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Full Length Research Paper

Study on relationship between microsatellite polymorphism and producing ability on litter size trait of Hu sheep in China

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Four microsatellite loci (OarAE101, BM1329, BM143 and OarHH55) linked to FecB gene on chromosome 6 and one microsatellite locus (OarHH55) on chromosome 4 were selected to study their correlation with litter size of Hu sheep breed. The results showed that the average polymorphism information content (PIC), heterozygosity (He) and effective allele number (Ne) were 0.7214, 0.7558 and 4.4094, respectively, and all 5 microsatellite loci reached high polymorphism (PIC > 0.5), and the five microsatellite loci can be used for genetic diversity evaluation in Hu sheep breed. The result of variance analysis showed that there were extreme significant differences among the different genotypes for the producing ability of litter size in OarAE101 locus and BM143 locus (P < 0.01), and OarHH35 locus (0.01 < P < 0.05). However, there was no significant difference among the different genotypes for the producing ability of litter size in BM1329 and OarHH55 loci (P > 0.05). Choosing by combining producing ability and special gene (gene type) will play an great role in improving selecting accuracy and breeding progress, and it is a wonderful and new analyzed thought in repeatable characters with low heritability which is affected by measuring frequencies. And this study will have an important role in MAS (marker-assistant selection) and molecular breeding in Hu sheep in future.

Key words: Hu sheep, microsatellite markers, litter size trait, producing ability of ewes.

INTRODUCTION

The profits of sheep production is dependent on litter size, and it is very difficult to improve litter size by normal breeding methods since its heritability ranges between 0.05 - 0.15 (Wang et al., 1994). There are many sheep breeds with fecundity traits in the world, but only the Australian Booroola merino sheep has been clearly known. The fecundity gene was located on 6th chromosome (Piper et al., 1982; Davis et al., 1982, 1983). In 1989, it was named Fec^B (Fec = fecundity, B = Booroola) (Gognosag, 1989), it was precisely located in the area of 10cM between OarAE101 and BM1329 (Montgomery et al., 1993, 1994; Lord et al., 1998). Fec^B has been used as a marker assisted selection to improve sheep fecundity (Gootwine et al., 1998). The high fecundity Hu sheep in China gives 2 lambs per birth, except those that are in the first lambing period, 6 - 8 lambs per birth was recorded. The average lambing rate of Hu sheep's was 261.09% (Editorial section of "Records of Sheep and Goat Breeds in China", 1989).

Currently, research on molecular mechanism of fecundity in Hu sheep is very few. Sheep's fecundity is affected by physiological condition, nutrition level and germ quality and other exterior factors. Phenotypic value of fecundity could not really be inherited. Producing ability on fecundity of ewes, obtained from calculating repeatability of litter size, is a good genetic parameter for choosing ewes, which could measure potential inheritability of fecundity. Therefore, it would be very meaningful to improve choosing accuracy and breeding progress by combining producing ability on fecundity of ewes and alleles (gene

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Table 1. Primer sequences, annealing temperature and $MgCl_2$ amount.

Marker	Primer sequences	Annealing temperature (℃)	$MgCl_2$ (μl)
OarHH55	5′-3′GTTATTCCATATTCTTTCCTCCATCATAAGC 3′-5′CCACACAGAGCAACTAAAACCCAGC	63	2.4
OarHHHH35	5′-3′AATTGCATTCAGTATCTTTAAACATCTGGC 3′-5′ATGAAAATATAAAGAGAATGAACCACACGG	63	2.4
BM143	5′-3′ACCTGGGAAGCCTCCATATC 3′-5′CTGCAGGCAGATTCTTTATCG	63	2.4
BM1329	5′-3′TTGTTTAGGCAAGTCCAAAGTC 3′-5′AACACCGCAGCTTCATCC	60	1.2
OarAE101	5′-3′TTCTTATAGATGCACTCAAGCTAGG 3′-5′TAAGAAATATATTTGAAAAAACTGTATCTCCC	59	2.4

type). In this paper, we focused on fecundity trait of Hu sheep and we selected microsatellites closely linked with FecB gene reference to Booroola sheep.

The aims of this study were to study the relationship between microsatellites and producing ability on fecundity trait of Hu sheep, and to provide scientific basis to further study the mechanism of fecundity and marker assisted selection.

MATERIALS AND METHODS

Experimental animals

The litter traits (lambs orn/ewe lambing) were collected from the records of 65 Hu sheep ewes from Suzhou sheep breeding farm in Jiangsu province, China. Blood samples were collected and DNA was isolated using deposit leukocyte phenol/chloroform extraction method (Sun et al., 2008).

Microsatellite analysis

Primers were selected based on the Boroola sheep data and synthesized by Sangon Biotech Company, Shanghai, China (Table 1). Four microsatellites (OarAE101, BM1329, BM143 and OarHH55) linked with fecundity gene on the 6th chromosome and one microsatellite (OarHH55) linked with production gene on the 4th chromosome were used. PCR were performed in 20 μl reaction volume containing 2 μl of 50 ng genomic DNA, 2 μl of 10×buffer, 1.2 - 2.4 μl 25 mmol/l $MgCl_2$, 0.4 μl 10 mmol dNTP, 1 μl 8 μmol/μl upper and lower primers, 0.2 μl of 5 U Taq DNA polymerase and ddH_2O to 20 μl as described by (Buchanan, 1992a, 1992b, 1993). The PCR protocol consisted of an initial denaturation at 94℃ for 5 min, 30 cycles of 1 min at 94℃, anneal for 1 min and extended 1min at 72℃, followed by a final extension at 72℃ for 10 min. PCR products were electrophoreses on denaturing polyacrylamide gel, silver stained and visualized under ultraviolet light using trans-illuminator. The length of amplified fragment relevant to pBR322/MspI marker was calculated by Kodak Digital Science ID Image Analysis Software.

Statistical analysis

Genetic polymorphism analysis

Allele frequencies were computed by gene counting method. Heterozygosity (He) (Nei, 1978), polymorphism information content (PIC) (Botstein et al., 1980) and effective allele number (Ne) (Kimura and Crow, 1974) were calculated using SAS package.

Assessment of producing ability of ewes

Repeatability:

$$r_e = \frac{MS_B - MS_W}{MS_B + (n-1)MS_W}$$

If there is difference in each population in observation number, weighted value n_0 should be taken into consideration:

$$n_0 = \frac{1}{D-1}\left(N - \sum {n_i^2}/{N}\right)$$

n_0 = weighted mean of measurement frequency of individuals, N = sum of measurement frequencies, D = number of individuals, and n_j = measurement frequency of each individual.

Producing ability of ewes:

Producing ability of ewes can be calculated earlier when we have the evaluation of repeatability, and when litter records are different from individuals, the mean repeatability should be calculated with more records $r_{e(n)}$.

$$r_{e(n)} = \frac{V_G + V_{Eg}}{V_{p(n)}} = \frac{V_G + V_{Eg}}{[1+(n-1)r_e].V_p/n} = \frac{nr_e}{1+(n-1)\,r_e}$$

The producing ability on special trait of individual can be calculated by the following formula (Wang et al., 1994).

$$P_X = (P_n - \bar{P})r_{e(n)} + \bar{p} = (p_n - \bar{p})\frac{nr_e}{1+(n-1)r_e} + \bar{p}$$

Table 2. Amplication of five microsatellite markers in Hu sheep.

Locus	Genotype (number of individuals)		Size of alleles (bp)	Allele frequency
***0arAE*101**	103/113 (4)	101/111 (3)	103	0.091 7
	107/113 (8)	113/113 (10)	101	0.083 3
	107/107 (9)	107/111 (2)	107	0.266 7
	101/101 (2)	103/107 (3)	109	0.016 7
	101/107 (1)	103/103 (2)	113	0.275 0
	115/115 (4)	115/117 (1)	115	0.075 0
	111/111 (7)	101/113 (1)	111	0.158 3
	101/125 (1)	125/125 (1)	125	0.025 0
	109/109 (1)		117	0.008 3
***BM*1329**	146/146 (12)	146/162 (15)	146	0.416 7
	164/164 (13)	158/158 (3)	164	0.233 3
	146/158 (9)	162/162 (3)	158	0.133 3
	148/148 (2)	148/158 (1)	148	0.041 7
	146/164 (2)		162	0.175 0
***OarHH*35**	139/139 (7)	125/125 (5)	139	0.475 0
	139/141 (4)	137/125 (3)	141	0.033 3
	127/127 (2)		127	0.225 0
	139/127 (23)		125	0.241 7
	139/125 (16)		137	0.025 0
***BM*143**	118/118 (3)	116/104 (1)	118	0.266 7
	118/116 (8)	112/114 (2)	116	0.116 7
	102/104 (10)	112/118 (1)	102	0.141 7
	102/106 (2)	112/102 (3)	104	0.125 0
	118/106 (12)	118/112 (1)	106	0.175 0
	118/102 (1)	112/104 (1)	110	0.016 7
	118/108 (1)	116/112 (4)	108	0.041 7
	108/108 (1)	108/102 (1)	112	0.100 0
	106/104 (3)	116/106 (1)	114	0.016 7
	106/106 (1)	108/106 (1)		
***OarHH*55**	131/131 (1)	147/127 (11)	131	0.058 3
	131/129 (3)	147/135 (2)	129	0.266 7
	129/129 (3)	147/125 (4)	135	0.033 3
	135/131 (1)	127/125 (4)	147	0.325 0
	147/147 (4)	127/129 (8)	127	0.216 7
	147/129 (13)	127/127 (1)	125	0.100 0
	147/131 (1)	125/125 (2)		
	135/129 (1)	129/127 (1)		

P_x = producing ability on special trait of individual x, $\overline{P}$ = mean value of traits in the populaton, and P_n = mean value of n records on special trait.

Relationship analysis between marked gene type and producing ability of Hu sheep in litter size

The difference between marked gene type and producing ability of Hu sheep litter size was compared with General Linear Model procedure using SAS package according to the following model:

$$y_{ij} = \mu + M_i + e_{ij}$$

y_{ij} = Producing ability of Hu sheep in litter size; μ =population mean value; M_i = Fixed effect of i marked gene type; e_{ij} = random error effect.

RESULTS

Amplications analysis of microsatellitemarkers

Allele distribution and frequencies are list in Table 2. From the tables, 17 genotypes and 9 alleles that ranged

Table 3. Polymorphism information content (mean), mean heterozygosity (H) and mean effective number of alleles (Ne) of microsatellite DNA sites in Hu sheep.

Locus	Polymorphism information content (PIC)	Heterozygosity (He)	Ne
OarAE101	0.7803	0.8062	5.1611
BM1329	0.6788	0.7218	3.5945
OarHH35	0.6073	0.6636	2.9726
BM143	0.8169	0.8366	6.1210
OarHH55	0.7236	0.7618	4.1978
Mean Value	0.7214	0.758	4.4094

between 101 to 125 bp were found in OarAE101. The dominant genotypes are 107/113, 107/107, 111/111 and 113/113, and the dominant alleles are 107, 113, 111. 9 genotypes and 5 alleles that ranged between 101 and 125 bp were found in BM1329. The dominant genotypes are 146/146, 164/164, 146/158 and 146/162, and the dominant alleles are 146, 164, 158 and 162. 7 genotypes and 5 alleles that ranged between 125 and 141 bp are found in OarHH35. The dominant genotypes are 139/139, 139/127 and 139/125, and the dominant alleles are 139, 127 and 125. Twenty genotypes and nine alleles that ranged between 102 and 118 bp are found in BM143. The dominant genotypes are 116/118, 102/104, 118/106, and the dominant alleles are 118, 116, 106, 104 and 102. 15 genotypes and 6 alleles that ranged between 125 to 147 bp are found in Oarhh55. The dominant genotypes are 147/129, 147/127 and 127/129, and the dominant alleles are 147, 129 and 127.

PIC, Ne and He of microsatellite sites

Information of 5 microsatellite markers on PIC, Ne and He is list in Table 3. From Table 7, PIC of OarAE101, BM1329, OarHH35, BM143 and OarHH55 are 0.7803, 0.6788, 0.6073, 0.8169 and 0.7236, respectively, with an average of 0.7214. He of OarAE101, BM1329, OarHH35, BM143 and OarHH55 are 0.8062, 0.7218, 0.6636, 0.8366 and 0.7618, respectively, with 0.758 as mean value. Ne of OarAE101, BM1329, OarHH35, BM143 and OarHH55 are 5.1611, 3.5945, 2.9726, 6.1210 and 4.1978, respectively, with 4.4094 as mean value.

The result shows that polymorphism exists in 5 microsatellite markers, which can be used to evaluate genetic diversity of Hu sheep in reproduction.

Association between microsatellite markers and producing ability of ewe in litter size

Result showed that there are extreme significant differences among the different genotypes for the producing ability of litter size in OarAE101 locus and BM143 locus ($P < 0.01$), there are significant differences among the different genotypes for the producing ability of litter size in OarHH35 locus ($0.01 < P < 0.05$), however, no significant difference among the different genotypes for the producing ability of litter size in BM1329 and OarHH55 loci ($P > 0.05$).Those genotype whose indivi-dual number are less than 2 are not included in our analysis. Least square means ± standard error of producing ability of ewe in litter size in 5 microsatellite markers with multiple comparisons was listed in the Table 4.

Table 4 showed that least squares mean for the producing ability of litter size for OarAE101 107/113 was the largest, and it is not significantly higher than those for OarAE101 103/113, 107/107, 101/111, 113/113, 107/111 and 103/103 ($P > 0.05$), however it is significantly higher than those for OarAE101 101/101 and 103/107($0.01 < P < 0.05$), and it is extremely significantly higher than those for OarAE101 115/115 and 111/111 ($P < 0.01$) in Hu sheep.

Table 5 shows that least square means of producing ability of ewe in 162/162 of BM1329 is the largest, but no significance with other markers. Table 6 showed that the least squares mean for the producing ability of litter size for OarHH35 139/139 was the highest, however it was not significantly higher than those for OarHH35 139/144, 139/127 and 137/125 ($P > 0.05$). It was significantly higher than those for OarHH35 127/127 and 125/125 ($0.01 < P < 0.05$), and extremely significantly higher than those for OarHH35 139/125 ($P < 0.01$) in Hu sheep.

Table 7 showed that least squares mean for the producing ability of litter size for BM143 118/118 was the highest, however it is not significantly higher than those for BM143, 118/106, 118/110, 106/104 and 112/114 ($P > 0.05$), it is significantly higher than those for BM143 118/116 and 102/106 ($0.01 < P < 0.05$), and it is extremely significantly higher than those for BM143 102/104, 112/102 and 116/112 ($0.01 < P < 0.05$) in Hu sheep. Table 8 shows that least square means of producing ability of ewe in 147/135 of OarHH55 are the largest, but no significance with other markers.

DISCUSSION

Microsatellite polymorphism

Ne, PIC and He are all used to evaluate population genetic

Table 4. Least square means of OarAE101 genotypes for the producing ability of litter size of ewes in Hu sheep.

Treat	*OarAE*101 genotypes	Number of individuals	Least square means (LSMs)
1	103/113	4	2.572 5±0.036 2 ^{abAB}
2	107/113	8	2.595 0±0.025 6^{aA}
3	107/107	9	2.585 6±0.024 1^{aAB}
4	101/101	2	2.460 0±0.051 2^{bcABC}
5	115/115	4	2.475 0±0.036 2^{bcBC}
6	111/111	7	2.430 0±0.027 3^{cC}
7	101/111	3	2.516 7±0.041 8^{abcABC}
8	113/113	10	2.588 0±0.022 9^{aAB}
9	107/111	2	2.490 0±0.051 2^{abcABC}
10	103/107	3	2.473 3±0.041 8^{bcABC}
11	103/103	2	2.555 0±0.051 2^{abcABC}

LSMs are expressed with their standard error values. Means with the different superscripts within the same column differ significantly (0.01 < P < 0.05); means with the different capital superscripts within the same column differ extremely significantly (P < 0.01).

Table 5. Least square means of BM1329 genotypes for the Producing ability of litter size of ewes in Hu sheep.

Treat	*BM*1329 genotypes	Number of individuals	Least square means (LSMs)
1	146/146	12	2.540 0±0.024 2^{a}
2	164/164	13	2.483 8±0.023 2^{a}
3	146/158	9	2.561 1±0.028 0^{a}
4	148/148	2	2.460 0±0.059 3^{a}
5	146/164	2	2.460 0±0.059 3^{a}
6	146/162	15	2.548 7±0.021 7^{a}
7	158/158	3	2.556 7±0.048 4^{a}
8	162/162	3	2.576 7±0.048 4^{a}

LSMs are expressed with their standard error values. Means with the same superscripts within the same column differ not significantly (P >0.05).

diversity. Ne of OarAE101, BM1329, OarHH35, BM143 and OarHH55 are 5.1611, 3.5945, 2.9726, 6.1210 and 4.1978 respectively, with average value of 4.4094. Montgomery et al. (1994) detected OarAE101, OarHH55 and BM143, and found 7, 11 and 11 alleles, respectively, on the markers. Chu et al. (2001) found 4 - 5 and 4 alleles, respectively on OarAE101 and BM1329. Such differences may result from the difference of populations and samplings.

PIC can be used to describe variation of microsatellite markers. Botstein et al. (1980) thought PIC was an index to measure genetic valuation. When PIC >0.5, the locus is of high polymorphism. When 0.25 < PIC < 0.5, the locus is of moderate polymorphism. When PIC <0.25, the locus is of low polymorphism. In this way, OarHH35, BM1329, OarHH55, OarAE101 and BM143 are all of high polymorphism. In genetic linkage analysis, microsatellite markers with PIC over 0.70 are the better markers (Barker et al., 1994). Under this condition, parents are usually heterozygous on the locus but alleles are separated in offsprings. PIC is high in this study, and average value is 0.7214, showing genetic abundance in fecundity trait of Hu Sheep.

The gene diversity (He) reflects population variation in multiple gene loci. Generally speaking, He is considered as the best genetic parameters. In our study, of 5 microsatellite markers, He of OarHH35 is the highest, and He of BM1329 is the lowest. The average He of Hu sheep is 0.758. Arranz et al. (1998) detected the genetic variation of 5 Spanish sheep breeds and Awassi sheep, and their result shows that the average He of these sheep is 0.713 - 0.771. Our study is close to the result.

Producing ability of ewe

Litter size is a character with very low heritability, about 0.1. Therefore, it is hard to use normal breeding methods to improve litter size, and the maker-assisted selection may accelerate the sheep breeding process. However, currently, few molecular researches on litter size of Hu sheep have been conducted, and only some lateral researches were done in the condition of Hu sheep as control population. Moreover, most researchers follow the

Table 6. Least square means of OarHH35 genotypes for the producing ability of litter size of ewes in Hu sheep.

Treat	*OarHH35* genotypes	Number of individuals	Least square means (LSMs)
1	139/139	7	2.592 9±0.030 6^{aA}
2	139/141	4	2.540 0±0.040 5^{abAB}
3	127/127	2	2.460 0±0.0573 0^{bB}
4	139/127	23	22.550 8±0.016 9^{aAB}
5	139/125	16	2.484 3±0.020 2^{bB}
6	125/125	5	2.481 7±0.033 1^{bB}
7	137/125	3	2.536 7±0.046 7^{abAB}

LSMs are expressed with their standard error values. Means with the different superscripts within the same column differ significantly (0.01 < P < 0.05); means with the different capital superscripts within the same column differ extreme significantly (P < 0.01).

Table 7. Least square means of BM143 genotypes for the producing ability of litter size of ewes in Hu sheep.

Treat	*BM143* genotypes	Number of individuals	Least square means (LSMs)
1	118/118	3	2.640 0±0.040 7^{aA}
2	118/116	8	2.514 4±0.023 5^{bcdABC}
3	102/104	10	2.454 0±0.022 2^{cdC}
4	102/106	2	2.490 0±0.049 8^{bcdABC}
5	118/106	12	2.586 3±0.021 2^{aAB}
6	118/110	2	2.590 0±0.049 8^{abABC}
7	106/104	3	2.536 7±0.040 7^{abcdABC}
8	112/114	2	2.555 0±0.049 8^{abcdABC}
9	112/102	3	2.450 0±0.040 7^{dC}
10	116/112	4	2.475 0±0.035 2^{bcdBC}

Means with the different superscripts within the same column differ significantly (0.01 < P < 0.05; means with the different capital superscripts within the same column differ extremely and significantly (P < 0.01).

Table 8. Least square means of OarHH55 genotypes for the Producing ability of litter size of ewes in Hu sheep.

Treat	*OarHH55* genotypes	Number of individuals	Least square means (LSMs)
1	131/129	3	2.533 3±0.050 0^{a}
2	129/129	3	2.450 0±0.050 0^{a}
3	147/147	4	2.572 5±0.043 3^{a}
4	147/129	13	2.547 7±0.024 0^{a}
5	147/127	11	2.520 9±0.026 1^{a}
6	147/135	2	2.590 0±0.061 2^{a}
7	147/125	4	2.525 0±0.043 3^{a}
8	127/125	4	2.492 5±0.043 3^{a}
9	127/129	9	2.485 6±0.028 9^{a}
10	125/125	2	2.525 0±0.061 2^{a}

LSMs are expressed with their standard error values. Means with the same superscripts within the same column do not differ significantly (P > 0.05).

thought of doing selection or predicting production according to the correction between special marker alleles (gene type) and phenotypic value (Chu et al., 2002a; Wang et al., 2003; Lei et al., 2003; Ji et al., 2003). However, litter size of sheep is affected by many exterior factors as physiological condition, nutrition level and

sperm quality of ram. Phenotypic value of fecundity do not pass down truly, thus it is not very efficient to use correction between marker and phenotypic value.

Producing ability is ewe's genetic potentiality on a repeatable character, which may be obtained by multiple measurement of litter size, and it is indeed an important reference to select excellent ewe. The data of producing ability of ewe can be used to evaluate ewe's potentiality on litter size. Thus, choosing by combining producing ability and special gene (gene type) will play a great role in improving selecting accuracy and breeding progress, and is a wonderful and new analyzed thought in repeatable characters with low heritability which is affected by measuring frequencies.

Association between microsatellite markers and producing ability of ewe in litter size of Hu sheep

In this study, there are extreme significant difference among the different genotypes for the producing ability of litter size in OarAE101 locus and BM143 locus ($P < 0.01$), and there are significant difference among the different genotypes for the producing ability of litter size in OarHH35 locus ($0.01 < P < 0.05$), however, no significant difference among the different genotypes for the producing ability of litter size in BM1329 and OarHH55 loci ($P > 0.05$). In the locus of OarAE101, the least squares mean for the producing ability of litter size of 107/113 genotype was the highest, but it was not significantly higher than genotype of 103/113, 107/107, 101/111, 113/113, 107/111 and 103/103 ($P > 0.05$), and the producing ability of litter size of 111/111 genotype was the least. But in the research of Chu et al, the allele of 107 and genotype of 107/111 showed a positive significant relationship with the litter size trait of Small Han tail Han sheep, two alleles (109 and 109) showed a negative relationship with the litter size trait (Chu et al., 2001). In the locus of BM1329, there was no significant difference in producing ability of litter size among the different genotype, but in the further study, we could find that least square means of producing ability of ewe in 162/162, 158/158 and 146/158 were the highest (2.5567 - 2.5767), and the least square means of producing ability of ewe in 148/148 and 146/164 are lowest. But in the research of Chu et al, the allele of 146 and genotype of 146/158 showed a positive significant relationship with the litter size trait of Small Han tail Han sheep, the allele (148) showed a negative relationship with the litter size trait (Chu et al., 2001). In comparison with our study and the precious study (Chu et al., 2001) we could find, that there were some same or similar result, while there were also some different result. In our study, there were too many genotype and alleles, which maybe because of different sample scale or sample area or different polyacrylamide gel system.

Currently, there are a little over 100 million sheep in China, ranking first in the world. However, number of slaughter is lower than world average. If we want to change the situation of low efficiency and low profit of sheep market in China, the most forceful method is to improve reproducing ability. This study builds a foundation for the maker-assistant selection in Hu sheep, and also provides new experiment thought in discussion of mechanism of reproductive ability of sheep.

Conclusion

Five microsatellite markers: OarAE101, BM1329, BM143, OarHH55 and OarHH35 could be used to study genetics in Hu sheep diversity. The result of variance analysis showed that there were extreme significant difference among the different genotypes for the producing ability of litter size in OarAE101 locus and BM143 locus ($P < 0.01$), there were significant difference among the different genotypes for the producing ability of litter size in OarHH35 locus ($0.01 < P < 0.05$), and there were no significant difference among the different genotypes for the producing ability of litter size in BM1329 and OarHH55 loci ($P > 0.05$).

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