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A. Ozaki · T. Sakamoto · S. Khoo · K. Nakamura
M. R. M. Coimbra · T. Akutsu · N. Okamoto

Quantitative trait loci (QTLs) associated with resistance/susceptibility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*)

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Abstract Infectious pancreatic necrosis (IPN) is a well-known acute viral disease of salmonid species. We have identified quantitative trait loci (QTLs) associated with resistance to this disease in rainbow trout. We searched for linkage among 51 microsatellite markers used to construct a framework linkage map in backcross families of rainbow trout (*Oncorhynchus mykiss*), produced by crossing IPN-resistant (YN-RT201) and -susceptible (YK-RT101) strains. Two putative QTLs affecting disease resistance were detected on chromosomes A (IPN R/S-1) and C (IPN R/S-2), respectively, suggesting that this is a polygenic trait in rainbow trout. These markers have great potential for use in marker-assisted selection (MAS) for IPN resistance and provide the basis for cloning of IPN resistance genes. Clarification of the genetic bases of complex traits has broad implications for fundamental research, but will also be of practical benefit to fish breeding.

Key words Infectious pancreatic necrosis (IPN) · Disease resistance · Microsatellite markers · Rainbow trout · Quantitative trait loci

Introduction

Infectious pancreatic necrosis (IPN) is a highly contagious viral disease which causes extensive mortality among young salmonid fishes, and has spread to various countries (Wolf et al. 1960; Sano 1971), thus becoming a major concern in hatcheries. IPN virus (IPNV) is the prototype virus of the Birnaviridae family; these are unenveloped, icosahedral animal viruses, approximately 60 nm in diameter, with bisegmented double-stranded RNA genomes (Dobos et al. 1979; Brown 1986). Specific differences in disease resistance have been demonstrated among salmonid species (Pilcher and Fryer 1980; Geert et al. 1996). In addition, as a result of extensive studies on IPN, variation in resistance has been found among cultivated varieties of rainbow trout (Hill 1982). Based on this variation, Okamoto et al. (1987, 1993) succeeded in isolating two strains of rainbow trout, one highly susceptible (YK-RT101), the other highly resistant (YN-RT201), to IPN. Furthermore, inter-strain hybridization of rainbow trout has been found to improve IPN resistance (Hanada and Ushiyama. 1985). However, no research has been done on the genes that are responsible for resistance and/or susceptibility to this disease, and their chromosomal locations are unknown.

The recent development of molecular markers has made it possible to identify individual loci controlling quantitative traits (Tanksley 1993). A number of studies have contributed to breeding programs by identifying loci influencing quantitative traits in domesticated animals (e.g., pigs and cattle) and food crops (e.g., tomato and maize) (Paterson et al. 1988; Edwards et al. 1992; Georges et al. 1993; Andersson et al. 1994).

In fish, the construction of a genetic map based on molecular markers at a large number of sites in the genome is necessary to permit the identification of individual loci controlling quantitative traits of economic significance to fisheries (e.g., disease resistance, fecundity, growth etc.). In rainbow trout, two independent linkage maps have been constructed using molecular markers. Young et al. (1998) developed a high-resolution and high-density AFLP (amplified restriction fragment length polymorphism) linkage map, using a doubled haploid line. The disadvantage of this method is that the use of dominant markers can not take account

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A. Ozaki · T. Sakamoto · S. Khoo · K. Nakamura
M. R. M. Coimbra · N. Okamoto (✉)
Department of Aquatic Biosciences,
Tokyo University of Fisheries,
Konan 4, Minato, Tokyo 108-8477, Japan
E-mail: nokamoto@tokyo-u-fish.ac.jp
Tel.: +81-3-54630547
Fax: +81-3-54630552

T. Akutsu
Fuji Trout Hatchery,
Shizuoka Prefectural Fisheries Experimental Station,
Inokashira, Fujinomiya, Shizuoka 418-0108, Japan

of two fundamental aspects of genetic mapping in salmonids: tetrasomic inheritance and sex-specific recombination rates. Sakamoto et al. (2000) overcame this problem by using co-dominant markers – microsatellites or simple sequence repeats (SSRs) – which are useful for rapidly surveying many individuals. They reported sex-specific SSR linkage map differences in recombination rates and tetrasomic inheritance. These linkage maps have facilitated the analysis of quantitative trait loci (QTLs) that control complex traits, such as upper temperature tolerance (Jackson et al. 1998) and spawning time (Sakamoto et al. 1999). Demand for QTL analyses of complex traits is increasing because of its potential to facilitate the manipulation of important traits in fish breeding. Rainbow trout is one of the most intensively studied fish species because of its importance as a food and game fish, and as a model for genome evolution following tetraploidization (Ohno et al. 1970). This species is also an ideal fish model for the genetic dissection of complex traits, including disease resistance. Because of the high reproductive capacity and relatively large clutch size, several generations of many rainbow trout families can be produced and characterized in a short period of time. Also, inbred lines and doubled haploid lines that display a variety of characteristics can be developed. Consequently, the unique characteristics of the rainbow trout model allow the use of contemporary biometrical techniques that have already proven successful in other vertebrates.

In this study, we describe the construction of a framework linkage map of the 97-K2-2 family [the backcross family resulting from the crosses: YK-RT101 (female) × YN-RT201 and YK-RT101 × F1 male] based on microsatellite markers, and report the first identification of QTLs associated with disease resistance in fish.

Materials and methods

IPNV strains, sample collection and preparation

The Buhl strain of IPN virus (IPNV), which is the dominant IPNV serotype in Japan, was used as a challenge virus. This virus was provided by Dr. K. Wolf (National Health Research Laboratory, Kearneysville, W.Va, USA). The IPNV had been passaged 20 times in RTG-2 cells (Wolf and Quimby 1962) and was cloned once before use. Techniques for viral propagation in RTG-2 cells were previously described by Okamoto et al. (1983).

Rainbow trout families

YN-RT201, an IPN-resistant strain (Okamoto et al. 1993), and YK-RT101, an IPN-susceptible strain (Okamoto et al. 1987), were cultured at the Yoshida Research and Training Station of Tokyo University of Fisheries (Table 1). Both strains were phenotypically selected for seven generations during the past 20 years. In order to find loci related to IPN resistance/susceptibility, we performed a cross between these two strains and generated an F1 hybrid family, 94-N515-K560. A male (that had not been challenged with IPNV) of the family 94-N515-K560 was then backcrossed to the susceptible strain (YK-RT101) to generate a backcross (BC) family termed 97-K2-2 (Fig. 1).

Table 1 Tests of resistance to IPN in the YN-RT201 and YK-RT101 strains of rainbow trout

Lot (year/month)	Cumulative mortality (%)[a]	
	YN-RT201[b]	YK-RT101[c]
1979/6	10.0	95.0
1979/12	1.7	95.0
1980/6	3.3	86.7
1980/9	3.3	98.3
1980/12	3.3	100
1981/6	23.3	100
1981/9	3.3	100
1981/12	1.7	95.0
1982/6	6.7	85.0
1982/9	1.7	100
1982/12	3.3	100
1983/6	0	98.3
1983/9	1.7	100
1983/12	15.0	100
1984/6	0	100
1984/12	0	100
1986/6	5.0	85.0
1986/9	0	100
1986/12	0	98.3
1989/2	5.0	86.0

[a] Artificially induced infections with IPNV were monitored at 15 °C for 28 days. The data for cumulative mortalities are averages of duplicate tests

[b] YN-RT201 is highly resistant to IPNV, as reported by Okamoto et al. (1993)

[c] YK-RT101 is highly susceptible to IPNV (Okamoto et al. 1987)

IPNV challenge and resistance tests

Resistance tests to IPNV were conducted on the F1 hybrid family 94-N515-K560 and the backcross family 97-K2-2, according to Okamoto et al. (1983). For each test of artificially induced infections, 100 fry weighing approximately 0.15 g were kept in a small beaker filled with 1 l of aerated water at 15 °C. Virus was added to a final concentration of $10^{5.0}$ $TCID_{50}$ (50% tissue culture infections dose)/ml of water. After 1 h, fish were divided into two groups of 50 each, and transferred to tanks where they were maintained for 30 days at 15 ± 1 °C. Controls were treated in the same manner except that no virus was added.

The phenotypic scoring system adopted uses two values, 1-dead (susceptible) and 0-survivor (resistant). Viral isolation from dead fish was performed according to the protocols of Okamoto et al. (1984) to confirm that the fish had been killed by IPNV.

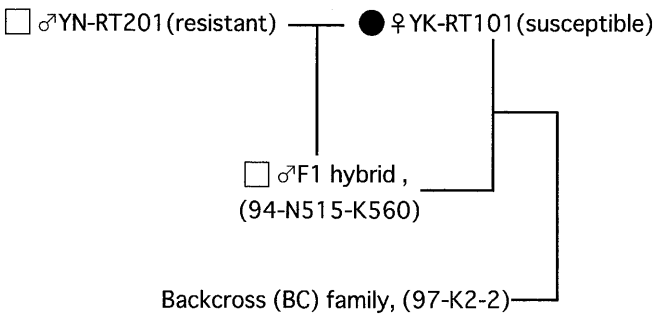

Fig. 1 Schematic representation of the rainbow trout population used in this study, which was derived from a cross between the strains YN-RT201 and YK-RT101. QTL analysis for IPN disease resistance was carried out using a backcross family

Microsatellite analysis

Genomic DNA was extracted from wholemount tissue from the 97-K2-2 BC progeny and their parents, using a modification of the procedure of Bardakci and Skibinski (1994). PCR was performed in a 25-µl reaction volume containing 0.5 µM of unlabeled forward primer and 0.05 µM of reverse primer labeled with [γ-^{33}P]ATP using T4 polynucleotide kinase, plus 0.4 mM of each dNTP, 10 mM TRIS-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 µg/ml BSA, 0.625 U of *Taq* DNA polymerase (Takara) and 50 ng of template DNA. A specific annealing temperature was used for each microsatellite marker. The PCR program consisted of an 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 s at 95 °C, and a final extension for 3 min at 72 °C. Amplicons were denatured by adding an equal volume (25 µl) of formamide buffer (98% formamide, 10 mM EDTA pH 8.0, 0.05% bromophenol blue, and 0.05% xylene cyanol) and heating at 95 °C for 10 min. Three microliters of each sample were loaded on a 6% polyacrylamide gel to obtain individual genotypes. Allele sizes were determined using a standard M13 sequencing ladder. The gel was dried and used to expose a Fuji Image Plate Bas 1000 (Fujix) overnight.

Linkage analysis

We used 121 loci selected from each linkage group of the genetic linkage map of rainbow trout (Sakamoto et al. 2000). Linkage groups and the order of markers were determined using Map Manager QT28b (Manly and Olson 1999), and chromosome names were according to Sakamoto et al. (2000). In order to construct the linkage map and map loci associated with IPN, we analyzed 52 individuals from the 97-K2-2 progeny, 26 IPN-susceptible and 26 IPN-resistant fish.

QTL analysis

The analysis used to identify QTLs associated with IPN was divided into three steps

Step 1 involved the identification of potential genomic regions with QTLs flanked by molecular markers. Backcross progeny, composed of 26 fish that died from IPN and 26 fish that survived IPN were typed at 51 loci spaced throughout the rainbow trout genome (Table 2). Simple associations between the genotypes of BC progeny at each marker locus and phenotypic values for IPN resistance/susceptibility were assessed using a χ^2 distribution. Marker loci producing a χ^2 distribution statistic with $P \leq 0.20$ ($df = 1$, $\chi^2 \geq 1.64$) were considered as potential QTLs. At Step 2, the suggestive markers detected in Step 1 were typed in the entire BC population (97-K2-2: $n = 100$, 54 dead fish and 46 survivors). Step 3 involved estimating (1) the approximate position, (2) the additive and dominant effects expected, and (3) the proportion of the variation in the IPN resistance/susceptibility trait explained by individual QTLs. These estimates were made using the interval-mapping and the likelihood-ratio statistic (LRS) program of Map Manager QT28b (Manly and Olson 1998). This statistic can be converted to the conventional base-10 LOD score by dividing it by 4.61 (twice the natural logarithm of 10). Putative QTL effects were determined by single-locus association using the general linear model.

Table 2 Microsatellite markers typed in the 97-K2-2 backcross family of rainbow trout

LG[a]	Number of markers used[b]	Number of markers with $P \leq 0.20$ at Step 1[c]	Genome coverage (cM)
Chr2	1	0	20
Chr5	2	2	20
Chr8	3	0	35.4
Chr15	1	0	20
Chr18	2	0	35.4
*Chr*A	2	2	20
*Chr*B	2	0	21.9
*Chr*C	3	3	50.7
*Chr*D	1	0	20
*Chr*E	1	0	20
*Chr*F1	1	0	20
*Chr*F2	2	0	20
*Chr*G	3	0	58.5
*Chr*H	1	1	20
*Chr*I	1	0	20
*Chr*J	2	0	66.2
*Chr*K	2	0	23.8
*Chr*L	2	0	20
*Chr*M	1	1	20
*Chr*N	4	0	70
*Chr*O1	5	0	21.9
*Chr*O2	2	0	20
*Chr*P	1	1	20
*Chr*Q	3	0	62.3
*Chr*R	1	0	20
*Chr*S	1	0	20
*Chr*T	1	1	20
Total	51	10	786.1

[a] LG, linkage group; Chr, chromosome number
[b] 26 IPN-resistant and 26 IPN-susceptible rainbow trout (total $n = 52$) from 97-K2-2 backcross progeny were analyzed
[c] Loci producing a χ^2 distribution statistic with $P \leq 0.20$ at Step 1 (see text)

Results

Phenotypic variation of resistance to IPN in rainbow trout

The frequency distribution of the parameter "days to die" among the progeny of the cross between YK-RT101 and YN-RT201 (F1 family 94-N515-K560) and the backcross family (97-K2-2) are shown in Fig. 2. Cumulative mortality in the 94-N515-K560 family was 36% and in the 97-K2-2 progeny 54%. These results suggested that the phenotypes of the backcross family were derived from the sum of effects of individual IPN QTL alleles of the parental lines (resistant/susceptible). No mortality was detected in the controls.

Linkage analysis and genome composition of the 97-K2-2 family

Among 121 markers used, 51 microsatellite markers were grouped into 27 linkage groups using the backcross mode of Map Manager QT28b (Fig. 3). Other markers were non-polymorphic in the family analyzed. The 27 linkage groups were further rearranged into 29 linkage groups based on known linkage information from the linkage map produced by Sakamoto et al. (2000). The male-linkage map of the 97-K2-2 family ($n = 52$) showed low recombination rates. A linkage map with a total length of 786.1 cM was constructed (Table 2). The

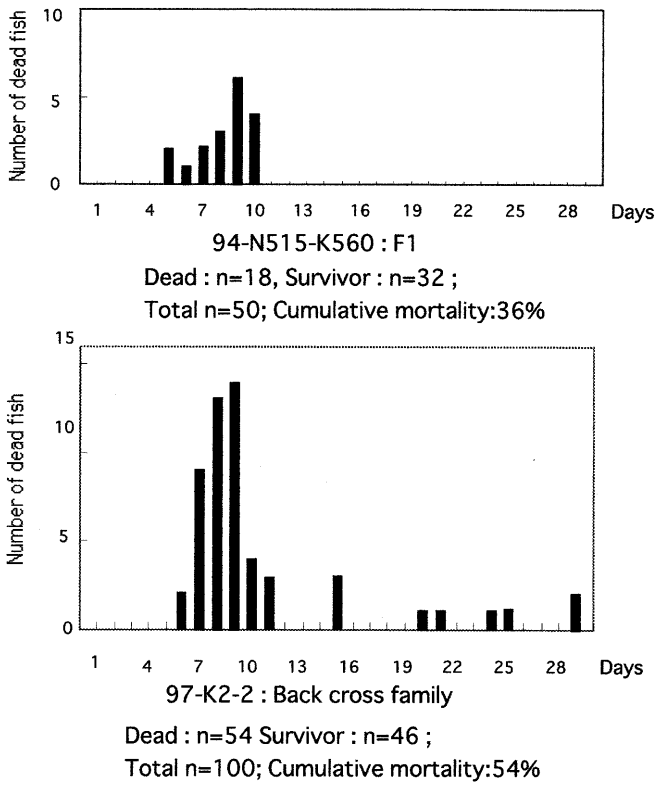

Fig. 2 Frequency distribution of "days-to-die" among susceptible progeny in the F1 (YN-RT201 × YK-RT101; family 94-N515-K560) and the backcross family (F1 × YK-RT101; family 97-K2-2)

average distance between markers was 15.4 cM. All determined marker groups agreed with those of the rainbow trout map.

QTLs for IPN disease resistance

Initially, 52 individuals of the 97-K2-2 family were analyzed at 51 loci spaced throughout the rainbow trout genome. These results are shown in Table 3. As a result, two highly significant QTLs associated with IPN disease resistance were detected based on the LRS. One QTL peak, designated as IPN R/S-1, was located close to the markers OmyRGT41TUF (LRS = 10.4) and Ssa4DU (LRS = 10.4) on chromosome A (Fig. 3, Table 4). The other QTL peak (IPN R/S-2) was located close to the markers OmyOGT4TUF (LRS = 10.4) and OmyRGT6/iiTUF (LRS = 8.4) on chromosome C (Fig. 3, Table 4). Each QTL explained about 17% of the total phenotypic variation in the 52 individuals screened (Table 4).

Later, we confirmed the linkage-group location of these two QTLs by screening 100 individuals from the 97-K2-2 family. One QTL peak, designated IPN R/S-1, was indicated by significant LRS values for two markers, OmyRGT41TUF (LRS = 16.9) and Ssa4DU (LRS = 16.9) (Fig. 3, Table 4). The other QTL peak (IPN R/S-2) was indicated by suggestive LRS values for two markers, OmyOGT4TUF (LRS = 13.7) and OmyRGT6/iiTUF (LRS = 10.9) (Fig. 3, Table 4). The amounts of total trait variance that could be explained by a QTL between the two groups of 52 and 100 individuals, did not show any significant differences (Table 4).

It is worth mentioning that for the QTL IPN R/S-1, the YN-RT201 allele was inherited by about 77% (20/26) of the survivors, and by about 35% (9/26) of the dead progeny in the case of the 52 fish tested, respectively. For the QTL region of IPN R/S-2, where only the YK-RT101 allele was inherited by the progeny, about 65% (17/26) of the fish survived and 23% (6 or 7/26) died (Fig. 4, Table 5). These results showed that the YN-RT201 allele acts as a dominant effect and the YK-RT101 allele has an additive effect on IPN disease resistance. Taking these results into account, we conclude that at least two loci are involved in IPN disease resistance.

Discussion

This study constitutes the first report on the mapping of QTLs associated with disease resistance/susceptibility, using molecular tools, in hatchery fish. We identified in rainbow trout two chromosomal regions containing QTLs that were associated with IPN disease resistance/susceptibility.

Two putative QTL associations, of medium to large effect, with several components of IPN disease resistance/susceptibility, were localized to the linkage groups *ChrA* and *ChrC*. On *ChrA*, the YN-RT201 allele exhibited a dominant effect on IPN disease resistance in the region of IPN R/S-1, and on *ChrC*, the YK-RT101 allele displayed an additive effect on resistance to IPN disease at IPN R/S-2. These two loci were responsible for a significant portion (27–34%) of the total phenotypic variation in a backcross family (Table 4). The fact that we could not predict, at the beginning of this study, that the susceptible strain carried a resistance allele (IPN R/S-2), must be attributed to the limitations imposed by the use of phenotypical selection alone for determining the number of genes involved in expression of the character of interest. In maize and tomato, QTLs detected in one environment were frequently detected in another environment, suggesting very little Environment × QTL interaction (Stuber et al. 1987; Paterson et al. 1991). In hatcheries, salmonid species are raised under relatively standard, controlled conditions that minimize the environmental component and increase the accuracy of phenotypic evaluations. The relatively large effect of the QTLs identified here should therefore be detectable in other hatchery experiments under similar conditions.

In comparison to other traits, disease resistance is an ideal candidate for the application of marker-assisted selection (MAS), due to its economic importance. In commercial hatcheries, the final product must be supplied as a crossbred animal so that the development of superior hybrids for IPN disease resistance and other

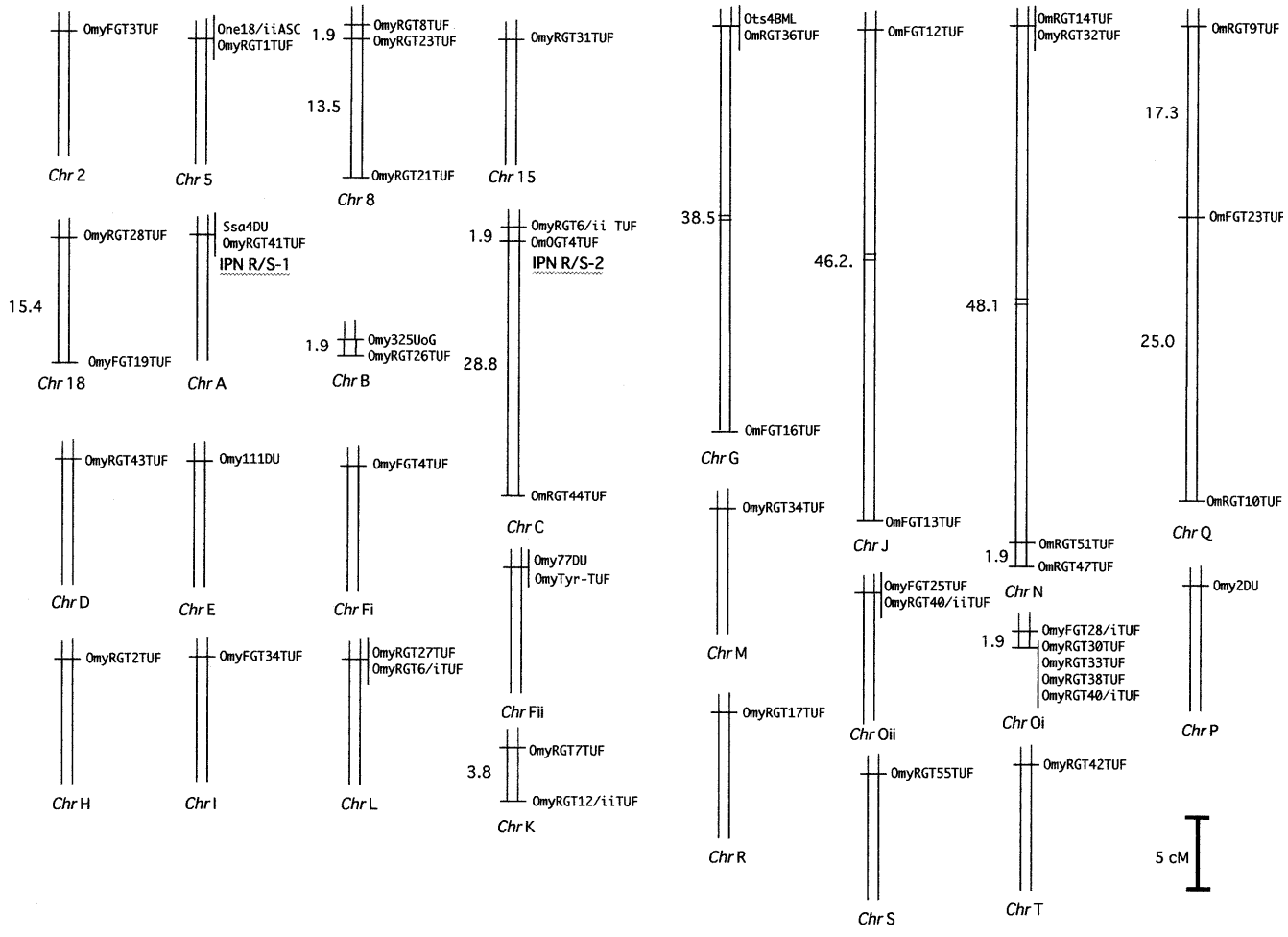

Fig. 3 Genetic linkage map constructed using microsatellite markers. The regions within which QTLs for IPN disease resistance were localized are indicated by the *wavy lines*. This linkage map was based on the analysis of the backcross progeny (97-K2-2: n = 52). Map distances were calculated using Map Manager QT28b ($P \leq 0.05$)

traits of economic relevance can be achieved by complementing MAS with standard breeding schemes. However, the effectiveness of MAS in the selection based on challenge with the pathogen depends on the proportion of additive genetic variance explained by markers and on the heritability of the disease trait. In rainbow trout, the paternal haplotype may be effective in improving IPN disease resistance. The sex-specific difference in recombination rates could partially account for the strong interaction. We observed a difference between the paternal and maternal effects on survival, following IPNV challenge (unpublished data). The contribution of each candidate locus to the phenotypic variation is at most 10∼20% in rice (Lin et al. 1998), in the same range as our results (approximately 17%), which implies that each has an effect on breeding. Therefore, our results suggest that it will be possible to improve the IPN disease resistance of rainbow trout.

However, many difficulties will have to be overcome before these ideas can be put into practice. Epistasis, or the interaction between loci, implies that different loci affecting the same trait interact so that the phenotype expressed is a function of the particular combination of alleles present at the different loci. The converse of epistasis is genetic heterogeneity, where two or more loci function independently as causes of disease and act via separate biological pathways (Vallejo et al. 1998). In this study, we could not investigate the role that epistasis might play in determining the expression of IPN disease resistance/susceptibility. It is not clear whether each QTL corresponds to only one locus or to a chromosomal region containing a cluster of genes, each having a relatively small genetic effect. Thus, it is necessary to deal with each QTL as a single Mendelian factor and to localize each QTL precisely on the linkage map. The populations previously used in QTL analyses of fish were mainly backcross or doubled haploid lines, which simultaneously segregate multiple genetic factors, dispersed over the whole genome, making it difficult to use them for the precise mapping of any one of the multiple QTLs. Moreover, determination of the true genetic action of a QTL is more difficult, because the genetic parameters of a given QTL are often affected by the segregation of other QTLs. In plant genetics, near-isogenic lines (NILs), developed by backcrossing, have been widely

Table 3 Linkage data for IPN R/S QTLs in the 97-K2-2 backcross family

LG[a]	Marker locus	LRS[b]	Contribution (%)[c]	*P*	Add[d]
*Chr*2	OmyFGT3TUF	0.1	0	0.78	–0.04
*Chr*5	Onel8/iiASC	2.0	2	0.16	0.19
	OmyRGT1TUF	2.0	2	0.16	0.19
*Chr*8	OmyRGT8TUF	0.3	0	0.57	0.08
	OmyRGT23TUF	0.1	0	0.78	0.04
	OmyRGT21TUF	0.3	0	0.57	0.08
*Chr*15	OmyRGT31TUF	0.3	0	0.57	–0.08
*Chr*18	OmyRGT28TUF	0.1	0	0.77	0.04
	OmyFGT19TUF	0.1	0	0.78	–0.04
*Chr*A	Ssa4DU	10.4	17	< 0.01	0.43
	OmyRGT41TUF	10.4	17	< 0.01	0.43
*Chr*B	Omy325UoG	0.8	0	0.37	0.13
	OmyRGT26TUF	1.5	1	0.22	0.18
*Chr*C	OmyRGT6/iiTUF	8.4	13	< 0.01	–0.39
	OmyOGT4TUF	10.4	17	< 0.01	–0.43
	OmyRGT44TUF	2.9	4	0.08	–0.24
*Chr*D	OmyRGT43TUF	1.3	1	0.25	0.16
*Chr*E	Omy111DU	0.7	0	0.40	0.12
*Chr*Fi	OmyFGT4TUF	0.3	0	0.57	0.08
*Chr*Fii	Omy77DU	0.3	0	0.57	0.08
	OmyTyr-TUF	0.3	0	0.57	0.08
*Chr*G	Ots4BML	0.0	0	1.00	0.00
	OmyRGT36TUF	0.0	0	1.00	0.00
	OmyFGT16TUF	1.3	0	0.26	–0.15
*Chr*H	OmyRGT2TUF	2.0	2	0.15	0.20
*Chr*I	OmyFGT34TUF	1.3	0	0.26	–0.15
*Chr*J	OmyFGT12TUF	0.1	0	0.77	0.04
	OmyFGT13TUF	0.1	0	0.78	0.04
*Chr*K	OmyRGT7TUF	0.1	0	0.78	–0.04
	OmyRGT12/iiTUF	0.1	0	0.78	0.04
*Chr*L	OmyRGT27TUF	0.0	0	1.00	0.00
	OmyRGT6/1TUF	0.0	0	1.00	0.00
*Chr*M	OmyRGT34TUF	2.0	2	0.16	–0.19
*Chr*N	OmyRGT14TUF	0.1	0	0.77	0.04
	OmyRGT32TUF	0.1	0	0.77	0.04
	OmyRGT51TUF	0.3	0	0.57	0.08
	OmyRGT47TUF	0.7	0	0.40	0.12
*Chr*Oi	OmyFGT28/1	0.1	0	0.78	–0.04
	OmyRGT33TU	0.0	0	1.00	OM
	OmyRGT40/1TUF	0.0	0	1.00	0.00
	OmyRGT38TUF	0.0	0	1.00	0.00
	OmyRGT30TUF	0.0	0	1.00	0.00
*Chr*Oii	OmyFGT25TUF	0.7	0	0.40	–0.12
	OmyRGT40/iiTUF	0.7	0	0.40	–0.12
*Chr*P	Omy2DU	5.3	8	*0.02*	0.32
*Chr*Q	OmyRGT9TUF	0.8	0	0.38	–0.12
	OmyFGT23TUF	1.3	0	0.25	–0.16
	OmyRGTIOTUF	0.1	0	0.78	–0.04
*Chr*R	OmyRGT17TU	0.7	0	0.40	–0.12
*Chr*S	OmyRGT55TUF	0.1	0	0.76	–0.04
*Chr*T	OmyRGT42TUF	2.9	3	0.09	0.23

[a] The data were obtained using the Map Manager QT Backcross Statistic ($P = 0.05$, 52 informative progeny, additive regression model with no control for other QTLs). LG, linkage group; Chr, chromosome number

[b] Likelihood ratio statistic (LRS) for the association of the trait with this locus

[c] Percentage of the total trait variance that would be explained by a QTL at this locus

[d] Additive regression coefficient for the association

used to perform accurate genetic analyses. Segregating populations for each QTL obtained from crossing NILs and their recurrent parents simplify genetic variations by excluding extra-genetic factors, making it easier to study the effects of a single QTL. This strategy will be useful for the analysis of epistasis, or interaction between loci, of IPN disease resistance/susceptibility in rainbow trout.

Candidate genes for disease resistance are quite often associated with major histocompatibility complex (MHC) haplotypes. Thus, in the case of chickens, MHC haplotypes B21 and B19 (Hepkema et al. 1993) affect susceptibility to Marek's disease virus. In rainbow trout, differences in MHC haplotypes may explain IPN disease resistance/susceptibility. The next step will be to determine the locations of MHC class I and II genes by using

Table 4 Single-locus associations for putative QTLs for IPN disease resistance

Number of progeny	QTL region	Marker locus	LG[a]	LRS[b]	Contribution[c]	TPV[d]	*P*	DPE[e]
52 (26 dead, 26 survivors)	IPN R/S-1	OmyRGT41TUF	ChrA	10.4	17		0.00125	N
		Ssa4DU	ChrA	10.4	17*		0.00125	N
	IPN R/S-2	OmyOGT4TUF	Chz-C	10.4	17*		0.00125	K
		OmyRGT6TUF	*ChrC*	8.4	13	17* + 17* = 34%	0.00380	K
100 (54 dead, 46 survivors)	IPN R/S-1	OmyRGT41TUF	ChrA	16.9	15		0.000039	N
		Ssa4DU	ChrA	16.9	15*		0.000039	N
	IPN R/S-2	OmyOGT4TUF	*ChrC*	13.7	12*		0.00021	K
		OmyRGT6TUF	*ChrC*	10.9	9	15* + 12* = 27%	0.00097	K

[a] LG, linkage group; Chr, chromosome number
[b] Likelihood ratio statistic (LRS) for the association of the trait with this locus
[c] Percentage of the total trait variance which would be explained by a QTL at this locus
[d] Total phenotypic variation of highly significant QTLs (*) in IPN R/S-1 and IPN R/S-2
[e] Direction of phenotypic effect. N or K indicates that YN-RT201 or YK-RT101 alleles increased these values, respectively

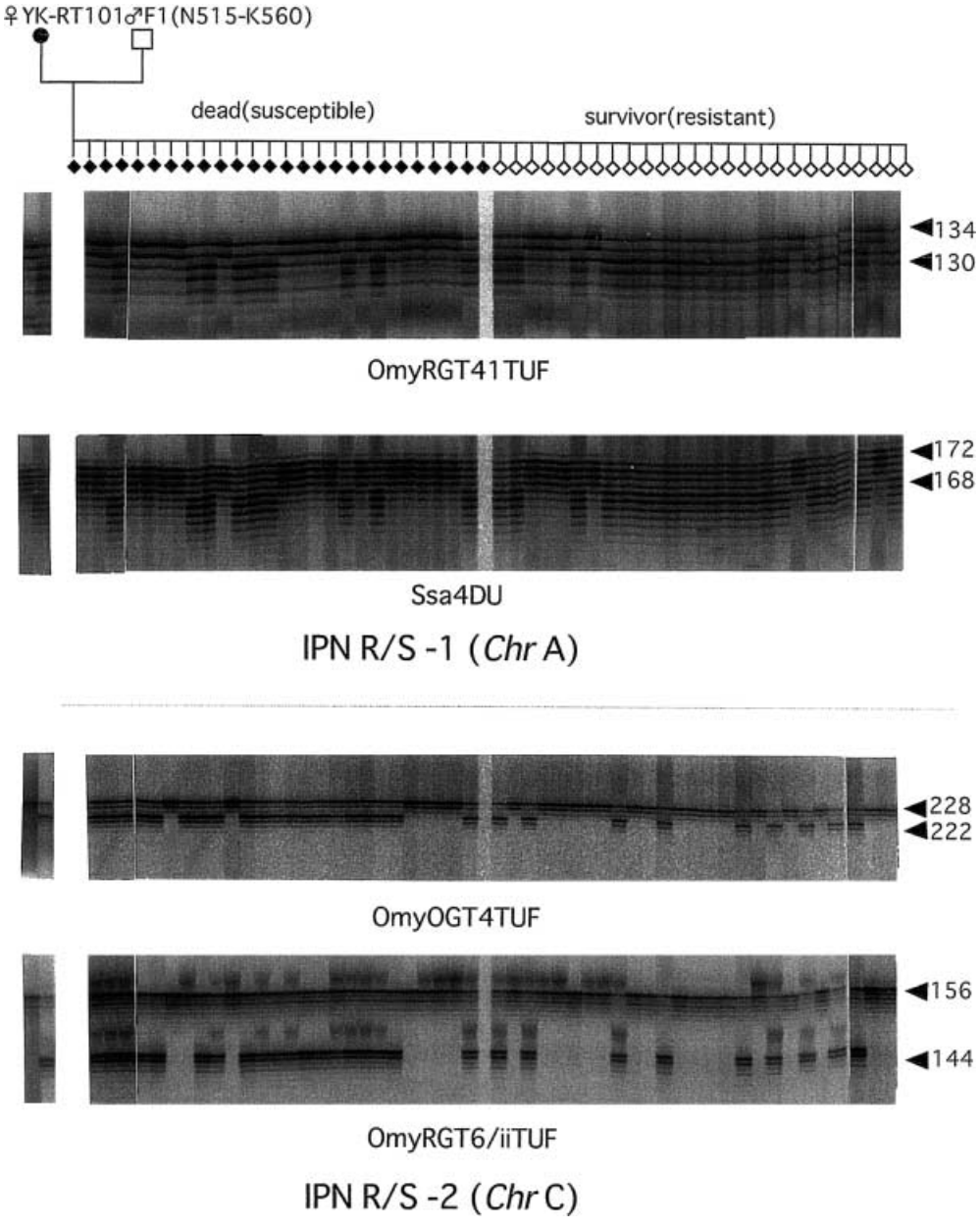

Fig. 4 Autoradiographs of markers associated with IPN disease resistance in QTL regions. IPN R/S-1 on *Chr*A. Two alleles of OmyRGT41TUF and Ssa4DU were associated with IPN resistance/susceptibility. The *upper* band is derived from YK-RT101, and the *lower* one from YN-RT201. IPN R/S-2 on *Chr*C. Two markers of OmyOGT4TUF and OmyRGT6/iiTUF were associated with the trait. The *upper* band is from YK-RT101, and the *lower* from YN-RT201

Table 5 Genotypes of the 97-K2-2 progeny at putative QTLs for IPN disease resistance

Number of progeny	QTL region	Marker locus	Marker genotypes detected	
			YK-RT101 allele (dead:survivor)	YN-RT201 allele (dead:survivor)
52 (26 dead, 26 survivors)	IPN R/S-1	OmyRGT41TUF	17:6	9:20
		Ssa4DU	17:6	9:20
	IPN R/S-2	OmyOGT4TUF	6:17	20:9
		OmyRGT6TUF	7:17	19:9
100 (54 dead, 46 survivors)	IPN R/S-1	OmyRGT41TUF	40:16	14:30
		Ssa4DU	40:16	14:30
	IPN R/S-2	OmyOGT4TUF	17:31	37:15
		OmyRGT6TUF	19:31	35:15

single-strand conformation polymorphism (SSCP) or cleavase I fragment length polymorphism (CFLP) methods. This approach may indicate the involvement of MHC haplotypes in IPN disease resistance/susceptibility. The main conclusion to be drawn from this discussion is that immunogenetics studies are likely to shift their main focus from MHC genes to QTLs – and, within QTLs, from structural to regulatory gene segments. This is not to say that the MHC will become a secondary approach, for it is the most important QTL. In tetraploid fish like salmonid species, especially, several MHC alleles/haplotypes exist, which makes immunogenetic analyses more difficult. This is the most important reason for choosing to focus on QTL analysis rather than immunogenetics in this species.

Microsatellite markers offer many advantages: they are abundant, and they show high levels of allelic variance and co-dominance for genome mapping of domesticated species. When enough segregating markers are scattered throughout an entire genome, it is theoretically possible to detect and characterize polygenes that quantitatively affect inherited characters. More than 190 microsatellite loci have now been mapped on rainbow trout maps. The number of QTLs involved in IPN disease resistance/susceptibility detected in this study should be considered as a minimum estimate, and future studies may identify additional genes associated with the traits, for many microsatellite markers are now available. This study is a first step towards the precise localization of the resistance genes. Linkage studies of genetic factors affecting complex traits only provide approximate chromosomal regions within which relevant genes are located. Through a combination of high-resolution QTL mapping, molecular marker mapping, analysis of candidate genes, strategies of positional cloning and further characterization of chromosomal location, will eventually allow the identification of IPN disease resistance/susceptibility genes and improve our understanding of the genetic mechanisms that underlie the trait.

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