Genetic Status of ESR Locus and Other Unidentified Genes Associated with Litter Size in Chinese Indigenous Tongcheng Pig Breed after a Long Time Selection*

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ABSTRACT : The Tongcheng pig breed is a famous Chinese indigenous breed. The Ministry of Agriculture of China has filed it as 1 of 19 national key conservation breeds selected from more than 100 Chinese indigenous pig breeds in 2000. In order to improve the reproductive performance, it has been intensively selected to increase the litter size for about 10 years. The population randomly sampled from conservation nucleus of eight families in the *Tongcheng* pigs was genotyped for identification of their estrogen receptor locus polymorphisms with the PCR-RFLPs method. Only AB heterozygotes and BB homozygotes were detected, and x^2 test demonstrated that the locus was in disequilibrium at a significant level ($p<0.05$). In the present paper, the litter sizes in different parities were regarded as different traits. Holistic status of other unspecific and unidentified genes was estimated by using the statistical methods. Coefficients of kurtosis and skewness showed that the litter size still presented segregating characteristic in the 2nd, 5th, 7th, 8th and 9th parities. Analysis of homogeneity of variance between families confirmed the results for the 5th, 7th and 8th parities. The heritability of litter size for the 1st to 10th parities was estimated with paternal half-sib model and individual estimated breeding values (EBVs) were evaluated by a single trait animal model as well. We found that the averages of EBVs for litter size in each parity did not differ significantly between genotypes, despite the significant difference for original phenotype records in the 3rd, 4th and 5th parities ($p<0.05$ or $p<0.01$). The results may be explained by the deduction that the polymorphisms of ESR locus are no longer the important genetic base of litter size variation when the frequency of allele B accumulated in the experience of selection procedure, and further conferring that there exist special genes associated with litter size in the recent Tongcheng pigs population can be made. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 5 : 598-602)***

Key Words : Chinese Tongcheng Pigs, ESR Locus, Litter Size, Heritability, Breeding Value

INTRODUCTION

Porcine production traits of modern commercial pig breeds have achieved remarkable progresses over the past decades, but the improvement of reproduction traits was quite restricted. The litter size in swine belongs to reproduction traits with low level of heritability with estimated value usually distributing around 0.1 (Okere and Nelson quoted from Rothschild et al., 2002), thus the significant genetic gain is ordinarily difficult to meet. The history of porcine breeding also indicates that litter size made little improvement under the normal breeding system, and how to improve porcine reproduction traits is now one of the important tasks of pig breeders.

Recently, the well-going project of porcine genome mapping accelerates research on the molecular mechanism of reproduction performance. Some candidate genes or markers linked with QTLs associated with litter size have been detected. Among these reports, the linkage between estrogen receptor (ESR) gene and loci controlling litter size was repeatedly identified in different populations (Rothschild et al., 1996; Short et al., 1997; Isler et al., 2002; Kaminski et al., 2002; van Rens et al., 2002). However, some reports showed that there was no detectable association of the ESR gene with litter size (Drogemuller et al., 2001; Linville et al., 2001; Gibson et al., 2002).

There are more than 100 indigenous pig breeds in China. Tongcheng pig is a famous representative of Central China Type named two-end black pig, and the photo of which was recently published on the cover of "journal of heredity" (2003, 94(5)). As good research material, Tongcheng pigs were already involved in some studies (Liu et al., 2002; Mo et al., 2003; Pan et al., 2003). The Ministry of Agriculture of China has filed it as 1 of 19 national key conservative breeds in 2000. Tongcheng pigs have many prominent idioplasmatic characters such as sound mothering ability, well adaptability to crude diet, nice anti-disease competence, excellent meat quality. However, their litter size is smaller

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than Taihu pigs. So, the conservation farm of *Tongcheng* pigs made an intensive selection strategy to enhance the litter size about 10 years ago, which was an opening selection scheme that made the core herd could continuously absorb superexcellent sows from commodious rural areas. Contrast to the reproduction performance before selection, the litter size of Tongcheng sows averagely increased 2 piglets per sow per litter (Xu et al., 2001). In order to investigate the characters of genes associated with litter size after a long time selection, our laboratory was requested by the farm manager to confirm whether those genes pertinent to litter size reported in the public literatures have the similar effect on litter size in the present Tongcheng sows. The objective of this article was to reveal the genetic status of ESR locus and other unidentified genes associated with litter size in Tongcheng pigs by conjointly using molecular investigations and statistical analyses.

MATERIALS AND METHODS

Sampling and traits

Sows were raised in houses with an open gateway to a hypaethral fenced yard, and were fed 11, 12.5 and 14% crude protein diets during pre-pregnancy, pregnancy and lactation. A within full-sib family random sampling method was used to select sows originated from eight paternal families in the core herd of Tongcheng pigs for jugular blood or hair root bulbs according to whether pregnancy or not. A total of more than 2,500 records of 254 sows from 1991 to 2002 were involved in this investigation. The data items contained pedigree, parities, total litter size at birth, and year season, which came from the overall data stored in farm database.

Isolation of DNA and genotyping

Until DNA isolation, the blood and hair root bulbs for ESR locus typing were stored in 10 ml plastic tubes containing normal temperature anticoagulant and absolute alcohol, respectively. Genomic DNAs were extracted from whole blood following a conventional method and DNAs from hair root bulbs were isolated through an ameliorated procedure partially according to Kim et al. (2002). Hair DNA extraction buffers include KCl 1.86 g, Tris 0.61 g, $MgCl_2 \cdot 6\ H_2O$ 0.25 g, Gelatin 0.05 g, NP40 2.25 g and Tween 20 2.25 g per 500 ml. Incubate hair bulbs at 56°C for 1 h and at 95°C for 10 minutes, make an instantaneous centrifugation after blend, and suck the supernatant for the further PCR amplification. The ESR primers were as follows: ESRF 5`-CCTGTTTTTACAGTGACTTTTACAG AG-3`, ESRR 5`-CACTTCGAGGGTCAGTCCAA TTAG-3`. The reactions were loaded onto a MJ PTC-100 TMP thermal cycler under the following parameter: 1 cycle at 94°C for 4 min, 55°C for 1 min and 70°C for 1 min; 31 cycles of 94°C, 55°C for 1 min, and 70°C for 1 min; and 1 cycle at 72°C for 8 min followed by a final extension step at 4°C. PCR products were cleaved by the restriction endonuclease reactioin of *Pvu*II. Agarose gel electrophoresis was used to separate the DNA segments. Molecular manipulations above were mainly according to the procedures elaborated by Rothschild et al. (1996).

Statistical and genetic analyses

Population genetic analyses : Genotype frequency, allele frequency and heterozygosity based on a single-locus were calculated according to Pamilo and Varvio-Aho (1984). The chi-square (χ^2) was used to test the genotypic departures of the whole population from Hardy-Weinberg equilibrium (Ledwina and Gnot, 1980).

Statistical analyses : Ordinary statistical parameters of phenotype records in different parities were estimated. Fisher kurtosis coefficient and skewness coefficient were calculated to characterize litter size. Levene's test was used for estimation of homogeneity of variances among families (Levene, 1960). Software package of SPSS 10.0 was used to conduct all of the work mentioned above.

Quantitative genetic analyses : Heritabilities of litter size in different parities were evaluated by the variance component of original sires (model I). In order to directly compare the results of significance test for the difference of the individual EBVs parallel with phenotypes between individuals with different ESR genotypes, EBV of each individual was estimated by a single trait animal model not directly including the genotypic information on ESR locus (model II). Such model was constructed also because individuals for EBVs estimation included the nonexistent individuals with data records but without genotypic information on ESR locus in order to elevate precision and veracity of estimation, and significance test for the difference of EBVs and phenotypes between genotypes was done by t-test.

The models were:

$$Y_{ijk}=\mu+YRS_i+Sire_j+e_{ijk} \quad (I)$$

$$Y_{ijk}=\mu+YRS_i+F_j+EBV_{ijk}+e_{ijk} \quad (II)$$

where, Y_{ijk}=individual observation of the ijk^{th} animal; μ=population mean; YRS_i=effect due to the i^{th} farrowing year season; $Sire_j$=effect of the j^{th} original sire; F_j=effect of the j^{th} paternal family; EBV_{ijk}=individual estimated breeding value of the ijk^{th} animal assumed to be $N\sim(0, \sigma_a^2)$; and e_{ijk}=random error associated with Y_{ijk} observation assumed to be $N\sim(0, \sigma_e^2)$. The litter sizes in different parities were estimated separately taken as different traits, and software *MatVEC* 1.03 (http://statistics.unl.edu/faculty/steve/software/matvec/) was involved in the model analyses.

Table 1. The parametric evaluation of Tongcheng pigs on the ESR locus by PCR-RFLPs

Genotypes	Genotype frequency	Allele	Allele frequency	Heterozygosity	p value of x^2 test
AB	0.20	A	0.10	0.18	p=0.00
BB	0.80	B	0.90		

Table 2. The averages, standard errors, standard deviations (SD) and ranges for litter sizes

Parities	1	2	3	4	5	6	7	8	9	10
Number of litters	254	248	252	250	234	212	192	174	164	128
Averages	9.16	10.77	11.78	11.78	11.94	11.64	12.23	11.765	11.46	12.01
Std. error of averages	0.32	0.25	0.21	0.28	0.32	0.32	0.25	0.39	0.45	0.48
SD	1.32	1.70	1.41	1.41	1.98	1.89	2.43	2.02	2.07	2.08
Ranges	6-12	8-17	9-15	9-15	8-16	7-16	9-18	8-15	9-15	9-15

Table 3. The kurtosis, skewness, standard errors of which and Levene's test for the litter size in different parities

Parities	1	2	3	4	5	6	7	8	9
Kurtosis	0.197	2.889	-0.496	-0.479	-0.506	0.627	-0.078	-0.959	0.333
Std. error of Kurtisis	0.304	0. 464	0. 419	0. 391	0.424	0.432	0. 223	0.442	0.423
Skewness	-0.010	1.263	0.059	0.059	0.315	0.027	0.608	-0.206	-1.167
Std. error of Skewness	0.198	0.351	0.284	0.283	0.387	0.262	0.341	0.332	0.427
Significance of Levene	0.310	0.262	0.148	0.146	0.021	0.098	0.048	0.050	0.201

Table 4. Estimations of heritability and EBVs for the litter size in different parities

Parities	1	2	3	4	5	6	7	8
Heritab-ility	0.106	0.161	0.168	0.176	0.098	0.096	0.201	0.164
Range of EBVs	-1.11-0.98	-0.87-1.06	-0.96-0.86	-1.12-1.02	-0.92-1.00	-0.99 -1.04	-1.34-1.10	-1.24-1.08

Note: Breeding values don't include the mean in above table. EBVs is abbreviated from estimated breeding value.

RESULTS

Results of ESR locus genotyping

The parametric estimation shown in Table 1 revealed the low-level polymorphisms of ESR locus in present Tongcheng pigs. Only two genotypes of AB and BB were detected with the frequencies of 0.20 and 0.80, respectively. The estimation of heterozygosity was on the low side of theoretical range, which confirmed the large difference between amounts of allele A and B. The result of chi-square test showed that ESR locus in present Tongcheng pigs was significantly in departure from Hardy-Weinberg disequilibrium.

Descriptive statistics of litter size

From Table 2, we could find that average of litter size in the 7th parity was highest. The estimation of standard deviation showed a trend that the more parities, the more magnitude of variation, which showed that the environmental effect is gradually strengthened along with the parities increasing. The litter size was comparatively stable within 10 parities, generally it increased from 1st to 2nd parities, and fluctuated parity by parity from the 3rd to 10th parities.

Parametric statistics for the exceptional normal distribution of litter size

The results of kurtosis, skewness and homogeneity of variance among families were displayed in Table 3. These estimated parameters have visible differences between parities except between the 3rd and 4th parities, which demonstrated that the litter size in different parities is regarded as different traits is meaningful. Skewness quantifies raw data by revealing the leaning direction of a distribution (e.g., left-leaning and right-leaning). The skewness coefficients attest that distributions of litter sizes closely fit positive skew in the 2nd, 5th and 7th parities, closely negative skew in the 8th and 9th parities, and non-leaning distribution in the 1st, 3rd, 4th and 6th parities. Kurtosis is the peakedness of a distribution. The fisher kurtosis coefficient showed the peakedness in the 2nd, 6th and 9th parities was apt to sharpness and was flatness in the 3rd, 4th, 5th and 8th parities. Seen from the last row in the Table 3, the significance of Levene statistics reached the 0.05 level in the 5th, 7th and 8th parities, respectively.

Results of heritability and estimated breeding value

The range of estimated heritability varied from 0.096 to 0.201 in Table 4, which showed large varying width and synchronously demonstrated that it is meaningful to regard litter size in different parities as different traits. The range of EBVs was also displayed in Table 4. The Table 5 contained significance test for the difference of phenotype and EBVs between heterozygotes and homozygotes by t-test. The means of phenotype in the 3rd, 4th and 5th parities between heterozygotes and homozygotes were significantly different ($p<0.05$ or $p<0.01$), but not significantly different in other parities. The means of EBVs were not significantly

Table 5. The significance tests for difference of phenotype and EBVs of the litter size averages among the genotypes in different parities

Genotypes \ Parities		1	2	3	4	5	6	7	8
Phenotype averages	AB	8.86	10.40	11.03a	11.17a	14.98A	11.49	12.89	11.96
	BB	9.31	10.84	12.02b	12.03b	11.14B	11.97	11.78	11.84
EBVs averages	AB	-0.022	-0.082	-0.018	0.084	-0.081	-0.012	0.089	-0.002
	BB	0.038	0.021	0.025	0.101	0.004	0.087	0.099	0.032

Different small letters indicate the averages differ significantly at $p<0.05$. Different capital letters indicate the averages differ significantly at $p<0.01$, and not significantly without letters between genotypes within the same column ($p>0.05$).

different between heterozygotes and homozygotes for each parity, but a trend showed that EBVs of homozygotes have higher value than that of heterozygotes. Because the animals for EBVs estimation included those individuals without genotypic information on locus ESR, which resulted in that means of EBVs of both the heterozygotes and homozygotes were greater than zero in the 4th and 7th parities.

DISCUSSION

The allele B of ESR locus used to play a role in the selection for increasing the litter size in Tongcheng pigs

Our results did not directly prove that allele B, the favorable allele, on estrogen receptor locus has significant genetic effect on litter size in present Tongcheng pigs, while indirect proof was made. The Tongcheng pigs have experienced a long time selection for the purpose of improving litter size, and its reproduction performance improved 2 piglets per sow per litter after selection (Xu et al., 2001). The living sows in farm were reserved according to the selection scheme, so the legionary allele B was enriched in present Tongcheng pigs and it resulted in no AA homozygotes were detected. Considering the long time selection history, significant genotypic departures from Hardy-Weinberg equilibrium showed that there was a pressure of concentrating of allele B in Tongcheng pigs, which hinted that the selection response of ESR locus corresponding with the executed selection had happened. So, we can conclude that improvement of Tongcheng sows' reproduction performance was parallel with clustering of allele B. Because of concentration of favorable allele B more and more, the low-level polymorphisms of ESR locus are no more the important genetic base of litter size variation. Thus, the indirect prove was made by us that the ESR locus used to link tightly with some QTLs controlling litter size variation before selection and in the early phase of selection.

There still are other genes or QTLs controlling litter size variation in present Tongcheng sows based on the parametric evaluation

Two common methodologies, the variance homogeneity test among families and analysis of kurtosis and skewness, were applied to reveal genetic status of genes or QTLs controlling litter size variation in present Tongcheng sows. The fitness detection for normal distribution is a simple estimation for the rough diagnosis of segregation of major genes or QTLs in the quantitative traits. According to this elements, coefficients of kurtosis and skewness in the Table 3 indicate that the litter size in the 2nd, 5th, 7th, 8th and 9th parities present non-normality distribution in the population, which formally hints the segregation action of major genes or QTLs. More detailedly speaking, the litter sizes in the 2nd, 5th and 7th parities were affected by the positive factors, and the negative factors were in action to the litter size in the 8th and 9th parities. The effect of factors in the 2nd, 6th and 9th parities was relatively concentrative, but dispersive in the 3rd, 4th, 5th and 8th parities. The further analysis of homogeneity of variance among families by the Levene's test confirmed the results of normality distribution estimation. Although the Bartlett's test is an older, traditional and widely used test, but it is dependent on meeting the assumption of normality. Therefore Levene's test has now largely replaced it (http://www2.chass.ncsu.edu/garson/pa765/assumpt.htm). According to the results of Levene's test, the litter size was proved that unequal variances emerge among families in the 5th, 7th and 8th parities. The results showed that some major genes or QTLs still exhibit to control the litter size variation in present Tongcheng sows, which was indirectly supported by the identification of other associated genes. Additionally, the results also indicated us that different genes controlling a specific trait may have different function modes in a specific genetic background.

The issues about further selection strategies on litter size in the present Tongcheng pigs

The genetic markers strongly associated with QTLs of highly desirable characteristics have aroused interests of researchers and breeders. Plastow et al. (1998) introduced a selection aimed at increasing the frequency of alleles with a positive effect on a given trait to us, and Rothschilds et al. (1996) firstly used the maker of allele B to increase the level of traits linked to the pig reproduction. As for the Tongcheng pigs, the frequency of allele B reached 0.90 after a long time selection, thus the selection strategy using the marker of allele B to increase the litter size is not

effective with genetic component controlled by the ESR locus decreasing to a certain extent. On the other hand, the variance homogeneity test among families and the values of kurtosis and skewness sustained the conclusion that the litter size is still controlled under other major genes or QTLs in present population, which illuminates that the litter size of Tongcheng sows still has a biggish selection potential. By way of parenthesis, the research shows us that different genes conjointly controlling a specific trait can have different response patterns under the same selection on the phenotype or on the estimates of breeding values derived from the phenotype, thus the optimal use of a specific major gene requires design of specific selection strategies. If molecular breeding scheme will be applied to resume and sustain the selection responses in present Tongcheng pigs, we must screen out novel detectable markers associated with the litter size variation in the further analyses. Due to inexistence of parental DNA material for most of the living individuals, the experiment design for detection of specific markers linked with major genes is quite different from the classic family design with parental molecular information and progeny phenotypic information, therefore specific method of identifying novel major genes controlling litter size in present Tongcheng pigs should be taken into account.

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