally, as suggested by Li *et al*. (2000), relationships between alleles cannot be assumed as identity by descent does not necessarily equate to identity by state (i.e. PCR product size).

Discussion

The genetic diversity and structure of five geographically separated feral pig populations and a commercial pig breed from mixed ancestry (Large White and Landrace) were evaluated using 14 microsatellite loci. Descriptive statistics were calculated and we found high levels of genetic variation, suggesting that feral pig populations from mainland Australia and Papua New Guinea (PNG) contain substantial genetic information not contained in the domestic stock we sampled. Populations also showed a considerable degree of differentiation from one another. Nei's standard genetic distances were used to construct a UPGMA dendrogram (figure 1), which showed that pigs from far northern Australia, PNG and commercial stock are most closely related, presumably reflecting the coexistence of domestic/wild stock with humans in these areas. Australian and PNG feral pigs are genetically diverse and provide compelling data that feral pigs will be difficult to control. These wild stocks may ultimately provide valuable information and resources for future agriculture, as these may no longer be retained in commercial pig lines.

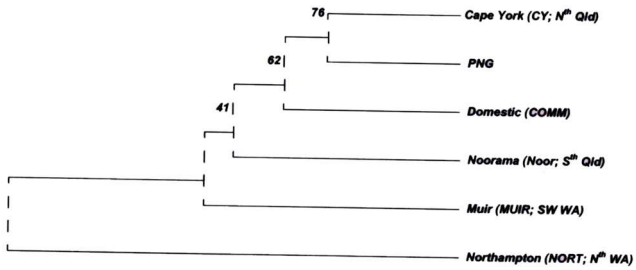

Figure 1. UPGMA dendrogram of five wild Australian, one Papua New Guinean, and a commercial breed (mixed Landrace and Large White) based on Nei's (1978) standard genetic distance (given in table 2). The bootstrap values (1000 replicates) are given as a percentage at nodes.

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