Investigation of Gene and Microsatellite Heterozygosities Correlated to Growth Rate in the Chinese Meishan Pig

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ABSTRACT : A total of 130 Chinese Meishan piglets were scored for their genotypes at five gene loci and five microsatellite loci. The average gene heterozygosity was 0.3338 and the average microsatellite heterozygosity was 0.2954, and the heterozygosity of the overall ten loci was 0.3146. The data of birth weight (BW) and body weight on day 35 (W35) were collected, average daily gain (ADG) for each individual was computed as the slope from the regression of weight on age. There was no significant correlation between individual heterozygosity and birth weight ($p>0.05$). Significant regressions were observed between ADG and the heterozygosity of loci ($p<0.01$). Similar results were observed in W35 and loci heterozygosity ($p<0.01$). Heterozygosity at these ten loci explained 43.62% of the total variation in ADG and 45.48% in W35. Significant correlations existed not only in the function of gene loci but also in neutral microsatellite loci, so it indicated that associative overdominance affected piglet growth significantly. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 7 : 927-932)***

Key Words : Associative Overdominance, Heterozygosity, Growth Trait, Pig

INTRODUCTION

Frydenberg (1963) introduced the term associative overdominance, which is 'due to non-randomized linkage between the observed pair of allelic units and the entire remainder of genetic alternatives present in the same chromosome pair'. Ohta (1971) developed an analytical model on association overdominance that depends in addition on selection coefficients of linked selected loci, also on genotypic correlations due to inbreeding and linkage. By the application of this model to data from *Drosophila*, Ohta concluded that associative overdominance 'is probably responsible for most of the observed superiority of heterozygotes'. Ever since then, associative overdominances have been observed in many aquatic species and allozyme/protein markers were widely used (reviewed by Hansson and Westerberg, 2002). These aquatic species were rainbow trout (Ferguson, 1992), scallops (Grant and Eleftherios, 1994), clams (Garton et al., 1984), mussels (Dichl et al., 1986), oysters (Koehn and Shumway, 1982) and so on. In the past decade, some mammalian species (e.g. red deer, seal, pig and yak) were included for studying correlations between heterozygosity and traits (Coltman et al., 1998; Coulson et al., 1998; Slate et al., 2000). Beside allozyme/protein marker, DNA marker (microsatellite, RFLP, etc) was employed in these researches (Danzmann et al., 1989; Liskauskas and Ferguson, 1990; Kark et al., 2001; Daniel et al., 2002).

Although pig is an important farm animal, associative overdominance was seldom probed in the past decades. Wu et al. (2001) discussed association of microsatellite genomic heterozygosity with inbred pig average daily gain and backfat depth. Liu et al. (2003) studied the relationship between individual heterozygosity and carcass and meat quality traits in a synthesized white pig population. The two literatures showed that positive relationships existed between loci heterozygosity and traits mentioned above. Generally, most of the Chinese native pig breeds were with relatively low growth rate. In order to improve their growth performance, many basic researches should be conducted firstly (Wang et al., 2004). This study describes genetic variability and the relationship between heterozygosity and early growth traits in Meishan pig population, a fecund but low growth rate pig breed distributed in Yangtze River Delta area. The objectives are (1) to serve as a basis for future studies on the relationship among genetic variability and growth, (2) to probe whether the association of Meishan piglet growth and loci heterozygosity can be explained by associative overdominance.

MATERIAL AND METHODS

Animals and growth traits

To avoid sire and litter effects, we selected two piglets randomly from each litter and the sires were also balanced. The growth data of 130 piglets was collected from 65 litters. The growth traits included birth weight (BW) and body weight on day 35 (W35). Average daily gain (ADG) for each individual was computed as the slope from regression of weight on age (days), using PROC REG of SAS (1998). Blood samples were collected from each animal's anterior vena cava. Genomic DNA was isolated from whole blood by standard salting-out procedures (Xiong, 1999).

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Table 1. Primer sequences, scored method and reference for 5 gene and 5 microsatellite loci

Locus	Primer sequence	Method	Reference*	Locus	Primer sequence	Reference*
OB	TGCAGTCTGTCTCCTCCAAA CGATAATTGGATCACATTTCTG	PCR- RFLP	(1)	*SW1332*	GCATATGCTGCAGGTACGG CAGCCTAAGCCAAGTATGTGG	(5)
ESR	CCTGTTTTTACAGTGACTTTTACAGAG CACTTCGAGGGTCAGTCCAATTAG	PCR- RFLP	(2)	*SW1301*	TGGATAAGCAATGAGGTCCC TAGTGGATTTATAATGTGCTAACCC	(5)
FSHB	AGTTCTGAAATGATTTTTCGGG TTTGCCATTGACTGTCTTAAAGG	PCR- RFLP	(1)	*SW1883*	CCATTTGGGGTCATTTTTTG CTATTGGTGGTAAAGGAGCAGC	(5)
INHBB	GGCGGTGGCTACAGCTCCGATTCA GCCTGCGACTGTCAAGAAATTCAC	PCR- RFLP	(3)	*S0035*	GGCCGTCTTATACTCTCAGCATA CCAAATAAACAGCAGGCAGCCT	(6)
INHBA	GGCAAGGTCAACATCAGCTGTA ACTCCTCCACGATCATGTTCTG	SSCP	(4)	*SW133*	GGCCTGAATTACATATGTTCCC AATGTGGCAACAAAACAAAAG	(7)

* (1) Korwin-Kossakowska et al., 2001; (2) Korwin-Kossakowska et al., 1999; (3) Weimann et al., 1995; (4) Lahbib-Mansais et al., 1996; (5) Alexander et al., 1996; (6) Ponsuksili et al., 2002; (7) Rohrer et al., 1994.

Table 2. Mean, standard deviation and ranges of birth weight (BW), body weight on day 35 (W35) and average daily gain (ADG) of piglet

Traits	N	Mean	Std	Min. value	Max. value
BW (kg)	130	0.83	0.22	0.40	1.30
W35 (kg)	128	8.72	2.34	4.00	16.74
ADG (kg/d)	128	0.2321	0.0689	0.0882	0.4645

Std: standard deviation of the mean; Min.: minimum.
Max.: maximum; N: number of individual measured.

Genotypes

A total of 5 microsatellite loci (Table 1) were genotyped with the following protocol. The PCR comprised a total reaction volume of 20 μl: 5.0 μl of template DNA, 0.2 μl of Taq polymerase (5 U/μl), 1.6 μl dNTPs (2.5 mmol/l), 1.0 μl of each primer (75 ng/μl), 7.6 μl of ddH_2O, 1.6 μl of $MgCl_2$ (25 mmol/l) and 2.0 μl 10×reaction buffer provided by the enzyme supplier. An Eppendorf Thermal Cycler was programmed for an initial incubation at 94°C for 3 min; 35 cycles each with denaturing at 94°C for 1 min, annealing at 56°C for 45 s and extension at 72°C for 1 min; and a final cycle at 72°C for 5 min. The PCR products were electrophoresis with polyacrylamide gel and silver stained with standard methods.

The five genes were scored as literatures described (see Table 1). We selected the genes and microsatellites listed in Table 1 because they were neutral loci for growth traits in the pig population (unpublished data). The protocol for microsatellite loci scoring was modified by the method described by Selvi et al. (2004).

Heterozygosity and its relationship with growth rate

Heterozygosity was averaged across all of the ten loci for each piglet, and the subsets of the five gene loci and the five microsatellite loci were averaged, respectively. Univariate regression analyses were used to estimate linear regression between the various growth traits (dependent variable) and mean heterozygosity (independent variable). All univariate relationships were visually inspected for possible nonlinear relationships using scatterplots. We applied tests for overdominance versus inbreeding effects as described by David (1995) to our heterozygosity-growth trait regressions. "Test A" is designed to compare the variance explained by univariate versus multivariate models using a likelihood ratio test: If significant, this test provides evidence for overdominance (David, 1995).

RESULTS

Means and standard deviation for growth traits were listed in Table 2. The performances of the three traits studied here were highly in correspondence with the same traits reported in literature (Zhang and Jiang, 1999). The standard deviation for birth weight (BW) was relatively high and the range varied widely (from 0.40 to 1.30 kg). The standard deviation and range for W35 and average daily gain (ADG) were very similar to those of BW, which suggested these early growth traits lacked selection and the native pig population contained sufficient genetic variability resource.

There were 3 alleles in each microsatellite locus and 2 alleles in each gene locus. The allele number for each locus was coincidence with literatures (Wu et al., 2000; Jiang et al., 2003; Yang et al., 2003). All the sampling variances of the loci scored were smaller than 0.1%.

The distributions of individual heterozygosity for 130 piglets are shown in Figure 1. Individual heterozygosity has a spiky appearance. The five gene loci were characterized by relatively small allelic variation across all piglets, just 2 allelic in each locus (Table 4). Heterozygosity varied among individuals (Figure 1a), indicating a wide range of genetic variation within this sample population, an important consideration when testing for genetic variation-growth relationships. Three of the five gene loci were found to be significantly out of Hardy-Weinberg equilibrium *(ESR*, *FSHb*, and *INHba*; see Table 3). All departures from Hardy-Weinberg equilibrium were due to an excess of homozygotes (Table 4).

The five microsatellite loci were characterized by relatively low allelic variation across all piglets. There were

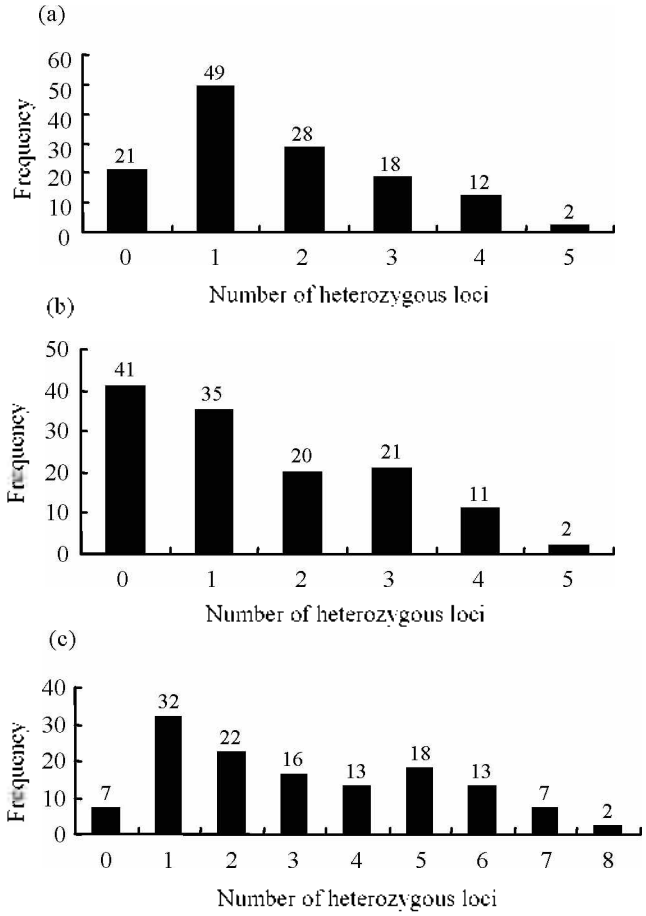

Figure 1. Distributions of loci heterozygous frequency for the 130 piglets scored in this study. (a), heterozygous frequency calculated by the five gene loci. (b), heterozygous frequency calculated by the five microsatellite loci. (c), heterozygous frequency calculated by the five gene and five microsatellite loci.

3 alleles in each locus (Table 3). Heterozygosity varied among individuals similar to those of the genes mentioned above (Figure 1b). indicating a wide range of genetic variation within this sample population. All the microsatellite loci except SW133 were found to be significantly out of Hardy-Weinberg equilibrium (see Table 3). All departures from Hardy-Weinberg equilibrium were due to an excess of homozygotes (Table 4). This phenomenon of microsatellite loci was observed in other literatures (Cho et al.. 2004; Li et al.. 2004; Sun et al.. 2004).

At eight loci the average daily gain of heterozygotes was larger than those of homozygotes. Partial *F* tests indicated that six positive differences (*ESR*. *FSHB*. *INHBB*, *SW*1332. *SW*1301 and *SW*1883) were statistically significant between heterozygotes and homozygotes ($p<0.01$). The sign test and the sequential Bonferroni test (Rice. 1989) suggest that there is overall trend for heterozygote means to exceed homozygote means.

The question of whether there is a systematic difference in average daily gain between homozygotes and heterozygotes for different classes of loci can be approached in this way. which is based on whether the polymorphism is scored from a function gene locus or not. According to this criterion, the loci were divided into two groups. one of five function gene loci and another five neutral microsatellite loci. We have used two approaches in testing the null hypothesis that there is no class-related effect on the difference in average daily gain among individuals with different degrees of heterozygosity. First. all 10 loci were treated as comprising one homogeneous class and average daily gain was regressed against the degree of individual heterozygosity. Then the loci were divided into two mutually exclusive groups and the two regressions were compared to each other and to the overall regression (see Table 5).

The univariate analyses yielded six significant regressions (see Table 5. $p<0.01$): they were W35 or GR

Table 3. Allele frequency, observed heterozygosity (H_o) and *D* for each of the five gene and five microsatellite loci *

Locus	Allele	Allele frequency	H_o	D	Locus	Allele	Allele frequency	H_e	D
SW1332	1	0.1808	0.2846	-0.5483	OB	a	0.4538	0.5385	0.0863
	2	0.3808				b	0.5462		
	3	0.4385							
SW1301	1	0.3231	0.2154	-0.5295	*ESR*	a	0.5692	0.2692	-0.4372
	2	0.6615				b	0.4308		
	3	0.0154							
SW1883	1	0.0462	0.2154	-0.6007	*FSHB*	a	0.6154	0.2538	-0.4617
	2	0.5192				b	0.3846		
	3	0.4346							
S0035	1	0.0077	0.4154	0.2175	*INHBB*	a	0.2692	0.3077	-0.3336
	2	0.7846				b	0.7308		
	9	0.2077							
SW133	1	0.0692	0.3462	0.0737	*INHBA*	a	0.4308	0.3077	-0.3726
	2	0.8115				b	0.5692		
	3	0.1192							

* H_o = observed heterozygosity, $D = (H_o-H_e)/H_e$, H_e = expected heterozygosity.

Table 4. Average daily gain (ADG), birth weight (BW) and body weight on day 35 (W35) for heterozygote and homozygote at each loci

Locus	Average daily gain				Birth weight				Body weight on day 35			
	N	Homozygote+SD	N	Heterozygote-SD	N	Homozygote-SD	N	Heterozygote+SD	N	Homozygote+SD	N	Heterozygote+SD
OB	60	0.2405±0.0687	70	0.2249±0.0688^{NS}	60	0.8317±0.2245	70	0.8343±0.2119	60	9.0058±2.3218	70	8.4803±2.3380
ESR	95	0.2010±0.0405	35	0.3163±0.0590**	95	0.8358±0.2068	35	0.8257±0.2457	95	7.6704±1.4059	35	11.5794±1.9367**
FSHB	97	0.2019±0.0405	33	0.3208±0.0577**	97	0.8397±0.2045	33	0.8136±0.2526	97	7.7031±1.4076	33	11.7203±1.9086**
INHBB	90	0.1978±0.0394	40	0.3092±0.0580**	90	0.8350±0.2087	40	0.8288±0.2372	90	7.5598±1.3612	40	11.3398±1.9186**
INHBA	90	0.2386±0.0686	40	0.2174±0.0683^{NS}	90	0.8322±0.2219	40	0.8350±0.2082	90	8.9442±2.3214	40	8.2248±2.3216
SW1332	93	0.2133±0.0582	37	0.2791±0.0720**	93	0.8113±0.2019	37	0.8878±0.2453	93	8.0641±1.9757	37	10.3786±2.3760**
SW1301	102	0.2237±0.0704	28	0.2624±0.0540**	102	0.8142±0.2135	28	0.9018±0.2196	102	8.4203±2.3749	28	9.8250±1.8373**
SW1883	102	0.2221±0.0677	28	0.2684±0.0616**	102	0.8123±0.2146	28	0.9089±0.2122*	102	8.3624±2.2880	28	10.0361±2.0537**
S0035	76	0.2293±0.0616	54	0.2360±0.0784^{NS}	76	0.8053±0.2189	54	0.8722±0.2100	76	8.6008±2.1034	54	8.8946±2.6408
SW135	85	0.2239±0.0737	45	0.2475±0.0564^{NS}	85	0.8188±0.2145	45	0.8600±0.2215	85	8.4305±2.4997	45	9.2751±1.8966*

** p<0.01, * p<0.05, NS: p>0.05

Table 5. Results of the univariate regression analysis for growth traits with individual heterozygosity

Trait	Gene heterozygosity			Microsatellite heterozygosity			Overall loci heterozygosity		
	Slope	R^2	P	Slope	R^2	P	Slope	R^2	P
BW	-0.0216±0.0765	0.0006	0.7776	0.1907±0.0673	0.0590	0.0053	0.1484±0.0899	0.0208	0.1014
W35	6.5546±0.5853	0.4949	0.0001*	3.3077±0.6875	0.1532	0.0001*	7.4683±0.7227	0.4548	0.0001*
ADG	0.1935±0.0173	0.4956	0.0001*	0.0891±0.0199	0.1353	0.0001*	0.2174±0.0218	0.4362	0.0001*

R^2: R squared value. Boldfaced values are those significant (* p<0.01).

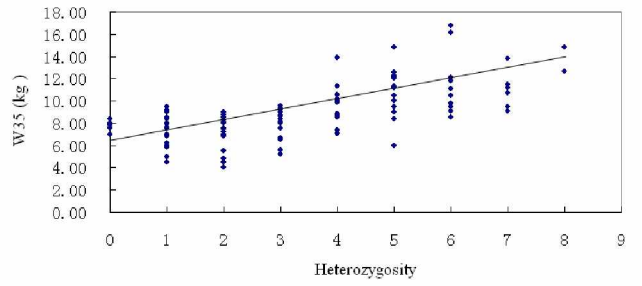

Figure 2. Body weight on day 35 (W35) of 130 piglets plotted against the degree of heterozygosity measured over the 10 loci listed in Table 1. Diamond circles indicate the mean body weight of each heterozygosity class. The solid line is the regression of individual body weight against the degree of heterozygosity.

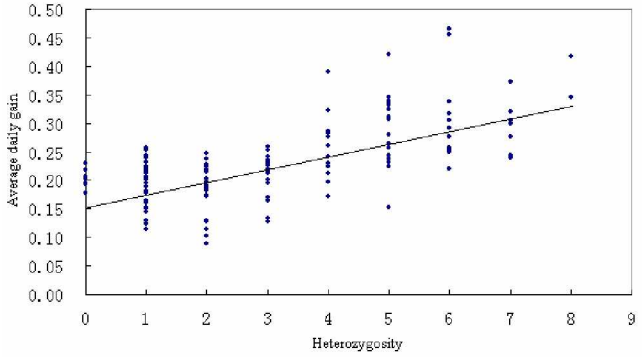

Figure 3. Average daily gain (ADG) of 130 piglets plotted against the degree of heterozygosity measured over the 10 loci listed in Table 1. Bold circles indicate the mean growth rate of each heterozygosity class. The solid line is the regression of individual body weight against the degree of heterozygosity.

with heterozygosity (including gene loci subclass, microsatellite loci subclass and over all loci heterozygosity). All the significant regressions had positive slope with *R* square values ranged from 0.1353 to 0.4956, which showed heterozygosity at these loci explained 13.53-49.56% of the total phenomenon variation in growth traits. Visual inspection of all univariate scatterplots showed no evidence of nonlinear relationships for any of the traits, although most univariate relationships had positive slope estimates (Table 5). There were no significant regressions for birth weight (BW) with heterozygosity (p>0.05).

Figure 2 plots body weight on day 35 (W35) of all 130 individuals against their degree of over all 10 loci heterozygosity. The correlations between these variables are significant and account for more than 45% of observed variance in W35.

DISCUSSION

Significant effects of heterozygosity on three growth traits were found in the native Meishan pig population. The univariate regressions explained the observed variance in the growth traits from 13.53% to 49.56%. It was a strong relationship between heterozygosity and piglet growth. However, to our knowledge, there has not been other study that used genetic markers to examine heterozygosity-growth traits correlations in pig. There were two studies that showed significant nonmammalian microsatellite-based genetic variation-fitness correlations involved growth rates in domestic shrimp (Bierne et al., 2000) and rainbow trout (Ferguson, 1992).

Associative overdominance is classically described using two categories of models: linkage disequilibrium in small populations or identity disequilibrium in infinite populations (Bierne et al., 2000). Either linkage disequilibrium or inbreeding alone can produce an apparent superiority of heterozygotes for a marker locus. The effect

of linkage disequilibrium on the difference between the heterozygote and homozygote values can be positive (associative overdominance) or negative (associative underdominance), depending on the frequencies of the marker alleles and the degree of their association with the deleterious gene. In the presence of both, the possibility of associative overdominance due to linkage disequilibrium is relatively higher (Zouros, 1993). In this study, both gene loci heterozygosity and microsatellite loci heterozygosity showed significant relationships with growth traits, which is a strong case to support associative overdominance hypothesis.

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