Construction of Genetic Microsatellite Maps for Some Chromosomes in Chinese Swine Reference Population

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ABSTRACT : In aiming to identify the genes or genetic regions responsible for quantitative traits, a swine reference population had been constructed using three Large White boars and seven Meishan dams as parents. Five F_1 males and 23 F_1 females were intercrossed to generate 147 F_2 offspring. Thirty-one microsatellite markers covering *Sus scrofa* chromosomes (SSC) 2, 4, 6 and 7 were genotyped for all members. Construction of genetic microsatellite maps was performed using the CRIMAP software package. The lengths of these chromosomes were longer than MARC maps. They were 158.6cM, 180.3cM, 197.3cM and 171.4cM, respectively. A two modified orders of markers were observed for SSC6 and SSC7. The female map on SSC6 was shorter than male map, and the contrary was on SSC 2, 4 and 7. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 10 : 1386-1390)***

Key Words : Swine, Chromosomes 2, 4, 6, and 7, Crimap Software, Linkage Maps

INTRODUCTION

Most of important economic traits in farm animals are quantitative traits and controlled by quantitative trait loci (QTL). The development of molecular biology techniques and the application of these techniques to farm animals have progressed rapidly and given an opportunity for researchers wishing to identify genes that control quantitative traits. The construction of linkage map is the first step for detection of QTL (Haley et al., 1992). Until now, there were three linkage maps of genetic markers published, i.e. the MARC map (Rohrer et al., 1996), the PiGMaP consortium map (Archibald et al., 1995), and the SCAND map (Marklund et al., 1996). In aiming to identify the genes or genetic regions responsible for quantitative traits, our university has constructed a chinese swine reference family using Large White and Meishan pigs. These two breeds are quite different in traits such as growth and litter size. Recently, we acquired several sets of swine microsatellite primers from Dr. MF Rothschild lab, Iowa State University. Prior to the detection of QTL, in the present study we constructed genetic microsatellite maps for *Sus scrofa* chromosomes (SSC) 2, 4, 6, and 7 which were chosen as previous work had revealed QTL on these chromosomes affected growth and fat traits (Andersson et al., 1994; de Koning et al., 1999; Knott et al., 1998; Wang et al., 1998).

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MATERIALS AND METHODS

Animals

The three-generation pedigree used in this study comprised three Large White and seven Meishan founders. Five F_1 males and 23 F_1 females were intercrossed to generate 147 F_2 offspring.

Microsatellite marker genotyping

Thirty-one microsatellite markers were chosen from the genetic markers on linkage map reported by the MARC (Rohrer et al., 1996) in order to cover evenly the genetic maps of SSC 2, 4, 6 and 7. The microsatellite primers provided kindly by Dr. MF Rothschild were used to PCR. PCR performed in a 20 μl volume containing 50 ng template DNA, 30 μmol dNTP, 5pmol of each primer, 1U *Taq* DNA polymerase in standard *Taq* DNA polymerase buffer, and $MgCl_2$ concentration showed in Table 1. The amplification reactions were as follows: 5 min at 94°C, 35 cycles of 94°C for 30 sec, annealing temperature (see Table 1) for 30 sec, and 72°C for 30 sec, finally followed by an extension step of 94°C for 5 min. All the amplification products separated by PAGE in 8% gels and stained with silver (Bassam et al., 1991; Su et al., 2000). These gels were photographed and dried for permanent reservation. The heterozygosity and polymorphic information content were calculated according to Ott (1993).

Linkage analyses

Linkage analyses were performed by CRIMAP version 2.4 (Green et al., 1990). Using the BUILD option, a multi-point linkage map was constructed. The orders of these

Table 1. PCR condition and characterization of microsatellite markers in this study

Marker name	SSC	$MgCl_2$ (mmol)	Ann. temp. (°C)	No. of allele	Size range (bp)	Inf. mei[1]	Hetero-zygosity	PIC[2]
SW2516	2	1.5	60	3	176-186	306	0.6384	0.6212
SW1201	2	1.5	58	4	213-223	306	0.6337	0.6015
S0170	2	2.5	60	3	154-160	306	0.6882	0.6552
SW1883	2	2.5	62	3	154-163	303	0.7772	0.7209
SW1879	2	2.5	58	3	203-217	297	0.6877	0.6454
SW2192	2	1.5	60	3	180-190	291	0.6941	0.6562
SW308	2	2.0	65	4	123-164	298	0.6771	0.6404
SW2404	4	1.5	62	3	130-149	294	0.6360	0.5600
SW835	4	1.5	60	4	120-240	294	0.6486	0.5745
SW752	4	1.5	60	3	112-124	294	0.5578	0.4987
SW270	4	1.5	60	3	137-145	113	0.4434	0.4026
SW841	4	1.5	60	3	158-170	265	0.4964	0.3733
SW445	4	1.5	58	4	181-203	303	0.6779	0.6249
S0161	4	1.5	65	5	130-160	302	0.5310	0.4216
S0035	6	1.5	65	3	180-208	254	0.6442	0.5810
SW2406	6	1.5	58	5	226-256	284	0.7643	0.7503
SW1841	6	1.5	58	5	180-240	265	0.7705	0.7555
SW1302	6	1.5	58	4	172-206	249	0.6525	0.6241
SW133	6	1.5	62	3	124-144	204	0.5351	0.4804
SW1473	6	1.5	60	2	174-186	263	0.4819	0.3658
S0121	6	2.0	58	4	180-188	281	0.5975	0.5165
SW322	6	1.5	64	2	120-126	35	0.0968	0.0921
SW607	6	1.5	58	2	160-176	120	0.4374	0.3417
SWR1343	7	2.0	60	6	120-150	163	0.4961	0.4929
SW2155	7	3.0	65	7	135-151	305	0.7688	0.7530
SW1856	7	1.5	58	4	173-197	287	0.6050	0.5790
SW859	7	1.2	60	4	101-119	216	0.4781	0.4778
SW352	7	1.5	55	4	104-112	196	0.5613	0.5333
SW252	7	2.0	60	6	143-191	298	0.7687	0.7507
SW581	7	1.5	57	2	201-205	161	0.4996	0.3748
S0212	7	1.5	55	5	232-250	207	0.7777	0.7626

[1] Informative meioses, [2] Polymorphic information content.

loci were checked using the FLIPS 2 to 5 procedure to test if the marker order needed to be revised. When all markers were included. the CHROMPIC option was used to identify unlikely crossover events. The recombination rates and marker distances were obtained using the FIXED procedure.

RESULTS AND DISCUSSION

Microsatellite genotyping

The microsatellite marker names selected for genotyping and the number of alleles, size range, informative meioses. heterozygosity, and polymorphic information content (PIC) observed in this study are showed in Table 1. For most of microsatellite primers. annealing temperatures and $MgCl_2$ concentration were different from the conditions reported by papers (Ellegren et al.. 1994; Alexander et al.. 1996) or at world-wide web site (http://sol.marc.usda.gov). Allele sizes of some microsatellites exceeded the size ranges reported before. For example. 7Sw352 was 143 to 191 bp in this study, while it was 149 to 179 bp at USDA site (http://sol.marc.usda.gov). Paszek et al. (1995) found that 6Sw1057 was 150 to 188bp in MARC map. 140 to 191bp in

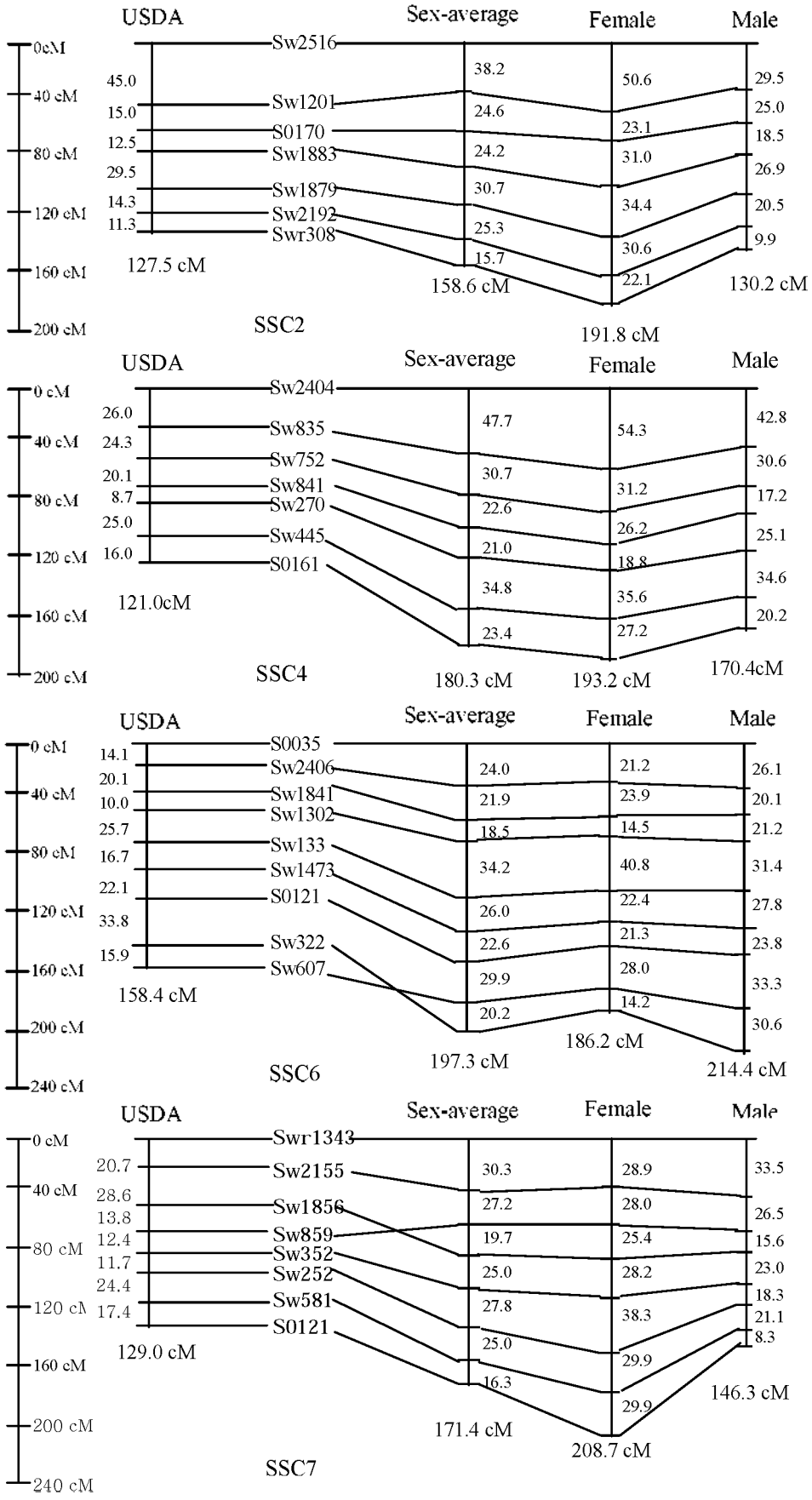

Figure 1. Porcine genetic linkage map based on estimated sex-average and sex-specific maps distances. USDA-MARC sex-averaged, sex-averaged, female-specific, and male-specific maps are shown from left to right in each chromosome. Map distances between two markers, in Kosambi cM, are shown to the left or right of each map.

PiGMaP map. The average informative meiosis for all microsatellite markers was 250.32, average heterozygosity 0.60, and polymorphic information content 0.56. According to these results, microsatellite markers in this study are highly polymorphic.

Microsatellite maps

Figure 1 presents the microsatellite maps of swine chromosomes 2, 4, 6, and 7. Genetic lengths of the sex-averaged maps are 158.6 cM, 180.3 cM, 197.3 cM, and 171.4 cM, respectively. Whereas the female maps were longer than the male maps on SSC 2, 4, and 7, the female map of SSC 6 was shorter than the male map. The difference between sex maps on SSC6 is small and not significant (p=0.2347) with χ^2 test using SAS (SAS, 1989). The orders of markers on chromosomes 2 and 4 were in agreement with maps published by Rohrer et al. (1996). There were differences in two regions between the present maps and the MARC maps on chromosomes 6 and 7. The log likelihood of the two alternative order of the markers were calculated in order to estimate the fidelity of the orders in this study.

The framework and comprehensive genetic linkage maps of porcine chromosome 6 have resulted from the first international effort to integrate genetic maps from multiple laboratories (Paszek et al., 1995). The comprehensive map on SSC6 is 166, 196 and 126 cM (for sex averaged, female and male maps, respectively). In this study, the sex-averaged map of chromosome 6 represented the marker order as S0121-SW607- SW322; whereas the MARC map had S0121- SW322-SW607. The $\log_{10}$ likelihood for the present and MARC orders were -16.494 and -16.881, respectively. Based on these calculations, the present order of the markers was not indicated to be more likely than the MARC order, it is impossible to conclude that one of the two orders is more likely because of the fact that the likelihood values for the two orders are approximately the same. Mikawa et al. (1999) reported that marker order on chromosome 6 was SWR1130-SW855-RYR1; whereas on the MARC map it was SWR1130-RYR1-SW855.

On SSC7, a comprehensive linkage map is slightly longer than the skeletal map, at 153.3, 215.3 and 183.8 cM for sex averaged, female and male maps, respectively (Rohrer et al., 1997). In our study, the best order was SW2155-SW859-SW1856, which is different from the reported order: SW2155-SW1856-SW859. The $\log_{10}$ likelihoods of the two different orders were -106.627 and -109.911. Based on these calculations, the present order of the markers was indicated to be more likely than the MARC order at least in our swine reference population of this study. The modified orders of markers for SSC7 were also reported by Rattink et al. (2000), either the p-arm or the q-arm.

The differences in the lengths of maps and marker orders are considered to be the result of differences in swine reference populations used to construction of linkage maps. The linkage maps in this study will lay the foundation for interval mapping of quantitative trait loci in this population.

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