RESEARCHREPORT

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ANIMAL SCIENCE

Title: Development of additional genetic markers for QTL in Swine
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1. ABSTRACT

This project involved the construction of porcine radiation hybrid maps, which is an very useful adjunct to genetic linkage mapping. Radiation hybrid panels are created by the use of x-ray radiation to physically break chromosomes of the species of interest into discrete fragments, the size of which are inversely proportional to the dose of radiation. These chromosomal fragments are then introduced into a rodent cell line for propagation and maintenance. The use of radiation hybrids avoids the necessity of markers having to be polymorphic in pedigrees. In addition, markers not resolved in genetic linkage groups due to an insufficient number of meioses can be resolved using radiation hybrids and the position of other markers with low number of meioses confirmed or correctly placed on the linkage map.

We have currently analyzed 901 markers on the radiation hybrid panel and have constructed framework maps of chromosomes with these markers. Due the increased resolution of radiation hybrid mapping the resultant maps consisted of 187 linkage groups compared to 19 on the genetic linkage map. The orders found are essentially the same as those indicated on our genetic linkage map (Rohrer et al. 1996). The power of radiation hybrid mapping has been demonstrated in that we have been able to resolve and order some markers that were clumped together on the genetic linkage map. Using this radiation hybrid panel markers can be mapped to a resolution of 61,000 base pairs compared to a minimum of 500,000 base pairs on the genetic map.

2. INTODUCTION

Current Status of the Problem.

Two major loci for birth weight on chromosome (SSC) 4 and ovulation rate (SSC 8) have been identified in a Meishan-Yorkshire swine cross by our group following a genomic scan using microsatellite (ms) markers selected from the USDA-MARC swine genetic linkage map (Rohrer *et al.* 1996). However, since all markers are not informative in all breeds and individuals within breed (Beattie, l994), the most current genetic maps for SSC4 and 8 are not of sufficient resolution to make effective use of either marker assisted selection (MAS) across breeds or identify the gene(s) accounting for the trait(s). Therefore, we propose to rigorously bracket these loci by identifying additional genetic markers using a cutting edge approach developed for similar purposes in the human genome mapping effort, radiation hybrid (RH)

panels. Radiation hybrid panels have the advantage that, once made, no additional animals are required for (genetic) linkage mapping.

Value of Proposed Research to the Swine Industry.

In order to maintain global competitiveness, American producers must maintain or increase carcass quality and yield in addition to improving their herds genetic merit and health. As part of the continuing programs in genome mapping at the University of Minnesota, the U.S.D.A. Meat Animal Research Center (MARC) and international collaborators, we have access to a radiation hybrid panel that, once characterized, will allow us to rapidly increase the density of markers on the available porcine maps in the region of QTLs. This use of RH to create high density maps in the region of a QTL would be the first such use of these reagents in swine genetics. The isolation and characterization of additional genetic markers which closely flank the loci we have identified will enable the industry to monitor families across breeds and incorporate the useful (gene) alleles into breeding schemes. The payoff of laboratory DNA-based marker-assisted selection to the producer engaged in day-to-day farm management is through increased efficiency of hog production which benefits all segments of the U.S. swine industry. It will permit virtual, real time, modification of production systems through enhanced selection.

3. OBJECTIVES

Two objectives were proposed.

(1) Increase the number of informative markers describing the QTLs of interest. Our immediate objective is to secure additional ms currently present on the swine genetic maps for chromosomal regions describing QTLs on SSC 4 and 8. We will use the following strategy to accomplish this objective. Interval analysis (Haley *et al*., l994) will continue to be used to improve the resolution about known QTLs with ms markers currently on the map, eg. SSC 8 between ms SW1037-S0086 that maximizes a QTL for ovulation rate. This region of SSC 8 currently comprises ~20cM, a region too large for successful application of either positional cloning of a gene or MAS. Approximately 22 ms lie in this interval. However, gaps > 5cM are present within this interval and not all ms are expected to be polymorphic either within the current QTL population (of Yorkshire and Meishan pigs) or any future population(s). Obviously, additional informative markers are essential, preferably without having to generate additional meioses within the current population to improve marker resolution. This is, of course, would also be true for any other chromosomal location we have identified.

(2) Characterize a Radiation Hybrid library. Radiation hybrid panels are created by the use of x-ray radiation to physically break chromosomes of the species of interest into discrete fragments, the size of which are inversely proportional to the dose of radiation. These chromosomal fragments are then introduced into a rodent cell line for propagation and maintenance. The use of radiation hybrids avoids the necessity of markers having to be polymorphic in pedigrees. In addition, markers not resolved in genetic linkage groups due to an insufficient number of meioses can be resolved using radiation hybrids and the position of other markers with low number of meioses confirmed or correctly placed on the linkage map.

4. PROCEDURES

Polymerase Chain Reactions (PCR) were performed on 118 radiation hybrids using standard conditions. Oligonucleotides for porcine markers were kindly provided by G Rohrer (USDA- Meat Animal-Research Center), M. Fredholm (The Royal Veterinary and Agricultural University, Denmark), C. Louis (University of Minnesota, St. Paul, MN USA) and the U.S. pig genome coordinator, or were synthesized at the Advanced Genome

Analysis Center (AGAC; University of Minnesota). Information concerning the majority of markers used in this study (microsatellites beginning with SW or S, and some genes) can be found in Rohrer et al (1994), Alexander et al (1996), Archibald et al (1995); or at http://www.marc.usda.gov/genome/swine/swine.html or http://www.ri.bbsrc.ac.uk/genome_mapping.html. Details of all other primer pairs can be found in Lahbib-Mansais et al. (1996) (INHBA), (Rettenberger et al. (1996) (IGLV, MX1, TGFB3, TCRA, CHAT, TCRB, IL4, RBP2, CCK, INHA, IGKC, TGFB2, CAST, IL6, ADRA2, PTH, ACO2, LIF, UOX), Groene et al. (1995) (S0116, S0117, S0118, S0119, S0331, S0333, S0334, S0335), Strahan et al. (1995) (GGTA1), Briley et al. (1996) (SKMC1), Sun et al. (1997) (RAF1, CP), Riquet et al. (1996) (S0368), Yerle et al. (1996) (S0365), and Joregesen et al. (1997) (marker names beginning SSC, SCC, or SSO).

5. RESULTS

The majority of work performed to date has focused on Objective 2. We have currently analyzed 901 markers on the radiation hybrid panel and have constructed framework maps of chromosomes with these markers. Due the increased resolution of radiation hybrid mapping the resultant maps consisted of 187 linkage groups compared to 19 on the genetic linkage map. The orders found are essentially the same as those indicated on our genetic linkage map (Rohrer et al. 1996). The power of radiation hybrid mapping has been demonstrated in that we have been able to resolve and order some markers that were clumped together on the genetic linkage map. Using this radiation hybrid panel markers can be mapped to a resolution of 61,000 base pairs compared to a minimum of 500,000 base pairs on the genetic map.. We are in the process of writing the manuscript describing the results of this study.

Now that we have a radiation hybrid framework map we are in a position to rapidly localize new markers on this map. We have begun Objective 1 by isolating yeast artificial chromosomes (YACs) that contain the genetic markers listed above. We are in the process of generating new microsatellite markers from YACs. One of the problems with YACs is that they can be chimeric , that is they contain insert DNA from two different parts of the genome. We will ensure that the new markers are from the region that we are interested in by testing them on the radiation hybrid panel to confirm their location. Once they location has been confirmed the new markers will be tested on our reference population and on other pig breeds to determine their degree of informativeness. This section of the work has been slowed due to the PI suffering a fractured vertebra and not being in the laboratory for 4 months. However, this section of the work is still continuing and results are expected later this year. These new ms will be used to confirm the positions of the QTL described above.

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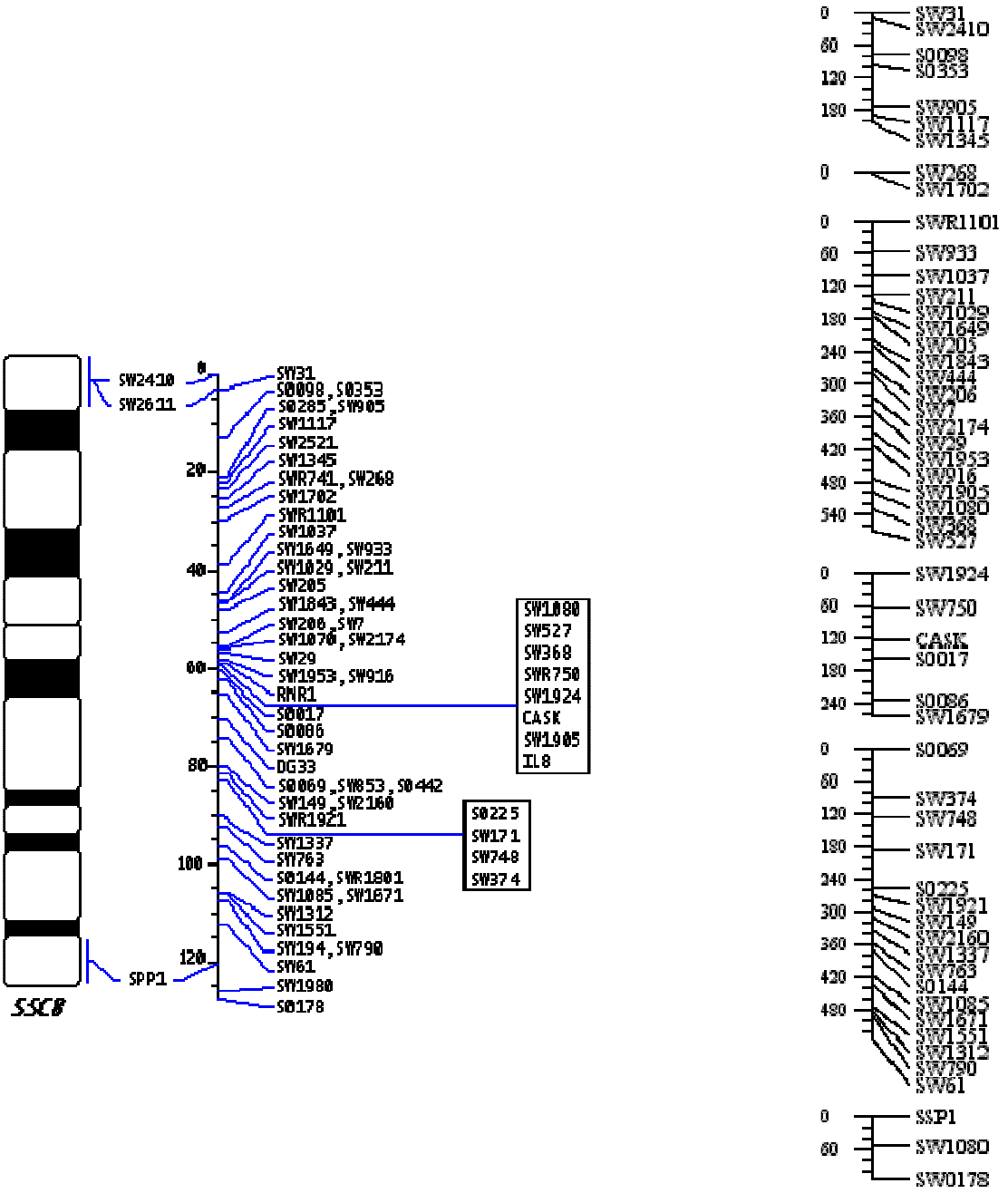

Figure1. Comparison of the genetic linkage map of swine chromosome 8 (Rohrer et al. 1996) on the right to the radiation hybrid amp on the left. Units on the genetic map are in centiMorgans (cM) and units of the radiation hybrid map are in centiRays (cRay).