

QTL Analysis of Teat Number Traits in an F₂ Intercross between Landrace And Korean Native Pigs

Hee-Bok Park^{1*}, Sang-Hyun Han^{2*}, Chae-Kyoung Yoo³, Jae-Bong Lee⁴, Sang-Rae Cho⁵, and In-Cheol Cho^{1†}

¹Subtropical Livestock Research Institute, National Institute of Animal Science, RDA, 63242 Jeju, Republic of Korea.

²Educational Science Research Institute, Jeju National University, Jeju, Republic of Korea. ³Institute of Agriculture and Life Sciences, Gyeongsang National University, 52828 Jinju, Republic of Korea. ⁴Korea Zoonosis Research Institute, Chonbuk National University, 54531 Iksan, Republic of Korea. ⁵Hanwoo Research Institute, National Institute of Animal Science, RDA, 25340.

ABSTRACT

The aim of this study was to identify quantitative trait loci (QTLs) influencing teat number traits in an F₂ intercross between Landrace and Korean native pigs (KNP). Three teat number traits (left, right, and total) were measured in 1105 F₂ progeny. All experimental animals were genotyped with 173 informative microsatellite markers located throughout the pig genome. We detect that seven chromosomes harbored QTLs for teat number traits: genome regions on SSC1, 3, 7, 8, 10, 11, and 13. Six of fourteen identified QTL reached genome-wide significance. In SSC7, we identified a major QTL affecting total teat number that accounted for 5.6 % of the phenotypic variance, which was the highest test statistic (F -ratio = 61.1 under the additive model, nominal $P = 1.3 \times 10^{-14}$) observed in this study. In this region, QTL for left and right teat number were also detected with genome-wide significance. With exception of the QTL in SSC10, the allele from KNP in all 6 identified QTLs was associated with decreased phenotypic values. In conclusion, our study identified both previously reported and novel QTL affecting teat number traits. These results can play an important role in determining the genetic structure underlying the variation of teat number in pigs.

(Key words: teat number, quantitative trait locus, genome-wide linkage analysis, Landrace and Korean native pigs)

INTRODUCTION

There are two kinds of Korean native pigs: the indigenous pig raised on the Korean Peninsula and the Jeju native pig inhabited on Jeju Island. Owing to being raised on an island that has been isolated for more than 1,000 years, the Jeju native pig (hereafter, the Jeju native pig is referred to as KNP) has exclusive genetic properties that are different from those of the pigs raised on the Korea Peninsula (Cho *et al.*, 2009). The coat color of KNP is black, and its feed efficiency and growth rate are low, as with most native breeds. However, KNP is famous for their meat quality features, such as reddish meat color and a high degree of marbling.

The teat number traits are important in swine industry due to the fact that it is a part of lactation organs and is related with the mothering ability and live litter size. Teat numbers vary between-breed and also within-breed. When the teat

numbers of Duroc, Large White and Landrace pig breeds were measured, the average values were 13.6, 14.3, and 14.4, respectively. Compared to the Western breeds, the teat number of KNP is lower; it is on average 13.2 for KNP (I-C. Cho, Unpublished data). The teat number traits show low (0.20) to medium (0.50) heritabilities (Ding *et al.*, 2009). Thus, it is important to elucidate the genetic foundation of this trait to improve genetic potentials of postnatal care of piglet. Several genome scanning analyses have been conducted to delineate the genome-wide map of QTL for teat number traits (left, right, and total) in pigs with the exception of SSC9, 13, 14, 18, and Y-chromosome (summarized in PigQTLdb: <http://www.animalgenome.org/cgi-bin/QTLdb/SS/index>). In this study, we identified QTL affecting teat number traits using an F₂ intercross between Landrace and Korean native pigs (KNP). We also present the identification of previously reported and novel QTL for teat number traits in pigs.

* Hee-Bok Park and Sang-Hyun Han contributed equally to this study.

† Correspondence: In-Cheol Cho

Tel: +82-64-754-5710

E-mail address: choic4753@korea.kr

MATERIALS and METHODS

1. Experimental animals and phenotypes

A three-generation resource population was generated and managed as described by Cho *et al.* (2011). Nineteen purebred KNP were crossed with 17 purebred Landrace. From these crosses, 91 F1 progeny and 1105 F2 progeny from 79 full-sib families were produced. Each of the F2 progeny, teat numbers at the left- (LeftTN) and the right- (RightTN) hand side were recorded by simple counting. The total teat number (TotalTN) was the sum of the teat number at both sides.

2. Genotypes and genetic map

A total of 173 informative microsatellite markers covering the autosomes and X chromosome were PCR-amplified in 1232 pigs. The map order and genetic distance were determined using the build option in the CRIMAP software version 2.4 (Green *et al.* 1990). The total map length was 2348.8 cM

3. Statistical analysis.

An interval mapping model based on least squares regression was used for QTL analysis, including the cofactors of sex, full-sib, along with additive and dominance effects (2 d.f.) for a putative QTL. We used a web-based GridQTL program to conduct QTL analysis (Seaton *et al.*, 2006). Identification of QTL was based on an *F*-ratio test statistic comparing a full model with a reduced model (with the cofactors only). The *F*-ratios were calculated at 1-cM intervals across the genome. Selection of the final genetic model for the QTL analysis was according to Cho *et al.* (2011). To detect a single QTL, genome-wide empirical significant thresholds of the test statistic (i.e., *F*-ratio) were obtained by 1,000 permutations of data (Churchill and Doerge, 1994). Genome-wide thresholds for highly significant ($\alpha=0.01$) and significant linkage ($\alpha=0.05$) were employed. Suggestive linkage was employed using a 5% chromosome-wide threshold. The 1.5-LOD (logarithm of odds) drop method was used to estimate support intervals for identified QTL at the suggestive and significant levels of significance (Dupuis and

Table 1. Summary of identified QTL for teat number traits

SSC	Traits	Position (cM)	<i>F</i> -ratio ^a	Mode of inheritance ^b	95% SI ^c		Additive (SE) ^d	Dominance (SE) ^e	%Var ^f
					cM	Marker			
1	TotalTN	49	15.7*	A	27-72	SW1515-SW2035	-0.23 (0.058) †		1.51
	LeftTN	49	13.0*	A	0-74	SW1514-SW2035	-0.13 (0.035) †		1.25
	RightTN	49	9.0	A	0-86	SW1514-S0331	-0.10 (0.034) †		0.87
3	TotalTN	1	9.6	A	0-55	APR22-SW487	-0.15 (0.049) †		0.93
	LeftTN	35	7.7	AD	21-59	SW2021-SW487	-0.11 (0.037) †	-0.19 (0.066) †	1.48
7	TotalTN	66	61.1**	A	60-78	S0102-SW147	-0.40 (0.051) †		5.63
	LeftTN	67	36.7**	A	56-80	207G8-4-SW147	-0.19 (0.031) †		3.46
	RightTN	65	48.3**	A	56-80	207G8-4-SW147	-0.21 (0.030) †		4.51
8	LeftTN	13	6.2	AD	0-32	SW2410-SW1345	-0.06 (0.030) *	-0.13 (0.045) †	1.21
10	TotalTN	99	8.5	A	82-135	SWR1849-SW2067	0.15 (0.052) †		0.82
	RightTN	115	7.9	A	79-135	SWR1849-SW2067	0.11 (0.041) †		0.76
11	LeftTN	62	6.1	A	0-81	SW1460-SW1135	-0.09 (0.035) *		0.59
13	TotalTN	67	9.9	A	38-106	SW864-S0215	-0.16 (0.052) †		0.96
	RightTN	80	13.0*	A	45-104	SW864-S0215	-0.11 (0.031) †		1.26

^a Test statistic and level of significance genome-wide significance (**1%, *5%) thresholds.

^b A represents additive effect; AD represents additive and dominance effects.

^c 95% support intervals estimated by the 1.5-LOD drop method. Flanking markers for the QTL support intervals.

^d Additive effect and standard error. A positive value means the Jeju native pig allele has an increase effect on a trait, and a negative value indicates that the Landrace allele has an increase effect on a trait.

^e Dominant effect and standard error.

^f Var % is the reduction in residual variance of the F2 population obtained by inclusion of a QTL at the given position.

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$

Siegmund, 1999). The verifications of novel QTL were done by comparing the flanking markers of previously reported QTL in the pig QTLdb (<http://www.animalgenome.org/QTLdb/pig>) with those of newly detected QTL in this study.

RESULTS and DISCUSSION

We identified six significant QTL and eight suggestive QTL in seven chromosomes (SSC1, 3, 7, 8, 10, 11, and 13) (Table 1). In SSC1, we detected two significant QTL for the TotalTN (nominal $P = 8.1 \times 10^{-5}$) and LeftTN (nominal $P = 0.0003$), and one suggestive QTL for RightTN at the same position (49 cM) (Fig. 1A). These QTL explained up to 1.51% of the phenotypic variance. At these QTL loci, alleles from the KNP were associated with decreased phenotypic values. QTL region affecting TotalTN overlapped with previously reported QTL regions in other studies (Wada *et al.*, 2000; Guo *et al.*, 2008; Ding *et al.*, 2009), while the previous studies did not report the QTL for LeftTN and RightTN. In SSC3, we identified two suggestive QTL for TotalTN (1 cM) and LeftTN (35 cM). The QTL at 35 cM showed decreased teat number with the estimated additive effect of -0.11 ± 0.037 and the dominance effect of -0.19 ± 0.049 . These QTLs accounted up to 1.48% of the phenotypic variance, and overlapped with QTL regions reported in previous studies (Guo *et al.*, 2008; Ding *et al.*, 2009). In SSC7, we identified most of the significant QTL (Fig. 1B). QTL for TotalTN (66 cM, nominal $P = 1.3 \times 10^{-14}$), LeftTN (67 cM, nominal $P = 1.9 \times 10^{-9}$), and RightTN (65 cM, nominal $P = 6.4 \times 10^{-12}$) were detected highly significant QTL and explained up to 5.63% of the phenotypic variance. With only an additive effect, the alleles at these QTL from KNP were associated with decreased phenotypic values. In SSC7, many QTL affecting teat number traits were reported in previous studies (Cassady *et al.*, 2001; Wada *et al.*, 2000; Sato *et al.*, 2006; Zhang *et al.*, 2007; Bidanel *et al.*, 2008; Guo *et al.*, 2008; Ding *et al.*, 2009). In our current study, QTL for TotalTN, LeftTN, and RightTN were overlapped with QTL in previous studies with exception of QTL reported in Cassady *et al.* (2001) and Sato *et al.* (2006). Sato *et al.* (2003), Mikawa *et al.* (2005), and Ding *et al.* (2009) were reported co-localization of QTL for vertebra number in a QTL region affecting teat number traits. The KNP alleles at these QTL were associated with decrease phenotypic value likewise QTL

affecting teat number traits. In SSC8, we identified one suggestive QTL for LeftTN at 13 cM. This QTL explained 1.21% of the phenotypic variance, and it was novel. The QTL at this position showed estimated additive effect of -0.06 ± 0.030 and the dominance effect of -0.13 ± 0.045 . In SSC10, we detected two suggestive QTL for TotalTN (99 cM) and RightTN (115 cM). QTL for TotalTN is overlapped with previous reported QTL (Rohrer, 2000; Hirooka *et al.*, 2001; Dragos-Wendrich *et al.*, 2003; Rodriguez *et al.*, 2005; Guo *et al.*, 2008), but QTL for RightTN was novel. At these loci, allele from KNP was associated with increased phenotypic value unlike other QTL. QTL affecting LeftTN was detected with suggestive level in SSC11 (62 cM), and it was novel. In SSC13, we identified QTL affecting TotalTN (67 cM) and RightTN (80 cM). QTL for RightTN was detected with significant level (F -ratio = 13.0 under the additive model, nominal $P = 0.0003$), explained 1.26% of the phenotypic variance; this QTL was novel.

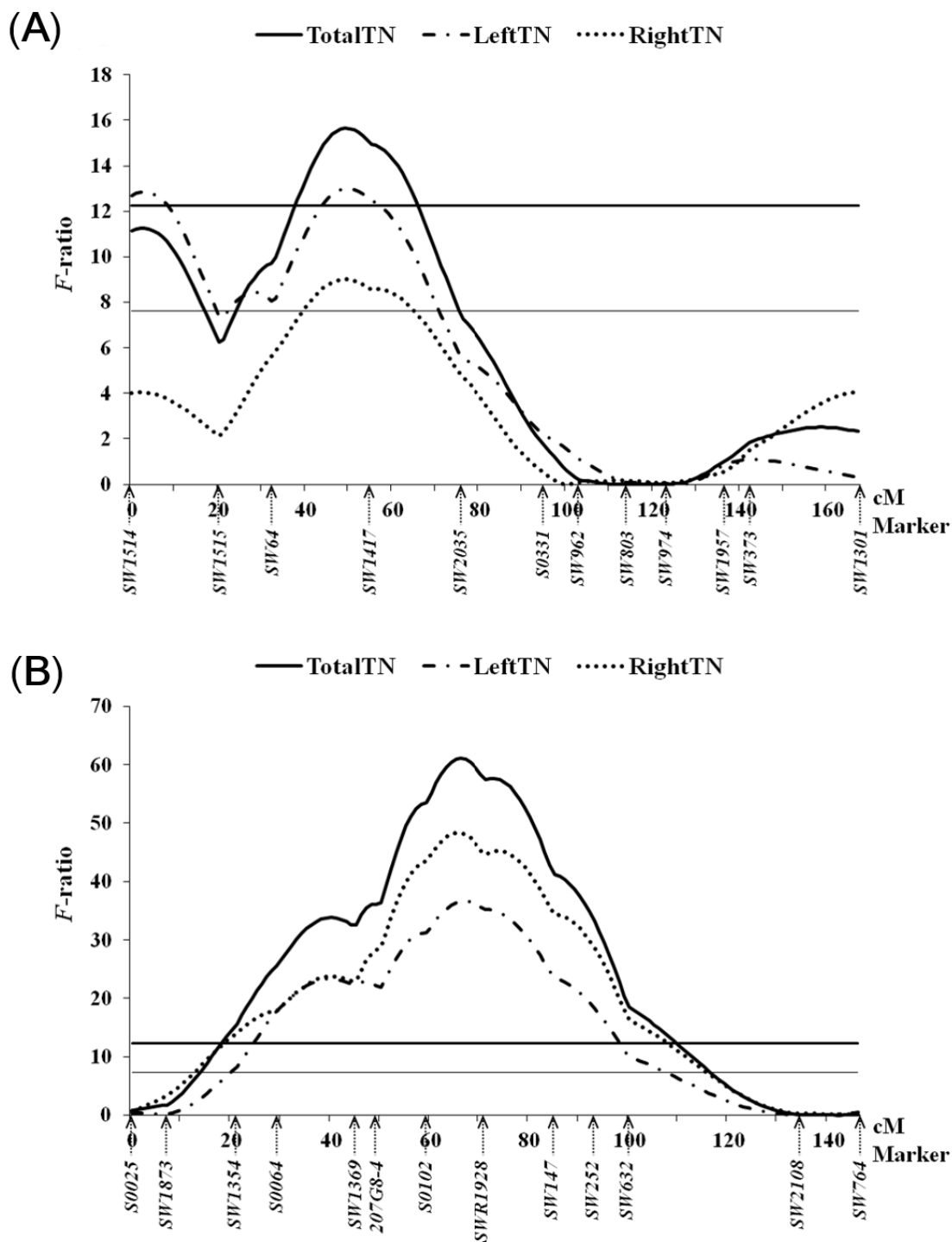
No QTL associated with teat number traits was detected in the X chromosome. In all of the identified QTL affecting teat number traits, the KNP allele was always associated with lower teat number, whereas the Landrace allele was associated with higher phenotypic values with the exception of QTL in SSC10. These results are consistent with the observed increase of teat number in Western breeds through selective breeding.

In conclusion, we conducted a genome-wide QTL analysis for teat number traits using an F2 intercross between KNP and Landrace pigs. A total of 14 QTLs were identified for teat number traits. The results from this study not only revealed novel QTLs but also verified previously reported QTLs. The results presented herein can play an important role in investigating the genetic structure of phenotypic variation of teat number traits in pigs.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institute of Animal Science (Project title: Study on breed preservation and applied technology development in Jeju native domestic animals; Project No.PJ01010502), Rural Development Administration, Republic of Korea.

Fig. 1 Test statistic profiles for teat number traits on (A) SSC1 and (B) SSC7 using an F2 intercross between Landrace and Korean native pigs. Marker map with genetic distance between microsatellite markers in Kosambi cM is given on the x-axis. The y-axis represents the *F*-value testing the hypothesis of a single QTL in a given position on the chromosome. The thick horizontal line indicate the 5% genome-wide significant threshold and thin horizontal line indicate the 5% chromosome-wise significant threshold. Trait abbreviations are listed in Supplementary table 1.



Supplementary table 1. Statistics of teat number traits in the F2 progeny of an intercross between Landrace and Korean native pigs

Trait	Abbr.	N	Mean	SD	Range	Correlation coefficients		
						TotalTN	LeftTN	RightTN
Total teat number	TotalTN	1105	13.7	1.3	10-18	1		
Left teat number	LeftTN	1105	6.8	0.7	5-9	0.872 [‡]	1	
Right teat number	RightTN	1105	6.9	0.7	5-9	0.875 [‡]	0.525 [‡]	1

‡ $P < 0.001$

REFERENCES

- Bidanel JP, Rosendo A, Iannuccelli N, Riquet J, Gilbert H, Caritez JC, Billon Y, Amigues Y, Prunier A and Milan D. 2008. Detection of quantitative trait loci for teat number and female reproductive traits in Meishan x Large White F2 pigs. *Animal* 2: 813-820.
- Cassady JP, Johnson RK, Pomp D, Rohrer GA, Van Vleck LD, Spiegel EK and Gilson KM. 2001. Identification of quantitative trait loci affecting reproduction in pigs. *J. Anim. Sci.* 79: 623-633.
- Cho IC, Park HB, Yoo CK, Lee GJ, Lim HT, Lee JB, Jung EJ, Ko MS, Lee JH and Jeon JT. 2011. QTL analysis of white blood cell, platelet and red blood cell-related traits in an F2 intercross between Landrace and Korean native pigs. *Anim. Genet.* 42: 621-626.
- Cho I.C, Han SH, Fang M, Lee SS, Ko MS, Lee H, Lim HT, Yoo CK, Lee JH and Jeon JT. 2009. The robust phylogeny of Korean wild boar (*Sus scrofa coreanus*) using partial D-loop sequence of mtDNA. *Mol. Cells* 28:423 - 430.
- Churchill GA and Doerge RW. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* 138: 963-971.
- Ding N, Guo Y, Knorr C, Ma J, Mao H, Lan L, Xiao S, Ai H, Haley CS, Brenig B and Huang L .2009. Genome-wide QTL mapping for three traits related to teat number in a White Duroc x Erhualian pig resource population. *BMC genet.* 10: 6-2156-10-6.
- Dragos-Wendrich M, Moser G, Bartenschlager H, Reiner G and Geldermann H. 2003. Linkage and QTL mapping for *Sus scrofa* chromosome 10. *J. Anim. Breed. Genet.* 120: 82 - 88.
- Dupuis J and Siegmund D. 1999. Statistical methods for mapping quantitative trait loci from a dense set of markers. *Genetics* 151: 373-386.
- Green P, Falls K and Crooks S (1990) Documentation for CRIMAP, Version 2.4. Washington Univ. School of Medicine, St. Louis, MO.
- Guo YM, Lee GJ, Archibald AL and Haley CS. 2008. Quantitative trait loci for production traits in pigs: a combined analysis of two Meishan x Large White populations. *Anim. Genet.* 39: 486-495.
- Hirooka H, de Koning DJ, Harlizius B, van Arendonk JA, Rattink AP, Groenen MA, Brascamp EW and Bovenhuis H. 2001. A whole-genome scan for quantitative trait loci affecting teat number in pigs. *J. Anim. Sci.* 79: 2320-2326.
- Mikawa S, Hayashi T, Nii M, Shimanuki S, Morozumi T and Awata T. 2005. Two quantitative trait loci on *Sus scrofa* chromosomes 1 and 7 affecting the number of vertebrae. *J. Anim. Sci.* 83: 2247-2254.
- Rodriguez C, Tomas A, Alves E, Ramirez O, Arque M, Munoz G, Barragan C, Varona L, Silio L, Amills M and Noguera JL. 2005. QTL mapping for teat number in an Iberian-by-Meishan pig intercross. *Anim. Genet.* 36: 490-496.
- Rohrer GA. 2000. Identification of quantitative trait loci affecting birth characters and accumulation of backfat and weight in a Meishan-White Composite resource population. *J. Anim. Sci.* 78: 2547-2553.
- Sato S, Oyamada Y, Atsuji K, Nade T, Sato S, Kobayashi E, Mitsuhashi T, Nirasawa K, Komatsuda A, Saito Y, Terai S, Hayashi T and Sugimoto Y. 2003. Quantitative trait loci analysis for growth and carcass traits in a Meishan x Duroc F2 resource population. *J. Anim. Sci.* 81: 2938-2949
- Sato S, Atsuji K, Saito N, Okitsu M, Sato S, Komatsuda A, Mitsuhashi T, Nirasawa K, Hayashi T, Sugimoto Y and Kobayashi E. 2006. Identification of quantitative trait loci affecting corpora lutea and number of teats in a Meishan x Duroc F2 resource population. *J. Anim. Sci.* 84: 2895-2901.
- Seaton G, Hernandez J, Grunchev JA, White I, Allen J, De Koning DJ, Wei W, Berry D, Haley C and Knott S. 2006. GridQTL: A grid portal for QTL mapping of compute intensive datasets. *Proceedings of the 8th World Congress*

on Genetics Applied to Livestock Production.

Wada Y, Akita T, Awata T, Furukawa T, Sugai N, Inage Y, Ishii K, Ito Y, Kobayashi E, Kusumoto H, Matsumoto T, Mikawa S, Miyake M, Murase A, Shimanuki S, Sugiyama T, Uchida Y, Yanai S and Yasue H. 2000. Quantitative trait loci (QTL) analysis in a Meishan x Gottingen cross population. *Anim. Genet.* 31: 376-384.

Zhang J, Xiong Y, Zuo B, Lei M, Jiang S, Li F, Zheng R, Li J and Xu D. 2007. Detection of quantitative trait loci

associated with several internal organ traits and teat number trait in a pig population. *J. Genet. Genomics* 34: 307-314.

Received August 23 2016, Revised September 30, 2016,

Accepted September 30, 2016