

# JOURNAL OF ANIMAL SCIENCE

*The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science*

## **Genetic variation and phylogenetics of Lanyu and exotic pig breeds in Taiwan analyzed by nineteen microsatellite markers**

W. H. Chang, H. P. Chu, Y. N. Jiang, S. H. Li, Y. Wang, C. H. Chen, K. J. Chen, C. Y. Lin  
and Y. T. Ju

*J ANIM SCI* 2009, 87:1-8.

doi: 10.2527/jas.2007-0562 originally published online August 15, 2008

The online version of this article, along with updated information and services, is located on  
the World Wide Web at:

<http://jas.fass.org/content/87/1/1>



**American Society of Animal Science**

[www.asas.org](http://www.asas.org)

# Genetic variation and phylogenetics of Lanyu and exotic pig breeds in Taiwan analyzed by nineteen microsatellite markers<sup>1</sup>

W. H. Chang,<sup>\*2</sup> H. P. Chu,<sup>†2</sup> Y. N. Jiang,<sup>\*</sup> S. H. Li,<sup>‡</sup> Y. Wang,<sup>‡</sup> C. H. Chen,<sup>§</sup>  
K. J. Chen,<sup>†</sup> C. Y. Lin,<sup>#</sup> and Y. T. Ju<sup>\*3</sup>

<sup>\*</sup>Department of Animal Science and Technology, National Taiwan University, Taipei, Taiwan 10673;

<sup>†</sup>Taitung Animal Propagation Station, Taitung, Taiwan 95444; <sup>‡</sup>Department of Life Science, National Taiwan Normal University, Taipei, Taiwan 11677; <sup>§</sup>Livestock Research Institute, Tainan, Taiwan 71246; and <sup>#</sup>Hualien Animal Propagation Station, Hualien, Taiwan 97362

**ABSTRACT:** The Lanyu pig is an indigenous miniature pig breed on Lanyu Islet near Taiwan, with a mitochondrial DNA genetic lineage remote from Asian and European pig breeds. The unknown population genetic structure and increased inbreeding among the small population of conserved Lanyu pigs is now of great conservation concern. Additionally, the presence for more than a century of exotic pig breeds in Taiwan has made gene introgression from exotic pig breeds into Lanyu pigs very possible. The present study thus aimed to investigate nuclear genetic variation within the conserved Lanyu pigs and the phylogenetic relationship and possible genetic introgression between Lanyu and exotic pig breeds by determining the polymorphism of 19 microsatellite loci. In the neighbor-joining tree constructed from 7 pig breeds based on Cavalli-Sforza and Edward chord genetic distances, 3 major clades were

recognized, in which the Asian and European breeds were separately clustered into 2 clades with a 93.0 and 99.9% bootstrap confidence value, respectively. All individuals of the Lanyu breed formed a unique subclade within the Asian clade based on the distance of the proportion of shared alleles,  $-\ln(ps)$ , suggesting that the Lanyu breed possesses a unique nuclear genetic structure and that no nuclear gene introgression from exotic breeds into the conserved Lanyu pigs has occurred in recent history. Fifteen of 19 microsatellite loci deviated from Hardy-Weinberg equilibrium (by Wright's statistic), suggesting a significant loss of heterozygosity in the conserved population. The valuable nuclear genetic structure and phylogenetic information should assist future conservation and population management of Lanyu pigs.

**Key words:** genetic variation, introgression, Lanyu breed, microsatellite, phylogenetics, pig

©2009 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2009. 87:1–8  
doi:10.2527/jas.2007-0562

## INTRODUCTION

The Lanyu pig is an indigenous miniature pig breed from Lanyu Islet, off southeast Taiwan, with a characteristic small body, narrow and straight head, small erect ears, and coarse black hair. Its phenotype differs from the domestic Asian and European pig breeds (Cheng, 1986). For the conservation of Lanyu pigs and to develop a laboratory pig stock, 2 herds of Lanyu

pigs were moved from Lanyu Islet to Taiwan, to the National Taiwan University (NTU) teaching farm in 1975, and to the Taitung Animal Propagation Station (TAPS) in 1980 (Chyr et al., 2001). Both herds were allowed panmixia from 1975 to 2004. Only 2 mitochondrial DNA haplotypes have been identified in all 44 heads of conserved Lanyu pigs: the type I Lanyu mitochondrial DNA (mtDNA) sequence is distinct from Asian and European breeds, whereas type II shows some intermediate features (Wu et al., 2007). The nuclear genetic structure within conserved Lanyu pigs remains unknown.

Many exotic pig breeds were introduced into Taiwan to improve the production performance of local pigs, by colonizers from 120 to 150 yr ago and by the Taiwan government in more recent times (Chyr et al., 2001). The Taoyuan breed was introduced from southern China into Taiwan around 1877. The Berkshire breed was imported from Japan in 1898. The Taiwan Livestock

<sup>1</sup>This work was supported by a grant from the National Science Council of Taiwan, Republic of China (NSC 94-2317-B-002-020 and NSC 96-2317-B-002-003), and Council of Agriculture of Taiwan, Republic of China (95AS-11.1.2-AD-U1 and 96AS-11.1.4-AD-U1). We thank Harry Wilson (Institute of Molecular Biology, Academia Sinica, Taiwan) for editing the manuscript.

<sup>2</sup>Both authors made equal contribution to this work.

<sup>3</sup>Corresponding author: ytju@ntu.edu.tw

Received September 5, 2007.

Accepted August 4, 2008.

Research Institute (TLRI) introduced the Hampshire and Yorkshire breeds in 1948, whereas the Landrace and Duroc breeds were imported by the Taiwan Sugar Corporation in 1960. Most recently, the Meishan breed was introduced from Japan by TLRI in 1994. Because most of those exotic breeds were introduced before the conserved Lanyu pigs were first reared in isolation, it is possible that some introgression of nuclear genes from exotic breeds into conserved Lanyu pigs has occurred.

Although the mitochondrial phylogenetics have been recently determined (Jiang et al., 2008), it remained of interest to determine whether the Lanyu breed possesses a unique nuclear genetic structure that matches that seen in the mitochondrial genome and to identify whether nuclear DNA from exotic breeds had introgressed into the conserved population of Lanyu pigs. A thorough investigation of the phylogenetics of this conserved Lanyu pig population was necessary for their future population management and to avoid further inbreeding.

## MATERIALS AND METHODS

The animal research protocols conformed to those approved by the National Taiwan University Animal Care and Use Committee.

### *Blood Sample Collection and Genomic DNA Purification*

Ten milliliters of each whole blood sample from all 44 conserved Lanyu pigs were collected (5 from NTU and 39 from TAPS) via venipuncture of a jugular vein. The exotic pig blood samples, from 36 Taoyuan, 35 Meishan, 32 Landrace, 31 Yorkshire, 32 Duroc, and 30 Berkshire animals, were obtained from TLRI. Genomic DNA was extracted and purified from collected blood samples with QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, CA; Ko et al., 2001). For this paper, the term "European pig breeds" comprises the Berkshire, Duroc, Landrace, and Yorkshire breeds, whereas the term "exotic Asian breeds" is used here for the Meishan and Taoyuan breeds.

### *Microsatellite Genotyping*

Nineteen microsatellite paired primers randomly located on 15 chromosomes, as recommended by the Domestic Animal Diversity Information System of the Food and Agriculture Organization of the United Nations, were chosen for genotyping (FAO, 2004; Table 1). The primers used for genomic DNA amplification were fluorescent end-labeled with carboxyfluorescein, tetrafluorofluorescein phosphoramidite, or hexachlorofluorescein phosphoramidite fluorescent dye. The fragments of microsatellite DNA in the genome were amplified by the PCR (PTC-200 Thermal Controller, M. J. Research, Waltham, MA) in 15- $\mu$ L reaction volumes with 50 ng of genomic DNA, 1.5  $\mu$ L of 10  $\times$  PCR buf-

fer, 0.375  $\mu$ L of 8 mM deoxynucleoside triphosphate, 4.5 pmol sense and antisense primers, and 0.6 units of DNA *Taq* polymerase (Amersham Biosciences, Arlington Heights, IL). The thermal cycling conditions were as follows: an initial 95°C for 5 min, then 37 cycles at 95°C for 30 s, 48 to 62°C for 30 s (depending on the locus), and 72°C for 45 s, with a final extension at 72°C for 7 min. The PCR products were resolved by electrophoresis with a MegaBACE 1000 DNA sequencer (Amersham Biosciences). The fluorescent-labeled marker ET-400 (Amersham Biosciences) was used as an internal size standard for length calibration. Allele sizes were determined using Genetic-Profiler software (version 2.2; Amersham Biosciences).

### *Data Analysis*

The Cavalli-Sforza and Edward chord genetic distances (Cavalli-Sforza and Edwards, 1967) between 7 breeds were obtained by the PHYLIP program package (version 3.6; University of Washington; <http://evolution.genetics.washington.edu/phylip/getme.html>; Felsenstein, 2006). Nei's  $D_A$  genetic distance between 7 breeds was obtained by a microsatellite analyzer (MSA; Dieringer and Schlötterer, 2003). Nei's

$$D_A = \sum_{k=1}^L \left( 1 - \sum_{i=1}^{q_k} \sqrt{x_{ik}y_{ik}} \right) / L, \text{ where } q_k = \text{the number of}$$

alleles of the  $k$ th locus,  $x_{ik} / y_{ik}$  = frequency of allele  $i$  in population  $x/y$ , and  $L$  = the number of loci examined. The proportion of shared alleles ( $ps$ ) is calculated

$$\text{as } ps = \sum_{k=1}^L \sum_{i=1}^{q_k} \min(x_{ik}, y_{ik}) / L, \text{ where } \min(x_{ik}, y_{ik}) \text{ repre-}$$

sents the minimum of  $x_{ik}$  and  $y_{ik}$ . The distance was taken as  $-\ln(ps)$ . The  $-\ln(\text{proportion of shared allele})$  distance within the 44 conserved Lanyu pigs and 196 individuals of 6 exotic breeds were determined by MSA. Allele frequencies at each locus, expected heterozygosity, and observed heterozygosity in conserved Lanyu pigs were calculated using CERVUS software (version 2.0, Field Genetics Ltd., Edinburgh, UK; Marshall et al., 1998). The GENEPOP (Genes in Population, version 3.4b, The Laboratoire de Genetique et Environnement, Montpellier, France) software package (Raymond and Rousset, 1995) was used to calculate Wright's  $F$ -statistic (fixation indices,  $F_{IS}$ ) and to test for deviation from Hardy-Weinberg equilibrium (HWE). It was found that  $F_{IS}$  was used to determine the reduction in heterozygosity in an individual within conserved Lanyu pigs. The fixation indices ( $F_{ST}$ ) as determined by MSA were used to determine genetic diversity among the 7 breeds. The effective number of alleles was estimated according to the formula of Kimura and Crow (1964). The neighbor-joining tree for the 7 breeds based on Cavalli-Sforza and Edward chord genetic distance was constructed using the PHYLIP (version 3.66) software package. Bootstrap values of 1,000 replicates were ob-

**Table 1.** The genetic variation of 19 microsatellite loci in 44 individuals of conserved Lanyu pigs in Taiwan<sup>1</sup>

Locus	Allele (n)	Effective allele	Allele size range	H <sub>o</sub>	H <sub>E</sub>	PIC	HWE test	F <sub>IS</sub>
SW857	3	1.90	104 to 114	0.286	0.480	0.391	**	0.408
IGF1	3	2.38	194 to 202	0.442	0.587	0.488	*	0.249
S0155	3	2.65	160 to 166	0.425	0.630	0.542	**	0.328
S0005	2	1.92	206 to 238	0.400	0.486	0.365	NS <sup>2</sup>	0.179
SW911	3	2.12	157 to 163	0.524	0.534	0.421	NS	0.020
S0068	4	3.07	244 to 256	0.571	0.682	0.614	**	0.176
S0002	5	3.33	174 to 210	0.452	0.694	0.633	**	0.351
S0228	3	2.24	227 to 243	0.419	0.559	0.459	*	0.254
SW024	3	1.45	98 to 118	0.295	0.313	0.282	NS	0.058
S0227	4	1.45	238 to 258	0.182	0.315	0.285	**	0.425
SW72	5	2.48	150 to 160	0.537	0.603	0.545	**	0.112
S0218	4	3.22	166 to 188	0.326	0.697	0.632	**	0.529
S0355	6	2.54	246 to 276	0.455	0.613	0.543	**	0.261
SW122	4	3.06	116 to 138	0.425	0.682	0.613	**	0.380
S0225	3	2.17	174 to 194	0.442	0.545	0.467	*	0.239
S0226	6	2.41	176 to 214	0.341	0.606	0.541	**	0.440
SW951	5	2.75	120 to 136	0.268	0.644	0.571	**	0.586
S0215	2	1.38	156 to 164	0.233	0.276	0.235	NS	0.158
S0386	3	2.92	156 to 172	0.095	0.666	0.584	**	0.858
Mean	3.74	2.39		0.375	0.559	0.485		0.332

<sup>1</sup>Table includes the number of alleles observed, effective alleles, allele size range, mean observed heterozygosity (H<sub>o</sub>), expected heterozygosity (H<sub>E</sub>), fixation indices (F<sub>IS</sub>), polymorphism information content (PIC), and loci deviating from Hardy-Weinberg equilibrium (HWE test, \**P* < 0.05; \*\**P* < 0.01).

<sup>2</sup>NS = not significant.

tained to test the robustness of the tree topology. MEGA3.1 (Molecular Evolutionary Genetics Analysis; Center of Evolutionary Functional Genomics Biodesign, Institute Arizona State University; <http://www.mega-software.net/mega.html>) software (Kumar et al., 2004) was used to construct another neighbor-joining tree based on  $-\ln(\text{proportion of shared allele})$  distance for 240 individuals from the 7 breeds.

## RESULTS

### *Allele Frequencies, Heterozygosity, and HWE Test of Conserved Lanyu Pigs*

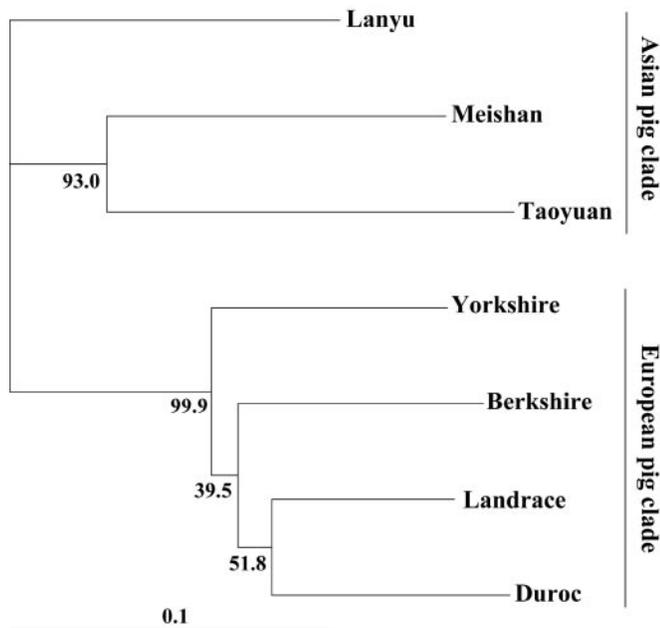
To determine the degree of nuclear genetic diversity within conserved Lanyu pigs, the allele frequencies and heterozygosity of 19 microsatellite markers recommended by the FAO and International Society for Animal Genetics were examined, and HWE tests were performed. The loci of all 19 microsatellite markers in the 44 conserved Lanyu pigs were polymorphic (Table 1). Total alleles per locus varied from 2 (S0005, S0215) to 6 (S0355, S0226), with a mean value at all loci of 3.74. The effective alleles ranged from 1.38 to 3.33, and the mean number of effective alleles was only 2.39 (Table 1). The polymorphic information content (PIC) was calculated to measure the allele diversity at each of the polymorphic loci (Botstein et al., 1980), and allelic PIC ranged from 0.235 to 0.633, with a mean value of 0.485.

To describe the genetic diversity and possible deviations from HWE within the population, the mean observed heterozygosities, mean expected heterozygosities, and HWE test were calculated (Table 1). The ob-

served heterozygosities of all loci (range 0.095 to 0.571) were less than expected heterozygosities (from 0.276 to 0.697) within conserved Lanyu pigs. Fifteen of 19 loci showed significant deviation from HWE (*P* < 0.05), with 12 loci showing severe deviation (*P* < 0.01). The F<sub>IS</sub> of each locus ranged from 0.020 (SW911) to 0.858 (S0386), and the mean F<sub>IS</sub> of all loci was 0.332. Fourteen private alleles located at 12 loci were identified, including 166 (S0155), 163 (SW911), 244 (S0068), 174 (S0002), 118 (SW024), 238 and 258 (S0227), 272 and 276 (S0355), 174 (S0225), 176 (S0226), 136 (SW951), 188 (S0218), and 227 bp (S0228).

### *Mean Genetic Diversity and Neighbor-Joining Tree of Conserved Lanyu and Exotic Pig Breeds*

To assess the genetic distance among conserved Lanyu, Taoyuan, Meishan, Berkshire, Landrace, Duroc, and Yorkshire breeds, the Cavalli-Sforza and Edwards chord distances ( $D_C$ ) and Nei's  $D_A$  distances based on the allele frequencies of 19 microsatellite loci were obtained (Table 2). The  $D_C$  between Duroc and Landrace breeds was 0.1324, similar to the result from Kim et al. (2005). Shorter  $D_C$  were found between Lanyu and Berkshire (0.2360), Yorkshire (0.2383), and Meishan (0.2405) breeds than between Lanyu and Taoyuan (0.2633), Duroc (0.2642), and Landrace (0.2675) breeds. The Nei's  $D_A$  between Lanyu and exotic breeds were similar to  $D_C$  (Table 2). Nei's  $D_A$  between Lanyu and Berkshire (0.6056), Yorkshire (0.6115), and Meishan (0.6172) breeds were shorter than against other exotic pigs (including Taoyuan, Duroc, and Landrace breeds). The genetic distances between European breeds (in-



**Figure 1.** Neighbor-joining tree of Lanyu, Taoyuan, Meishan, Landrace, Yorkshire, Duroc, and Berkshire constructed from Cavalli-Sforza and Edward chord genetic distance by 19 microsatellite marker polymorphisms. The numbers at the branch nodes are the percentages of a group occurrence in 1,000 bootstrap replications of resampled loci.

cluding Landrace, Yorkshire, Duroc, and Berkshire) were shorter than to Asian breeds (including Lanyu, Meishan, and Taoyuan). To understand the phylogenetic relationship between Lanyu and exotic breeds, a neighbor-joining tree was constructed based on  $D_C$ . All European pig breeds were clustered in 1 major clade with a 99.9% bootstrap value after 1,000 resamplings (Figure 1). The Lanyu breed formed a unique clade. The Meishan was clustered with Taoyuan with a bootstrap value of 93.0%. To address whether Lanyu pigs with type I or II mtDNA haplotypes (Wu et al., 2007; Jiang et al., 2008) have different relatedness to exotic breeds,  $D_C$  distances of haplotype type I and type II and different exotic breeds were calculated (Table 3). The  $D_C$  between mtDNA type I haplotype and type II in Lanyu pigs was 0.0225. The  $D_C$  between type I Lanyu haplotype and Berkshire breed and between haplotype type II Lanyu and Berkshire were 0.2408 and 0.2304, respectively; type II Lanyu sequence is closer to the

Berkshire breed than type I. In addition, the  $D_C$  from type II Lanyu to European pig breeds was closer than type I (Table 3).

### *Genetic Variation and Neighbor-Joining Tree of 240 Individuals of Conserved Lanyu and Exotic Pig Breeds*

To determine whether the genetic variation of the 44 conserved Lanyu pigs could demarcate them from all exotic breeds, as seen in the polymorphism of 19 microsatellite markers, a neighbor-joining dendrogram of 240 individuals from 7 breeds was established based on the distance of  $-\ln(\text{proportion of shared alleles})$ . All of the conserved Lanyu and exotic pig breeds (except 1 Berkshire and 1 Yorkshire pig) were classified into their own cluster, indicating the absence of nuclear gene introgression between conserved Lanyu pigs and 99% of exotic breed pigs examined here (Figure 2). Although the NTU Lanyu pigs clustered with TAPS Lanyu pigs to form a unique clade, the NTU Lanyu pigs formed a unique subclade separated from the major Lanyu pig clade.

## DISCUSSION

### *Genetic Diversity Within Conserved Lanyu Pigs*

In total, 71 alleles were identified in 19 microsatellite loci within the Lanyu pig genome. The PIC value is used to evaluate the informativeness of each microsatellite marker under study. Here, the mean measured PIC value of 19 microsatellite markers was 0.485, ranging from 0.235 to 0.633, indicating that 10 markers were highly informative ( $\text{PIC} > 0.5$ ), 8 markers were reasonably informative ( $0.5 > \text{PIC} > 0.25$ ), and the final marker was only slightly informative (S0215,  $\text{PIC} < 0.25$ ; Botstein et al., 1980). The range in size of the alleles was 98 to 276 bp, consistent with the result of Yang et al. (2003), who used the same microsatellite markers to study genetic variation among 18 Chinese indigenous pig breeds. The number of effective alleles at the 19 loci ranged from 1.38 (S0215) to 3.33 (S0002), based on the Kimura and Crow (1964) formula. All detected allele numbers at the 19 loci were greater than their effective

**Table 2.** Cavalli-Sforza and Edward chord (below the diagonal) and Nei's  $D_A$  (above the diagonal) genetic distances between Lanyu, Taoyuan, Meishan, Landrace, Yorkshire, Duroc, and Berkshire pig breeds by 19 microsatellite marker polymorphisms

Breed	Lanyu	Meishan	Taoyuan	Landrace	Yorkshire	Duroc	Berkshire
Lanyu		0.6172	0.6757	0.6863	0.6115	0.6780	0.6056
Meishan	0.2405		0.6028	0.7515	0.7320	0.7039	0.7391
Taoyuan	0.2633	0.2347		0.7804	0.7524	0.7959	0.8134
Landrace	0.2675	0.2927	0.3042		0.3413	0.3395	0.3747
Yorkshire	0.2383	0.2852	0.2932	0.1331		0.4473	0.4255
Duroc	0.2642	0.2743	0.3101	0.1324	0.1743		0.4165
Berkshire	0.2360	0.2880	0.3170	0.1461	0.1659	0.1623	

**Table 3.** Cavalli-Sforza and Edward chord genetic distances between type I and type II haplotype mitochondrial DNA of Lanyu, Taoyuan, Meishan, Landrace, Yorkshire, Duroc, and Berkshire pig breeds by polymorphisms of 19 microsatellite markers

Breed	Lanyu I	Lanyu II	Meishan	Taoyuan	Landrace	Yorkshire	Duroc
Lanyu II	0.0225						
Meishan	0.2422	0.2455					
Taoyuan	0.2646	0.2660	0.2347				
Landrace	0.2736	0.2625	0.2927	0.3042			
Yorkshire	0.2425	0.2372	0.2852	0.2932	0.1331		
Duroc	0.2713	0.2537	0.2743	0.3101	0.1324	0.1743	
Berkshire	0.2408	0.2304	0.2880	0.3170	0.1461	0.1659	0.1623

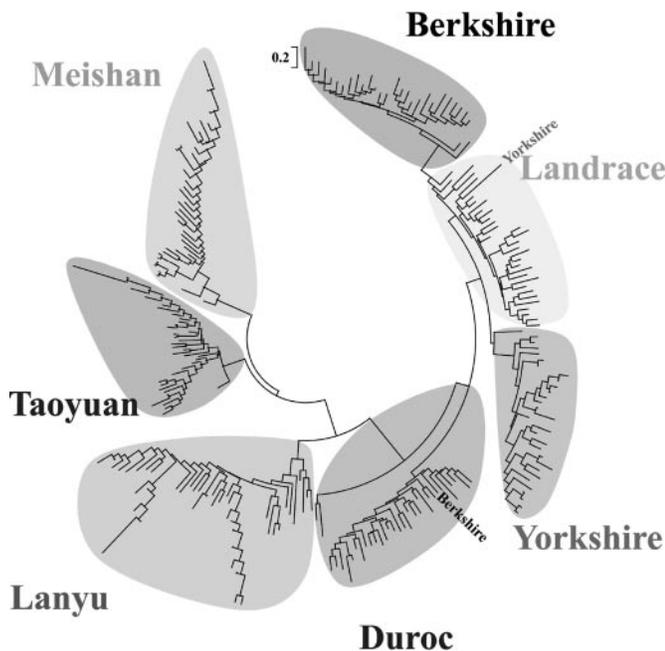
allele number, which suggests that the 19 markers used in the present study were suitable for determination of genetic polymorphisms and diversity within the Lanyu pigs. The average number of alleles (3.74) for 19 loci in the conserved Lanyu pigs is less than the mean of 13.31 alleles present in 26 microsatellite loci of 18 Chinese breeds (Yang et al., 2003) and 8.19 alleles present in 20 microsatellite loci of 10 Chinese breeds (Li et al., 2004), suggesting either homogenous selection or gene flow restriction in the Lanyu pig population.

The observed heterozygosities (mean 0.375) of all 19 microsatellite loci were less than expected (mean 0.559). Fifteen of 19 loci deviated significantly from Hardy-Weinberg equilibrium ( $P < 0.05$ ), including 12 loci with very significant disequilibria ( $P < 0.01$ ). All  $F_{IS}$  values (mean 0.332) of the 19 microsatellite loci in conserved Lanyu pigs were positive, suggesting a severe loss of heterozygosity in individuals due to panmixia within the conserved population. In addition, our previous studies (Wu et al., 2007; Jiang et al., 2008) of the

diversity of mitochondrial cytochrome b sequences in conserved Lanyu pigs showed significant deviation from neutrality (Fu and Li's test = 1.44055,  $P < 0.05$ ), low haplotype number ( $h = 0.384 \pm 0.066$ ), and low nucleotide diversity ( $\pi = 0.0037 \pm 0.00064$ ). Taken together, the less number of alleles by locus, less mean observed heterozygosity than expected heterozygosity, and positive  $F_{IS}$  values detected in microsatellite DNA and loss of the diversity of mtDNA haplotype demonstrated a severe loss of heterozygosity both in mitochondrial and nuclear genomes within the population of conserved Lanyu pigs. The current Hardy-Weinberg disequilibria of conserved Lanyu pigs may be a consequence of the original narrow genetic basis (5 males and 19 females were used to found the 2 herds before 1980), and this founder effect may have been exacerbated by the small conserved population and consanguineous mating.

**Genetic Distance and Phylogenetic Relationship Among Lanyu and Other Pig Breeds**

The  $F_{ST}$  statistic was calculated to estimate the genetic differentiation between Lanyu and 6 exotic pig breed populations (refer to Suppl. Table S1; <http://jas.fass.org/content/vol87/issue1/>). The  $F_{ST}$  value of each microsatellite locus ranged from 0.2171 (SW122) to 0.6052 (S0215), and all 19 microsatellite loci deviated significantly from 0 ( $P < 0.01$ ). The overall  $F_{ST}$  value obtained from 19 microsatellite loci from the 7 breeds was 0.3988 (refer to Suppl. Table S1). This mean  $F_{ST}$  value indicates that about 39.9% of the total genetic variation can be attributed to differences between populations and 60.1% to differences within populations, indicating a clear genetic structuring of breeds used in this study. Nevertheless, small differentiation indices ( $F_{ST}$  from 0.077 to 0.22) have also been found in studies of 18 and 10 Chinese breeds (Yang et al., 2003; Li et al., 2004), with a greater level of genetic differentiation between 11 European breeds (0.27, Laval et al., 2000). A contrary result was obtained in an analysis of the genetic diversity of 52 European and 46 Chinese pig populations by 39 microsatellite markers based on a pooled DNA samples method, indicating the measured differentiation of breeds might be very dependent on the type (including number) of breeds and the meth-



**Figure 2.** The neighbor-joining tree was constructed based on  $-\ln(\text{proportion of shared alleles})$  distances among 240 individuals from 7 pig breeds by 19 microsatellite marker polymorphisms.

odological approach (Megens et al., 2008). The breeds used in this study have a high level of genetic differentiation and are very distinct from each other. The high differentiation indices detected in the present analyses may be due to the natural genetic variability of the breeds studied.

The Cavalli-Sforza and Edward  $D_C$  and Nei's  $D_A$  (Nei et al., 1983) genetic distances are more accurate ways of measuring the genetic distance from microsatellite frequency data (Takezaki and Nei, 1996). Both  $D_C$  and Nei's  $D_A$  genetic distances between Lanyu and exotic breeds were calculated; both genetic distances showed consistent results (Table 2). The closest genetic distance was determined to be between Lanyu and Berkshire breeds ( $D_C$  0.2360 and Nei's  $D_A$  0.6056), followed by, in turn, the Yorkshire, Meishan, Taoyuan, Duroc, and Landrace breeds. The genetic distances between European pig breeds (including Berkshire, Duroc, Landrace, and Yorkshire) were much shorter ( $D_C$  range 0.1324 to 0.1743) than the distance ( $D_C$  range 0.2347 to 0.2633) between Asian pig breeds (including Lanyu, Meishan, and Taoyuan breeds). The distances between Lanyu versus Berkshire or Yorkshire ( $D_C$  0.2360 or 0.2383) were closer than between Lanyu and Meishan or Taoyuan breed ( $D_C$  0.2405 or 0.2633), suggesting minimal gene flow between Lanyu, Meishan, and Taoyuan breeds in recent times. The relationship of  $D_C$  and Nei's  $D_A$  genetic distance between Lanyu and the exotic breeds is not consistent with the time of importation of each exotic breed into Taiwan.

The Berkshire and Yorkshire are categorized as Old English pigs. Their original lop-ear appearance was changed to a prick-ear phenotype before the late 19th century. Historical records point conclusively to genetic introgression of Chinese pigs into the Berkshire and Yorkshire breeds during their development (Jones, 1998; Rothschild and Ruvinsky, 1998), but there is a lack of any genetic evidence to support the historical record. Our previous study revealed a very close pairwise genetic distance of type II mtDNA to Yorkshire mtDNA (Wu et al., 2007; Jiang et al., 2008), with a difference of 1 nucleotide in cytochrome b (1,140 nucleotides in length) and 4 nucleotides in the control region (1,163 nucleotides in length). The cytochrome b and control region of Berkshire mtDNA clustered with type II mtDNA in a cluster of Asian breeds based on pairwise distances (Wu et al., 2007; Jiang et al., 2008). The close genetic distances in both mtDNA and microsatellite loci between Lanyu versus Berkshire and Yorkshire suggests a close genetic lineage between Lanyu and the 2 exotic breeds. The development of the Yorkshire and Berkshire breeds in the early 19th century saw a documented continued decrease in body size that has been suggested to be the result of introgression of pig breeds from China (Porter, 1993). As a corollary, a synthetic breed referred to as the Lee-sung pig interbred from the Landrace and Lanyu breeds shows prick ears and white coat color (refer to Suppl. Figure S1). In consideration of the sum of the short genetic distances,

prick ears, and historical records, we hypothesize that the historical development of the Berkshire and Yorkshire breeds might indeed have involved introgression from ancestors of the Lanyu pig. The Yorkshire has mtDNA similar to type II haplotype mtDNA in Lanyu pig, whereas the Berkshire may have seen introgression of mitochondria from other breeds from mainland Asia. Nevertheless, although there is no documentary evidence that the Lanyu pig was previously present on the main island of Taiwan, we cannot rule out the possibility that the close genetic distance between Lanyu and Berkshire was due to genetic introgression from Berkshire into Lanyu pigs when the Japanese occupied Taiwan. A closer genetic distance was also identified between Lanyu and Meishan than between Lanyu and Taoyuan. The phenotypes of the Taoyuan and 2 extinct breeds (Tingshuanghsi and Minung breeds) in Taiwan are similar to those of the Meishan and Lower Changjiang River Basin breeds in Mainland China (Cheng, 1986; Chyr et al., 2001); hence, we could not rule out the possibility that the Meishan and Lower Changjiang River Basin breeds were introduced into Taiwan before the Taoyuan breed.

### *Interpopulation Structure Among Lanyu and Exotic Breeds*

The  $D_C$  genetic distances for 19 microsatellite loci among 7 breeds were used to construct their neighbor-joining tree (Figure 1). Three major clades were recognized. The Lanyu breed formed a unique clade, the Meishan and Taoyuan breeds formed the second clade, and the European breeds (including Yorkshire, Berkshire, Landrace, and Duroc) formed the remaining clade. The European breeds were clustered and separated from Asian breeds (Lanyu, Meishan, and Taoyuan breeds) with a high bootstrap value (99.9%). In addition, the nucleotides in positions 109, 131, 145, 390, and 1089 of the control region of Lanyu mtDNA sequences were identical to Asian pigs, a feature suggested to be diagnostic between Asian and European pigs (Gongora et al., 2004; Wu et al., 2007). Together, characterization of mtDNA and diversity of microsatellite loci suggest that the Lanyu breed should be classified as an Asian breed. Nevertheless, the Lanyu pig and European breeds were separated from Meishan and Taoyuan breeds with a confidence bootstrap value of 93.0%, suggesting that the Lanyu breed possesses a distinct nuclear genetic lineage to Taoyuan and Meishan breeds. Together, these results suggest minimal gene flow between these breeds in recent times, that the Lanyu breed has a unique genetic lineage in its nuclear genome, and that it is remote from Asian and European-type breeds.

### *Genetic Structure of 240 Individual Lanyu and Exotic Pigs*

The neighbor-joining tree based on  $-\ln(\text{proportion of shared alleles})$  distance (Bowcock et al., 1994) was

constructed from 240 individuals of 7 breeds by the polymorphism of 19 microsatellite loci. Only 2 out of 240 individual pigs clustered with different breeds, indicating that 99.2% of the individuals can be assigned to their own breeds. This suggests that the polymorphism of the 19 microsatellite loci used in the present study is useful for breed demarcation. The 44 Lanyu pigs were clustered into a single clade, and no exotic pig was clustered in the Lanyu pig clade, again suggesting that no interbreeding occurred between Lanyu and exotic breeds in recent times. The 5 Lanyu pigs from the NTU teaching farm formed a subclade within the Lanyu pig clade, which may be due to the small inbred sample from the NTU teaching farm (Suppl. Figure S2). Nevertheless, we could not exclude the possibility of a different genetic lineage of the original founders of the Lanyu pig population (1 male and 3 females) at the NTU teaching farm in 1975 to the TAPS Lanyu pigs (originally 4 male and 16 female founders in 1980).

### ***Genetic Relationship of mtDNA Haplotype and Microsatellite Loci in Conserved Lanyu Pigs***

The maternal genetic structure of conserved Lanyu pigs based on the polymorphism of mitochondrial genomic sequences was analyzed in our previous study. Only 2 specific mtDNA haplotypes (type I and II) were identified (Wu et al., 2007; Jiang et al., 2008). In the present study, the nuclear genetic distance and lineage within the conserved Lanyu pig population was determined based on the polymorphism of 19 microsatellite loci. Controversially, a close  $D_C$  genetic distance (0.0225) was identified between pigs possessing type I and Type II mtDNA haplotypes, and the phylogenetic structure based on  $-\ln(\text{proportion of shared alleles})$  distance from all 44 conserved Lanyu pigs did not correlate with the phylogenetic structure based on the pairwise distance of their mtDNA sequences (Suppl. Figure S2). This lack of correlation may reflect differences in the mechanisms and rate of mtDNA and nuclear genome replication, evolution, and reassortment (Gamow, 2004).

### ***Formation of the Unique Genetic Structure and Their Speciation***

How does the Lanyu pig possess both nuclear and mitochondrial unique genomes? The tiny Lanyu Islet measures only 45.74 km<sup>2</sup> overall. Nearly 3,000 aborigines reside on this small islet today. When we examined the haplotypes of mtDNA of the extant pigs on the Lanyu Islet in 2005, only type I and exotic pig mtDNA were identified (Jiang et al., 2008). No additional haplotype of mtDNA was obtained from the Lanyu Islet, leading us to hypothesize that the Lanyu breed might have developed from a small founder population when they were originally domesticated on the Lanyu Islet. After that, a combination of the geographic barrier limiting

gene flow and genetic drift led to the speciation of the Lanyu breed. In addition, the founder effect was again enhanced after they were further isolated as conserved herds from a very small founder population (5 males and 19 females) after 1975. Here again, our conclusions should be considered relative to the limited sample of breeds examined and should not preclude conclusions that might be obtained from a more comprehensive set of breeds.

In conclusion, the Lanyu pig possesses unique genetic signatures in both nuclear and mitochondrial genomes and is distinct from both Asian and European domestic breeds. The serious loss of nuclear genetic diversity in the conserved Lanyu pig population identified in this study parallels that found in the mitochondrial genomes in our earlier study, suggesting that significant attention must be directed to management of the remaining animals to maintain their genetic diversity. Specific knowledge of the genetic identity of each animal presented here should greatly assist such a goal. This miniature pig breed, nonetheless, has much potential for future development as a novel animal model to study mechanisms of genetic disease in mammals.

## **LITERATURE CITED**

- Botstein, D., R. L. White, M. Skolnick, and W. R. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32:314–331.
- Bowcock, A. M., A. R  iz-Linares, J. Tomfohrde, E. Minch, J. R. Kidd, and L. L. Cavalli-Sforza. 1994. High resolution human evolutionary trees with polymorphic microsatellites. *Nature* 368:455–457.
- Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: Models and estimation procedures. *Am. J. Hum. Genet.* 19:233–257.
- Cheng, P. 1986. Description of Chinese pig breeds. Page 150–155 in *Pig Breeds in China*. Z. Zhang, ed. Shanghai Scientific and Technical Publishers, Shanghai, China.
- Chyr, S. C., K. J. Lin, H. L. Chang, T. S. Yang, and H. T. Yen. 2001. Guidelines in Animal Science-Swine Production. Chinese Society for Animal Science, Taipei, China.
- Dieringer, D., and C. Schl  tterer. 2003. Microsatellite analyzer (MSA): A platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes* 3:167–169.
- Felsenstein, J. 2006. PHYLIP Version 3.66 Executables for Power-Mac. University of Washington, Seattle, WA.
- FAO. 2004. Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD): Recommended Microsatellite Markers. <http://dad.fao.org/cgi-bin/getblob.cgi?sid=59253a437cbea40e38a81682a1e805ee,50005882> Accessed Apr. 6, 2005.
- Gamow, G. 2004. Pages 115–131 in *Philosophies and Methods of Molecular Data Analysis*. Sinauer Associates Inc., Sunderland, MA.
- Gongora, J., P. Fleming, P. B. S. Spencer, R. Mason, O. Garkavenko, J. N. Meyer, C. Droegemueller, J. H. Lee, and C. Moran. 2004. Phylogenetic relationships of Australian and New Zealand feral pigs assessed by mitochondrial control region sequence and nuclear GPIP genotype. *Mol. Phylogenet. Evol.* 33:339–348.
- Jiang, Y. N., C. Y. Wu, C. Y. Huang, H. P. Chu, M. W. Ke, M. S. Kung, K. Y. Li, C. H. Wang, S. H. Li, Y. Wang, and Y. T. Ju. 2008. Inter-population and intra-population maternal lineage genetics of Lanyu pig (*Sus scrofa*) by analysis of mitochon-

- drial cytochrome b and control region sequences. *J. Anim. Sci.* 86:2461–2470.
- Jones, G. F. 1998. Genetic aspects of domestication, common breeds and their origin. Pages 17–50 in *The Genetics of the Pig*. M. F. Rothschild and A. Ruvinsky, ed. CAB International, Wallingford, UK.
- Kim, T. H., K. S. Kim, B. H. Choi, D. H. Yoon, G. W. Jang, K. T. Lee, H. Y. Chung, H. Y. Lee, H. S. Park, and J. W. Lee. 2005. Genetic structure of pig breeds from Korea and China using microsatellite loci analysis. *J. Anim. Sci.* 83:2255–2263.
- Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. *Genetics* 49:725–738.
- Ko, Y., J. Abel, V. Harth, P. Bröde, C. Antony, S. Donat, H. P. Fischer, M. E. Ortiz-Pallardo, R. Their, A. Sachinidis, H. Vetter, H. M. Bolt, C. Herberhold, and T. Brüning. 2001. Association of CYP1B1 codon 432 mutant allele in head and neck squamous cell cancer is reflected by somatic mutations of p53 in tumor tissue. *Cancer Res.* 11:4398–4404.
- Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief. Bioinform.* 5:150–163.
- Laval, G., N. Iannuccelli, C. Legault, D. Milan, M. A. Groenen, E. Giuffra, L. Andersson, P. H. Nissen, C. B. Jorgensen, P. Beeckmann, H. Geldermann, J. L. Foulley, C. Chevalet, and L. Ollivier. 2000. Genetic diversity of eleven European pig breeds. *Genet. Sel. Evol.* 32:187–203.
- Li, S. J., S. H. Yang, S. H. Zhao, B. Fan, M. Yu, H. S. Wang, M. H. Li, B. Liu, T. A. Xiong, and K. Li. 2004. Genetics diversity analyses of 10 indigenous Chinese pig populations based on 20 microsatellites. *J. Anim. Sci.* 82:368–374.
- Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7:639–655.
- Megens, H. J., R. P. M. A. Crooijmans, M. S. Cristobal, X. Hui, N. Li, and M. A. M. Groenen. 2008. Biodiversity of pig breeds from China and Europe estimated from pooled DNA samples: Differences in microsatellite variation between two areas of domestication. *Genet. Sel. Evol.* 40:103–128.
- Nei, M., F. Tajima, and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *J. Mol. Evol.* 19:153–170.
- Porter, V. 1993. Page 256 in *Pigs: A Handbook to the Breeds of the World*. Cornell University, Ithaca, NY.
- Raymond, M., and F. Rousset. 1995. Genepop (version 3.4): Population genetics software for exact tests and ecumenicism. *J. Hered.* 896:248–249.
- Rothschild, M. F., and A. Ruvinsky. 1998. *The genetics of the pig*. CAB International, London, UK.
- Takezaki, N., and M. Nei. 1996. Genetic distance and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* 144:389–399.
- Wu, C. Y., Y. N. Jiang, H. P. Chu, S. H. Li, Y. Wang, Y. H. Li, Y. Chang, and Y. T. Ju. 2007. Type I Lanyu pig has a maternal genetic lineage distinct from Asian and European pigs. *Anim. Genet.* 38:499–505.
- Yang, S. L., Z. G. Wang, B. Liu, G. X. Zhang, S. H. Zhao, M. Yu, B. Fan, M. H. Li, T. A. Xiong, and K. Li. 2003. Genetic variation and relationships of eighteen Chinese indigenous pig breeds. *Genet. Sel. Evol.* 35:657–671.

## References

This article cites 19 articles, 6 of which you can access for free at:  
<http://jas.fass.org/content/87/1/1#BIBL>