A microsatellite linkage map of rainbow trout and its application in QTL analysis

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SUMMARY: The majority of species and strains reared globally for aquaculture are relatively unimproved for commercially important traits. The potential for genetic improvement in fish species compared with domestic livestock, is very high. Therefore, we are integrating molecular genetic technologies into aquaculture to help solve some of the major genetic problems. Our long-term goal is to use genetic markers to increase the efficiency of artificial selection in fish stock improvement. To do this, marker-assisted selection (MAS) has been proposed. MAS can be carried out with an understanding of the linkage relationships between quantitative trait loci (QTL) and markers. To identify QTL controlling traits of economic importance, a genetic linkage map is required, with variable markers distributed throughout the genome. We have constructed a genetic linkage map for rainbow trout using 192 microsatellite, 3 RAPD, 5 ESMP, and 7 allozyme markers in three backcross families. As a first step towards MAS, some QTLs associated with economically important traits have been identified using this linkage map. The genetic linkage map based on microsatellites could be useful for QTL analysis in aquaculture.

KEYWORDS: microsatellite, linkage map, QTL, rainbow trout, MAS

INTRODUCTION

The ability to identify chromosomal regions influencing quantitative traits has recently been improved by the development of detailed linkage maps based on DNA markers. Microsatellite or simple sequence repeat (SSR) marker type maps have been constructed in many organisms,1,2,3,4) and have been used to help locate genes for hereditary diseases and quantitative trait loci (QTL) controlling traits of economic importance.5,6) The use of DNA markers also facilitates the eventual positional cloning of the functional genes contributing to the quantitative trait.7) Construction of a genetic map, based on DNA markers at a large number of sites in the fish genome is necessary to identify QTL controlling traits of economic importance for fisheries, i.e., spawning time, maturation timing, disease resistance and growth. The utilization of strains differing in performance levels for a given quantitative trait or a strain highly polymorphic for loci controlling a quantitative trait is required to map markers of performance genes.

The first comprehensive salmonid linkage map was based on 54 allozyme loci from several salmonid species and their hybrids.9) The utility of this map was however limited, because only a few markers were characterized for any one species. Recently, genetic linkage maps based on a wide variety of new molecular markers have been constructed. In fish, most linkage maps have been constructed using randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), or probes homologous to nuclear interspersed elements.9,10,11) These markers, although applicable in a rapid and cost-effective manner for a single cross, are limited by the inability to use the same markers across different strains, without cloning and sequencing the markers.12)

SSR markers are largely co-dominantly expressed, evenly distributed throughout the genome, and surveyed rapidly in many individuals using PCR techniques.12) Furthermore, a large advantage of SSR markers is that they are hypervariable which very often results in the detection of all four segregating chromosome regions (including null alleles) in the progeny, thus facilitating a direct comparison of sex-specific recombination rates within related full-sib genomic backgrounds. Few linkage maps based on microsatellites in fish are available, with a notable exception being the SSR genetic linkage map for zebrafish and tilapia.11,12)

In this paper, we report the first comprehensive male and female-specific SSR linkage map in rainbow trout. In salmonids, microsatellite markers are often conserved among salmonid species.13,14) Thus, we further provide information on where SSR markers from eight salmonid species are located within the rainbow trout map. Large sex-specific differences in recombination rates are expected to occur between the sexes in salmonids due to the differential sex-specific alignment of chromosomes during meiosis.15)

Some QTLs associated with economic importance (eg. upper temperature tolerance,16,17) spawning time,18) and IPN disease resistance19) in rainbow trout have been identified using this microsatellite linkage map. The
genetic linkage map based on microsatellites could be useful for genetic analysis of QTL in aquaculture.

MATERIALS AND METHODS

Rainbow trout backcross families
We used three backcross families (lot 25, n = 48; lot 41, n = 48; and lot 44, n = 90) previously utilized to detect QTLs for upper temperature tolerance\(^\text{16,17}\) and spawning time.\(^\text{18}\)

Microsatellite analysis

Genomic DNAs were extracted from muscle, liver, or gill tissue from the backcross progeny and their parents by the method of Bardakci and Skibinski (1994).\(^\text{20}\) PCR was performed in a 11μl reaction volume containing 5 pmol of unlabeled primer and 0.17pmol of primer end-labeled with \([γ-33P]\) ATP using T4 polynucleotide kinase, plus 0.175 mM of each dNTP, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 – 2.5mM MgCl2 (dependent upon the primers), 0.1mg/ml BSA, 0.25 unit Taq DNA polymerase and 30 ng template DNA. A specific annealing temperature was used for each microsatellite marker. Specific PCR programs used to amplify SSR DNA regions varied according to lab of origin but were similar to the following: initial denaturation at 95°C for 2 min, followed by 35 cycles consisting of 1 min at the annealing temperature, 1 min at 72°C, 30 seconds at 95°C, and a final extension of 3 min at 72°C. PCR products were separated in a 6% polyacrylamide 7 M urea gels. DNA fragment sizes were determined using the products obtained from an M13 sequencing reaction. Autoradiographs were produced following gel drying by exposure to Kodak Biomax™ MR film.

Linkage analysis

We had to use several families for genetic mapping because not all markers were informative in the parental combinations tested. Map construction was not sex-averaged due to the large differences in recombination rate detected between the sexes. Thus, sex-specific linkage maps were generated.

Linear assignments of markers within linkage groups were aided by the program MAPORD. (Danzmann RG, unpubl. data, 1999). When possible, map construction involved using LOD adjusted recombination estimates between anchor markers (i.e., segregation data obtained from all families for a pair of adjacent markers) present in all mapping families. Map distances were assigned using raw recombination estimates between adjacent markers as salmonids have almost complete interference during meiosis.\(^\text{21}\) Map distances for solo markers located within anchor markers were adjusted using the LOD weighted recombination estimates between anchor markers with MAPDIS (Danzmann RG, unpubl. data, 1999). For a family with lower than average recombination, the map distance will be adjusted upwards, while a family with higher than average recombination will have the map distances adjusted downwards. Two approaches can be adopted for placing solo markers on the map when they are located outside of anchor marker intervals (i.e., at the ends of the linkage group). Either the raw recombination estimate may be used, or the LOD weighted recombination estimate of the nearest anchor pair may be used to readjust the map distance of the marker. The latter approach was used in constructing the female map. In cases where anchor markers were not present in a given linkage group, the unadjusted recombination estimates obtained from each respective family was used as the map interval estimate. Pairwise recombination estimates used as input into MAPORD and MAPDIS were obtained with LINKMFE4 (Danzmann RG, unpubl. data, 1999) which tests for independent segregation of male and female-specific alleles across marker regions (i.e., fully out-crossed genomes). Estimates of the differences in sex-specific recombination rates along chromosome intervals were conducted using the program RECOMDIF (Danzmann RG, unpubl. data, 1999).

RESULTS

Microsatellite linkage map

We have constructed a genetic linkage map for rainbow trout (Oncorhynchus mykiss) with 207 markers, comprising 191 SSR, 7 allozyme, 3 RAPD, and 6 ESMPs in three backcross families. In addition segregation data were obtained for 6 SSR in lot 25, and 6 SSR markers in lot 44, which remain unlinked to the other markers tested.

We detected 29 linkage groups using segregation data obtained from female parents that span approximately 10 Morgans. An additional 3.5 Morgans were detected using the segregation data from males for markers that were not polymorphic in females, but these distances may be biased by their relative chromosomal position. A large number of markers that are assigned to the male map remain unassigned to the female map. Since the female map is relatively incomplete and female recombination rates are much higher than those observed in males, these markers may reside in the same linkage groups as described for males. Based on the results of seven allozyme markers and the sex determining locus, it is possible to identify five linkage groups (2, 5, 8, 15 and 18) previously reported.\(^\text{8}\)

Differences in recombination rate between males and females
The sexes show substantial differences in recombination rate for the same pairs of linked markers (Fig. 1). The ratio of female: male recombination rates among all adjacent markers is 3.25:1. Individual pairwise Female: Male recombination differences varied from infinity to 0.00 (Fig. 2). Female recombination rates exceeded male recombination rates for many homologous regions compared. Female recombination rates around the centromere were much higher than those of males. Conversely, male recombination rates appeared to be higher in telomeric regions. For example, male map distances between the terminal two markers on linkage groups N are substantially greater than those of the female. Terminal markers (OmyFGT47TUF / OmyFGT51TUF) in linkage groups N have estimated gene-centromere distances of 50 (unpublished data) confirming their telomeric location based on additive linkage distances in the female map (Fig. 1). The recombination ratio in telomeric regions is estimated to be 0.14:1 (Female: Male), while regions proximal to the centromere have approximately a 10:1 (Female: Male) recombination ratio.

**DISCUSSION**

**Sex-specific recombination rates**

In human, mouse, cattle, pig, and indeed most vertebrates studied thus far, recombination rates show significant differences between the sexes. Female map distances are usually greater than those in the male.\(^{3,4,22}\) Ratios (Female: Male) average from approximate unity to 1.8:1 within many species, including humans.\(^{12,23,24}\) Birds may show a reversal of this trend, with slightly higher rates in males compared with females,\(^{1}\) which appears consistent with Haldane's prediction\(^{25}\) that the heterogametic sex shows slightly lower recombination rates. Recombination rates in male salmonids are also repressed relative to females, presumably because of structural constraints imposed on crossing over within multivalent pairings. Such pairings often involve metacentric chromosomes resulting from Robertsonian fusions of ancient acrocentric chromosomes which in turn may pair with their respective acrocentric homeologues.\(^{15,16,26,27}\) We were able to assess recombination rate differences between the sexes by using outcrossed families and gene centromere mapping approaches\(^{28}\) to localize centromeres within linkage groups. Using this approach, we report the largest sex-specific recombination differences for any known vertebrate. These findings are consistent with previous reports of large Female: Male recombination differences among salmonid species detected with allozymes.\(^{27}\)

![Fig. 1 Comparative female (left) and male (right) linkage map of rainbow trout based primarily upon SSR markers in linkage group R and N. Markers linked in the male map but unlinked in the female map are indicated by M<.](image)

**QTL analysis in aquaculture**

In rainbow trout, some QTLs associated with economic importance have been identified using this microsatellite linkage map. Two selected lines of rainbow trout divergent for temperature tolerance were used to map several QTLs for upper temperature tolerance (UTT) in seven linkage groups.\(^{16,17}\) Eight chromosomal regions spanning seven linkage groups show an association with spawning time,\(^{18}\) additionally two putative QTL associated with IPN disease resistance/susceptibility have been mapped in rainbow trout.\(^{19}\) These QTL markers have potential for use in marker-assisted selection (MAS) in aquaculture.

Once the linkage phases of marker and QTL alleles are established within family lines, the selection of future parents can be made on the basis of choosing 15 individuals with a maximal number of high performance QTL alleles.\(^{29}\) Identification of a large number of DNA markers linked to QTL controlling traits of economic significance will contribute to the application of DNA marker-assisted selection in aquacultural breeding.\(^{30}\) By identifying markers of high performance QTL in different strains or species, it may also be possible to successfully improve the performance of such traits in other strains.
(having lower performance QTL) through introgression of the desired QTL. However, the performance of QTL alleles may be altered in foreign genomic backgrounds due to epistatic interactions with other genes, and thus performance estimates must be obtained for QTL regions in outbred genomes prior to the ‘blind faith’ initiation of such breeding programmes.

REFERENCES


