

GENETIC STRUCTURE OF FORMOSAN MINIATURE PIGS AND THEIR CORRECT INDIVIDUAL ASSIGNMENT APPROACH

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Lanyu pig has a distinct maternal genetic lineage remote from Asian and European pigs

The Lanyu pig is an indigenous breed from Lanyu Islet, located south-east of Taiwan, with quite distinct phenotypic characteristics from other pig breeds in Asia and Europe. From geographic considerations, the Lanyu pig may have originated from mainland China, Austronesia or the Ryukyu Islands. Two herds of Lanyu pigs were imported from Lanyu Islet into Taiwan and reared in isolation on two different farms before 1980. In the present study, polymorphism of the mitochondrial DNA control region sequence was used to clarify phylogenetic relationships among Lanyu, Asian and European pigs. The conserved Lanyu pigs had two distinct haplotypes of mitochondrial control region divergence. The type I Lanyu sequence appeared independently as a unique clade different from Asian and European pig sequences, while type II Lanyu sequence was clustered in the major Asian clade. The pairwise distances between the major Asian clade versus type I Lanyu clade and European clade are 0.01726 ± 0.00275 and 0.01975 ± 0.00212 , respectively. Estimates of divergence time suggest that type I Lanyu sequence split from the major Asian pig clade in prehistoric times. The type II Lanyu mtDNA shares a close genetic lineage with Japanese Satsuma and New Zealand Kune Kune mtDNA with 0.00095 ± 0.00000 and 0.00192 ± 0.00000 pairwise distances respectively, indicating gene flow between Lanyu Islet, Japan and Oceania in recent times. Together these results indicate that the Lanyu pig (type I sequence) has a remote genetic lineage from Asian-type pigs, while the type II Lanyu sequence may represent a more recent introgression of modern Asian pigs.

Inter-population and intra-population maternal lineage genetics of Lanyu pig (*Sus scrofa*) by analysis of mitochondrial cytochrome b and control region sequences

The Lanyu pig possesses a distinct maternal genetic lineage remote from Asian and European pigs. The part II aimed to understand the phylogenetic relationship among conserved Lanyu, Asian and European type pigs based on the *cytochrome b* coding gene, to ascertain the maternal lineage and genetic diversity within the conserved Lanyu pigs, and to address whether genetic introgression from exotic or Formosan wild pigs had occurred in the conserved Lanyu pigs. When Maximum Likelihood trees were constructed, the Type I Lanyu mitochondrial genes formed a unique clade with a large pairwise distance of both *cytochrome*

b and control region from Asian and European type breeds, Formosan wild pigs and exotic breeds. Significant loss of genetic diversity of mtDNAs within the conserved Lanyu pigs was demonstrated by low haplotype and nucleotide diversities, supported by Fu and Li's D^* neutrality test (1.44055 ; $P < 0.05$). These results indicate no maternal lineage mtDNA gene introgression from Formosan wild pigs and introduced exotic pigs to conserved Type I Lanyu pigs, and a severe loss of heterozygosity of mtDNA in conserved Lanyu pigs. The remaining extant pigs in the Lanyu Islet have been introgressed with exotic breeds. Strategies for the future conservation of native Lanyu pigs are now even more urgent and important.

Genetic variation and phylogenetics of Lanyu and exotic pig breeds in Taiwan analyzed by 19 microsatellite markers

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 Part III thus aimed to investigate nuclear genetic variation within the conserved Lanyu pigs, and study the phylogenetic relationship and any genetic introgression between Lanyu and exotic pig breeds by determining the polymorphism of 19 microsatellite loci. In the Neighbor-Joining tree constructed from seven pig breeds based on Cavalli-Sforza and Edward chord genetic distances, three major clades were recognized, where the Asian and European breeds were separately clustered into two clades with a 93.0 and 99.9 % bootstrap confidence value, respectively. All individuals of the Lanyu breed formed a unique subclade within (near to) the Asian clade based on $-\ln(\text{proportion of shared alleles})$, 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. 15 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 the future conservation and population management of Lanyu pigs.

Genetic structure of Formosan miniature pigs and their correct individual assignment approach

The Lanyu is an indigenous miniature pig breed in Taiwan. It has been used to generate two separate lines (Spotty Lanyu and Binlang) and crossbred with Landrace and Duroc to produce two hybrids (Lee-Sung and Mitsai, respectively) for use in laboratories. Keeping track of the genetic origins of laboratory animals and stud stock is an important function of genetic databases. In this paper, these pigs and the following breeds: Berkshire, Duroc, Landrace and Yorkshire, Meishan and Taoyuan, TLRI Black Pig No.1, Kaohsiung Animal Propagation Station Black pig are studied to build a genetic reference database. Nineteen microsatellite markers were used to give information on genetic structure and the array of genetic variation and differentiation among the studied breeds. The method ranked microsatellite markers based on high heterozygosity, high F_{ST} , allelic number or high D_{scores} . Assignment was done using the Bayesian method with ≥ 99.4 % assignment accuracy being obtained. 99 % of the 352 animals studied were assigned into their correct reference

populations based on 14 markers for ranking high F_{ST} , 10 markers for high H_E , 10 markers for high K, and 9 markers for high Dscores. To reduce error of assignment among the Lanyu and its derived lines and breeds, the Lanyu breeds' characteristics of high polymorphic, heterozygosity, F_{ST} and Dscores were used to correct individual assignments. Ranking for highest H_E , K, and F_{ST} , only 6 markers for high H_E , 7 markers for high F_{ST} and 8 markers for high K were required to obtain 99 % assignment accuracy.

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