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Phylogenetic relationships of Australian and New Zealand feral pigs assessed by mitochondrial control region sequence and nuclear *GPIP* genotype

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Abstract

Pigs were introduced into Australia and New Zealand in the 18th and 19th centuries, with some establishing feral populations. With few records of pig introductions into these two countries, molecular phylogenetic analysis was used to assess their origins. Mitochondrial (mt) control region sequence and nuclear glucosephosphate isomerase pseudogene (*GPIP*) restriction fragments were used, as distinct European and Asian domestic pig and Wild Boar control region clades and *GPIP* genotypes can be recognised. Feral pig control region sequences clustered with either European or Asian domestic pig sequences and both Asian and European *GPIP* alleles were segregating. It was not possible to distinguish direct importation of Asian domestic animals into Australia and New Zealand from indirect introgression of Asian domestic sequences via Europe. However, the clustering of three feral control region sequences of pigs from northern Australia with Asian Wild Boar implies unrecorded introduction of Wild Boar or crossbred animals into Australia. However, two of these feral pigs had European *GPIP* alleles. In combination, analyses of control region and *GPIP* markers suggest that both European and Asian pigs have contributed in similar frequencies to the origins of Australian feral pigs.

Keywords: Feral pigs; Domestic pigs; Control region; *GPIP* genotype

1. Introduction

Feral pigs (*Sus scrofa*) are pest animals in Australia and New Zealand, causing agricultural and environmental damage (Choquenot et al., 1996). There is no evidence that pigs were present in Australia or New Zealand before the arrival of Europeans, but they were found across the Pacific islands (Cassels, 1983). Europeans brought domestic pigs into Australia and New Zealand during the 18th and 19th centuries, some of which were deliberately released or escaped to establish feral populations (Clarke and Dzieciolowski, 1991; Epstein and Bichard,

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1984; Pullar, 1953; Tisdell, 1982). The deliberate release of these animals was an attempt to naturalise them and provide food for early European settlers such as explorers, sealers, and whalers (Tisdell, 1982). The origins of the pigs introduced during the early exploration and colonisation of Australia and New Zealand are uncertain due to poor documentation or absence of records. Australian and New Zealand feral pig populations are generally believed to be directly or indirectly descended from European and Asian domestic animals (Badger, 1988; Challies, 1976; Clarke and Dzieciolowski, 1991; Dunmore, 1969; Reed and Reed, 1951). Feral pigs could also be influenced by the introgression of Asian pig genetic material into European pig populations during the development of European swine breeds, when pigs from Asia were imported into Europe and crossbred with European pig breeds during 18th and 19th centuries (Epstein and Bichard, 1984; Jones, 1998; Porter, 1993).

Pigs were brought into Australia with the European settlement of Sydney in 1788 (Pullar, 1953; Tisdell, 1982). Also, pigs from Timor were released into the Northern Territory (Australia) in 1827 after a settlement was abandoned (Pullar, 1950, 1953). It is possible that pigs were introduced directly from China during the mass immigration of Chinese people in the Gold Rush, and from New Guinea into North Queensland before 1900 (Pullar, 1950, 1953). Pigs were imported from the island of Kisar (Indonesia) to the Northern Territory in 1838, as well as from New South Wales (Australia) in 1838 and 1843 (Letts, 1962). Pigs were also released on Kangaroo Island (South Australia) in 1803 (Cooper, 1954). The Australian Westran inbred line has been derived from this feral population and used for biomedical purposes (Lee et al., 2002; McIntosh and Pointon, 1981).

No association has been found between aborigines and pigs before European arrival (Pavlov, 1992; Pullar, 1953), but it is probable that the Malays from Timor and adjacent islands would have carried pigs with them and traded these pigs to the aborigines of the northern coast of Australia (Pullar, 1953).

There is no archaeological (Davidson, 1984) evidence of the presence of pigs in New Zealand before European arrival. Captain Cook presented domestic pigs to the Maori people on his second and third voyages in 1773 and 1777 (Clarke and Dzieciolowski, 1991). Clarke and Dzieciolowski (1991) suggest that the Kune Kune pig was introduced to the North Island between 1795 and 1840 by Spanish and Portuguese mariners sailing from Chinese seas. However, it has been suggested that Kune Kune pigs were kept by the Maori people of the North Island of New Zealand before the arrival of Europeans, as similarities in the words used by Polynesians and Maoris to name pigs have been found (Clarke and Dzieciolowski, 1991; Tipene, 1980).

Different pig releases into the wild have been made in New Zealand such as those made on the subantarctic Auckland Island by European expeditions between 1807 and 1840, Maori settlers between 1842 and 1843 and at different times since 1850 (Challies, 1975). Auckland Island feral pigs probably originated from domestic pigs brought from the Australian or New Zealand mainland and have been isolated on this subantarctic island for up to 200 years (Challies, 1975).

With few records of pig introductions into Australia and New Zealand, molecular phylogenetic analysis of relationships between these pigs and others around the world might aid in assessing their origins. Joint mitochondrial control region and nuclear glucosephosphate isomerase pseudogene (*GPIP*) analyses have been used to assess European and Asian pig contributions to pig breeds and populations (Giuffra et al., 2000; Gongora et al., 2003; Ishiguro et al., 2002). Phylogenetic analyses of the mtDNA control region sequence from pigs around the world group them into two main clades, European and Asian, each including both domestic pig and Wild Boar (Giuffra et al., 2000; Kim et al., 2002; Okumura et al., 2001; Watanobe et al., 2001, 2002). The level of molecular divergence between Asian and European haplotypes lead to estimates of time of divergence ranging from 58,000 to 500,000 YBP, clearly establishing independent domestications from European and Asian Wild Boar ancestors about 9000 YBP (Giuffra et al., 2000; Kim et al., 2002; Okumura et al., 2001). The clustering of some European pig breed control region sequences with Asian pigs has also been useful in corroborating the introgression of Asian pigs into European breeds in 18th and 19th centuries (Giuffra et al., 2000; Kim et al., 2002; Okumura et al., 2001). These conclusions from control region sequences have been consistent with analyses of other mt and nuclear markers including microsatellites and whole mt genome studies (Alves et al., 2003; Clop et al., 2004; Giuffra et al., 2000; Kijas and Andersson, 2001; Li et al., 2000; Paszek et al., 1998; Yang et al., 2003).

Nuclear sequences such as the glucosephosphate isomerase pseudogene (*GPIP*) gene have also been used to analyse European and Asian domestic pigs and Wild Boar (Giuffra et al., 2000). *GPIP**1 and *GPIP**3 alleles have been associated with Asian domestic pig and Asian Wild Boar, while *GPIP**4, and *GPIP**4a alleles have been associated with European domestic pig and European Wild Boar (Giuffra et al., 2000; Ishiguro et al., 2002). The utility of the *GPIP* marker to understand the influence of Asian genes in European pigs and vice versa has also been demonstrated by recent studies (Gongora et al., 2003; Ishiguro et al., 2002; Naya et al., 2003).

In the present study, we analyse both mtDNA control region and nuclear *GPIP* markers from 40 animals of feral and domestic pig breeds to assess European and Asian contribution to the diversity of Australian and New Zealand feral pigs.

2. Methods

2.1. DNA sampling

Sampling feral pigs in Australia is difficult, as it requires hunting these animals in remote areas. Consequently samples collected for other studies, including pig serum samples collected by Mason and Fleming (1999) were used. Many of these serum samples failed to yield useable quality DNA. Eventually 21 DNA samples were obtained from 88 feral pig serum and ear samples from 14 localities in Queensland (Qld), New South Wales (NSW), Western Australia (WA) and South Australia (SA) (Fig. 1). New Zealand feral pig samples were obtained from animals on the North Island and subantarctic Auckland Island. DNA was extracted using QIAamp DNA mini kits (Qiagen Pty, Clifton Hill). To generate a phylogenetic framework suitable for feral pig comparison, DNA samples from 11 European (Tamworth, Large Black, Wessex Saddleback, Angeln Saddleback, Bentheim Black Pied, German Large White, German Landrace, Red Angeln Saddleback and Mangalitza), six Chinese (Tibetan, Ganzhongnan Spotted, Guizhou Xiang, and Guan Xiang) and two synthetic Asian-European (Gottingen minipig) pig breeds were also analysed.

2.2. PCR conditions

Porcine primers for L-strand (5′-CCAAGACTCA AGGAAGGAGA-3′) and H-strand (5′-GGCGCGGA TACTTGCATGTG-3′) were used to amplify the complete mtDNA control region (Kim et al., 2002). PCR was performed in 25 μl reaction volumes for all primer pairs, containing 50 mM KCl, 10 mM Tris–HCl, pH 9.0, 0.1% Triton X-100, 1 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 μM of each primer, 1 U of *Tli* DNA polymerase (Promega, Sydney), and 100 ng of template DNA. PCR conditions included an initial denaturation at 95 °C for 5 min followed by 35 cycles of 1 min at 95 °C, 1 min at 60 °C, and 5 min at 72 °C, and a final extension for 15 min at 72 °C. Addition of 3′A-overhangs post amplification was performed using 1 U of *Taq* polymerase (Promega) and 0.2 mM dATP for 10 min at 72 °C. Replicate experiments were performed using the PCR protocol described above except that the *Pfu* DNA polymerase (Promega) was used in the initial PCR.

2.3. Cloning and sequencing

PCR products were gel-purified using an UltraClean DNA purification kit (Mo Bio Laboratories, Solana Beach) and cloned using TOPO TA cloning kits

Fig. 1. Map indicates the geographical location of sampled feral pigs. In Australia: Kowanyama Qld (36), Koolatah Qld (34), Rutland Qld (35), Vanrook Qld (39), Mount Larcom Qld (37), Julia Creek Qld (40), Dirranbandi Qld (44), Cooktown Qld (48), Oberon NSW (38), Taralga NSW (50), Tooraweenah NSW (41 and 42), Churchmans WA (46), Dandalup WA (45), Canning WA (47, 48, and 49), and Kangaroo Island SA (32). In New Zealand: Auckland Island (51, 52, and 53), and North Island (33). The numbers for feral animals correspond to their identification number in Figs. 2 and 3. Black circles on the map indicate where other feral pig samples were taken, but DNA could not be extracted.

(Invitrogen Life Techonologies, Melbourne). Clones were checked for inserts by PCR under the same conditions mentioned above, using *Taq* polymerase. Plasmid DNA from positive clones was purified using Ultra-Clean Mini Plasmid Prep kit (Mo Bio Laboratories). An aliquot was digested with *Eco*RI (Promega) to release the entire insert and confirm its length.

A SequiTherm EXCEL II sequencing kit-LC (Epicentre Technologies, Madison) was used to sequence the insert using universal vector primers M13 reverse and forward. Reaction products were loaded in a 4% polyacrylamide gel on a LI-COR sequencer, model 4200 (LI-COR, Lincoln). Forward and reverse sequences were overlapped to obtain a single sequence for each animal. To avoid PCR artefacts, two independent PCR reactions were used to amplify each mitochondrial and nuclear sequence for cloning. Inserts from two to four different clones were sequenced from each amplification and animal.

2.4. PCR-restriction fragment length polymorphism analysis (PCR-RFLP)

The primer pair GPIP1 (5′-TGCAGTTGAGAAGG ACTTTACTT-3′)-GPIP6 (5′-GAAGTTACAGGGCA TCATCTTG-3′) was used to amplify 507 bp of the *GPIP* gene (Giuffra et al., 2000; Ishiguro et al., 2002). PCR conditions were as described by Ishiguro et al. (2002), except that the *Tli* proof reading DNA polymerase (Promega) was used in the PCR to minimise amplification sequence artefacts. *GPIP* fragments were digested in two independent reactions using the restriction enzymes *Bgl*I and *Hha*I (Promega) for 1 h at 37 °C to recognise nucleotide polymorphism at positions 223 and 316, respectively (Ishiguro et al., 2002; Naya et al., 2003). These restriction enzymes can differentiate Asian *GPIP* alleles from European *GPIP**4 and *GPIP**4a alleles but do not discriminate within Asian alleles. All Asian alleles were denominated as *GPIP**3. Two replicate PCR-RFLP experiments for each sample and enzyme were electrophoresed in 2% agarose gels, stained with ethidium bromide, and photographed. All pig samples mentioned above were genotyped, including a Westran pig DNA sample.

2.5. Data analyses

Thirty-one published sequences from the control region (Kim et al., 2002; Okumura et al., 1996, 2001; Takeda et al., 1995; Ursing and Arnason, 1998; Watanobe et al., 2001) were aligned with 40 novel Australian and New Zealand feral and European and Asian domestic pig sequences. Novel control region sequences from this study were deposited in GenBank with Accession Nos. AY463061 through AY463096 and AY486115 through AY486118.

Published sequences correspond to: Erhualian (AF276922), Tongcheng (AF276923), Wan'an (AF276924), Yanxin (AF276927), Gandonghei (AF276928), Neijang (AF276929), Jinghua (AF276930), Putian (AF276931), Wanhua (AF276932), Wannanhua (AF276925), Cheju (AF276933), Moncai (AB041481), Westran (AF276921), Berkshire (AF276936 and AB041484), Welsh (AF276937), Meishan (D17739), Okinawa (AB010592), Satsuma (AB015091), Japanese Wild Boar (AB015085), Ryukyu Wild Boar (AB015087), Landrace (AB041496), Duroc (AB041486), Large White (AB041492 and D42180), Pietrain (AB041489), Yucatan miniature (AB015093), Middle White (D42182), Hampshire (AB041488), Swedish commercial (AJ002189), and Italian Wild Boar (AB015094). A warthog sequence (AB046876; Okumura et al., 2001) was used as an outgroup. Phylogenetic analyses on European and Asian Wild Boar and domestic pig control region sequences (including the published sequences used in the present study) and other mt sequences mentioned above were performed using maximum likelihood and neighbor-joining (NJ) with different parameters and substitution models which are supported by bootstrap analyses (Giuffra et al., 2000; Kim et al., 2002; Okumura et al., 2001; Watanobe et al., 2001, 2002).

Seventy-one sequences from the control region were aligned using the ClustalW software (Thompson et al., 1994). Except for the first unit, the tandem repeat motif of the control region sequences was excluded from the analysis due to its high degree of heteroplasmy (Ghivizzani et al., 1993). Phylogenetic analyses were performed using NJ in PAUP software version 4.0 beta (Swofford, 2000). NJ tree construction was based on pairwise genetic distances using HKY85 (Hasegawa et al., 1985) and Kimura-2 parameter (Kimura, 1980) substitution models, taking into account all nucleotide substitutions. These two models assume that nucleotide substitutions occurred at different rates but with equal and unequal base frequency, respectively. The statistical confidence of each node was estimated by 1000 bootstrap replicates, resampling all characters of the control region in each replicate (Felsenstein, 1985). Molecular diversity indices were calculated using the ARLEQUIN program (Schneider et al., 2000).

3. Results

3.1. Control region sequence

About 1047 bp of the control region sequence from 40 pigs were analysed after exclusion of the repeat motif. We identified 104 polymorphic sites that were observed in the novel and published sequences (Fig. 2). Eighty-eight transitions, 16 transversions, and four single nucleotide deletion/insertions were observed. The transition

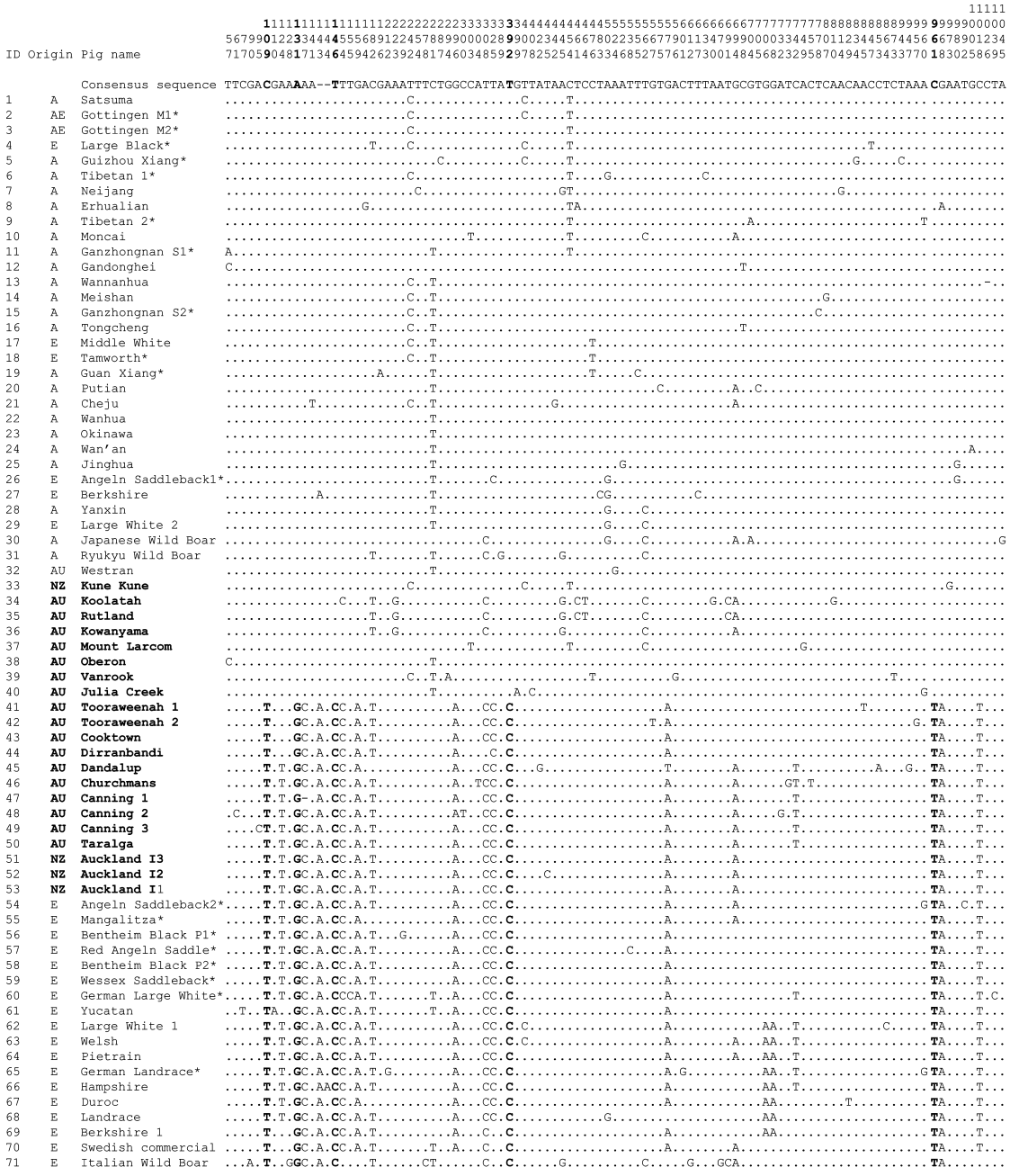

Fig. 2. Variable sites of control region sequences from Australian and New Zealand feral pigs and European and Asian domestic pigs and Wild Boar. Names in bold correspond to novel feral pig sequences and those with asterisk (*) to novel European and Asian pig breed sequences generated in the present study. Leftmost column indicates identification number (ID) used in the phylogenetic analysis in Fig. 3. The column second from left indicates the geographical origins of these animals: Europe, E; Asia, A; Asia/Europe, AE; Australia, AU; and New Zealand, NZ. The Yucatan miniature pig is from the American continent, but is derived from European breeds. Dots (·) and dashes (-) indicate matches and gaps respectively with the consensus sequence. Nucleotide position numbers on the top of the figure correspond to those in pig control sequence AB015087. Diagnostic nucleotide positions 109, 131, 146, 392, and 961, which distinguish Asian from European clades are in bold. Right brackets indicate the bipartition of the Asian and European pig control region haplotypes.

to transversion ratio was 5:1. Among the 71 pig sequences 65 haplotypes were found. Four of these haplotypes were shared between novel and published sequences and 2 haplotypes were shared between published sequences: Satsuma and Gottingen Minipig 1, Middle White, and Tamworth; Auckland Island pigs 2 and 3; Bentheim Black Pied 2 and Wessex Saddleback; Wanhua and Okinawa; and Yanxin and Large White 2.

Positions 109, 131, 146, 392, and 961 were diagnostic in distinguishing between Asian and European clades since specific substitutions for each clade were observed there. Positions 137, 143, 154, 159, 182, 296, 308, 325,

577, 968, and 1018 were informative, but they were not diagnostic for these clades since the same substitution or single nucleotide deletion was observed in at least one sequence from within the other pig clades.

3.2. Control region clustering and GPIP genotypes

Two major clades were recognised using NJ, namely Asian versus European domestic pig and Wild Boar (Fig. 3). Pairwise genetic distances within European (average distances ± SD, 0.0053 ± 0.0034) and Asian (average distances ± SD, 0.0061 ± 0.0030) showed that they are closely related within each clade, but distinct from each other as average pairwise genetic distances between clades is 0.0205 (±0.0023), consistent with results reported by Kim et al. (2002).

The same major clades and nodes were recovered using KY85 and Kimura-2 parameter substitution models. Control region sequences from German Large White, German Landrace, Red Angeln Saddleback, Wessex Saddleback, Angeln Saddleback 2, Mangalitza, and Bentheim Black Pied 1-2 clustered with European domestic pigs. Gottingen Minipig, Tibetan 1-2, Ganzhongnan Spotted 1-2, Guan Xiang and Guizhou Xiang pig breeds clustered with Asian domestic pig sequences as did the Large Black, Tamworth, and Angeln Saddleback 1 pig sequences (Fig. 3).

The novel feral pig sequences from Tooraweenah (NSW), Taralga (NSW), Dirranbandi (Qld), Vanrook (Qld), Cooktown (Qld), Canning (WA), Dandalup (WA), and Churchmans (WA) clustered with European domestic pig breeds, while feral sequences from Oberon (NSW), Julia Creek (Qld) and Mount Larcom (Qld) clustered with Asian domestic pig sequences (Fig. 2). Feral pig sequences from Kowanyama, Koolatah, and Rutland (Qld) clustered with Asian Wild Boar, being more closely related to Ryukyu Wild Boar (*Sus scrofa riukiuanus*) than Japanese Wild Boar (*Sus scrofa leucomystax*). Westran sequence (SA) clustered with Asian domestic pigs as reported previously (Kim et al., 2002). Kune Kune and Auckland Island pig sequences from New Zealand clustered, respectively, with Asian and European domestic pigs, which is consistent with earlier and preliminary analyses of some of these sequences (Gongora et al., 2002). Feral pig control region sequences are widely dispersed among the branches of the pig breed phylogenetic framework. However, within the European clade, twelve of the feral sequences and six European rare pig sequences (Angeln Saddleback, Red Angeln Saddleback, and Bentheim Black Pied from Germany; Mangalitza from Hungary; Wessex Saddleback from the United Kingdom) clustered in a distinct subclade from common commercial domestic pig breed sequences. Also, within this subclade there is an unexpected clustering of nine feral sequences in a subclade with two rare domestic breeds, Angeln Saddleback and Mangalitza. Further, these feral sequences are predominantly from Western Australia and New Zealand at the geographical limits of sampling. In the present study, *GPIP**4/*GPIP**4 and *GPIP**4/*GPIP**4a genotypes were observed in European pig breeds, and *GPIP**3/*GPIP**3 genotypes were observed in Asian domestic pig samples as expected for these breeds (Fig. 3) and as was observed in previous studies (Giuffra et al., 2000). Many of the *GPIP* genotypes from feral pigs were heterozygous for Asian (*GPIP**3) and European (*GPIP**4 or *GPIP**4a) alleles.

The *GPIP* genotypes observed in Australian and New Zealand feral pigs were not concordant with control region sequence clustering (Fig. 3), although the *GPIP**3 allele is more common in feral animals with Asian control region, and *GPIP**4 and *GPIP**4a alleles are more common in feral pigs with European control region sequence (Fig. 3).

4. Discussion

The clustering of individual Tamworth, Large Black, and Angeln Saddleback control region sequences within the Asian clade adds them to the list of European breeds already including Middle White, Berkshire, and Large White known to be segregating Asian control region sequences (Giuffra et al., 2000; Kim et al., 2002; Okumura et al., 2001). In contrast, their *GPIP* genotypes are consistent with a European origin for their genomic DNA, although more sampling is required to address this issue adequately. The introgression of Asian genes into the European domestic pig population has been documented and supported elsewhere by mitochondrial and nuclear sequence studies (Giuffra et al., 2000; Kijas and Andersson, 2001; Kim et al., 2002).

The clustering of feral sequences as separate subclades within the European clade suggests that many of the pigs that escaped or were released to form feral populations in Australia and New Zealand were derived from domestic breeds that are now rare or possibly extinct. The Australian feral pig may therefore contain important genetic material for animal production management. For example, commercial domestic pigs in Australia are now dominated by Large White and Landrace and synthetic derivatives of these white breeds whereas feral pigs are almost all pigmented (Choquenot et al., 1996). This characteristic might be useful for free-range piggeries, where white pigs are more susceptible to sunburn (Cameron, 1984).

The predominant clustering of feral pig control region sequences from NSW and WA within the European clade is consistent with the importation of mainly, if not exclusively, European domestic pigs during the European settlement there (Choquenot et al., 1996). Three feral sequences from Qld clustered with

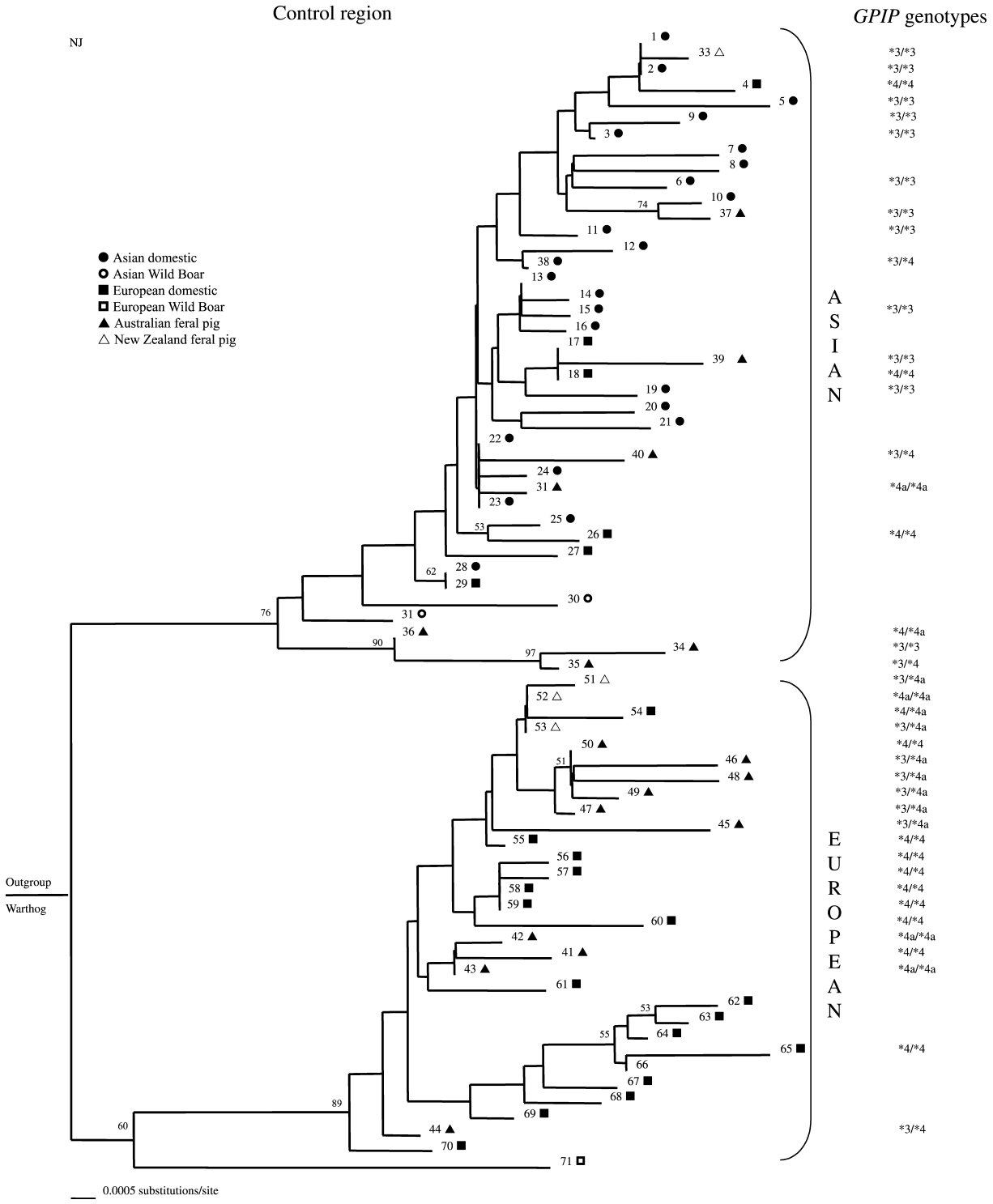

Fig. 3. NJ dendogram based on HKY85 pairwise genetic distances estimated from control region sequences from European and Asian domestic pig and Wild Boar, and Australian and New Zealand feral pigs. The number at the end of each branch corresponds to the animal ID used in Fig. 2 for control region haplotypes. A warthog sequence was used as an outgroup. Bootstrap values (%; 1000 replicates) greater than 50% are indicated on branches. *GPIP* genotypes are mapped onto the corresponding animals in the control region phylogeny used in the present study.

Asian domestic pigs and two of them with European domestic pigs. Some European domestic breeds have similarly high frequencies of Asian mitochondrial sequences (Okumura et al., 2001).

For feral pigs with Asian domestic pig control region sequences it is impossible to distinguish between direct importation of Asian animals into Australia and New Zealand or introgression via Europe of Asian sequences.

This indicates that the control region sequence is not a useful marker for this purpose, but used in conjunction with nuclear markers provides better understanding of the Asian/European and Asian domestic pig contribution into the feral herd.

The discordance between control region sequence and *GPIP* genotypes makes it more difficult to interpret Australian and New Zealand feral pig origins, other than to say that the genetic contributions from the Asian and European origins are quite well mixed. However, one result does provide unequivocal evidence for a direct Asian contribution to the Australian feral pig population. The unexpected clustering of three mtDNA sequences of feral pigs from Cape York in north Qld with Asian Wild Boar implies unrecorded and relatively recent introduction of Wild Boar or their crossbreds into the Australian feral pig herd. These sequences are related to the mtDNA lineage of Ryukyu Wild Boar which is considered to be a descendent of the Asian continental boar distinct from those from northeast Asia and Japanese main islands (Watanobe et al., 1999, 2003). These three feral pigs could have a number of sources: the previous unrecorded release of related lineage of Asian Ryukyu Wild Boar during the early settlements or during the recent migrations of people from Asia (Pullar, 1953); the release of pigs, derived or crossbreeds from southeast Asian Pacific *Sus scrofa vivatus*, perhaps containing Wild Boar genes (Groves and Grubb, 1993; Oliver et al., 1993), sourced from Torres Strait islands, New Guinea or Indonesia; or the release of pigs traded with indigenous Australians by south-east Asian farers, e.g., Macassan trepangers (Macnight, 1976). Of course, the animals with these control region sequences are clearly not ''Asian Wild Boar'' in external phenotype and indeed two of them had European *GPIP* alleles, confirming their hybrid origins. The Westran pig similarly has Asian domestic pig control region sequence, but European *GPIP* alleles, indicative of a similar and mixed origin.

Allen et al. (2001) and Robins et al. (2003) have independently researched the origins of the Auckland Island and Kune Kune feral pigs based on 120 and 394 bp, respectively, of the control region sequence, but were too small to use in the phylogenetic analyses reported here. However, the clustering of the Auckland Island pigs with European pigs reported by Robins et al. (2003) using NJ analysis was consistent with the present study.

The Auckland Island pig control region sequences are consistent with documented European introduction in the 18th century (Choquenot et al., 1996; Clarke and Dzieciolowski, 1991), but the presence of Asian *GPIP* alleles suggests that some released animals were influenced by the Asian pig introgression into European breeds. Their high control region sequence similarity could be explained by founder effects and isolation as a small population in these islands for up to two centuries, and the higher rate of drift for mitochondrial sequences. However, this contrasts with the heterozygosity of *GPIP* genotypes.

The Kune Kune control region sequence and *GPIP* genotype results are consistent with a Pacific/Asian origin. However, it is not possible to determine if Polynesians or Europeans brought this pig into New Zealand.

The present results indicate that feral pigs possess valuable porcine genetic diversity that could be useful for sourcing future breeds for livestock production. Generally, considered an agricultural, environmental, and exotic disease threat (Choquenot et al., 1996), feral pigs can be useful in studies of environmental adaptation and disease resistance, which could be important for commercial pig production. Also, feral animals that have bred in the wild for a long time can be useful for generating new biomedical resources, such as the Westran inbred line (McIntosh and Pointon, 1981).

Further studies of feral pig populations and domestic pig breed origins could be conducted by PCR-RFLP surveys of both *GPIP* and control region markers. Partial amplification of the control region sequence and subsequent restriction digestion using enzymes *Mae*III or *Tsp*45I for position 109, *Mse*I for position 131, *Tsp*509I for position 146 and *Bsa*JI and *Bst*4CI for position 392 would be informative to distinguish between Asian and European origins and could be implemented in larger scale population screening.

Additional samples from a wider geographical area and analyses of *Sus* species and *Sus scrofa* subspecies from northern Australia, southeast Asia, New Guinea and smaller Pacific islands and concurrent archaezoological, historical, and anthropological studies may help to further understanding of the origins of the ''Asian Wild Boar'' control region sequences in these feral animals. Better understanding the origins of feral pigs in Australia will require population sampling, including pigs from southeast Asia and Pacific islands, and analysis of microsatellite markers in the framework of the marker biodiversity databases being accumulated on populations and breeds throughout the world (Li et al., 2003).

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