ORIGINS OF KUNE KUNE AND AUCKLAND ISLAND PIGS IN NEW ZEALAND

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INTRODUCTION

Migrating Polynesians first introduced pigs from Asia to the Pacific islands (Diamond, 1997), but it is not clear whether they reached New Zealand. European sailors and settlers introduced pigs into New Zealand in the 18th and 19th centuries, many of which became feral, but few records were kept of these introductions (Clarke and Dzieciolowski, 1991a; 1991b). It is believed that the European settlers introduced contemporary domestic animals originating either directly or indirectly from Europe (Challies, 1976). However it is known that Captain Cook released pigs on the South Island in 1773, obtained from Tonga and Tahiti, and undoubtedly of Polynesian origin (Clarke and Dzieciolowski, 1991a). Mitochondrial DNA (mtDNA) sequence may help distinguish between different possible origins for feral populations of pigs from New Zealand and outlying islands, as distinct European and Asian clades can be recognised on mitochondrial sequence (Giuffra *et al*., 2000; Kim *et al*., 2002). Of particular interest in this study are two phenotypically and geographically distinct populations, the Kune Kune "breed" and an isolated population from Auckland Island.

Kune Kune pigs are now distributed across the North and South Islands**.** They display a placid temperament, with small to moderate body size, and are quite rotund and stocky, with short ears, thick legs, snout turned-up and a coarse coat of bristly hair of many possible colors (Clarke and Dzieciolowski, 1991b). Kune Kune are characterised by distinctive long cylindrical appendages (wattles) that hang from the lower jaw. There have been claims that the Kune Kune were brought to New Zealand by Maoris but no archaeological evidence have been found to support this (Tipene, 1980; Davison, 1984). It has even been speculated that they originated in China and were brought by Spanish and Portuguese mariners (Clarke and Dzieciolowski, 1991a). The Auckland Islands are a remote group of uninhabited subantarctic islands in the Pacific Ocean, three hundred and fifty nautical miles south of New Zealand. The pigs there were similar in physical appearance to feral pigs of the main New Zealand islands, being black or white to brown with black markings, although having longer snout, and higher hair and bristle coverage for the cold conditions. (Challies, 1975). Auckland Island pigs were released there between 1807 and 1850 during failed European and Maori settlements, but not all them survived (Challies, 1975; Taylor, 1971). Pigs were removed from Auckland Island in 1999 to restore the environment to its pristine state and were brought to the mainland of New Zealand in order to preserve the breed (MAF, 1999).

Mitochondrial D-loop DNA has a high rate of substitution, making it useful for analysis of subspecific phylogenetic relationships and has been useful in determining the origin and

history of pig populations and breeds (Okumura *et al*., 2001; Giuffra et *al*.; Kim *et al*., 2002). The aim of this paper is to assess the phylogenetic relationship of the Kune Kune and Auckland Island pig populations, by comparing their mitochondrial D-loop DNA sequences with published domestic and wild boar pig sequences to help determine their genetic backgrounds and origins.

MATERIALS AND METHODS

Sampling, PCR, cloning and sequencing. Template DNA was extracted from muscle biopsy from one Kune Kune pig (originally provided by Dr. Matisoo-Smith from Auckland University Anthropology Department) and from blood of one Auckland Island pig using Puregene kit (Gentra systems) according to the manufacturer protocol. Pig primer pairs, PCR conditions and cloning were the same as described by Kim *et al*. (2002). Cloned inserts were sequenced and loaded in a 4% polyacrylamide gel on a LI-COR sequencer (model 4200). To avoid PCR artefacts, two to four different clones were sequenced from each animal or a second independent cloning was done to confirm the results.

Data analyses. 17 D-loop sequences described fully by Kim *et al*. (2002), accession number AF276921 through AF276937 [eleven Chinese (Eurhualian, Tongcheng, Wan'an, Yanxin Gandonghei, Neijang, Jinghua, Putian, Wanhua and Wannanhua), three Korean (Cheju), one Australian Kangaroo Island feral (Westran) and two European (Berskshire and Welsh)] along with thirteen published Asian, European and South American sequences [Meishand39 (D17739), Meishand81 (D42181), Okinawa (AB01592), Satsuma (AB01591), Moncai8 (AB041481), Japanese Wild Boar (AB015085), Ryuku Wild Boar (AB015087), Swedish (AJ002189), Landrace (D16483), Duroc (D42179), Large White (D42180), Yucatan miniature (AB015093), European Wild Boar (AB15094)] were aligned with the novel New Zealand sequences using the ClustalW multiple alignment software (Thompson *et al*., 1994). Except for the first unit, the tandem repeat motif was excluded from the analysis due to its high degree of heteroplasmy (Ghivizzasni *et al*., 1993). Phylogenetic analyses were performed using PAUP software version 4.0 beta (Swofford, 2001). Pairwise distances were estimated using the maximum-likelihood method (HKY85 model) after elimination of nucleotide gaps (Hasegawa *et al*., 1985). A Neighbor-Joining (N-J) tree was generated based on maximum-likelihood distances (Saitou and Nei, 1987). The statistical confidence of each node was estimated by 1000 random bootstrap resamplings of the data (Felsentein, 1985).

RESULTS AND DISCUSSION

1045 base pairs of the mtDNA control region were sequenced for one Kune Kune pig and one Auckland Island pig. Comparison of these sequences with 30 previously reported sequences identified 30 different haplotypes with 61 variable positions (Figure 1a). Three positions were insertion/deletion. The polymorphic sites show fifty one nucleotide transitions and seven nucleotide transversions. The Kune Kune sequence has one unique substitution and the Auckland Island sequence has five. The Neighbor-Joining tree shows two main groups of European or Asian pigs as found by Kim *et al*. (2002). The Kune Kune sequence clustered with Asian pig mitochondrial DNA, being most similar to Satsuma sequence (bootstrap value 89%, genetic distance of 0.00096) and separated by distances of 0.00482 and 0.00579 with respect to Eurhualian and both Neijang and Gandonghei pigs respectively. On the other hand, the

Auckland Island pig D-loop haplotype clustered with the European domestic pig clade (bootstrap value 96%), with a genetic distance of 0.00675 to Landrace breed sequence and 0.00772 with respect to both the Welsh and Duroc pigs (Figure 1b). Average pairwise HKY85 distances (± SD) of Kune Kune with respect to Asian and European sequences are 0.00591 (±0.00172) and 0.02000 (±0.00205) respectively. Comparison of the Auckland Island pigs with European and Asian sequences gives average pairwise distances of 0.01008 (±0.00481) and 0.02179 (±0.00120) respectively. For the Auckland Island sequence, the average pairwise distance with respect to European sequences is inflated by the inclusion of European Wild Boar, which branches early from the European clade, and also by the five unique substitutions, but it is still clearly more similar to European sequences than Asian sequences.

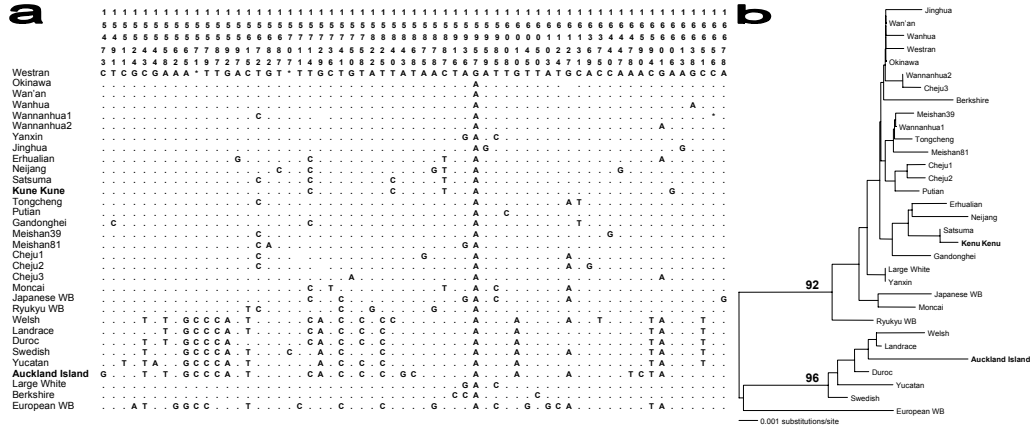

Figure 1. a, Nucleotide substitutions and gaps found in 32 porcine mtDNA D-loop sequences. Dots (.) and asterisks (*) indicate matches with reference nucleotide sequence (Westran pig) and gaps, respectively. Nucleotide position numbers on the top of the figure correspond to those in AJ002189. b, N-J tree of 32 pig mtDNA D-loop. The Kune Kune clusters with Asian domestic pigs, being most closely related to Chinese and Japanese breeds. The Auckland Island sequence clusters with domestic European breeds

The unequivocal Asian origin of the Kune Kune mitochondrial sequence is consistent with the pigs being taken from Asia to New Zealand by the Polynesian ancestors of present day Maoris, but may be better supported by the well documented introduction of Polynesian pigs into New Zealand by Captain Cook in 1773. Also possible is the introduction of this Asian mitochondrial sequence via a European breed, which acquired Asian mitochondria by introgression in the 18th century in Europe. Such introgression explains the clustering of the Large White and Berkshire sequences with Asian pigs in Figure 1b. Analysis of additional Kune Kune sequences as well as more Polynesian sequences may help distinguish the first two possibilities from the third. Finding unambiguous Polynesian sequences may be difficult though, as Giuffra *et al*. (2000) found that a feral pig sequence from Cook Island in Polynesia clustered with European domestic pig sequences. Analyses of nuclear gene sequences in conjunction with

mtDNA sequences will also help in discriminating between European and Asian origins as for the porcine *GPIP* gene in the study of Giuffra *et al*. (2000).

Analysis of microsatellite marker allele frequencies using the standard ISAG/FAO marker set (Li *et al*., 2000) will also assist in deciphering the relationships of these populations of pigs and are already underway for the Auckland Island population and are planned for the Kune Kune pigs. Jointly these studies will illuminate the history of Pacific island pigs, their geographic origins and genetic diversity.

CONCLUSION

Auckland Island pig mitochondrial DNA has a European origin. Kune Kune pigs have Asian mitochondrial DNA but at this stage we cannot distinguish between i) Polynesian introduction of Asian pigs, ii) European introduction of pigs from Asia/Polynesia or iii) introgression of Asian mtDNA into European pigs in Europe in 17th century and subsequent introduction of these "European" pigs into New Zealand.

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