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A genome scan for quantitative trait loci affecting male reproductive traits in a White Duroc × Chinese Erhualian resource population[1]

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ABSTRACT: Chinese Erhualian boars have dramatically smaller testes, greater concentrations of circulating androgens, and fewer Sertoli cells than Western commercial breeds. To identify QTL for boar reproductive traits, testicular weight, epididymal weight, seminiferous tubular diameter at 90 and 300 d, and serum testosterone concentration at 300 d were measured in 347 F_2 boars from a White Duroc × Chinese Erhualian cross. A whole genome scan was performed with 183 microsatellites covering 19 porcine chromosomes. A total of 16 QTL were identified on 9 chromosomes, including 1% genome-wide significant QTL for testicular weight at 90 and 300 d and seminiferous tubular diameter at 90 d on SSCX, and for epididymal weight and testosterone concentration at 300 d on SSC7. Two 5% genome-wide significant QTL were detected for testicular weight at 300 d on SSC1 and seminiferous tubular diameter at 300 d on SSC16. Nine suggestive QTL were found on SSC1, 2, 3, 5, 7, 13, and 14. Chinese Erhualian alleles were not systematically favorable for greater reproductive performance. This study confirmed the previous significant QTL for testicular weight on SSCX and for epididymal weight on SSC7, and reported QTL for seminiferous tubular diameter and testosterone concentration at the first time. The observed different QTL for the same trait at different ages reflect the involvement of distinct genes in the development of male reproductive traits.

Key words: boar, quantitative trait loci, reproductive trait

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INTRODUCTION

The extensive implementation of AI in swine production requires selection on boars for reproductive traits. However, it is difficult to measure boar reproductive traits, and hence is difficult to estimate breeding value of boars. During recent years, considerable interest has been directed toward finding candidate genes affecting boar reproduction for marker-assisted selection (Chen et al., 2004; Nonneman et al., 2005; Wimmers et al., 2005). Unfortunately, candidate gene studies are often nonreproducible, and effects of candidate markers are sometimes population-specific (Georges, 2007). As an initial step to decipher genes underlying complex traits in farm animals, QTL mapping has been widely performed using experimental and commercial pedigrees. So far, only limited QTL affecting boar reproductive characteristics have been mapped. Significant QTL for several male reproductive traits have been identified on porcine chromosomes (Bidanel et al., 2001; Ford et al., 2001; Rohrer et al., 2001; Sato et al., 2003). However, QTL for seminiferous tubular diameter and serum testosterone concentration remains unexplored.

Chinese Erhualian and Meishan pigs are different strains in Taihu pigs. Boars from these breeds have dramatically smaller adult testes, greater concentrations of circulating FSH, LH, and androgens, fewer seminiferous tubules, fewer Sertoli cells per gram of testicular tissue, and less total daily sperm production compared with Western commercial breeds (Wise et al., 1996; Lunstra et al., 1997), but the genetic basis of the differences remains unknown. We have constructed a large 3-generation resource population by crossing White Duroc boars and Chinese Erhualian sows. Male reproductive traits have been recorded in this resource population. The objective of this study was to identify QTL for testicular weight, epididymal weight, seminiferous tubular diameter, and serum testosterone concentration in boars.

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Table 1. Phenotypic means, SD, and ranges of F_2 boars in a White Duroc × Erhualian resource population

Trait	No.	Mean	SD	Range
300 d				
Testicular weight, g	205	464.61	145.41	156.41 to 914.41
Epididymal weight, g	205	190.50	60.29	58.99 to 482.23
Seminiferous tubular diameter, μm	189	277.90	4.70	95.30 to 307.50
Serum testosterone concentration, ng/mL	195	27.86	22.40	2.00 to 133.00
90 d				
Testicular weight, g	142	65.74	45.27	9.30 to 303.34
Epididymal weight, g	142	25.19	16.55	6.43 to 153.19
Seminiferous tubular diameter, μm	134	134.30	2.70	30.03 to 182.64

MATERIALS AND METHODS

Animals

All procedures involving animals followed the guidelines for the care and use of experimental animals established by the Ministry of Agriculture of China.

A 3-generation resource population was created and managed as described by Ren et al. (2006b). Briefly, 2 White Duroc sires and 17 Erhualian dams were mated to produce F_1 animals in 2001, from which 9 F_1 boars and 59 F_1 sows were intercrossed (avoiding full-sib mating) to produce 983 F_2 males and 929 F_2 females in 6 batches from 2003 to 2006. To obtain large full-sib families, each F_1 sow was usually mated to the same boar in different batches. All piglets were raised at the experimental farm in Jiangxi Agricultural University (Nanchang, P. R. China) until 120 d; after that, some of animals were then transferred to Jiangxi Swine Performance Test Station (Nanchang, P. R. China) for measurements of feeding behaviors and feed consumption. Male reproductive traits were measured in 347 F_2 boars from 55 families in the experimental farm, including 205 F_2 boars at 300 d and 142 F_2 boars at 90 d (Table 1).

Phenotypic Measurement

Testicular and epididymal weights of F_2 males were recorded when they were castrated at 90 and 300 d under anesthetization by an intraperitoneal injection of 2 mg/kg of BW of xylazole hydrochloride (Zhengfeng, Lanzhou, China), respectively. After orchidectomy, testicular tissues were dissected into blocks of about $1 \times 1 \times 0.5$ cm^3 from the central parenchyma of each testis and fixed with 10% formaldehyde solution for overnight at 4°C. After dehydration through serial concentrations of ethanol (70, 80, 95, 100%; 2 × 1 h each), tissues were embedded into paraffin wax. Sections (3 to 5 μm) were cut and stained with hematoxylin and eosin on glass slides according to the routine histological procedure. Subsequently, sections were subjected to the measurements of seminiferous tubular diameter by using a Leica DMR microscope (Leica, Wetzlar, Germany) with 250-fold enlargement. In each assay, at least 5 tubular cross sections were randomly measured. For tubules with an oval cross-section on the slide, the diameter was determined by averaging the longest and shortest distances of the cross sections. To determine serum testosterone concentration in boars, 3 blood samples were collected from each animal at a 2-d interval from the lateral auricular vein. Approximately 3 mL of each blood sample was collected into sterile test tube and stood for 6 h at room temperature, and then centrifuged at 2,795 × *g* at 4°C for 10 min. Serum was transferred into a 1.5-mL Eppendorf tube and stored at −80°C. Testosterone concentrations were analyzed by a VICTOR2 D 1420 Multilabel Counter (PerkinElmer, Turku, Finland) with the AutoDELFIA Testosterone kit (PerkinElmer) according to the manufacturer's instruction.

Microsatellites and Genotyping

Microsatellite markers were initially selected from the USDA-MARC linkage map (Rohrer et al., 1996). A final set of 183 informative markers at approximate 20-cM intervals covering the whole porcine genome was selected and genotyped across the entire White Duroc × Erhualian resource population. Genomic DNA was isolated from tail or ear samples. The PCR primers for microsatellite markers were labeled with fluorescent dyes including FAM, HEX (Aoke, Beijing, China), or NED (ABI, Foster City, CA). Amplifications were performed in a 15-μL mixture containing 40 ng of genomic DNA, 0.3 U Taq polymerase (Takara, Dalian, China), 10 × supplied buffer, 1.5 m*M* $MgCl_2$, 0.2 m*M* each dNTP, and 10 pmol of each primer. The PCR conditions were as follows: predenaturation at 94°C for 3 min; 38 cycles of 94°C for 20 s, optimal annealing temperature for 20 s and 72°C for 30 s; and a final extension at 72°C for 8 min. The PCR products were recorded with a 3130XL Genetic Analyzer and analyzed by GeneMapper Software Version 3.7 (ABI).

Statistics

Genotypic data were first analyzed with CRIMAP version 2.4 (Green et al., 1990) to construct a whole-genome linkage map. The QTL analysis for each trait

was performed by a method of composite interval mapping based on the least-squares regression approach (Haley et al., 1994) and implemented via the QTLexpress at http://qtl.cap.ed.ac.uk; last accessed Oct. 25, 2007. Factors significantly affecting male reproductive traits were determined by using PROC GLM (SAS Inst. Inc., Cary, NC) and were included in the models as fixed effects or covariates. Family and batch were included as the fixed effects in the model for all traits measured. Body weight and age at castration were the covariates in the QTL model for testicular weight; testicular weight was the covariate for epididymal weight and for seminiferous tubular diameter; and average testicular and epididymal weight were covariates for serum testosterone concentration. The QTL analysis was fitted at 1-cM intervals along each chromosome and the *F*-value for the QTL effect was calculated at each point. The position reaching the greatest *F*-value was considered as the position of the QTL. The detected QTL in the current population were fixed as the genetic background when the next-round QTL identification was carried out. We assumed that the founder breeds were fixed for alternative alleles at a QTL, and 2 alleles at a putative QTL at a given location were denoted by Q and q. Probabilities of QTL genotypes, denoted by Prob(QQ), Prob(Qq), and Prob(qq), were computed from the observed genotypes of markers linked to the QTL. For a given location on the sex chromosome, dominance and imprinting effects were excluded from the analyses. Empirical threshold values for QTL mapping were estimated by using the genome-wide permutation test with 1,000 random data shuffles as described by Churchill and Doerge (1994). The empirical 95% confidence intervals were evaluated by the bootstrapping approach with 2,000 iterations (Visscher et al., 1996). The information content of each marker was calculated as described by Knott et al. (1998).

RESULTS AND DISCUSSION

Phenotypic means, standard deviations, and ranges are presented in Table 1. A whole-genome linkage map was constructed with a length of 2,350.3 cM and an average marker interval of 14.3 cM (data not shown). The marker order was consistent with that on the USDA-MARC linkage map (Rohrer et al., 1996). The largest and smallest intervals were located between *S0009* and *SW2413* on SSC 11 (47.2 cM), and between *SW0664* and *SW0666* on SSC7 (0.9 cM). The information content of each marker was more than 0.5. A total of 16 QTL for the 7 reproductive traits measured were identified on 9 chromosomes (Table 2), including 1% genome-wide significant QTL for testicular weight at 90 and 300 d and seminiferous tubular diameter at 90 d on SSCX, and for epididymal weight and testosterone concentration at 300 d on SSC7. Two 5% genome-wide significant QTL were detected for testicular weight at 300 d on SSC1 and seminiferous tubular diameter at 300 d on SSC16. Nine suggestive QTL were found on SSC1, 2, 3, 5, 7, 13, and 14. The statistic *F*-curves indicating the genome-wide significant QTL are shown in Figure 1.

QTL for Testicular Weight

Testes are important to the spermatogenesis and secretion of androgen in mammals. Two 1% genome-wide significant QTL for testicular weight at 90 and 300 d were evidenced in the region flanked by *SW259* and *SW1943* on SSCX (Figure 1), which are the most significant QTL detected in this study and explained 20.61 and 14.71% of the phenotypic variances, respectively. This genomic region has been previously characterized as QTL for testicular weight at 220 d of age in a Meishan × White composite resource population (Rohrer et al., 2001), for testicular weight at 2 mo of age in a Meishan × Duroc intercross (Sato et al., 2003), and for testicular weight at 180 d of age in Meishan × Large White boars (Bidanel et al., 2001). Both Chinese Erhualian and Meishan alleles increased testicular weight at younger ages (60 and 90 d), but decreased testicular weight at older ages (180, 220, and 300 d). The distinct QTL effects on testicular weight at different ages could be caused by the greater proportion of Sertoli cells undergoing proliferation in Erhualian and Meishan boars during the early postnatal period, resulting in heavier testicular weight (McCoard et al., 2001).

Rohrer et al. (2001) detected a significant QTL for FSH concentration in a neighboring region of SSCX mentioned above. The QTL alleles of the founder breeds had opposite effects on testicular weight and FSH concentration. It is consistent with the finding that FSH concentration in blood is negatively correlated with testicular size in mature boars (Ford et al., 1997). Whether the 2 traits are affected by the same gene or different loci in the QTL region needs further investigation.

The QTL region on SSCX is orthologous to human chromosome Xp11.23–21 and the distal end of mouse chromosome Xp where the recombination rate is particularly low (McCoard et al., 2002), making fine mapping of the QTL and characterization of causal gene(s) difficult. The *SERPINA7*, previously known as *TBG*, was mapped to this chromosomal region. The *SERPINA7* regulates availability of thyroid hormones within tissues and plays a critical role in the development of testes. Strong associations between a missense mutation (His226Asn) in *SERPINA7* and testicular size have been consistently found (Nonneman et al., 2005; Ren et al., 2006a). However, additional genetic evidence is required to determine if the His226Asn polymorphism of *SERPINA7* is a closely linked marker or the causative mutation underlying the testicular size because of the extensive linkage disequilibrium on SSCX.

Another significant QTL for testicular weight at 300 d and a suggestive QTL for testicular weight at 90 d were mapped to an adjacent region on SSC1 (Table 2). The different significant levels of the QTL suggest that the

Table 2. QTL for male reproductive traits identified in a White Duroc × Erhualian resource population

Trait	Chr,[1] No.	Position, cM	95% confidence interval position	*F*-ratio[1]	Additive effect ± SE	Dominant effect ± SE	Variance[2] (%)
Testicular weight							
300 d	X	58[3]	55 to 71	30.44***	96.49 ± 17.49	—	14.71
	1	86	27 to 125.5	8.06**	58.41 ± 13.85	−55.47 ± 23.88	9.16
	7	125	0 to 129	4.82*	4.08 ± 6.52	11.18 ± 8.56	5.13
	5	91	0 to 114	4.69*	−44.49 ± 13.60	−25.07 ± 23.32	4.80
90 d	X	56[3]	51 to 60	40.76***	−41.50 ± 6.50	—	20.61
	1	139	0 to 139	6.90*	−8.96 ± 4.61	28.07 ± 7.75	9.07
Epididymal weight							
300 d	7	59	48.5 to 73	23.76***	−31.07 ± 3.93	9.08 ± 5.75	27.33
	3	95	11 to 106	4.75*	−15.56 ± 5.03	11.20 ± 7.50	4.50
90 d	2	93	1.5 to 144	4.95*	−0.19 ± 0.47	2.71 ± 0.75	9.98
Seminiferous tubular diameter							
300 d	16	43	34 to 55.5	9.79**	0.04 ± 0.11	1.05 ± 0.20	14.78
90 d	X	56[3]	47 to 82	14.93***	−0.67 ± 0.17	—	11.05
	14	102	14 to 102	5.51*	−0.02 ± 0.09	−0.32 ± 0.12	10.21
	13	139	13 to 145	5.22*	0.04 ± 0.09	−0.50 ± 0.13	9.57
	5	21	1.0 to 114	4.72*	−0.09 ± 0.11	−0.53 ± 0.24	8.44
Serum testosterone concentration							
300 d	7	71	4.5 to 75	10.68***	6.87 ± 2.05	−10.15 ± 2.66	14.30
	13	68	37.5 to 145	5.96*	−5.73 ± 1.79	−8.18 ± 2.58	7.33

[1]Significance levels determined by permutation test: *5% chromosome-wide significant level, **5% genome-wide significant level, ***1% genome-wide significance level.

[2]Percentage of the phenotypic variance explained by the QTL.

[3]Location on sex average chromosome.

corresponding quantitative trait gene could have more profound effects on testicular weight in mature boars. To our knowledge, this is the first time to identify the QTL for testicular weight on SSC1, though several QTL affecting correlated reproductive traits including age at puberty, gestation length, FSH concentration, and the weights of seminal vesicles, bulbo-urethral glands, and uterine horns were detected at positions near the QTL (Bidanel et al., 2001). Moreover, 2 suggestive QTL for testicular weight at 300 d were first identified on SSC5 and on the distal end of SSC7. However, Sato et al. (2003) mapped a significant QTL for testicular weight at 60 d on SSC3, which was not detected in this study and other populations (Bidanel et al., 2001; Rohrer et al., 2001). These results may be due to the different genetic basis in founder breeds or different ages when testicular weights of boars were measured.

QTL for Epididymal Weight

The epididymis plays a critical role in sperm maturity and storage, and greater epididymal weight may result in greater capacity for sperm storage and improve male fertility (Walker et al., 2004). The QTL for epididymal weight at 180 d have been previously detected on SSC4, 10, 13, 15, and X (Bidanel et al., 2001), which were not confirmed in this study. A 1% genome-wide significant QTL ($F = 23.76$) for epididymal weight at 300 d on SSC7 proximal to *SW1856* was detected, which explained 27.33% of the phenotypic variance. A suggestive QTL was detected for epididymal weight at 300 d on SSC3 and for epididymal weight at 90 d on SSC2, respectively (Table 2). The alleles from the Erhualian breed at these loci were associated with larger epididymal weight. In the previous QTL analysis of epididymal weight at 180 d in the Meishan × Large White intercross, the Chinese Meishan alleles are favorable at the QTL on SSC3, but unfavorable at the QTL on SSC4, 7, 10, and 15 (Bidanel et al., 2001). The reason for the discrepancy remains unknown.

Numerous QTL for diverse traits have been mapped around the QTL region on SSC7 (http://www.animalgenome.org/QTLdb/; last accessed Jan. 18, 2008), and more markers were selected from this region at the beginning of this study to diminish the confidence interval of the expected QTL for male reproductive traits. A high-resolution human-pig comparative map of this region (Demars et al., 2006) allows the identification of positional candidate genes. A promising positional candidate gene is the *SAM pointed domain-containing ETS transcription factor* (***SPDEF***) gene, which plays a crucial role in the development of reproductive tract in mouse and humans (Oettgen et al., 2000; Yamada et al., 2000).

QTL for Seminiferous Tubular Diameter

The percentage of testis volume occupied by seminiferous tubules is 83 to 85% in domestic boars and 87% in wild boars (Almeida et al., 2006), and the appearance of lumen in seminiferous tubule is evidence of fluid secretion and the formation of blood-testis barrier (Tindall et al., 1975). A 1% genome-wide significant QTL at 56 cM on SSCX (Figure 1) and 3 suggestive QTL on

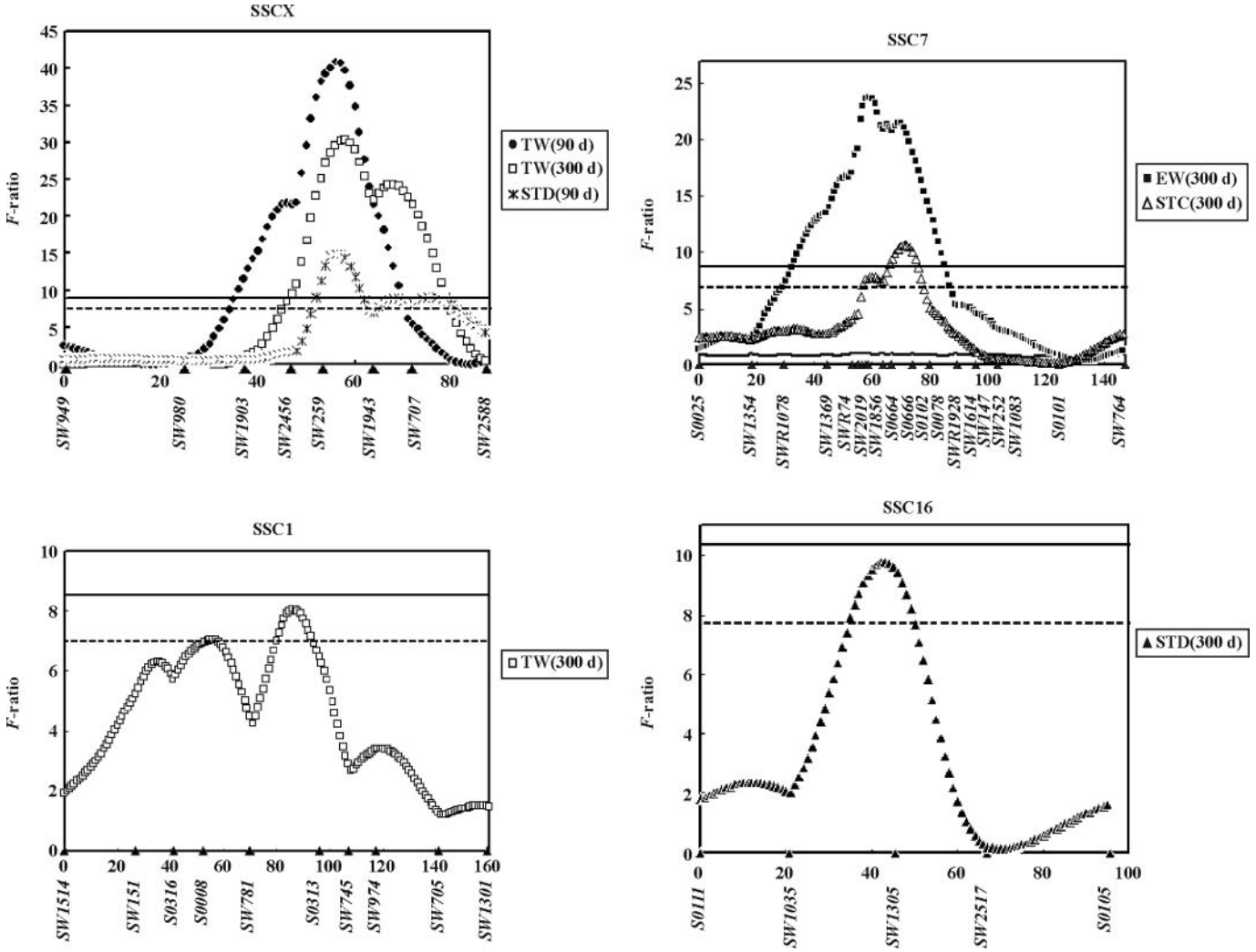

Figure 1. The statistic *F*-curves indicate genome-wide significant QTL for male reproductive traits on SSCX, SSC1, SSC7, and SSC16. Markers and distance in centimorgan are given on the x-axis, and *F*-ratios are indicated on the left y-axis. The 5 and 1% genome-wide significant levels are indicated by dashed lines and thick solid lines, respectively. TW (90 d) = testicular weight at 90 d; TW (300 d) = testicular weight at 300 d; EW (300 d) = epididymal weight at 300 d; STD (90 d) = seminiferous tubular diameter at 90 d; STD (300 d) = seminiferous tubular diameter at 300 d; STC (300 d) = serum testosterone concentration at 300 d.

SSC5, 13, and 14 for seminiferous tubular diameter at 90 d were detected. The favorable alleles were inherited from the Erhualian breed. The QTL region on SSCX overlapped with the QTL for testicular weight (Figure 1). In most domestic animals, the hypertrophy caused by unilateral castration is associated with the increased diameter and length of seminiferous tubules (Boockfor et al., 1983; Kosco et al., 1989). However, a low correlation was found between seminiferous tubular diameter and testicular weight in this population ($r = 0.16$, $P < 0.05$), which supported the previous hypothesis that the seminiferous tubular diameter may not determine total tubular volume and testicular weight (Zanella et al., 1999). Hence, there might be different genes underlying phenotypic differences of seminiferous tubular diameter and testicular weight in the overlapping QTL region.

Only one significant QTL for seminiferous tubular diameter at 300 d was detected (Figure 1), located at 43 cM on SSC16, and explained 14.78% of the phenotypic variance. The reason for a single QTL detected could be caused by the nonsignificant difference in seminiferous tubular diameter between the founder breeds as reported between Whitecross and Meishan (Okwun et al., 1996). Both QTL for seminiferous tubular diameter at 300 and 90 d were located in different genomic regions. Two QTL for epididymal weight at 300 and 90 d were also observed. This indicates the effect of different genes at different developmental stages.

QTL for Serum Testosterone Concentration

Testosterone, synthesized in the Leydig cells of testes, is essential for the maintenance of spermatogenesis and development of reproductive tract. In this study, we first detected QTL for serum testosterone concentration of boars at 300 d. Evidence was found for a 1% genome-wide significant QTL in the central region peaked at 71 cM on SSC7 and a suggestive QTL at 68 cM on SSC13. We noted that the QTL for serum testosterone concentration was located at a position near the

QTL for epididymal weight at 300 d on SSC7 (Figure l). Development of epididymis is under the control of testosterone prenatally and its growth increases around the onset of puberty when testosterone concentrations increase (Robaire and Hermo, 1988). Boars from the high-testosterone line had significantly larger epididymal weights than boars from the low-testosterone line (Walker et al., 2004). In addition, androgen-binding protein transports testosterone to the epididymis (Hermo et al., 1998), and the testosterone values obtained here were correlated with epididymal weight ($r = 0.30$, $P < 0.01$). Taken together, it is likely that QTL on SSC7 may contain gene(s) regulating the concentration of testosterone and the growth of epididymis simultaneously.

On SSC7 and SSC13, QTL for length and weight of uterine horns have been previously detected at a position close to the QTL for testosterone concentration, respectively (Bidanel et al., 2001). Testosterone is also responsible for female-associated characteristics such as the maturity of reproductive organs, and some uterine functions were inhibited by utilizing small dosages of androgen receptor agonist (Pope and Cárdenas, 2006). The genetic mechanisms concerning the correlation between testosterone concentration and development of the reproductive tract require further investigation.

It has been shown that genes residing on SSCX play an important role in the testicular development (Bidanel et al., 2001; Rohrer et al., 2001; Sato et al., 2003). However, plasma testosterone concentration was not influenced by breed of origin of the X chromosome in Meishan × White composite crossbred boars (Ford et al. 2001). It is consistent with observations from this study that no QTL affecting testosterone concentration was detected on SSCX.

In conclusion, this study detected a total of 16 QTL for testicular weight, epididymal weight, seminiferous tubular diameter at 90 and 300 d, and serum testosterone concentration at 300 d. Different QTL for the same trait at different ages reflect the involvement of distinct genes in the development of the reproductive traits. Further studies are required to fine-map these QTL with additional markers and populations.

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