Correlations of Genic Heterozygosity and Variances with Heterosis in a Pig Population Revealed by Microsatellite DNA Marker*

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ABSTRACT : Correlation of microsatellite heterozygosity with performance or heterosis was reported in wild animal populations and domestic animal populations, but the correlation with heterosis in a crossbreeding F_1 pig population remained uncertain. To explore this, we had random selected and mated Yorkshire×Meishan (F, n = 82) and their reciprocal (G, n = 47) to F_1, and used the two straightbreds as control groups (Yorkshire = 34, Meishan = 55), and observed the heterosis of birth weight (BWT), average daily gain (ADG) and feed and meat ratio (FMR). Two Kinds of measurement-individual heterozygosity (*IH*) and individual mean d^2 (lg value, *ID*) were used as index of heterozygosity and variance from 39 microsatellite marker loci to perform univariate regression analysis against heterosis. We detected significant correlation of *IH* with BWT in all of F_1 (F+G) and in F. We observed significant correlation of *ID* with ADG in all of F_1 (F+G), and with FMR in all of F_1 (F+G) and in F. There was significant maternal effect on heterosis, which was indicated by significant difference of means and distribution of heterosis between F and G. This difference was consistent with distributions of *IH* and *ID*, and with difference of means in F and G. From this study, it would be suggested that the two kinds of genetic index could be used to explore the genetic basis of heterosis in crossbreeding populations but could not determine which is better. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 620-625)***

Key Words : Pig, Heterosis, Microsatellite, Individual Heterozygosity, Individual Mean d^2

INTRODUCTION

Heterosis, or hybrid vigor, refers to the phenomenon that progeny from crossbreeding of inbred breed exhibit better than their parents in many performances including growth, biomass, individual development, reproduction and ability of adapting to environment. The genetic phenomena have been used in the diversity of agricultural production for many years, and the genetic basis has been discussed for almost a century (Shull, 1908; Bruce, 1910), but little consensus has emerged.

The recent advances in genome research have generated considerable advantage of conditions to reveal the genetic basis of the phenomenon, and that turn to predict hybrid performance in production using molecular marker. Large number of studies have been conducted in plant breeding program (Qifazhang et al., 1994, 1996; Yu et al., 1997; Sun et al., 2002; Reif et al., 2003), and papers about different animals which could give us some clue to recognize the progress in this field (Gavora et al., 1996; Wu et al., 2001; Shikano and Taniguchi, 2003).

In recent years, the genomic research programs of pig have made great progress in building linkage map by molecular marker especially by microsatellite DNA marker, and by which, generated a number of papers and results of QTL mapping (Bidanel et al., 2002; Zuo et al., 2003) and genetic diversity (Laval et al., 2000; Behl et al., 2002; Li et al., 2004; Wang et al., 2004) to help us to recognize genetic characters of economic traits in swine. However the paper about investigation of genetic basis of heterosis by way of DNA molecular marker is remaining scarce.

As our common recognizing from papers of the past years, performance is better along with the increasing of heterozygosity in crossbreeding population from inbred breed. But the correlation of heterozygosity inferred from molecular marker with economic traits was not in accordance, contrast to that, including positive correlation (Nicolas et al., 1998) and negative correlation (Zouros et al., 1984; Mallet et al., 1985) of heterozygosity with heterosis. But this situation was remaining uncertain in pig.

In this paper, we attempt to examine the relationship between molecular marker heterozygosity and heterosis of two main growth traits and birth weight by two different genetics indexes-individual heterozygosity and individual mean d^2 in F_1 population from Yorkshire×Meishan and its reciprocal. This two different measurement of heterozygosity had been used in several papers about animal fitness (Health et al., 2002) or animal molecular ecology (Coulson et al., 1998; Hansson et al., 2001) in nature animal population, and had been used in pig population to explore the correlation of heterozygosity with growth trait (Jiang et al., 2003), as well as been used in horse population for morphological trait (Curik et al., 2003). But used in F_1 for

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probing directly the genetic correlation of individual genic heterozygosity and variance with heterosis had not found.

MATERIALS AND METHODS

Population and traits

The Yorkshire (Y) and the Meishan (M) used in this experiment was imported from England and Jiading County (Shanghai), respectively, and they were kept straightbred for more than three years in our herd specific for experimental use before mating to build the population. The experimental pig population composed of 4 subpopulation: the two straightbred as control group; Yorkshire×Meishan (F) and their reciprocal (G) to F_1 for understanding the genetic basis of heterosis. Estrus and mating was controlled so that progeny was about 90 kg in late of summer or early autumns in 2002. The pig population had been raised at a herd with the same equipment and with the same ordinary food standard. Variables measured in this study were birth weight (BWT), average daily gain (ADG), feed and meat ratio (FMR). Birth weight was recorded within 24 h after birth. Average daily gain was the body weight gain during the period of measurement divided by number of days. Feed and meat ratio was the ratio of food intaking divided by body weight increasing during the period of measurement. All of them were recorded according to the method of Xiong and Deng (1999).

Describing statistical analysis of animal trait data

Phenotype and heterosis were analyzed with TTest procedure by SAS software (SAS Institute Inc, Version 6.12) for examining the variation among the different crossbred population.

The amount of heterosis or hybrid vigor (H) was evaluated as follow:

$$H = (F_1-P)/P$$

Where F_1 is the individual trait value of crossbred, and

$$P = (\overline{P}_1+\overline{P}_2)/2$$

In which $\overline{P}_1$ and $\overline{P}_2$ are the means of the corresponding straightbred that was representative of the means of their parents.

Microsatellite and PCR amplification

Thirty nine microsatellites on SSC4, SSC6, SSC7, SSC8, and SSC13 were selected based on the USDA-MARC Genome Database map (http://www.genome.iastate.edu/maps/), on which the loci position of the SSR was picked out by the interval of about 20-30 cM, and not having too many or less number of alleles so as to be apt to scoring. All of the microsatellite DNA primer was presented by US Pig Genome Coordinator (Max F. Rothschild). PCR of microsatellites were carried out on PE intergraded thermalcycler (PE-9600, USA). The reaction volume of each PCR was 20 μl containing 50 ng genomic DNA (from blood leukocyte), 1×PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 5 pM of each primer and 1 *U Taq* DNA polymerase (Biostar International, Canada). The thermalcycling conditions were: pre-denaturation for 4 min at 94°C, followed by 35 cycles of reaction at a fixed annealing temperature according to different microsatellite primer: annealing 45 s, 72°C extension 45 s, 94°C denaturing 45 s, with final extension at 72°C for 10 min. Each PCR product was added 3-4 μl loading buffer, 6-8 μl each pooled sample was loaded upon 8% polyacrylamide gels and taken electrophoreses for 10-12 h at constant power of 70-90 volt and sliver-stained before genotype scoring.

Genotype score, Genetic characteristic analysis, Individual heterozygosity (*IH*) and Individual mean d^2 (*ID*) calculation

Genotype score of individual was inferred by their parents' at the same polyacrylamide gels to avoid redundant (to expel from disturbing of nonspecific band by referring to the parental genotypes), the different microsatellite DNA fragment at the same loci on different polyacrylamide gels was distinguished by standard DNA marker (pBR322/Msp I). Allele size of microsatellite PCR amplification production was determined by method of Rickwood and Hames (1982) and referred to the standard DNA marker, so there was some error, but it was consistent and identical in all population, and the results was credible in this study.

Genetic characteristic analysis was performed by MAS software (Dieringer and Schlötterer, 2003).

Individual heterozygosity was calculated across all scored loci, if an individual was heterozygous at a locus it was scored as a '1' and if homozygous as a '0', the number of heterozygous loci divided by the number of loci examined; the mean across all loci scored was then taken.

Individual mean d^2 was calculated as the squared distance in repeat units between the two alleles an individual had at a microsatellite locus, averaged over all loci at which an individual was scored.

i.e.

$$\text{Mean } d^2 = M \frac{(i_a-i_b)^2}{n}$$

where i_a and i_b are the lengths in repeat units of alleles a and b at locus i, and n is the total number of loci at which an individual was scored (Coulson et al., 1998). The distribution of mean d^2 was highly skewed at all loci, in

Table 1. The performance and heterosis of growth traits and birth weight in pig population in this study (Means±SE)

	Y (34) Performance	M (55) Performance	F (82) Performance	G (47) Performance	F Heterosis	G Heterosis	Average heterosis
BWT	1.33±0.05	1.02±0.03	1.15±0.04^{a}	1.29±0.03^{b}	-0.02±0.03^{A}	0.10±0.03^{B}	0.040
ADG	0.70±0.01	0.46±0.02	0.64±0.01^{a}	0.72±0.02^{b}	0.09±0.01^{A}	0.22±0.02^{B}	0.155
FMR	2.33±0.05	3.40±0.10	2.81±0.06	2.69±0.06	-0.02±0.02	-0.06±0.02	-0.040

Y = Yorkshire straightbred; M = Meishan straightbred; F = Yorkshire Meishan.
G = Meishan×Yorkshire; BWT = birth weight; ADG = average daily gain; FMR = feed and meat ratio.
^{a,b} indicate $p<0.05$ significant differences; ^{A,B} indicate $p<0.01$ significant differences.

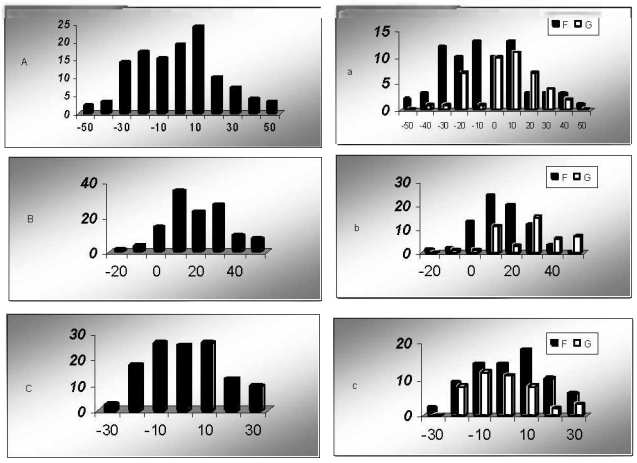

Figure 1. Distributions of midparent (representative by purebrid) heterosis of growth traits in this study. A, B, C indicate distribution of BWT, ADG, FMR in all F_1 (F−G), respectively; a, b, c indicate distribution of BWT, ADG, FMR in Yorkshire× Meishan (F) and reciprocal (G), respectively. X axis-value of heterosis; Y axis-individual count.

order to improve normality, it was transformed by $\log_{10}$ (mean d^2), and subsequent analyses were performed on the transformed value (Hansson et al., 2001). The two measurement results were presented by distributing figure to give us information of variation.

Regression analyses of *IH* and *ID* against heterosis

The two measurements were used for calculating the correlations of heterozygosity and variance with heterosis by univariate regression. The analysis was carried out with REG procedure of SAS software, and the results were presented by tables and figures of linear regression.

RESULTS

Heterosis of the hybrid

Highly significant ($p<0.01$) differences in heterosis of birth weight and average daily gain was observed between F and G. progeny from G has better performance and heterosis than from F, but the significant difference was not found for FMR, although there was better heterosis in progeny from G than from F (Table 1). The highest heterosis (22%) was observed in average daily gain in G, and this heterosis was best (15.5%) in all of F_1 (F+G) among the three traits.

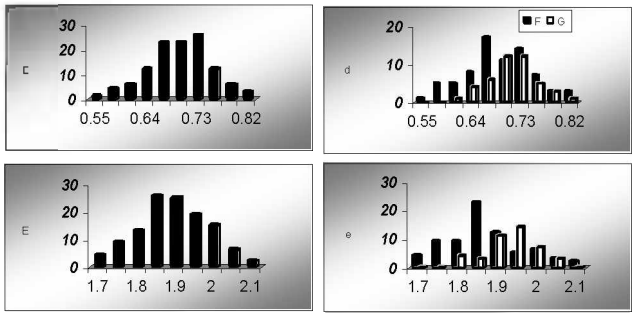

Figure 2. Distribution of individual heterozygosity (*IH*) and individual mean d^2 (l g value, *ID*). D, d-distribution of *IH* in all of F_1 (F−G), and in F and G respectively; E, e-distribution of *ID* in all of F_1 (F−G), and in F and G respectively; X axis-individual heterozygosity (*IH*) (D, d) or individual mean d^2 (lg value, *ID*) (E, e); Y axis-individual count.

The significant difference between the YM and MY (F vs. G) was observed from the distribution of the heterosis for BWT and ADG (Figure 1a, b): more proportion of individual number from G than from F distributed in the region of positive heterosis (the region of more than 0 in x axis). For FMR it was reverse (Figure 1c), but the negative heterosis indicate better feed efficiency. All of the distribution were nearly normal, and exhibited considerable range for the three traits (Figure 1A, B, C).

Polymorphism, Individual heterozygosity (*IH*) and Individual mean d^2 (lg value, *ID*) of microsatellite marker loci

Among the 39 microsatellite DNA markers used in this study, considerable allelic variations were observed in allele size range, as well as in number of alleles and heterozygosity across all of F_1 (Table 2). Average observed heterozygosity was range from 0.19 (sw864) to 0.72 (sw2440 and s0121), and average observed $\log_{10}$ (mean d^2) was range from 0.33 (sw935) to 2.45 (sw2406) in all of F_1 population. The *IH* across all loci was 0.53-0.82, and the *ID* was 1.67-2.07 (not presented).

The distributions of *IH* and *ID* in all of F_1 (Figure 2D, E), and in F and G (Figure 2d, e), were showed in Figure 2. The distributions of the two different kinds of genetic index were near normal in all of F_1, as well as in F and G respectively. But in the later, there were some interested character: a) the distribution of *IH* in F and G was different, that was the average *IH* in G higher than that in F, although

Table 2. The microsatellite DNA marker used in this study and their genetic characteristics observed in all of F_1 (F+G)

SSR*	Allele obs.	H_o	Allele size (bp) obs.	$lg(d^2)$
sw489	4	0.68	153-177	1.99
sw835	4	0.65	225-240	1.91
sw2409	2	0.39	87-92	1.38
sw2454	5	0.34	106-133	2.28
s0023	4	0.48	76-106	2.16
sw1996	2	0.36	156-161	0.47
sw445	6	0.71	178-209	2.17
s0161	4	0.61	140-163	2.36
sw2406	4	0.64	221-256	2.45
sw1841	6	0.69	174-231	2.27
sw1302	4	0.51	168-185	1.43
swr1130	6	0.71	112-137	1.88
sw1129	6	0.61	127-155	1.35
sw1473	4	0.47	169-183	1.69
s0121	6	0.72	164-191	2.08
sw322	4	0.54	111-119	0.82
swr1343	3	0.51	127-141	1.98
sw2155	4	0.46	135-145	0.99
sw1856	5	0.62	181-198	1.75
swr2036	4	0.59	155-173	2.27
sw352	3	0.49	107-113	0.93
sw252	4	0.44	151-169	1.65
sw581	3	0.40	197-205	0.74
s0212	4	0.60	229-242	1.43
sw2410	4	0.37	110-124	1.43
s0098	4	0.34	94-113	1.62
sw268	4	0.57	122-144	1.82
sw1037	4	0.38	125-142	1.97
sw1953	3	0.43	151-167	1.97
sw2160	3	0.55	171-185	1.99
sw1085	3	0.53	121-137	1.73
sw1980	3	0.46	181-193	2.05
sw1691	2	0.32	128-139	1.81
sw935	2	0.26	196-199	0.33
sw864	3	0.19	169-178	0.93
sw1495	3	0.33	155-168	1.14
sw520	4	0.43	102-120	2.07
sw2440	5	0.72	137-151	1.73
s0291	4	0.38	166-183	1.45
Overall	3.9	0.57		1.68

* http://www.genome.iastate.edu/maps/

no significant was detected; b) the distribution of *ID* in F and G was observed highly significant difference (Table 3). This characteristic was consistent with the distributions of the heterosis mentioned above.

Correlation of *IH* and *ID* with heterosis

Correlations of the two measurements with heterosis were presented in Table 4 and illustrated in Figure 3. A highly significant correlation of *IH* with heterosis for BWT was observed in all of F_1 (F+G) (r = 0.2763, p = 0.0025) and in F (r = 0.3346, p = 0.0063), but that was not exist for ADG and FMR. We detected significant correlation of *ID*

Table 3. Comparison of means *of IH* and *ID* in F and G population (Means±SE)

	F Means±SE	G Means±SE	t value	Pr>|t|
IH	0.6809±0.0075	0.6977±0.0070	-1.64	0.1035
ID	1.8395±0.0110	1.9093±0.0102	-4.64	<0.0001

F = Yorkshire×Meishan; G = Meishan×Yorkshire.

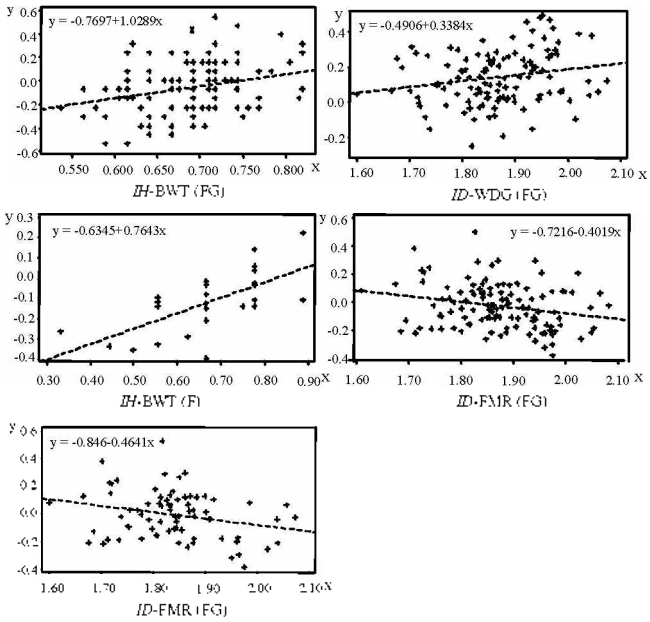

Figure 3. Illustrations of results of univariate linear regression of *IH* and *ID* against heterosis of F_1 for significant items.

with heterosis for ADG (r = 0.2053, p = 0.0251) and FMR (r = -0.2321, p = 0.0111) in all of F_1, and for FMR in F (r = -0.2593, p = 0.0247). There were no significant correlation of the two measurements with heterosis of the three traits in G was detected.

The significant correlations of the two genetic indexes with heterosis for the three traits and its R values were generally small. Meanwhile, all of the significant correlation was positive correlation, that was along with the increase of *IH* and *ID*, the heterosis was better (Figure 3).

DISCUSSION

Performance and heterosis in this study

Yorkshire and Meishan are representative of different kinds of elite germplasm in pig. The crossbreeding between them is representative of typical heterogeneous combination, and the progeny would be highly heterozygosity and heterosis. We observed from the results in this study that the performance and heterosis was different between Yorkshire×Meishan and Meishan×Yorkshire, the later was significant higher than the former, especially heterosis for BWT and ADG. This was another instance of heterosis affected by maternal effect which was reported by Falconer

Table 4. Correlation of *IH* and *ID* with heterosis of F_1

Group		BWT			ADG			FMR		
		Corr.	R value	P	Corr.	R value	P	Corr.	R value	P
F+G	*IH*	0.2763	0.0763	0.0025	0.1392	0.0194	0.1310	-0.1066	0.0114	0.2483
	ID	0.1474	0.0217	0.1112	0.2053	0.0421	0.0251	-0.2321	0.0539	0.0111
F	*IH*	0.3346	0.1120	0.0036	0.0544	0.0030	0.6427	-0.0730	0.0053	0.5335
	ID	0.0867	0.0075	0.4628	0.1103	0.0673	0.3462	-0.2593	0.0673	0.0247
G	*IH*	-0.0108	0.0001	0.9444	0.1726	0.0298	0.2626	-0.1249	0.0156	0.4192
	ID	0.0115	0.0001	0.9411	0.0376	0.0014	0.8084	0.0376	0.0014	0.8084

F = Yorkshire×Meishan; G = Meishan×Yorkshire; BWT = birth weight; ADG = average daily gain; FMR = feed and meat ratio.

and Mackay (1996). There was crossbreed inferior for BWT in Yorkshire×Meishan, which was instance of maternal effect too. The crossbreeding inferior had been reported by Zouros et al. (1984) in bivalve molluscs, and Mallet et al. (1985) in marine bivarine.

Distributions of *IH* and *ID* in different animal population

Microsatellite has been used as marker in many population genetic studies, owing to their abundance genetic varieties and high heterozygosity in most eukaryote genomes. The proportion of loci which are heterozygous versus homozygous should be correlated with the extent of recent inbreeding or outbreeding in animal population. In general, mean d^2 focuses on events deeper in the pedigree than individual heterozygosity, that was why mean d^2 has more extensive distribution range than heterozygosity in our study and most of papers (Hansson et al., 2001; Heath et al., 2002). But there is difference situation for distribution of the two measurements between nature population and domestic animal population in which the genetic structure is disturbed by factitious factors such as nonrandom mating, especially in crossbred F_1 population as our study. It was understandable that the distributions of the two genetic indexes were nearer normal distribution than that from reported by papers (Coulson et al., 1998; Hansson et al., 2001; Heath et al., 2002), this is due to homogeneous genetic structure resulted by crossbreeding of two purebred. It was interested that the distributions of the two kind measurements reported by Heath et al. (2002) and Curik et al. (2003) (from domestic animal population) had more similar and nearer normal distribution than reported by Coulson et al. (1998) and Hansson et al. (2001) (from nature population), but less than that of this study (from crossbred F_1 population).

Difference of means and difference of distribution of the two measurement (*IH* and *ID*) between YM and MY was consistent with distribution of performance and heterosis. This inferred that genome heterozygosity, not only microsatellite loci heterozygosity such as *IH* and *ID*, but also heterozygosity of functional gene loci affecting the relative traits, would be the genetic basis of heterosis (Coulson et al., 1998) in this crossbred population.

Correlation of *IH* and *ID* with heterosis

The present study was intended to investigate the extent of correlation between marker heterozygosity and heterosis in pig population. We found several instances of significant effect of genetic variation (both *IH* and *ID*) on heterosis of three traits in this study, all of them were positive correlation, although the correlation was low. However most of them had little correlation was detected between heterozygosity and heterosis, especially in MY population which had good heterosis. Other studies had shown the same results, such as Wu et al. (2001) in inbreeding pig population; Coulson et al. (1998) and Slate et al. (2000) in nature red deer; Health et al. (2002) in domestic chinook salmon and so on. There has other study that used microsatellite markers to examine heterozygosity–fitness correlations in fish, such as Blanchfield et al. (2003) in brook trout (*Salvelinus fontinalis*), which reported no significant relationships. The reason that did not detect the significant correlation in MY population may be could not reach the statistical power due to no enough individual number in this study.

Which is more suitable to do the evaluations

Some papers had drawn the conclusion that, the individual mean d^2 provides better measure of individual genetic variability than heterozygosity for microsatellite data, and could be a convenient tool for assessing the effects of inbreeding and outbreeding in nature population (Coltman et al., 1998), or in small and closed population (Curik et al., 2003). Other papers could not draw the conclusion of which is better to do the evaluations in nature population (Coulson et al., 1998) or in domestic animal population (Health et al., 2002). In our crossbred F_1 pig population, when economic traits or hetersis was estimated by molecular genetic index of microsatellite, it would be have relationship with genetic base of pedigree, number and selection of microsatellite marker loci used in the research, number of animal engaged in statistical analysis and so on, so it is difficult to determine which is better.

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