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Genetic differentiations between randomly and selectively bred pig populations in Yunnan, China

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Abstract: To assess the genetic diversity between randomly and selectively bred populations, we sequenced 438 bp of the mitochondrial DNA control region from 102 pigs. These samples represented four native pig breeds, one nucleus and one conservation herd from Yunnan, China. Twenty haplotypes with sixteen polymorphic sites were identified. The number of haplotypes in the nucleus herd of Saba pig and the conservation herd of Banna miniature pig were restricted to three and one, respectively, while the randomly bred pig populations exhibited over six haplotypes. Notably, haplotype diversity in randomly bred populations was significantly greater than the selectively bred populations (h=0.732 vs. 0.425 and 0, exact test, $P \leq 0.0036$). These findings demonstrate that selective breeding generated low genetic diversity compared to randomly bred pig breeds. A timely intervention and well programmed breeding approach would stop further genetic diversity reduction in the nucleus and conservation herds of native pig breeds. Otherwise, selective breeding would dramatically reduce genetic diversity in only several years, indicating that sharp contradictions exist between breeding, conservation and genetic diversity. Genetic relationships are discussed based on net genetic distances among pig populations.

Key words: Yunnan pig breeds; Genetic diversity; Randomly bred population; Selectively bred population

云南地方猪种随机群体与选育群的遗传差异

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摘要：为估计随机群体与选育群间的遗传差异, 测定了云南地方猪种 102 个个体 438 bp 的线粒体 DNA D-loop 片段, 涉及 4 个随机群体、1 个保种群和 1 个核心群。检测的 16 个多态位点界定了 20 个单倍型, 撒坝猪核心群和版纳微型猪保种群的单倍型数量仅为 3 个和 1 个, 而 4 个随机群体的单倍型数量都在 6 个以上, 4 个随机群体的单倍型多态度极显著高于这两个选育群(h=0.732 对 0.425 和 0.000, exact test, $P \leq 0.0036$)。结果发现, 选育会导致遗传多样性过低, 尽管在随机群体中有较高的遗传多样性, 基于此, 及早干预和合理的育种计划将会有效地阻止核心群和选育群的遗传多样性下降。否则, 选育甚至会在短短几年内使遗传多样性程度急剧下降, 表明育种、保种与遗传多样性保存之间存在着尖锐冲突。该文还讨论了几个群体的遗传关系。

关键词：云南猪种; 遗传多样性; 随机群体; 选育群
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All mitochondrial DNA (mtDNA) variations originate from mutations (Brown et al, 1979; Wallace, 1995), which makes it ideal for studying genetic diversity of wild and domestic animals. Over the years mtDNA has become an efficient marker for analyzing the genetic relationships and divergence of intra- and inter-mammalian populations (Brown et al, 1982; Watanabe et al, 1986,1999,2001; Disotell et al, 1992; Zhang & Oliver, 1993; Luikart et al, 2001). The genetic relationship between wild boar and the domestic pig was reported by comparing the mtDNA (Watanabe et al, 1986, 1999; Giuffra et al, 2000; Okumura et al, 2001). The complete mtDNA sequence of the pig (*Sus scrofa*) was published (Ursing & Arnason, 1998). Ancient pig mtDNA was amplified and sequenced to clarify its origin and transition (Watanabe et al, 2001). Some authors held that Chinese pigs were introduced to improve European pig breeds during the 18th and early 19th centuries (Brown et al, 1982; Jone, 1998; Giuffra et al, 2000; Kijas & Anderson, 2001; Kim et al, 2002). Recent finding revealed that pigs were domesticated in the Mekong region and in the middle and downstream regions of the Yangtze River in East Asia (Wu et al, 2007). Although genetic diversity is a vital index for species diversity and conservation, humans depend on crossbreeding to improve the productivity and adaptability of livestock. Due to its harsh climate, geographical condition, and inconvenient transport, several local pig breeds were formed in the Yunnan Province of China (Lian et al, 2003). To protect and utilize the local pig resources, breeders have tried selection and crossbreeding (Lian et al, 2003). However, whether genetic diversity in maternally inherited mtDNA affects the success or failure of selection and conservation after selective breeding for some generations remains unclear. Consequently, we analyzed 102 pig samples for the 438 bp mtDNA control region to study the genetic diversity in randomly and selectively bred pig breeds from Yunnan.

1 Material and Methods

1.1 Breeding background of native Yunnan pig breeds

A breeding herd of Saba pigs was established in 1991 at the Pig Breeding Farm of Chuxiong Prefecture, consisting of four boars and twenty sows from Luquan County in Yunnan. In 1993, fifteen boars and fifty-five sows from Shuangbai County in Yunnan were added to the breeding herd. After observation and testing, eight boars and thirty-five sows were selected to remain in the breeding herd. In 1996, the nucleus herd was established, in which one boar and four sows from Yao'an County were introduced. Systematic selective breeding was used and high quality animals were selected for repeated reproduction. The nucleus herd was opened periodically. Random mating and organized mating were equally practiced. A sow yielded for every generation annually (Lian et al, 2003). Similarly, in the conservation herd of Banna miniature pigs from the Pig Breeding Farm of Xishuangbanna Prefecture, eight boars and forty sows were introduced from the remote countryside in 1987. A proportion of the population was moved to Yunnan Agricultural University of Kunming in 1990 as part of a conservation program. Systematic breeding was also conducted on the Banna miniature pig. The herd was closed after the base population was grouped, and randomly mating was practiced, with the exception of sib mating. Closed herd breeding was subsequently carried out (Lian et al, 2003). The initial base population of the Baoshan pigs consisted of three boars and forty-seven sows brought from mountainous villages of Shidian County in Baoshan Prefecture. In 2001, six boars and thirty-seven sows were introduced from Longyang District, Shidian County, and Changning County to join the base population. The nucleus herd was opened periodically (Lian, 2005) and an open nucleus breeding system was also applied to the Baoshan pigs. High quality animals were retained and used to reproduce repeatedly.

Randomly bred populations were comprised of native pig breeds from the remote countryside. These included Saba pigs from Lufeng County, Diqing Tibetan pigs from Zhongdian County, and Banna miniature pigs from Xishuangbanna Prefecture (Fig. 1).

1.2 DNA isolation, PCR amplification and automated DNA sequencing

Blood or muscle tissue samples were collected from pigs from different regions of Yunnan Province (Fig. 1). We randomly sampled inter-families, and focused on morphological characteristics to exclude hybrids. The DNA was isolated and purified (Huang et al, 1999), and 20−50 ng genomic DNA was used as a template for polymerase chain reaction (PCR) amplification of the mtDNA control region. The primers mitL99 (5'-^{15336}CCCAAAGCTGAAATTCTAAA-3') and mitH451 (5'-^{15822}GGTGAGATGGCCCTGAAGTAAG-3') were designed (Takeda et al, 1995) using a reference sequence of *Sus scrofa* (Accession nos. AF304201).

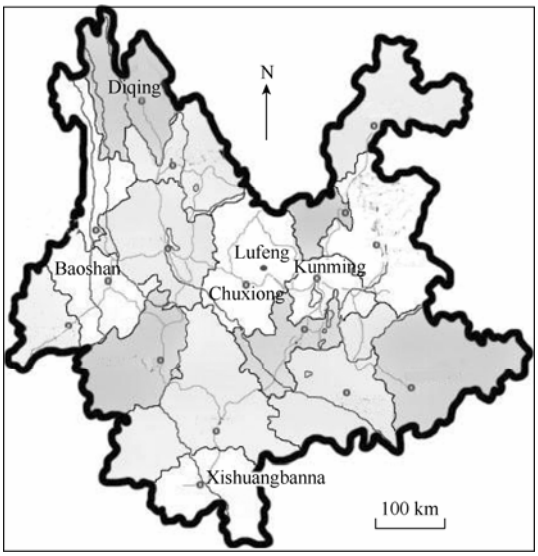

Fig. 1 Map of Yunnan, China, showing sampled prefectures/counties

The PCR amplifications were performed in 50 μL of 2.5 mmol/L of $MgCl_2$, 200 μmol/L of each dNTPs, 1 μmol/L of each primer, and 0.25 U of *Taq* polymerase. Amplification was carried out using 35 cycles of denaturation for 3 min at 95 °C and extended by 1 min at 94 °C. Annealing was done for 1 min at 60 °C, followed by 1 min at 72 °C, and a final extension of 5 minutes at 72 °C. The PCR products were purified using spin columns (Watson BioTechnologies Inc., Shanghai). Purified amplicons were sequenced directly for both the DNA strands using the ABI PRISM BigDye™ Terminator Cycler Sequencing Ready Reaction Kit (Applied Biosystems, CA USA). The resulting raw sequences were checked using DNAStar 5.0 (DNAStar Inc., Madison, WI) and submitted to GenBank (Accession numbers: AY178254~AY178265, AY178267~AY178274). Polymorphic sites and haplotypes were determined using MEGA software, Version 2.1 (Kumar et al, 2001) and DNASP software, Version 3.00 (Rozas & Rozas, 1999). Population diversity, population differentiation and exact test were performed using Arlequin Version 2.00 (Schneider et al, 2000). Nucleotide diversity and net genetic distance for inter- and intra-populations were computed using DNASP, Version 3.00 (Rozas & Rozas, 1999). A neighbor-joining tree was constructed by the net genetic distance matrix among populations using MEGA software, Version 2.1 (Kumar et al, 2001), under the Kimura 2-parameter substitution model.

2 Results

2.1 Genetic diversity of Yunnan pig breeds

Four hundred and thirty-eight base pairs of mtDNA control region were identified by comparison with the control region of *Sus scrofa,* GenBank (Accession nos. AF304201). Twenty haplotypes, including sixteen polymorphism sites (Tab. 1 and Tab. 2), were detected, of which five were singleton polymorphic sites and eleven were parsimony informative sites. Fifteen transition and two transversion substitutions were identified. No deletion/insertion was found in the entire 102 sequence dataset. Haplotype H23 was dominant (83.3%) in both the conservation herd of Banna miniature pig and the nucleus herd of Saba pig. The same maximum haplotype was shared (40.20%) in the samples. There were seven haplotypes (H3, H6, H7, H8, H16, H18, and H21) with single occurrence (0.98%). Some specific haplotypes, such as H7 in Baoshan pigs, and H8 and H13 in Diqing Tibetan pigs, were also found. Further, haplotypes H16, H17, H18, and H19 and H3 and H4 were found in the randomly bred Banna miniature pig population and the Saba pig population, respectively. Two more haplotypes (H21 and H22) were restricted exclusively to the nucleus herd of Saba pig (Tab. 2). The numbers of haplotypes in the conservation herd of Banna miniature pig and nucleus herd of Saba pig were one and three, respectively. The number of haplotypes found in the randomly bred populations ranged from six to ten. Haplotype diversity (h) and nucleotide diversity (π) in the randomly bred populations were significantly higher than the selectively bred populations (P<0.01) (Tab. 3).

2.2 Genetic relationship among six pig populations

Nucleotide diversity and net genetic distance between inter- and intra-populations are shown in Tab. 4. A neighbor-joining tree constructed by net genetic distances demonstrated the genetic relationship among the six pig populations. The nucleus herd of Saba pig and the conservation herd of Banna miniature pig initially clustered together; then they clustered with randomly bred population of Saba pig and Baoshan pig. All these pigs had similar morphological and physiological characteristics and belonged to Southwest-type of Chinese pig breeds (Lian et al, 2003). There was, however, very distinct genetic distance between the randomly bred Banna miniature pig population and the other populations (Fig. 2).

3 Discussion

The evolution rate of the mitochondrial DNA control region is several times higher than those of other mtDNA regions and nuclear genes. Because of this, the

Tab. 1 The sixteen polymorphic sites of mitochondrial DNA control region

	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	3	4	5	5	5	5	6	6	6	6	7	7	7	7	7	7
	9	1	2	5	6	8	1	1	4	7	5	5	5	7	7	8
	3	8	7	0	1	3	0	7	8	5	7	8	9	4	6	7
H5	G	T	G	C	T	C	C	G	C	T	A	A	T	C	G	A
H10	.	.	.	.	.	T	.	.	T	.	.	.	C	T	.	.
H23	.	.	.	.	.	T	.	.	.	.	.	.	.	T	.	.
H6	.	.	.	.	.	T	.	.	.	.	.	.	.	T	C	.
H14	.	.	.	.	.	T	T	.	.	.	.	.	.	T	.	.
H11	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
H9	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.
H20	.	.	.	.	.	T	T	.	T	.	.	.	.	T	.	.
H7	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.
H12	V	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T
H8	.	.	.	.	.	T	.	.	.	.	.	.	C	T	.	.
H13	.	.	.	.	.	T	T	.	T	.	G	.	.	T	.	.
H22	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.
H21	.	C	.	.	.	T	.	.	.	.	.	G	.	T	.	.
H4	.	.	.	.	.	T	.	.	.	C	.	.	.	T	.	.
H3	.	.	.	.	.	T	.	A	.	.	.	.	.	T	.	.
H19	A	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.
H17	.	.	.	.	.	T	T	.	T	.	.	.	C	T	.	.
H16	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.
H18	A	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.

H for haplotype. '.' denotes similar nucleotide base with reference to the reference sequence H5.

Tab. 2 Distribution of haplotypes in the pig populations

Haplotype	Baoshan pig	Diqing Tibetan pig	Randomly bred population of Saba pig	Nucleus herd of Saba pig	Randomly bred population of Banna miniature pig	Conservation herd of Banna miniature pig	Total
H5	2	—	1	—	—	—	3(2.94)
H10	1	3	1	—	—	—	5(4.90)
H23	6	2	11	12	2	8	41(40.20)
H6	1	—	—	—	—	—	1(0.98)
H14	3	2	1	—	—	—	6(5.88)
H11	2	1	—	—	—	—	3(2.94)
H9	1	1	—	—	—	—	2(1.96)
H20	1	3	3	—	3	—	10(9.80)
H7	1	—	—	—	—	—	1(0.98)
H12	1	1	—	—	—	—	2(1.96)
H8	—	1	—	—	—	—	1(0.98)
H13	—	2	—	—	—	—	2(1.96)
H22	—	—	—	3	—	—	3(2.94)
H21	—	—	—	1	—	—	1(0.98)
H4	—	—	7	—	—	—	7(6.86)
H3	—	—	1	—	—	—	1(0.98)
H19	—	—	—	—	9	—	9(8.82)
H17	—	—	—	—	2	—	2(1.96)
H16	—	—	—	—	1	—	1(0.98)
H18	—	—	—	—	1	—	1(0.98)
Sum	10	9	7	3	6	1	20

Number of animal sharing haplotypes and their frequencies is depicted in brackets. The bottom line shows haplotype found per population.

Tab. 3 The diversity indices in the six pig populations from Yunnan

Breeds	Haplotype diversity (h)	Nucleotide diversity (π)
Baoshan pig	0.883±0.056**	0.00390±0.00523
Diqing Tibetan pig	0.925±0.039**	0.00518±0.00482
Randomly bred population of Saba pig	0.737±0.065**	0.00297±0.00423
Nucleus herd of Saba pig	0.425±0.133	0.00205±0.00275
Randomly bred population of Banna miniature pig	0.732±0.096**	0.00595±0.00398
Conservation herd of Banna miniature pig	0.000±0.000	0.00000±0.00000

**denotes highly significant level (P<0.01).

Tab. 4 Estimates of interpopulational (dxy), intrapopulational (dx or dy), and net nucleotide diversity (dA) among the six pig populations in Yunnan

Breeds	Baoshan pig	Diqing Tibetan pig	Nucleus herd of Saba pig	Randomly bred population of Banna miniature pig	Conservation herd of Banna miniature pig	Randomly bred population of Banna miniature pig
Baoshan pig	0.390	0.497	0.315	0.369	0.228	0.687
Diqing Tibetan pig	0.043	0.518	0.469	0.468	0.371	0.785
Nucleus herd of Saba pig	0.018	0.108	0.205	0.284	0.114	0.660
Randomly bred population of Banna miniature pig	0.026	0.061	0.033	0.297	0.174	0.665
Conservation herd of Banna miniature pig	0.033	0.112	0.011	0.025	0.000	0.545
Randomly bred population of Banna miniature pig	0.194	0.228	0.259	0.219	0.248	0.595

All values were multiplied by 100. The figures on the diagonal refer to dx or dy, those above the diagonal represent dxy, and those below the diagonal represent the values of dA=dxy−(dx+dy)/2 (Nei, 1987).

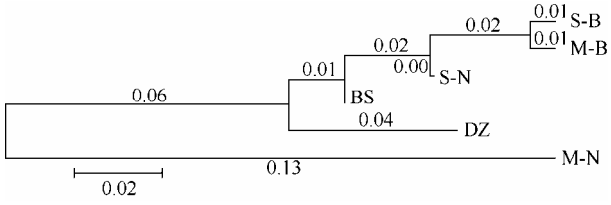

Fig. 2 N-J tree constructed by net genetic distances among the six populations

S-B for nucleus herd of Saba pig, M-B for conservation herd of Banna Miniature pig, S-N for randomly bred population of Saba pig, BS for Baoshan pig, DZ for Diqing Tibetan pig, M-N for randomly bred population of Banna Miniature pig. The numbers represent branch length.

D-loop region has been used extensively to study relationships among populations (Brown et al, 1979; Watanabe et al, 1986, 1999; Lin et al, 1999; Giuffra et al, 2000; Luikart et al, 2001). The sequences of 438 bp of the mtDNA control region in 102 pigs revealed twenty haplotypes with sixteen polymorphic sites. The number of haplotypes in Baoshan pig and Diqing Tibetan pig, and randomly bred Saba and Banna miniature pigs were 10, 9, 7, and 6, respectively (Tab. 2). Similarly, the number of haplotypes in the nucleus herd of Saba pig and the conservation herd of Banna miniature pig were confined to three and one (h=0.732 via 0.425 and zero), respectively. Low haplotype diversity indicated that long-term intensive breeding has significantly reduced diversity in the selectively bred pig populations (exact test, P≤0.0036). It was suggested that randomly bred populations possessed high diversity. But the population differentiation between the nucleus herd of Saba pig and the conservation herd of Banna miniature pig was not significant (P=0.688). Historically, pig breed resources in Yunnan Province were formed due to the wider fields, greatly different climates and people's living customs (Lian et al, 2003). Over the years geographical, climatic, and cultural factors have restricted the gene flow between pig populations and resulted in the inheritance of few selected variable sites (or haplotypes) in subsequent generations. Compared to the nucleus herd of Saba pig, gene flow has been restricted in the base population of Baoshan pig. This is mainly due to the grouping of the Baoshan pig for breeding in 1998 and sampling in 1999, which led to the fixation of many extant haplotypes in the region. After seven years of

selection, some lineages were eliminated after only a few generations of breeding in the nucleus herd of Saba pig, which led to the loss of some haplotypes before breeding. Moreover, during the fifteen-year conservation program of Banna miniature pig, haplotype diversity has reduced to a single lineage (Lian et al, 2003). Haplotype diversity in the nucleus herd of Saba pig was 0.425, significantly lower than the lowest haplotype diversity (0.732) found in the randomly bred population (Tab. 3) (exact test, of $P<0.01$). Furthermore the Diqing Tibetan pig exhibited a haplotype diversity of 0.925, double that found in the nucleus herd of Saba pig. Compared to the four randomly bred populations, nucleotide diversity was similar for the conservation and nucleus herds and fell in the range of 0.00205-0.00595 (Tab. 3). Our results clearly suggest that human induced factors have generated great differences in diversity among native pig breeds. Our study also reinforces the finding that population fragmentation and captive breeding greatly reduces diversity, as reported in blue chaffinch, Hector's dolphin, cheetah, and sea otter (Menotti-Raymond & O'Brien, 1993; Pestano et al, 2000; Pichler & Baker, 2000; Freeman et al, 2001; Larson et al, 2002; Yildiz et al, 2002).

It is interesting to note that two bred populations (the nucleus herd of Saba pig and the conservation herd of Banna miniature pig) were initially clustered together in the N-J tree. We concluded the reasons were: (1) It was possible that mitochondrial haplotypes were linked with or related to some husbandry economic traits (Bell et al, 1985; Toelle et al, 1986; Brown et al, 1988; 1989), and the two pig populations may act to increase genetic homogeneity by paying more attention to litter size improvement for undertaking breeding programmes (Lian et al, 2003) and keeping captive populations (Nei, 1987; Menotti-Raymand & O'Brien, 1993; Freeman et al, 2001). Litter size improvement was mainly emphasized in the early period of selection and grouping (Lian et al, 2003) and dams possessing high litter size were selected and kept. At the same time, maternally inherited mitochondrion and special haplotypes were reasonably fixed in the populations and the frequency of special haplotypes increased. (2) Founder effect. The populations that first grouped and were selected accommodated a wider haplotype-pool, such as observed in the Baoshan pigs. Consequently, we traced the frequency fluctuation after some generations. Furthermore, dams possessing high litter size kept the same haplotypes as their mother and grandmother, and the haplotypes were inclined to simplification. Saba and Baoshan pigs, which belong to the Southwest type of Chinese pig breeds according to morphological and physiological characteristics, clustered together with the bred populations. We concluded that gene exchange with the Diqing Tibetan pig during their formation had occurred because of sharing four haplotypes (Data not shown). The Banna miniature pig was located furthest away in the southern area of Yunnan Province, and also showed the furthest relationship with other Yunnan native pig breeds. The neighbor-joining tree showed that the relationship between Yunnan native pig breeds was consistent with their geographical distribution and physiological characteristics.

In regards to domestic animal breeding programs, farmers have pursued genotype homozygosity to obtain expected heterosis. Meeting some productive requirements, however, comes at the cost of diversity. A possible link between mitochondrial haplotypes with some economic traits in animal husbandry couldn't be excluded (Bell et al, 1985; Brown et al, 1988,1989; Mannen et al, 1998,2003). Improvement in litter size was emphasized in early selection and grouping, with higher litter size sows selected and kept (Lian et al, 2003). Mitochondrial haplotypes inherited maternally to the next generation undergo expansion in the offspring easily. So, timely intervention and well programmed breeding approaches can stop further reduction in the genetic diversity of nucleus and conservation herds of native pig breeds. Hence, animals that were first grouped and selected overlaid more haplotypes. The Baoshan pig exhibited a similar pattern, and hence it became necessary to estimate haplotype frequency after only a few generations of breeding. Taking into account the single genetic marker, probing by Y-chromosomal and autosomal markers in the future will clarify the genetic differentiation associated with native pig breeds of Yunnan.

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