Genome Mapping and Genomics in Animals

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Genome Mapping and Genomics in Fishes and Aquatic Animals

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1 Salmonids

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1.1 Introduction

The modern salmon industry has had an explosive growth rate since the 1980s (Dunham et al. 2001), especially in Norway and the United Kingdom. Salmon and trout production requires cold waters with high oxygen content and low levels of pollutants, with a preference for protected coastal sectors far from big urban centers. So, even though cultured salmonid species originated in the Northern Hemisphere, close to 40% of the salmon produced in captivity today comes from the Southern Hemisphere, where they have been successfully introduced and cultivated.

1.2 History of Salmon Culture

1.2.1 Early History of Salmon Culture

The first attempts to artificially reproduce these species began in the middle of the fourteenth century in France. The French monk Dom Pinchon incubated trout eggs that he collected from the rivers where the fish bred. However, most authors attribute the development of artificial fertilization of trout and salmon eggs to Prussian Stephan Ludwing Jacobi (1711–1784) who published his experiences in 1763 in the "Hannoverschem magazinn" (Huet 1972). These findings were used in 1842 by Remy and Gehin in Vosges, France. They fertilized rainbow trout eggs and developed fry and fingerling production in ponds to restock streams in the Moselle River basin. In 1848, the French Academy sent a scientific commission to corroborate the findings, and eventually professor Coste, a specialist in embryology, obtained support of the French government for the construction of a hatchery in Huningue (Alsace) in 1851 (Blanco 1995). In the United States, the first hatchery was inaugurated in Maine in 1871. A second US hatchery was constructed in 1872 on the McCloud River, a tributary of the Sacramento River in California. The first artificial incubation of salmonids in Japan began with a lot of 17,000 eggs obtained from the Nakagawa River in 1876. Experiments in artificial incubation and release into different rivers of the main Japanese island, Honshu, continued until 1888 but without great success.

France, Germany, England, the United States, and Japan led the efforts to establish artificial reproduction of salmonids. In addition, these countries made great efforts to transplant salmonids to other places and latitudes. First was England, which distributed salmonids to its colonies in New Zealand and Australia. Although salmonids did not naturally exist in the Southern Hemisphere, the English government, with the support of the United States, sent eggs of Pacific salmon to be released into the southern English possessions. Their persistence finally resulted in the introduction of salmons in a wild state in New Zealand, now recognized as the first successful introduction of salmon in the Southern Hemisphere. Beginning in 1870, and continuing for more than 60 years, the United States led efforts to introduce eggs of Pacific salmon to different countries in Europe and the Southern Hemisphere, including Chile and New

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Zealand. These eggs were obtained from the McCloud River hatchery.

1.2.2 From 1890 to 1975

In 1890, Danish trout farmers began the development of trout culture in a system of earthen ponds, with freshwater flux through each fishpond. This system radically improved fish yield and reduced disease. This breakthrough led to the beginning of the commercial trout farming industry. Norway tried to implement the Danish system, but was not successful, due to the low temperatures of freshwaters in Norwegian winters. The seas around Norway are warmed by the influence of the Gulf Stream, which is an advantage for faster fish growth (Sedgwick 1988). In 1912, the Norwegians made the first attempts to cultivate rainbow trout in the sea. But it was not until the middle of the 1950s that the culture of salmon and rainbow trout in the sea began to grow. The industry became profitable and reached a production level near 500 MT in 1965 and 2,200 MT in 1974. The Norwegian system using floating cages to culture salmonids in sea water was adopted around the world (Willoughby 1999).

Table 1 Main salmonid producing countries in the world (Source: FAO global databases, Aquaculture production 2005a)

Country	Production 2005 (Metric Tons)
Norway	641,174
Chile	598,251
United Kingdom	142,613
Canada	103,164
Turkey	49,282
Denmark	37,001
United States of America	36,905
France	35,001
Iran (Islamic Rep. of)	34,760
Italy	30,564
Spain	26,132
Japan	24,461
Faeroe Islands	23,455
Germany	19,343
Australia	16,317
Poland	15,700
Ireland	15,378
China	14,507
Finland	13,713
Russian Federation	8,800
Other	64,057
Total World Production	1,886,521

1.2.3 From 1975 to the Present Time

With the decline of capture fisheries for wild salmon and trout in the Northern Hemisphere, aquaculture of salmonid species became increasingly important around the world. The development of culture systems that reproduced the complete life cycle in captivity, and the incorporation of artificial dry pellet diets in 1964 (Halver 1972), allowed the culture of salmonid species to become industrialized. Chile and Norway now produce 76% of the world's aquaculture salmon and trout. Other relevant producers are the UK, Canada, Turkey, Denmark and the US (Table 1). From 1980 to 1991, world production of farmed salmon grew from 7,149 MT to almost 325,563 MT, an increase of 4,600% (FAO 2005). At present, the world salmon and trout aquaculture production is more than two million MT per year, having tripled with respect to production at the beginning of the 1980s (Fig. 1). Farmed salmon, which is recognized in the market for its homogenous quality and constant supply, represents more than two thirds of the total. Atlantic salmon (*Salmo salar*) is the most important farmed species, followed by rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*) (Fig. 2).

1.3 Taxonomic Status and Distribution

Salmonids have a Holarctic distribution throughout Eurasia and North America (Scott and Crossman 1973) and are probably of freshwater origin, based on the evidence that all salmonids spawn in fresh water. Tchernavin (1939) suggested that ancestral salmonids were "small brightly colored fishes living in cool streams and lakes of the northern hemisphere. Using fresh water routes, they spread over a wide area. The environmental diversity of regions over which they spread favored the formation of numerous species" (Neave 1958). This suggests that a trout-like fish was ancestral to a salmon. Pacific salmon prob-

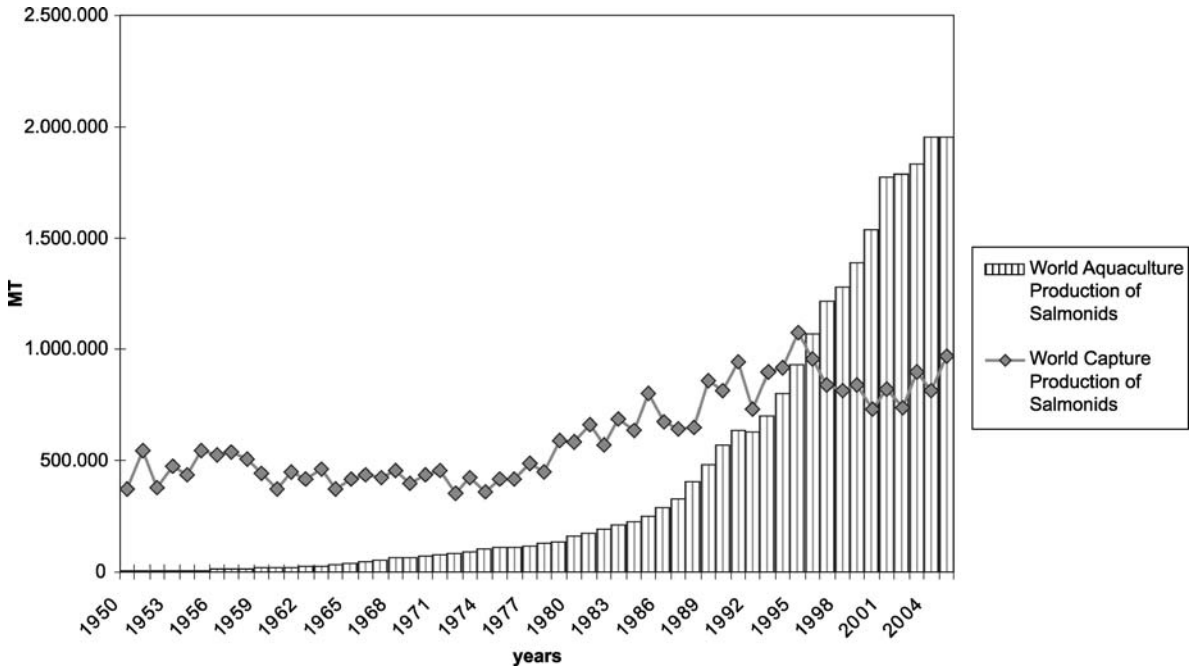


Fig. 1 A comparison of the world capture production and world aquaculture production of salmonids. (Source: FAO global databases 2005a, 2005b: Aquaculture production: Quantities 1950–2005 and Capture production 1950–2005)

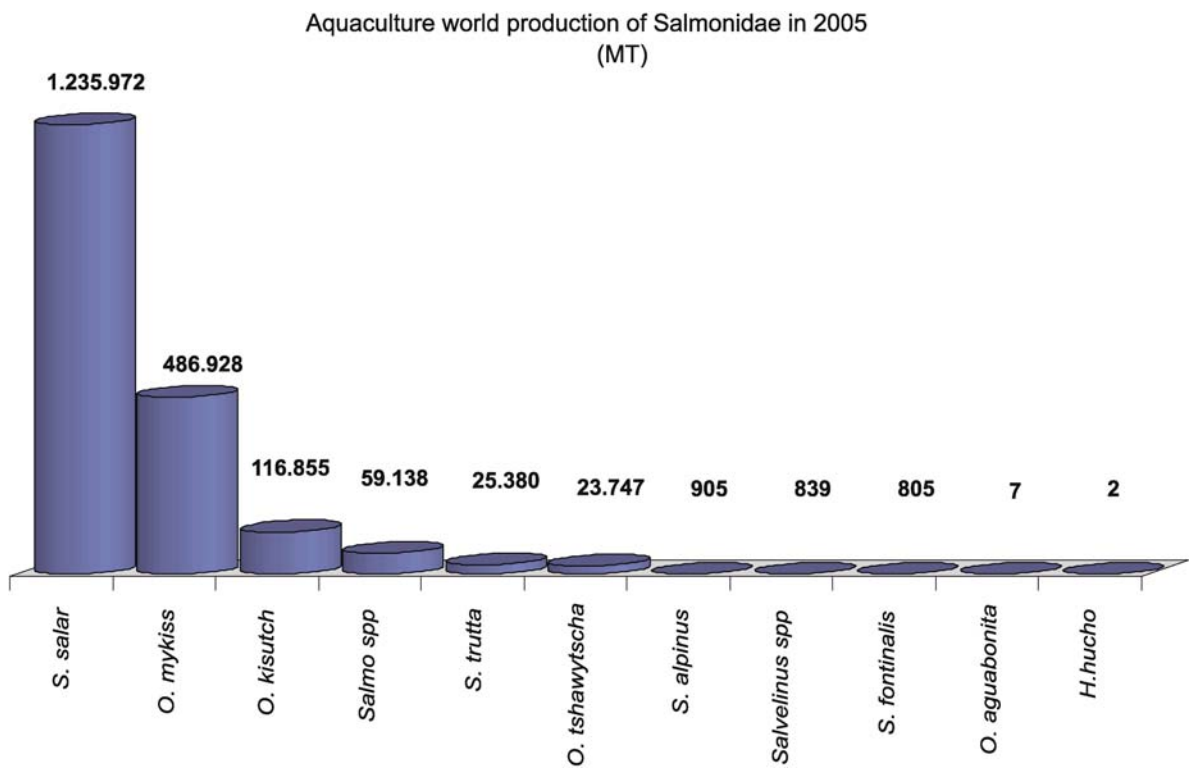


Fig. 2 The main species of farmed salmon and trout in the world in metric tons (MT). (Source: FAO global databases: Aquaculture production 2005a)

ably descend from a *Salmo* ancestor (Neave 1958). It is thought that separate Atlantic and Pacific salmon species arose after the closure of the Arctic link between the North Atlantic and the North Pacific oceans, around one million of years ago.

There are three subfamilies of salmonid fishes: *Coregoninae* (whitefishes and ciscoes), *Thymallinae* (graylings), and *Salmoninae* (lenoks, huchen, trouts, charrs, and salmons). The taxonomic classification of salmon is shown below:

Phylum: *Chordata*
Subphylum: *Vertebrata*
Superclass: *Osteichthyes*
Class: *Actinopterygii*
Order: *Salmoniformes*
Suborder: *Salmonoidea*
Family: *Salmonidae*
Subfamily: *Coregoninae*
Subfamily: *Thymallinae*
Subfamily: *Salmoninae*

Phylogenetic relationships among salmonid species are complex and sometimes difficult to resolve. Phillips and Oakley (1997) suggest the following reasons: (1) these fishes underwent a rapid adaptive radiation following tetraploidization around 50–100 million years ago (Allendorf and Thorgaard 1984), (2) hybridization and introgression have been common in this group (Utter and Allendorf 1994), and (3) recolonization of lakes released from glaciations within the past 10,000 years has resulted in assemblages of sympatric morphotypes or ecotypes with different degrees of reproductive isolation in Northern Hemisphere lakes.

The subfamily *Salmoninae* includes six genera of trout, salmon, and charr: *Brachymystax, Hucho, Salmothymus, Salvelinus, Salmo,* and *Oncorhynchus*. In this section, we describe the most important characteristics of cultivated salmonids in their natural environment.

1.3.1 Genus *Salmo*

This group doesn't necessarily die after spawning, and therefore repeated spawnings can be obtained from the same broodstock over several years. Good care of farmed broodstock can lead to high survival rates.

Atlantic Salmon (*S. salar*)

Natural Range: Atlantic salmon are found from north of the Hudson River to southern Greenland and Iceland on the western side of the Atlantic. On the eastern Atlantic, they are found from northern Portugal to the Kara Sea in Russia, including the Baltic Sea. **Life Characteristics:** Atlantic salmon are migrant, returning to freshwater during the 12 months preceding spawning, usually between October and December in the Northern Hemisphere. The female excavates a nest in silt-free gravel, usually in a tributary close to the salt water. On hatching, fry remain in the gravel using their remaining yolk sac. Fingerlings are carnivorous. After one or two years as freshwater "parr," the surviving fish undergo physiological and behavioral changes (smoltification) and migrate to begin the seawater phase of their life cycle. Salmon may return to their native rivers, after spending one winter and a few months at sea, but most return to freshwater after two or more winters at sea. Female salmon usually weigh between 3 and 6 kg at maturity and can produce around 1,200 and 2,000 eggs/kg of body weight.

Brown Trout (*S. trutta*)

Natural Range: Brown trout originate in the mountain waters of Central and Western Europe. Life Characteristics: The brown trout is a very polymorphic fish, with several subspecies that constitute simple geographic races or populations of different behavior. There are subspecies of brown trout that remain in rivers (*Salmo trutta fario*) and one less-abundant subspecies (*Salmo trutta trutta*) that migrates to the sea. Brown trout are a stenothermal coldwater fish that need freshwater and do not withstand high temperature variations. They spawn in the autumn or at the beginning of winter, leaving their normal habitat (seas, lakes, streams, or brooks) to swim upstream and spawn on gravel beds. They are an important sport fish around the world.

1.3.2 Genus *Oncorhynchus*

The main characteristic of this genus is that they generally die after spawning.

Pink Salmon (*O. gorbuscha*)

Common Names: pink, humpback (USA and Canada), karafutomaru (Japan), gorbuscha (Russia). **Native Range:** Pink salmon live in large rivers in North America and Asia. **Life Characteristics:** This is the most common Pacific salmon and also the most cold tolerant. Females produce around 2,000 relatively small eggs. Seaward migration takes place a few days after hatching. The young fish may spend 3–5 months in estuaries and coastal waters. They return to home rivers after 16–20 months, averaging 1.5 kg.

Chum Salmon (*O. keta*)

Common Names: chum, dog (USA and Canada), sake (Japan), and keta (Russia). **Native Range:** This species has a wide geographical distribution, from Oregon to the Arctic coast of Alaska in the eastern Pacific, and from Japan to the Arctic coast of Siberia in the western Pacific. **Life Characteristics:** Chum salmon are the second-most abundant Pacific salmon and also very cold tolerant. Spawning usually takes place in the lower reaches of rivers, within 150 km of sea. Eggs are laid between October and July on the Northern Hemisphere, depending on the latitude of the river. Females lay around 3,000 eggs. Fry migrate to sea within 1–3 months after emerging from the gravel. They spend up to 4 years in sea and may complete two or three ocean circuits.

Sockeye Salmon (*O. nerka*)

Common Names: sockeye (Canada and USA), red (Alaska), blueback (Columbia River), Benimasu (Japan), nerka (Russia). **Native Range:** Sockeye salmon are found from the Klamath River in California to the Yukon River in Alaska. In the western Pacific they range from the northern Bering Sea to the northern shore of the Okhosk Sea (Russia). **Life Characteristics:** The average spawning weight is 3.5 kg, and the female lays 3,500–4,000 small eggs. Fry can migrate to the sea, but usually migrate first to freshwater lakes where they grow slowly, feeding on planktonic crustaceans for 1–3 years. They eventually spend about 3 years at sea before returning to spawn.

Coho Salmon (*O. kisutch*)

Common Names: coho (Canada and Alaska), blueback (Canada), silver (USA), ginmaru (Japan), kizhuch (Russia). **Native Range:** This species is found in coastal streams from California to Norton Sound in Alaska, and from northern Hokkaido (Japan) to the Anadyr River (Russia). **Life Characteristics:** Similar to that of Atlantic salmon. In the wild, coho salmon stay in freshwater for 1–2 years. Most coho salmon spend 2 years at sea and grow fast during the second year. Spawning takes place in the autumn or early winter. Females produce between 1,000 and 2,000 eggs/kg of body weight.

Chinook Salmon (*O. tshawytscha*)

Common Names: king, (USA), spring (Canada), masunosuka (Japan), chavycha (Russia). **Native Range:** This species is found from the Ventura River in southern California to Point Hope in Alaska on the eastern rim and from Hokkaido to the Anadyr River (Russia) on the Asian side. **Life Characteristics:** This is the least abundant of all the Pacific salmon. Females lay about 3,000–12,000 eggs. Fingerlings migrate to sea after about 120 days and spend between 1 and 5 years in sea, mostly near shore or in inshore waters. The average weight at the end of marine life is about 10 kg.

Rainbow Trout (*O. mykiss*)

Common Names: steelhead (USA). **Native Range:** The natural range for rainbow trout extends from Alaska to Mexico and includes British Columbia, Washington, Oregon, California, Idaho, and Nevada. **Life Characteristics:** Trouts are found in cold, clear waters of creeks, rivers, and lakes (steelhead may be found in estuaries or oceans) with complex structures, such as riffles, submerged wood or boulders, and aquatic vegetation. Steelhead trout is the same species as rainbow trout, but steelhead is a migratory form, and rainbow is a landlocked form. During the course of artificial cultivation, this species has been widely crossbred. Because of crossbreeding, there is an impressive variety of types or strains of *O. mykiss*, where the main difference is the spawning season. Most populations spawn between January and May, but some strains have been obtained that start spawning in December or earlier in the Northern Hemisphere, and even, there are twice annually spawning strains with two spawns in one year (spring and autumn).

1.3.3
Genus *Salvelinus*

Arctic Charr (*S. alpinus*)
Common Names: omble chevalier (France), seesaibling (German), salvelino (Spanish), goylets (Russia), and arupusuiwana (Japan). **Native Range:** This species lives in the cold lakes of the northern slopes of Europe, Asia, and North America. **Life Characteristics:** Charr bear resemblance to salmon, but with a longer and more colorful body. They can weigh more than 4 kg and spawn in the autumn in the same way as other European salmonids.

Brook Trout (*S. fontinalis*)
Native Range: The brook trout is native to eastern and central North America, especially the Great Lakes and upper Mississippi River basin. **Life Characteristics:** Spawning generally occurs in the months of October and November. Females can produce between 100 and 400 eggs, depending upon the size and age of the individual. Depending upon water temperatures, the eggs hatch after 2–3 months of development. Brook trout take 2–3 years to mature and usually do not live longer than 6 years.

1.3.4
Genus *Coregonus*

Lake Whitefish (*C. clupeaformis*)
Native Range: Lake whitefish are widely distributed in North American freshwaters from the Atlantic coast across Canada and the northern United States to British Columbia, the Yukon Territory, and Alaska. **Life Characteristics:** This species inhabits lakes and large rivers, including brackish water. Whitefish spawn in early winter, and fry hatch the following spring. Some ecotypes of lake whitefish can reach a size of more than 9 kg and an age of more than 25 years. Other species of this genus occur across the northern land mass of North America, Asia, and Europe. Across this vast geographical range, this species exists in numerous forms or ecotypes, which has resulted in great nomenclatural confusion.

1.3.5
Genus *Hucho*

Danube Salmon (*H. hucho*)
Native Range: This is a continental salmon originally from the Danube basin. **Life Characteristics:** The Danube salmon live in fast-running water and migrate over short distances upstream at the spawning season. They can grow to more than 1 mt in length.

1.4
Genetic Improvement

Genetic improvement of aquaculture species did not begin until sophisticated varieties of *koi* carps were developed in Japan during the 1800s (Dunham et al. 2001). Fish improvement programs became more important in the 1900s with the rediscovery of Mendelian genetics and eventually grew into modern selection programs in the 1960s. Intensive genetic improvement programs began in 1970 coincident with the introduction of advanced methodologies and concepts of animal production. The continuing development of molecular genetics has led to the application of diverse biotechnologies and techniques to improve cultured fish stocks. Selection on quantitative characters of economic interest generally has produced much higher gains in aquatic species than those registered for domestic animals. For growth rate, for example, average genetic gains per generation of 15% for tilapia, of 14% for Atlantic salmon, 9–10% for coho salmon, and 14–20% for channel catfish have been reported (Bondari 1983; Gjerde 1986; Durham 1987; Hershberger et al. 1990; Gjerde and Korsvoll 1999; Rye and Eknath 1999; Neira et al. 2006a). These large gains can be explained by the enormous genetic variation and comparatively high fecundity of aquatic organisms, allowing high selection intensities (Gjedrem 1997). In contrast, for dairy cattle, the maximum expected genetic gain is about 2% per generation, with generation intervals of 6 years, while in pigs and birds, the genetic gains per generation can reach 2.5–4.5%. The generation interval for salmon in the Northern Hemisphere is about 3 years for Pacific salmon and rainbow trout and 4 years for Atlantic salmon. In Chile, a 2-year cycle has been achieved for coho salmon, while 3 and 4 years is the norm for rainbow trout and Atlantic

salmon, respectively. Shorter generation intervals are possible in Chile because of the availability of water with a higher temperature that allows the production of smolts within one year. This shorter generation interval is a significant advantage and translates into rates of growth gain of 4–5% per year in coho salmon.

1.4.1 Genetic Improvement Programs in Salmon Aquaculture

There are a limited number of experimental or commercial genetic improvement programs of aquatic species. In salmonids, there are results published on Atlantic salmon (Gjerde 1986; Gjerde and Korsvoll 1999), coho salmon (Hershberger et al. 1990; Gall and Neira 2004; Neira et al. 2006a), and rainbow trout (Kinkaid 1983; Gjerde 1986; Kause et al. 2003). Other breeding programs have been developed commercially in Scotland, Canada, Chile, the United States, the Netherlands, Iceland, Finland, Norway, and New Zealand.

Most of the established genetic improvement programs focus on salmonids. Data obtained from the First Fish Breeders Round Table meeting at the Akvaforsk Institute of Aquaculture Research, in Norway, indicated that there are ten genetic programs for Atlantic salmon (four in Norway, three in Chile, and one each in Canada, Ireland, Iceland, and Scotland), three for coho salmon (two in Chile and one in Canada), and five for rainbow trout (two in Norway and Chile and one in Finland) (T. Gjedrem unpublished).

Growth rate traditionally has been included as selection criteria in all salmonid programs, with very good results. Other characteristics of interest have been to improve carcass quality traits, such as flesh color, fat content, and texture of flesh in rainbow trout, Atlantic salmon, and coho salmon (Gjerde and Gjedrem 1984; Rye and Refstie 1995; Neira et al. 2004); early sexual maturity in Atlantic salmon (Gjerde 1986; Gjerde and Korsvoll 1999); skin color in rainbow trout (Kause et al. 2003); and early spawn date in coho salmon and rainbow trout (Siitonen and Gall 1989; Sadler et al. 1992; Su et al. 1999; Neira et al. 2006b). In general, there is great interest in the genetics of disease resistance, and promising results on infectious diseases such as furunculosis, infectious pancreatic necrosis virus (IPNV), and parasitic infection have been obtained for Atlantic salmon and rainbow trout (Gjedrem 1979; Gjedrem et al. 1991; Okamoto et al. 1993; Kolstad et al. 2005).

Despite these successes, there is still a lot of opportunity to improve the efficiency of the methods in salmonid selection programs. Several of these programs are applying methodologies of genetic evaluation designed for terrestrial animals, such as the BLUP system (best linear unbiased predictor) for breeding value prediction (Gall et al. 1993; Gall and Bakar 2002; Neira et al. 2006a). Also, even when a series of DNA markers and specific genes associated with productive traits have been published, fish breeding program have not incorporated marker-assisted selection (MAS) or gene-assisted selection (GAS) in their routine genetic evaluation systems.

Limitations of traditional selection are evident when the trait under selection is hard to measure, the individuals need to be sacrificed to measure the trait, the trait is sex-limited, or the heritability is low. In all these cases, the use of molecular markers linked or associated with the trait and accounting for a significant proportion of the variation (∼10% or more) are good candidates for MAS (Dekkers and Hospital 2002). The practical applicability of MAS or GAS involves an additional cost of genotyping at least 500 individuals every generation. Actually, the few molecular applications published in salmon breeding are limited to parental assignment or analysis (Norris et al. 2000) or to early sex identification using SSR (simple sequence repeat or microsatellite) markers in experimental populations (Reid et al. 2005).

1.5 Genetic Mapping in Salmonids

Genetic maps of salmonid species are still under development. The first blueprint of a salmonid map was published in 1990. It was a composite map for "all salmonid fishes," based on combined recombination rates obtained from different species and studies, with a small number of 54 polymorphic allozyme loci marking 22 chromosomal arms (May and Johnson 1990). There are four published genetic maps for rainbow trout, three partial maps for Atlantic salmon,

one for Arctic charr, one for lake whitefish and one for brown trout. A preliminary linkage map for pink salmon (*Oncorhynchus gorbuscha*) was presented at a genome conference in San Diego, California (Linder et al. 1999; Spruell et al. 1999), but these data have not been formally published in a map. So, currently there is published map information for four genera of salmonid fishes (*Oncorhynchus*, *Salmo, Salvelinus,* and *Coregonus*), and two of these (*Oncorhynchus* and *Salmo*) are commercially important. In addition to these more complete maps, there are also partial maps of specific linkage groups developed as a byproduct of quantitative trait loci (QTL) mapping for different traits in different species, principally rainbow trout.

It is interesting to note that linkage mapping in salmonid fishes is more similar to mapping in plants than mapping in other vertebrates. For example, family sizes are large, like in plants, and therefore only a small number of families are necessary for mapping. In addition, endogamic strains, isogenics, and doubled haploid (DH) lines can be constructed for mapping. As in plants, anonymous molecular markers, such as amplified fragment length polymorphism (AFLP, Vos et al. 1995), have been extensively used in fish (Moen et al. 2004a). In general, the standard nomenclature for AFLP markers is to use a code of six letters and a number representing the fragment size in base pairs, where the first three letters correspond to +3 nucleotides for the first restriction-enzyme-specific primer and the second three letters represent +3 nucleotides for the second restriction-enzyme-specific primer (Young et al. 1998). For SSR (simple sequence repeat) or microsatellite markers, a standard locus identification used in salmonid studies was proposed by Jackson et al. (1998). The designation begins with a three-letter acronym for the salmon species the SSR was isolated from (*Omy = O. mykiss*, *One = O. nerka*, *Ssa = S. salar*, *Str = S. trutta*, etc.) followed by a laboratory-specific designation for each marker and a suffix acronym describing the lab of the origin of the primers (Table 2). Duplicated loci amplified by one primer set are identified by an "/i" or "/ii"

Table 2 Source of the SSR primers used for mapping in salmonid fish*

Laboratory suffix acronym	Complete Name of Laboratory
ASC	Alaska Science Center, USA.
BFRO	Biotechnical Faculty, University of Ljubljana, Groblje Slovenia.
BML	Bodega Marine Laboratory, University of California, Davis USA.
CNRS	Centre National de la Recherche Scientifique, Chantal Poteaux France.
DIAS	Danish Institute for Animal Science, Tjele Denmark.
DU	Dalhousie University, Halifax, Nova Scotia Canada.
INRA	Institut National de la Recherche Agronomique, Jouy-en-Josas France.
LEE	National Fish Health Research Laboratory, Leetown, West Virginia USA.
Lav	Université Laval, Québec Canada.
OSL	Norwegian College of Veterinary Medicine, Oslo Norway.
NUIG	National University of Ireland, Galway Ireland.
NVH	National Veterinary Hospital, Norway.
OVIE	Universidad de Oviedo, Oviedo Spain.
SSBI	SeaStar Biotech Incorporated, Victoria, British Columbia Canada.
TUF	Tokyo University of Fisheries, Tokyo Japan.
UCH	Universidad de Chile, Santiago Chile.
UoG	University of Guelph, Ontario Canada.
UoS	University of Stirling, Scotland.
UoV	University of Victoria, British Columbia Canada.
UoP	University of Porto, Portugal.
UW	University of Washington, USA.

* Adapted from Sakamoto et al. (2000) and Gharbi (2001)

suffix within the marker name (Woram et al. 2004). In some cases, several SSR markers were published before this standard nomenclature was used, and for this, original names of the markers from the publications are adopted; for example, the designation SSOSL corresponds to a marker developed by Slettan et al. (1997) for Atlantic salmon.

1.5.1 First Generation Map

Rainbow Trout (*Oncorhynchus mykiss*)

The construction of genetic maps in salmonids is difficult due to a long generation interval in these fishes, normally 2–4 years depending on the species and culture conditions. However, in these fishes, it is possible to produce doubled haploid (DH) lines in a single generation using androgenesis (Pearson and Thorgaard 1985), and this approach was used to develop the first genetic map in rainbow trout. Today, this is the salmonid species with the best characterized genetic map and the most QTLs mapped (Table 6). The first genetic map was published by Young and colleagues in 1998. It included 332 AFLP markers and a few codominant markers (Table 3). Doubled haploid fishes were produced by the team of Gary Thorgaard at Washington State University (Young et al. 1996). The DH lines were produced from the F_1 hybrids of a cross between isogenic lines from Oregon State University (OSU) and Arlee (ARL) National Fish Hatchery (Montana, USA). Linkage groups identified in this hybrid used the acronym OA, due to the parental cross origin (OSU $\times$ ARL), and to distinguish them from linkage groups derived from crosses among other rainbow trout strains. AFLP markers were used instead of microsatellites because they took less time to develop and were cheaper to score. Two years later, the first map with predominantly codominant markers (191 SSR polymorphic loci; Sakamoto et al. 2000; Table 3) by the team of Roy Danzmann at the University of Guelph (Ontario, Canada) was published. The strategy used for mapping was to use the three backcross families initially used for QTL mapping for upper temperature tolerance (UTT) by Jakson et al. (1998). These families were obtained from crosses between fish with high and low tolerance to high temperature, from trout strains produced at Maple (Ontario, Canada) by the Ontario Ministry of Natural Resources. Finally, both groups produced a consolidated map with a length of 4,590 cM (Fig. 3), principally combining markers used in previous studies (Nichols et al. 2003a). This updated map was developed using the DH rainbow trout OA and AFLP used by Young et al. (1996), adding codominant markers (SSR) from Sakamoto et al. (2000) and some allozymes from May and Johnson (1990). Other interesting loci anchored in this map are 29 type I polymorphisms detected using single-strand conformation polymorphism (SSCP), indel, restriction fragment length polymorphism (RFLP), or single nucleotide polymorphism (SNP) screening. These loci were mapped to 20 different linkage groups. The 33 genes and allozyme loci mapped are listed in Tables 4 and 5, and from this, *SOD*, *bGLUA** and *CBR1* were mapped to the sex chromosome of this species. The allozymic locus *bGLUA**, formerly *HEX*, had been previously mapped to the sex chromosome by Allendorf et al. (1994) in this same species.

A fourth map for rainbow trout has been published recently by a French team lead by René Guyomard. The primary objective was to cover the overall 52 chromosome arms from the base karyotype of this species (Guyomard et al. 2006). The mapping panel consisted of two DH families, and the map includes 901 SSR loci, with an important fraction (389 markers) developed from expressed sequence tag (EST) libraries, and two SNP markers (Table 3). Also in this work, 369 transcript EST sequences of rainbow trout together with blastn were used to identify 49 ESTs syntenic with ESTs from zebrafish (*Danio rerio*) in 20 different linkage groups of this model fish.

Atlantic Salmon (*Salmo salar*)

For this species, there were three draft maps published in 2004 by two different laboratories. One map, developed principally by a team from Scotland, is based on 64 codominant loci with 50 SSRs effectively anchored in 15 linkage groups (Table 3), with 11 SSRs and 3 allozyme loci remaining unlinked (Gilbey et al. 2004). The approach used for mapping in this study was a backcross design using two families obtained from a cross between an F_1 hybrid male and a female from River Don (Anberdeenshire, Scotland). The parents of the F_1 male were a non-anadromous male Atlantic salmon obtained from Bristol Cove River (Newfoundland, Canada) and a female salmon from River Don (Scotland). An interesting finding of this study was the identification in silico (using the BlastX algorithm) that flanking sequences of marker *SS11* (GenBank AJ133370) anchored in linkage group 10 showed 91%

Table 3 Comparative information on salmonid genetics maps available until 2006

Reference	Map Size[1] (cM)	Linkage Groups	Number and type of molecular markers anchored in the map								*SEX* mapped to Linkage group[5]
			SSR	AFLP	RAPD	VNTR[2]	SINE	Allozymes	Genes[3]	Other[4]	
Oncorhynchus mykiss											
Young et al. 1988	2627.5	31	2	332	5	96	40				I
Sakamoto et al. 2000	1134	29	191		3			7	7		18
Nichols et al. 2003	4590	40	226	973	5	84	38	4	29		OA-I (18)
Guyomard et al. 2006	2750	31	901						2		RT-1
Oncorhynchus gorbuscha											
Linder et al. 1999	Not informed	55	28	393	35					164	Not mapped
Salmo salar											
Gilbey et al. 2004	630.5	15	50								Group 1
Moen et al. 2004	103–901	31–33	54	473							Not mapped
Salmo trutta											
Gharbi et al. 2006	346.4–912.5	35	288					13	4		BT28
Salvelinus alpinus											
Woram et al. 2004	390–992	46	184	129					5		Not mapped
Coregonus clupeaformis											
Rogers et al. 2001	1462	29		119							Not mapped

[1] When two values are reported, first value corresponds to male map and second value to female map.

[2] Includes VNTR detected as multilocus by probe hybridization and minisatellites amplified by PCR.

[3] Genes identified using SNP, SSCP, or SSR located in its flanking regions or introns.

[4] Includes Paired Interspersed Nuclear Elements (PINEs) and Comparative Anchor Tagged Sequences (CATS).

[5] Name of the linkage group that contained SEX locus obtained from original references.

Table 4 Allozyme loci mapped in different salmonid genomes

Locus	Allozyme name	IUPAC Enzyme Commission	Location in Linkage Groups on map of Rainbow Trout[1]	Brown Trout[2]
*bGLUA**	b-N-acetylhexosaminidase	3.2.1.52	OA-I	
*sSOD-1**	Superoxide dismutase 1	1.15.1.1	OA-I	
*sMDH-B1,2**	Malato dehydrogenase 1	1.1.1.37	OA-VI	BT-23
*sLDH-B2**	L-lactate dehydrogenase 2	1.1.1.27	OA-XV	
*sIDHP-3**	Isocitrate dehydrogenase 3	1.1.1.42	OA-XVI	
*mMEP-2**	Malic enzyme	1.1.1.40	OA-XVI	
*PGK-2**	Phosphoglycerate kinase	2.7.2.3	OA-XX	
*mIDHP-2**	Isocitrate dehydrogenase 2	1.1.1.42	OA-XXVII	BT-31
*sG3PDH-1**	Glyceraldehyde-3-phosphate dehydrogenase 1	1.1.1.8	OA-XXIX	BT-1
*sAAT-1,2**	Aspartate aminotrasferase	2.6.1.1		BT-1
*sMDH-A2**	Malato dehydrogenase 2	1.1.1.37		BT-8
*sIDHP-1**	Isocitrate dehydrogenase 1	1.1.1.42		BT-9
*PGDH**	Phosphogluconate dehydrogenase	1.1.1.44		BT-28
*EST-1**	Esterase 1	3.1.1.-		BT-7
*FH-1**	Fumarate hydratase 1	4.2.1.2		BT-23
*FH-2**	Fumarate hydratase 2	4.2.1.2		BT-35
*MPI-2**	Mannose-6-phosphate isomerase	5.3.1.8		BT-7
*TF-1**	Transferrine	Not enzymatic protein		BT-16

[1] Combined data from Sakamoto et al. (2000) and Nichols et al. (2003)

[2] Data from Gharbi (2001)

of sequence similarity with Aralar 1 protein (calcium-binding mitochondrial carrier protein) from several species, including *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus,* and *Rattus norvegicus.*

A second team of Norwegian researchers developed two more complete sex-specific maps using both SSR and AFLP markers (Table 3). The female map included 33 linkage groups and 230 markers, while the male map included 31 linkage groups with 251 markers. There were 22 linkage groups in common between sexes (Moen et al. 2004a). These maps were developed using one parental half-sib family from an AquaGen commercial breeding population, class year 1999. This family was subdivided into two groups of 69 and 67 offspring, and not all markers were genotyped in all 136 offspring. This study detected the greatest difference of recombination frequencies between sexes reported for any vertebrate: 8.26 in favor of female. This difference was translated in a difference in map size between male and female, 103 cM vs. 901 cM, respectively.

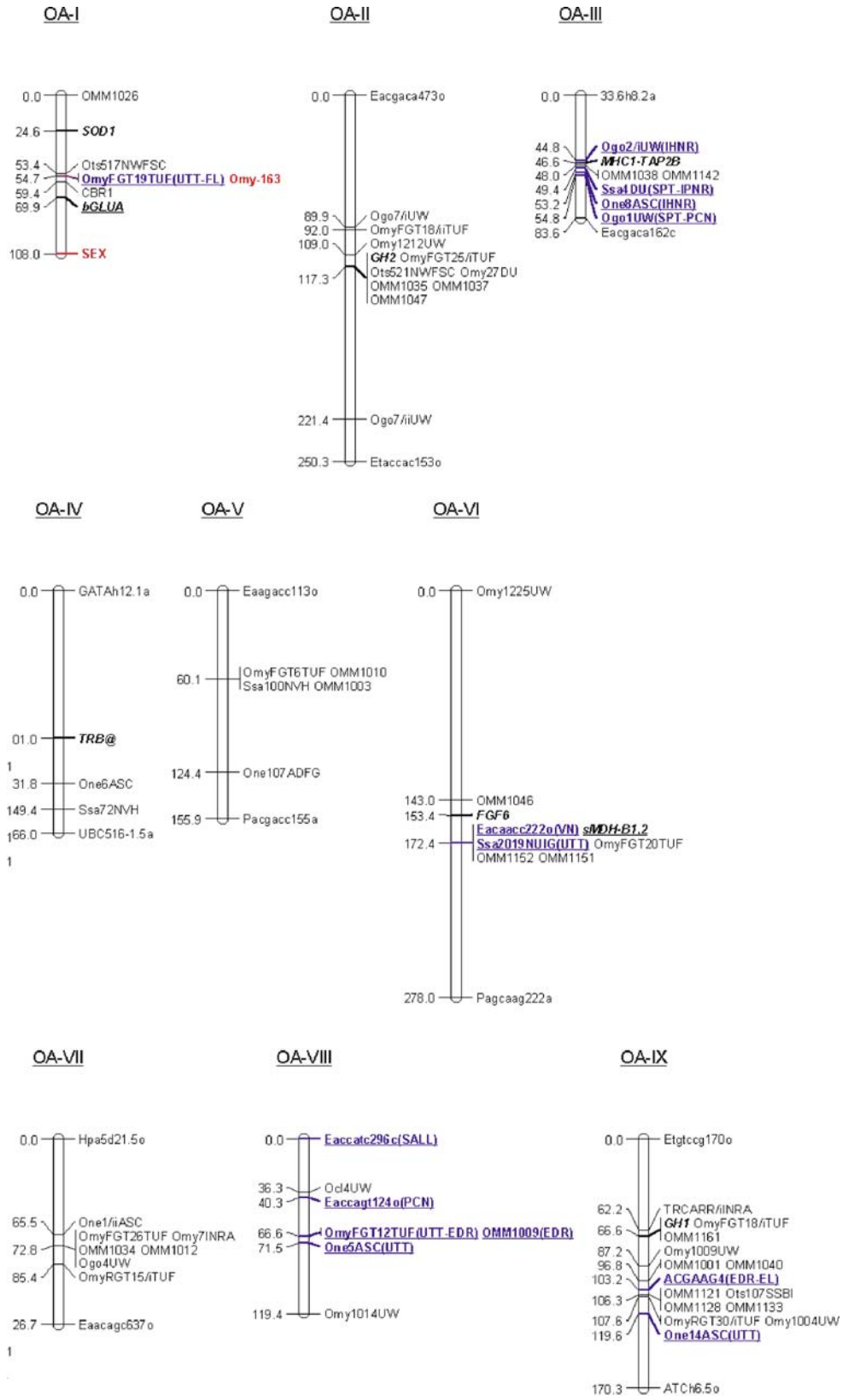
An examination of Atlantic salmon maps shows some shared markers that could be used to develop a consolidated map for this species. For example, Moen et al. (2004a) mapped the locus *Ssa197* in the linkage group 1, and Gilbey at al. (2004) mapped this marker in the linkage group 12. Similar patterns occurred for the locus *SSOSL438* that mapped in the linkage groups 9 and 8 in the respective maps (Fig. 4). In both cases, the maps have different markers on the homologous linkage groups (Fig. 4). It is, therefore, possible to combine the marker information and increase the map saturation. These maps for Atlantic salmon are two good approximations to produce a complete map for the species; however, more research will be necessary to produce a complete map. It is possible that the true map length should be similar to the rainbow trout map, because these species share a common ancestor and similar haploid chromosome number (Table 9). A consolidated map will be possible in a near future due to the cGRASP initiative to obtain the complete sequence of the Atlantic salmon genome and to develop more codominant markers (SNPs and SSRs) for mapping in this species (Davison et al. unpublished).

Table 5 Genes mapped in different salmonid genomes

Locus	Gene name	Location in Linkage Groups on map of		
		Rainbow Trout[1]	Brown Trout[2]	Arctic Char[3]
SOD1	Cu/Zn-superoxide dismutase 1	OA-I		
CBR1	Carbonyl reductase	OA-I		
GH2	Growth hormone 2	OA-II		AC-20
MHC1-TAP2B	MHC I transport and activating protein 2B	OA-II		
TRB@	T-cell receptor beta	OA-IV		
FGF6	Fibroblast growth factor 6	OA-VI		
WT1-b	Wilms tumor – type 1a	OA-VI		
GH1	Growth hormone 1	OA-IX		
hsc70	71 kD heat shock cognate protein	OA-IX		
CYP1A2	Cytocrome P50 subfamily I polipeptide 2	OA-X		
TRCARRi/ii	Trout red cell arrestin	OA-XI & OA-XX	BT-27 & BT-30	AC-20
TCL-1	C-type lectin	OA-XIV		
RB1	Retinoblastoma	OA-XVI		
5′ETS	External transcribed spacer for 18/S28S rDNA	OA-XVII		
GTH2B	Gonadotropin hormone II β subunit	OA-XVII		
CTSD	Cathepsin D	OA-XVIII		
MYC	C-myc proto-oncogene	OA-XIX		
VIM	Vimentin	OA-XIX		
TP53	p53 proto-oncogene	OA-XX		
TCL-2	ITIM-bearing C-type lectin	OA-XXI		
ID1	Inhibitor of DNA binding/differentiation 1	OA-XXII		
PRL	Prolactin	OA-XXIV		
HRAS	Ras-1 oncogene	OA-XXVII		
MT1B	Metallothionein B	OA-XXVII		
RAG1	Recombination activation gene-3′ UTR	OA-XXVII		
WT1-a	Wilms tumour – type 1b	OA-XXVII		
NRAMP-α	Natural resistance associated macrophage protein α	OA-XXIX		
UQCRCI	Ubiquinol-cytochrome c reductase core I protein	OA-XXIX		
ZNFN1A1	Zinc finger protein, subfactor 1 A	OA-XXX		
SL	Somatolactin	OA-XI	BT-14	AC-22
PPAR-γ	Peroxisomal proliferator activator receptor			AC-7
SOX-9	SRY-related high mobility group – box protein 9			AC-20
MYODi/ii	Myogenic differentiation antigen 1		BT-13 & BT-21	
TFi/ii	Transferrin		BT-16	

[1]Combined data from Sakamoto et al. (2000) and Nichols et al. (2003)
[2]Data from Gharbi (2001)
[3]Data from Woram et al. (2004)

Fig. 3 Consolidated map of rainbow trout (OSU × ARL). Only codominant markers are represented along with the most distal SSR or AFLP markers on every linkage group. *Markers* linked to QTLs are indicated in *blue* and *underlined* on corresponding linkage groups. *UUT* upper temperature tolerance, *SP* spawning date, *EDR* embryonic development rate, *EL* embryonic length, *FL* fork length, *BM* body mass, *PCN* pyloric caeca number, *VN* vertebrate number, *SALL* scales above lateral line, *AFR* anal fin ray counts, *IPNR* resistance to IPNV, *IHNR* resistance to IHNR, *NK* NK-like cytotoxicity. (Modified from Nichols et al. 2003; QTLs references in Table 6; Graphics made using MapChart, Voorrips et al. 2002)


OA-I
0.0 OMM1026
24.6 SOD1
53.4 Ots517NWFSC
54.7 OmyFGT19TUF(UTT-FL) Omy-163
59.4 CBR1
69.9 bGLUA
108.0 SEX
OA-II
0.0 Eacgaca473o
89.9 Ogo7/iUW
92.0 OmyFGT18/iTUF
109.0 Omy1212UW
GH2 OmyFGT25/iTUF
117.3 Ots521NWFSC Omy27DU
OMM1035 OMM1037
OMM1047
221.4 Ogo7/iiUW
250.3 Etaccac153o
OA-III
0.0 33.6h8.2a
44.8 Ogo2/iUW(IHNR)
46.6 MHC1-TAP2B
48.0 OMM1038 OMM1142
49.4 Ssa4DU(SPT-IPNR)
53.2 One8ASC(IHNR)
54.8 Ogo1UW(SPT-PCN)
83.6 Eacgaca162c
OA-IV
0.0 GATAh12.1a
01.0 TRB@
1
31.8 One6ASC
149.4 Ssa72NVH
166.0 UBC516-1.5a
1
OA-V
0.0 Eaagacc113o
60.1 OmyFGT6TUF OMM1010
Ssa100NVH OMM1003
124.4 One107ADFG
155.9 Pacgacc155a
OA-VI
0.0 Omy1225UW
143.0 OMM1046
153.4 FGF6
Eacaacc222o(VN) sMDH-B1,2
172.4 Ssa2019NUIG(UTT) OmyFGT20TUF
OMM1152 OMM1151
278.0 Pagcaag222a
OA-VII
0.0 Hpa5d21.5o
65.5 One1/iiASC
OmyFGT26TUF Omy7INRA
72.8 OMM1034 OMM1012
Ogo4UW
85.4 OmyRGT15/iTUF
26.7 Eaacagc637o
1
OA-VIII
0.0 Eaccatc296c(SALL)
36.3 Ocl4UW
40.3 Eaccagt124o(PCN)
66.6 OmyFGT12TUF(UTT-EDR) OMM1009(EDR)
71.5 One5ASC(UTT)
119.4 Omy1014UW
OA-IX
0.0 Etgtccg170o
62.2 TRCARR/iINRA
66.6 GH1 OmyFGT18/iTUF
OMM1161
87.2 Omy1009UW
96.8 OMM1001 OMM1040
103.2 ACGAAG4(EDR-EL)
106.3 OMM1121 Ots107SSBI
OMM1128 OMM1133
107.6 OmyRGT30/iTUF Omy1004UW
119.6 One14ASC(UTT)
170.3 ATCh6.5o

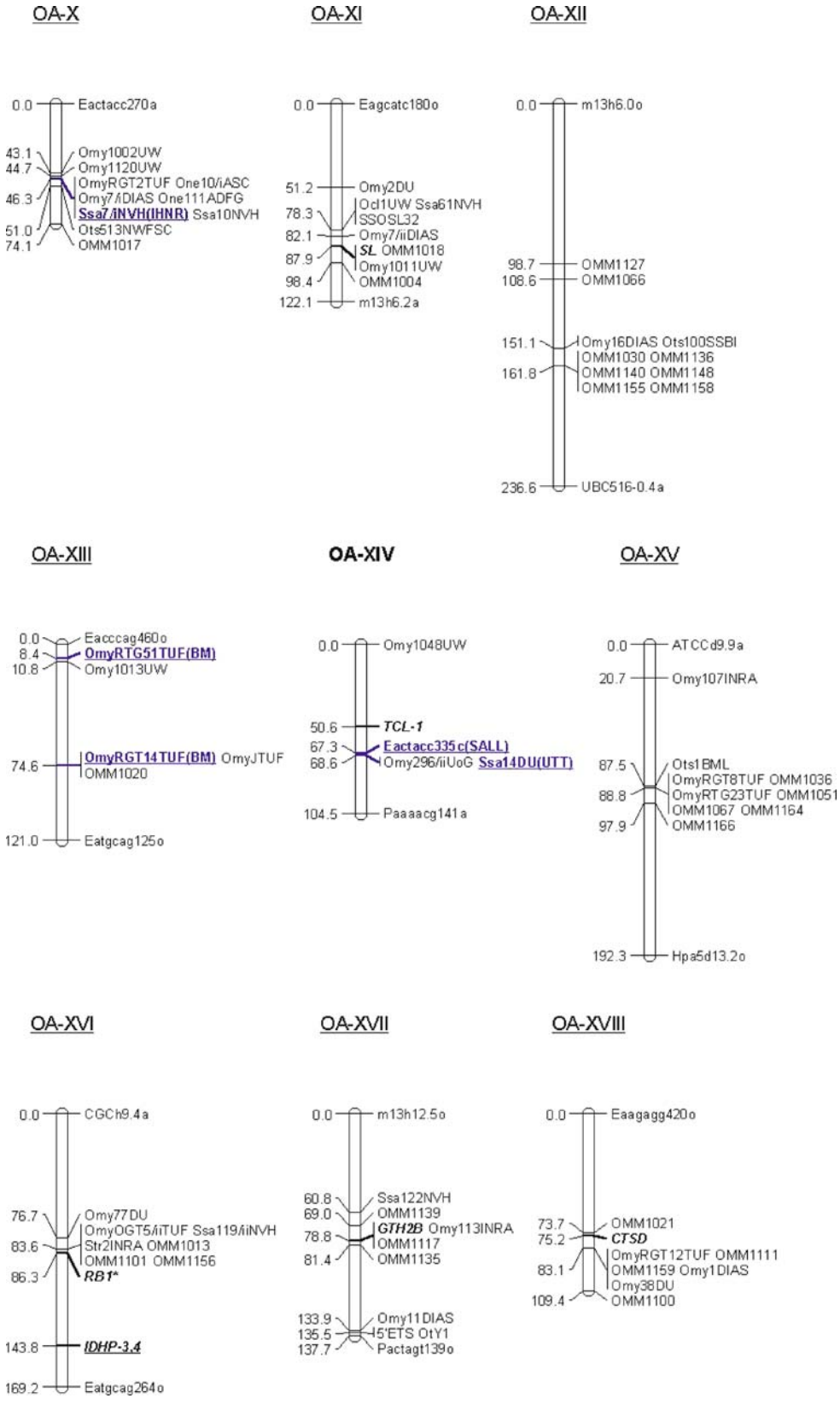


Fig. 3 (continued)

Fig. 3 (continued)

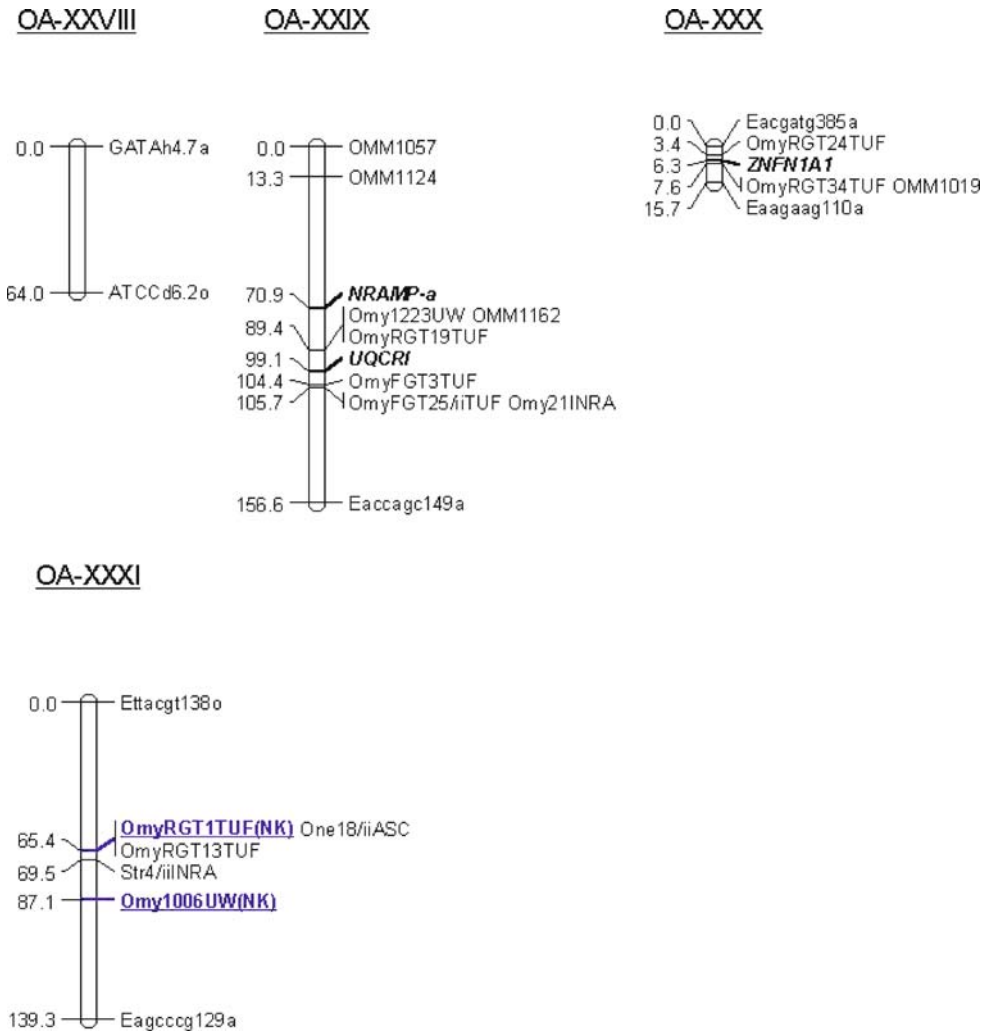


Fig. 3 (continued)

Arctic Charr (*Salvelinus alpinus*)

The first detailed linkage map for this species was published by a team from the University of Guelph, Ontario, Canada (Woram et al. 2004). This map has 46 linkage groups with 322 markers including SSRs, AFLPs, and SNPs (Table 3). It was developed using two commercial but not genetically improved strains of Arctic char, Nauyuk and Fraser. These strains were collected in Canada from Nauyuk Lake (Nunavut) in 1978 and the Fraser River (Labrador) from 1980 to 1984, from a reduced number of wild parents (Woram et al. 2004). For map construction, two backcross families were obtained from crossing a Fraser male with an F_1 hybrid (Fraser × Nauyuk) female and the reciprocal cross, an F_1 hybrid (Fraser × Nauyuk) male with Fraser female. From each family, 48 progeny were genotyped for mapping.

In this map, it was possible to anchor genes (type I markers) using SNP polymorphisms (detected by heteroduplex analysis) and SSR markers located within intron regions of trout red cell arresting (*TRCARR*), somatolactin (*SL*), growth hormone 2 (*GH2*), and peroxisome proliferator-activated receptor (*PPAR-γ*) genes. *SOX9* was mapped by a cluster of markers located in the introns 1 and 2 of this gene (Table 5). Three of these genes (*SOX9*, *TRCArr,* and *GH2*) were mapped to the same linkage group AC-20, and it is interesting that these same genes are located in a single chromosome in the mouse, suggesting conserved orthologous synteny (Woram et al. 2004).

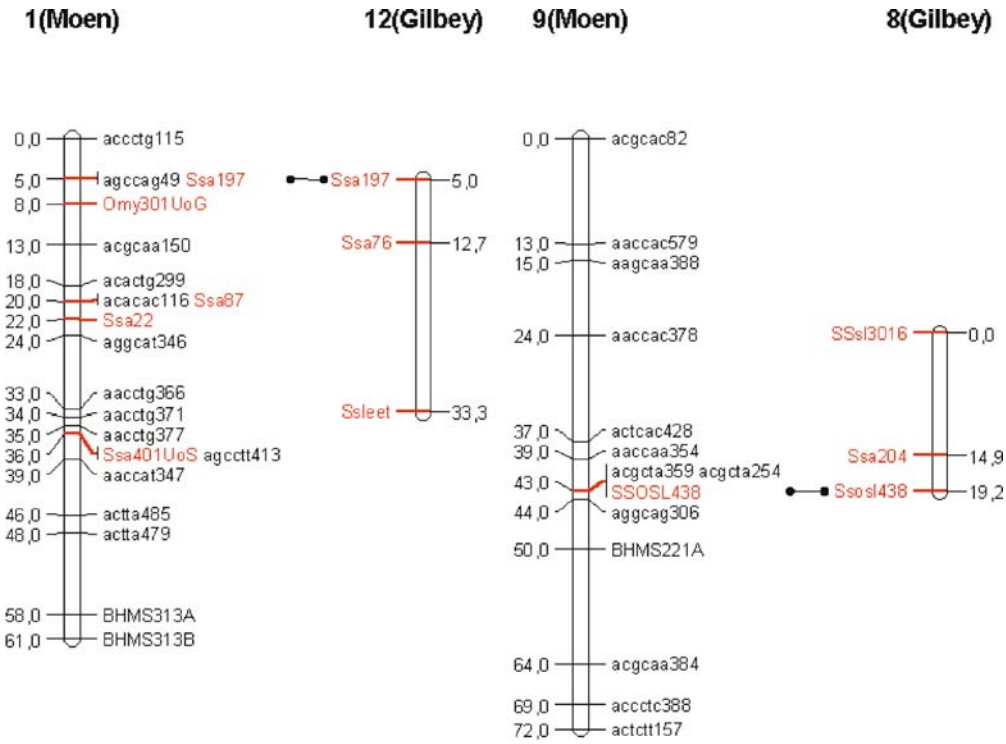


Fig. 4 Comparison of homologous linkage groups from Atlantic salmon maps from Moen et al. (2004a) and Gilbey et al. (2004) with the same loci mapped (*Ssa197* and *SSOSL438*). (Graphics made using MapChart, Voorrips et al. 2002)

Brown Trout (*Salmo trutta*)

The partial map for this species was developed by Karim Gharbi in his Ph.D. thesis work at the Institut National Agronomique Paris-Grignon, France (Gharbi 2001; Gharbi et al. 2005, 2006). This map has 302 codominant SSR and allozyme markers in 37 linkage groups (Table 3). The four mapping families used to develop this map were raised by a classical backcross design, where parental fish from different phylogenetic groups of brown trout (i.e., Atlantic, Mediterranean, or *marmoratus*) were used to produce an F_1 male that was mated with females from pure Atlantic origin. Finally, 45 or 48 progeny from each family (186 progenies in all) were genotyped for different numbers of different types of markers (fluctuating between 42 and 279) in the mapping experiment. Sex-specific maps were estimated to cover 346.4 and 912.5 cM of the male and female genomes, respectively.

Lake Whitefish (*Coregonus clupeaformis*)

For this salmonid species, there is a partial genetic map developed based on 119 AFLP markers by Rogers et al. (2001) as part of research on the genetic architecture and population divergence in this fish due to ecological shifts which occurred as a consequence of Pleistocene glaciation. Five divergent lineages in the *Coregonus* genus arose during this glaciation, and at present there is a secondary contact zone where different ecotypes coinhabit lakes located from southeastern Québec (Canada) to northern Maine (USA). The mapping effort in *Coregonus* has been made by crossing two ecotypes, dwarf (limnetic) and normal (benthic), where the main differences are the age at sexual maturity and adult size. Dwarf fishes generally mature at the first year of age and seldom exceed a body size of 20 cm and weight of 100 g, while normal fishes mature between two and five years of age and frequently exceed 40 cm and 1,000 g (Rogers and Bernatchez 2005).

The map for this species was developed using a classical backcross design, where the parental fishes were dwarf whitefish from Témiscouta Lake (Québec, Canada) and normal whitefish from Aylmer Lake (Northwest Territories, Canada) collected in

1996. From these stocks, an F_1 hybrid progeny dwarf/normal was produced using a complete diallele cross with 20 males and 20 females (Lu and Bernatchez 1998). The mapping family consisted of 60 progenies derived from a cross between an F_1 hybrid female with a pure dwarf male, and it produced a map of 1,462 cM and 29 sex-specific linkage groups (Table 3), where the average size of linkage groups was 50.5 cM and the number of markers per linkage groups ranged from 2 to 17. Future mapping efforts in this species should use codominant markers to increase the coverage of the genome (Rogers et al. 2001).

Pink Salmon (*Oncorhynchus gorbuscha*)

The construction of the genetic map in this species was made using 620 molecular markers of different types: AFLP, paired interspersed nuclear elements (PINE), randomly amplified polymorphic DNA (RAPD), and comparative anchor-tagged sequences (CATS) (Table 3). Preliminary results of mapping in this species were presented at the Plant & Animal Genome Conference in San Diego (California, USA) by Linder et al. (1999) and Spruell et al. (1999). However, a partial or complete map has not been formally published. In this preliminary map, 563 markers were anchored to 55 linkage groups, more than twice the haploid number of this species. A set of 310 loci were mapped in relation to their centromeres using gynogenetic progeny.

1.5.2 Second Generation Map

The construction of an integrated map with high resolution and saturation is a pending task in salmonids. However, the integration of maps will be a natural consequence of the gene mapping efforts in all salmonid species, principally because these fish evolved from a common ancestor 25–100 million years ago (Allendorf and Thorgaard 1984). There is mounting evidence from QTL mapping of conserved synteny of chromosomal regions, in spite of the differences in chromosome number and chromosome arm heterogeneity between and within species. For example, three QTLs for body weight have been detected in homologous chromosomal regions of rainbow trout and Arctic charr (Reid et al. 2005), and in our laboratory, we have suggestive evidence that three SSR loci linked to QTL for spawning date in rainbow trout to show allelic heterogeneity between coho salmons (*O. kisutch*) selected for early and late spawning date, as indicative of association with spawning time QTL (Diaz et al. unpublished). The mapping of the sex-determining locus also shows some evidence of SSR loci conserved between the Y chromosomes of salmonids (Woram et al. 2003), although the linkage of markers to the Y chromosome in rainbow trout has been highly variable and strain specific (Iturra et al. 1998), indicating that the differentiation of sex chromosomes is a more complex phenomenon in salmonid fishes. At the nucleotide sequence level, in general, it is possible to amplify SSR loci in different species of salmon, indicating the conservation of priming sites in these genomes. This feature is favorable to identification of homologous chromosomes between salmonid species. For example, in the Sakamoto et al. (2000) map for rainbow trout, SSR markers obtained from eight other species of salmon were used with cross-amplification with *Oncorhynchus mykiss*: cutthroat trout (*O. clarki*), pink salmon (*O. gorbuscha*), chum salmon (*O. keta*), sockeye salmon (*O. nerka*), chinook salmon (*O. tshawytscha*), marble trout (*Salmo marmoratus*), Atlantic salmon (*S. salar*), and brown trout (*S. trutta*). This phenomenon of cross-amplification has made it possible to map SSR loci from different species in all salmonid genetic maps that have incorporated these types of markers (Table 3). Additionally, Rexroad et al. (2005) showed cross-species amplification in all 89 polymorphic SSR markers developed from the rainbow trout genome. This cross-amplification included species of *Salvelinus*: Arctic charr (*S. alpinus*) and brook trout (*S. fontinalis*).

Until 2005, the main type of markers used for mapping in salmon were anonymous type II markers. However, based on EST sequences principally obtained from a normalized cDNA library (NCCCWA 1RT) developed in the USDA National Center for Cool and Cold Water Aquaculture in West Virginia, it has been possible to develop and map new SSR markers in rainbow trout (Rexroad et al. 2005). These SSR markers are particularly useful as type I makers for comparative mapping. It is noteworthy that a fraction of these markers could be mapped to chromosomes of freshwater puffer fish (*Tetraodon nigroviridis*), mice, and humans. Other ESTs containing

SSRs were also identified in the genome databases of fugu (*Takifugu rubripes*) and zebrafish (*Danio rerio*) but without any mapping information (Rexroad et al. 2005). This and other cDNA libraries are being used to develop SSR and SNP type I markers for mapping in rainbow trout and Atlantic salmon (Davison et al. unpublished).

Allozyme markers have been mapped in only two species of salmon (rainbow trout and brown trout, Table 4), and polymorphisms in type I markers have been mapped in only three salmon species (rainbow trout, brown trout, and Artic charr, Table 5). For both types of markers, only six loci have been mapped in more than one species, and only trout red cell arrestin (*TRCARR*) and somatolactin (*SL*) genes have been mapped in three species of salmons (Table 5). This lack of information about type I markers has so far prevented the development of integrated maps in salmonids.

1.5.3 Mapping Consequences of Ancestral Tetraploidy of Salmonids

Another phenomenon that complicates the map construction in salmonid fishes is that fishes of the *Salmonidae* family descend from a presumed autotetraploid ancestor (Allendorf and Thorgaard 1984), and from this event, the genomes of salmonids have been returning to diploid state. This event of tetraploidization and the restoration of diploidy have consequences in the map construction, because it involves the evolution from four homologous chromosomes in the ancestral fish to two new pairs of homologous and homeologous chromosomes. It is believed that this process of diploidization is not complete in males, because some chromosomes in the male fish still pair with their homeologous counterparts by multivalent formation during meiosis. From segregation analysis of duplicate allozymic locus *MDH-B1,2** in more than 8,000 rainbow trout progeny, it was evident that females always produce diploid gametes. Males, on the other hand, showed variable levels of tetrasomic segregation with a minimum of 25% (Allendorf and Danzmann 1997). The separation of multivalents and recombination may produce "pseudolinkage" in males. That is to say, it produces an aberrant pattern of nonrandom assortment, where an excess of nonparental type of progeny is observed when the phase of alleles is known (Wright et al. 1983). As a consequence, males have a lower recombination rate in comparison with females, as has been seen in all salmonid genetic recombination maps. Differences in recombination rates between sexes are well documented and have been explained by lower frequencies of crossing over in the heterogametic sex. Apparently, in salmonid fishes, this is increased by the formation of multivalents only in males. Evidence from rainbow and brown trout shows that in males, large chromosomal regions proximal to the centromere have recombination frequencies close to zero, in comparison with telomeric regions that exhibit higher recombination rates (Sakamoto et al. 2000; Gharbi et al. 2006). In rainbow trout, the recombination rate is 10:1 in favor of females close to the centromere and 0.14:1 in favor of males close to the telomere (Sakamoto et al. 2000). In Atlantic salmon, the difference in the recombination rate between the sexes is 3.92 (Gilbey et al. 2004) and 8.26 (Moen et al. 2004a) in favor of females. In the brown trout map, this difference is 6.4 in favor of females (Gharbi et al. 2006), while in the Arctic charr map, it is only 1.69, the lowest published in a salmon (Woram et al. 2004).

As a consequence of residual tetraploidy, some salmonid loci exhibit a tetrasomic inheritance pattern. Evidence of this phenomenon involving the allozyme loci has been reported in several studies since Bailey et al. (1970). For example, in rainbow trout, cytosolic isocitrate dehydrogenase loci (*sIDHP-3,4**, formerly *Idh-3* and *Idh-4*) share alleles. A similar phenomenon occurs for hepatic malate dehydrogenase (MDH) loci (*sMDH-1,2**, formerly *Mdh-1* and *Mdh-2*), and for the skeletal muscle form of MDH (*sMDH-3,4**, formerly *Mdh-3* and *Mdh-4*). These results are indicative of tetrasomic inheritance (Thorgaard et al. 1983). At the DNA marker level, duplicate loci have been detected for *Ogo2UW* SSR that detected loci in linkage groups OA-III and OA-XXV of a rainbow trout genetic map (Sakamoto et al. 2000). We also have evidence that this locus shows tetrasomic inheritance in coho salmon (C. Araneda unpublished data). A similar pattern was detected for SSR loci *OmyFGT-1TUF* during the construction of a rainbow trout map by Young et al. (1998). The phenomenon is rather general because O'Malley et al. (2002) detected homeolo-

gous regions among thirteen pairs of linkage groups, with 26 SSR loci being duplicated. In the brown trout map, 23 duplicated chromosomal regions were identified on 35 linkage groups, with more than 50 duplicated markers (Gharbi 2001; Gharbi et al. 2005, 2006).

1.6 Detection of Quantitative Trait Loci

Several QTLs have been mapped to different linkage maps on the salmon genome. The mapping strategies used have been composite interval mapping (CIM) to scan an AFLP-based map (Robison et al. 2001), and single marker analysis, which separately tests every segregating maternal or paternal SSR allele (Jackson et al. 1998). The QTLs for several normally distributed quantitative traits are furnished in Tables 6 and 7. Single markers associated with monogenic or polygenic traits without mapping data will be discussed in the next part of this chapter (Table 8).

1.6.1 Upper Temperature Tolerance

The first QTL described for any salmon was published by Jackson and colleagues (1998) for upper temperature tolerance (UTT) in rainbow trout based on the segregation of 24 loci in three backcross families (Table 6). This trait corresponds to the length of time that a fish is able to survive at 25.7 °C. The families used were obtained by crossing F_1 males with high-temperature-tolerant (two families) and intolerant parental females (one family). Hybrid F_1 progenies were obtained by crossing a high-temperature-tolerant female with an intolerant male. This study could detect two main chromosomal regions explaining 13% and 9% of the within-family variance of UTT, linked to *Omy325UoG* and *Ssa14DU*, respectively. These markers were anchored to linkage groups B and D on the Sakamoto et al. (2000) map (Table 6). It is interesting to note that these two linkage groups have some homology, because these SSR loci had been mapped to linkage groups OA-XXI and OA-XIV, respectively, in the consolidated map. However, the group OA-XIV carries a combination of markers formerly mapped to groups B and D in Sakamoto's map. Later studies in this species using the same BC design (three families) showed that the SSR locus *Ssa20.19NIUG* maps near a QTL with a stronger effect on UTT than those reported previously by Jackson et al. (1998), and the other locus *One14ASC* shows a weaker effect (Danzmann et al. 1999, Table 6).

Further evidence of an association of *Ssa2019NIUG* with UTT QTL was obtained using a diallel panel derived from a cross of two outbred rainbow trout strains unselected for temperature tolerance. This study confirmed the association of the locus *Ssa20.19NIUG* with UTT, explaining 7.5% of the phenotypic variance of this trait in these outbred crosses (Perry et al. 2001). However, it was not possible to detect any effect on temperature tolerance on chromosomal regions close to *Omy325UoG* and *Ssa14DU* loci. The authors attributed this lack of association to the different population designs, where the overall phenotypic variance in the outbreed study is considerably less than in the backcross design. Because of this, only the QTLs with a stronger effect will be detected in outbred populations (Perry et al. 2001).

In addition to these autosomal QTLs, evidence was recently obtained for a sex-linked QTL close to the locus *OmyFGT19TUF,* accounting for 9.6% of phenotypic variance of UTT, and also having a pleiotropic effect on fork length (FL) in rainbow trout (Perry et al. 2005). This work was performed using two designs, a backcross with three half-sib families and an outbred diallel panel with 40 full-sib families (Perry et al. 2005). Recent evidence shows that the duplicate uncoupling protein genes (*UCP2A* and *UCP2B*) map close to the SSR linked to UTT QTLs in both linkage groups in rainbow trout and are candidate genes to explain this temperature tolerance phenotype (Coulibaly et al. 2006). In mammals, the *UPC2* gene is involved in multiple functions, including energy balance. The protein encoded by this gene is located in the inner mitochondrial membrane (Coulibaly et al. 2006).

Arctic charr (*Salvelinus alpinus*) has been used to test UTT QTL effects in homologous chromosomal regions of rainbow trout on the basis of genetic maps available for both species (Somorjai et al. 2003). This study used a backcross family obtained from crossing of two strains characterized by different thermal selection regimens. One strain was obtained

Table 6 QTLs mapped on rainbow trout (*Oncorhynchus mykiss*) genome

Trait	Markers detected as linked to QTLs and its respective linkage group in brackets	References
Upper temperature tolerance	*OmyFGT19TUF* (OA-I*); *Ssa2019NIUG* (OA-VI*), *One14ASC* (OA-IX*); *Ssa14DU* (OA-XIV*); *Omy325UoG*, *OmyFGT2TUF* (OA-XXI*)	Jackson et al. 1998, Danzmann et al. 1999, Fishback et al. 2000, Perry et al. 2001
Spawning date	*Ssa4DU*, *OmyRGT41TUF*, *Ogo1UW* (OA-III*); *OmyFGT12TUF, Ssa311NCVM*, *One5ASC* (OAVIII*); *OmyFGT34TUF*, *Ssa103NVH* (OA-XIX*); *Ssa289DU*, *Ssa439NCVM* (OA-XXII*); *One2ASC*, *One19ASC*, *Ots4BM*L, *Ssa85DU*, *OmyRGT36TUF* (OA-XXIV*); *OmyRGT7TUF* (OAXXV*)	Sakamoto et al. 1999, Fishback et al. 2000, O'Malley et al. 2002
Embryonic development rate	ACGAGA5 (R6**); *OmyFTG12TUF*, *Omy18INRA*, *OMM1009* (OA-VIII*); ACGAAG4 (OA-IX*)	Robison et al. 2001, Sundin et al. 2005
Embryonic length	AGCCTC1 (R6**); ACGAAG4 (OA-IX*)	Robison et al. 2001
Embryonic weight	ACGAGA5 (R6**); AGCCGC4 (R11**)	Robison et al. 2001
Fork length	*OmyFGT19TUF* (OA-I*)	Perry et al. 2001
Body mass	*OmyRTG51TUF*, *OmyRTG47TUF, OmyRTG14TUF* (OA-XIII*); *OmyRGT1TUF* (OA-XXXI*)	Martyniuk et al. 2003
Pyloric ceaca number	AGCATA.4.355.hc, *Ogo1UW*, AGCATA.4.355.hc (OA-III*); EACCAGT124o, ACCACA.12.62.osu (OA-VIII*); AGCAGA.15.235.osu, ACCATC.12.249.hc (OA-XXIII*)	Zimmerman et al. 2005
Vertebrate number	ACGATC1, ACGAAG15 (OA-VI*)	Nichols et al. 2004
Scales above lateral line	ACGAAG12, AGCAGC16 (OA-VIII*); ACGAGA93ac, ACGATG7c (OA-XIV*)	Nichols et al. 2004
Anal fin ray counts	AGCACG150o, AGCACA8 (OA-XXII*)	Nichols et al. 2004
IPNV Resistance/susceptibility	*OmyRGT41TUF*, *Ssa4DU* (OA-III*); *OmyOGT4TUF*, *OmyRGT6/iiTUF* (OA-XXII*)	Ozaki at al. 2001
IHNV Resistance	GCTCTC178, GATCAG291, *One8ASC*, *OMM1054*, *Ogo2Ui/W* (OA-III*); *Ssa7NVH, OtsG78* (OA-X*); GGTCCC60, GATCC510, *OmyFGT-1TUF*, *Ssa94NVH*, *OtsG3* (OA-XXVII*)	Rodriguez et al. 2004
NK-like cytotoxicity	Aagaag.17.120.osu, Eacgaac97o, *Omy1006UW*, *OmyRGT1TUF*, accagt.11.274.hc (OA-XXXI*)	Zimmerman et al. 2004
Ceratomyxosis resistance	Acgact12 (OC17****)	Nichols et al. 2003b

* Current linkage groups identification after Nichols et al. (2003), consolidated rainbow trout OSU × ARL map.
** Original linkage group nomenclature from Robison et al. (2001).
*** Original linkage group nomenclature from Rodriguez et al. (2004).
**** Original linkage group nomenclature from Nichols et al. (2003b).

Table 7 QTLs mapped on different salmons genomes (excluding rainbow trout)

Specie	Trait	Markers detected as linked to QTLs and its respective linkage group in brackets	References
Salvelinus alpinus	Upper temperature tolerance	*Ssa14DU* (AC-12); *Ssa189NVH*, *Ssa85DU*, *Ssa185 NV Ssa119NVH* (AC-13); *SsaF43NUIG*, *One10ASC* (AC-26).	Somorjai et al. 2003
Salmo salar	Body weight	*Ssa401UoS* (AS-8); *Ssa417UoS*, *OmyRGT32 TUF*, *BHMS211* (S-11).	Reid et al. 2005
	Fulton's condition factor	*BHMS159* (AS-2); *Str58CNRS* (AS-5); *BHMS211* (AS-11); *OtsG249UCD* (AS-14).	Reid et al. 2005
	ISA resistance	aaccac74, agccta290 (7f)	Moen et al. 2004b
Coregonus clupeaformis	Absolute growth rate	GGTG199, GGT060n, GGTG104, GGTG107 (Lg1-m); ACTA135, CTTC220 (Lg6-f); AGAC126, AGAC175 (Lg12-m)	Rogers and Bernatchez 2005

from Nauyuk Lake (Northwest Territories, Canada) and is adapted to cooler temperatures with less tolerance to upper temperatures. The other strain originates from Fraser Lake (Labrador, Canada) and is considered more tolerant of high temperature. Significant associations with UTT were detected close to loci *Ssa189NVH* and *SsaF43NUIG*, and suggestive associations were detected for SSR loci *Ssa85DU*, *Ssa185NVH*, *One10ASC*, *Ssa14DU,* and *Ssa119NVH* (Somorjai et al. 2003; Table 7). Unfortunately, the authors did not report the contribution of each QTL to the overall phenotypic variance for UTT. In relation to QTLs reported for this trait in rainbow trout, the marker *Ssa14DU* shows association in both species. Also, the marker *Ssa119NVH* maps syntenically to *Omy77DU* in a region that in rainbow trout shows physiological epistasis with *Ssa2019NIUG* and *Ssa14DU* (Danzmann et al. 1999).

In summary, evidence from different populations, different experimental designs, and different species of salmon confirms at least three QTLs for UTT that map close to the loci *Ssa2019NIUG, OmyFGT19TUF*, and *Ssa14DU*. This evidence, obtained from different sources, argues strongly in favor of the idea that true QTLs for UTT have been detected in salmonid fishes (Complex Trait Consortium 2003). These studies of UTT QTLs have been made by Roy Danzmann's team using a compendium of visual basic programs (LINKMFEX) developed by him and designed to perform linkage analysis in outcrossing mapping panels (Danzmann 2005).

1.6.2 Spawning Date

In salmonids, spawning date (ovulation) is an important life-history trait determining the time of fertilization, date of progeny emergence, and even the probability of survival and the growth rate of the fry (Quinn et al. 2002). In salmon farming, extending the reproductive period allows for better management of fish production according to season, as well as increasing the period during which eggs are available on the market (Gall and Neira 2004).

The spawning date of rainbow trout is a trait with high additive genetic variation ($0.53 \leq h^2 \leq 0.65$; Siitonen and Gall 1989; Su et al. 1999). There are strains which spawn in spring (February to April, in the North Hemisphere), and strains which spawn in fall (September to December). The first evidence of chromosomal regions affecting spawning time was presented by Sakamoto et al. (1999), who used 54 SSR markers in a backcross family of 45 female progeny for mapping. This family was produced by crossing an F_1 hybrid male fish with a fall spawning female. The F_1 hybrid was obtained from mating of spring spawning male with a fall spawning female. Eleven SSR loci on five linkage groups showed significant association with spawning date QTLs: *OmyFGT12TUF*, *Ssa311NCVM,* and *One5ASC* on linkage group OA-VIII; *One2ASC*, *One19ASC*, *Ots4BML,* and *Ssa85DU* on group OA-XXIV; *Ssa4DU* on group OA-III; *Ssa289DU* and *Ssa439NCVM* on group OA-XXII; and *OmyFGT34TUF* on group

OA-XIX (Table 6). The effects on phenotypic variance of these QTLs were not reported in this study, but the approximate extent of the QTL regions on each linkage group was 4.6, 0, 17.8, 13, and 21 cM, respectively (Sakamoto et al. 1999). Later evidence obtained from a commercial trout farm where fish were spawning in different seasons (spring and fall) shows that females maturing at different times showed heterogeneity in the distribution of allelic frequencies for loci linked with spawning date QTLs *OmyFTG12TUF*, *OmyRGT41TUF* (linked to *Ssa4DU*), and *SSOSL439* (formerly Ssa439NCVM in Sakamoto et al. 1999; Table 6), compared to other loci not linked to QTL (Fishback et al. 2000). Fish spawning at the same time of year but in different years showed little heterogeneity in allele frequencies on QTL-linked loci (Fishback et al. 2000).

Another study performed by the same laboratory detected evidence of six QTL regions in rainbow trout, confirming the presence of four strong spawning date QTLs on four different linkage groups. This work used 201 SSR markers and 90 progeny of a backcross family made between two outbred strains of rainbow trout spawning in fall and spring, where a hybrid F_1 male was crossed with a fall spawning female (O'Malley et al. 2002). This study, like Sakamoto et al. (1999), confirmed the presence of one spawning date QTL on linkage group OA-XIX, in the interval between *OmyFTG34TUF* and *Ssa103NVH*, and a second QTL on linkage groups OA-VIII near the centromere and spanning the region from *OmyFGT12TUF* to *One5ASC*. A third region, also identified in previous studies, was detected on linkage group OA-XXIV in the interval flanked by *OmyRGT36TUF* and *Omy2ASC*. Another QTL region was confirmed on linkage group OA-III, close to the locus *Ogo1UW* and linked to *OmyRGT41TUF* and *Ssa4DU*. Finally, there was marginal evidence for two more QTLs, near marker *Ssa298DU* on linkage group OA-XXII (also detected by Sakamoto's work) and OA-XXV (marker *OmyRGT7TUF;* O'Malley et al. 2002).

In coho salmon (*Oncorhynchus kisutch*), three loci linked to QTL in rainbow trout (*One2ASC*, *One19ASC*, *OmyFTG34TUF*), show strong differences between female fish divergently selected for early and late spawning date for three generations (N. Diaz et al. unpublished data). This evidence supports the presence of QTLs close to these loci and is indirect evidence of conservation of chromosomal segment with QTLs for spawning time on salmonid genomes, although due to the lack of a linkage map for coho salmon, it is not possible to confirm the synteny of coho salmon and rainbow trout linkage groups.

In general, these studies indicate that spawning date is a highly polygenic trait in salmonid genomes, with at least four QTLs confirmed (Table 6). Again, mapping of QTLs for spawning time was performed using LINKMFEX software for single marker analysis (Danzmann 2005) and MultiQTL (version 2.1.2) for the interval mapping study by O'Malley et al. (2002).

1.6.3 Embryonic Development Rate

The embryonic development rate, evaluated principally as the time from fertilization to hatching, plays a significant role in the life history of wild and cultured salmon stocks. In nature, this trait determines the time of fry emergence, resulting in reduced predation rates, optimal availability of exogenous food, and appropriate conditions for fry migration (Robison et al. 1999). Mapping of QTLs linked to this trait has been performed using two homozygous clonal (DH) lines of rainbow trout with divergent hatching times, using composite interval mapping (CIM) and principally AFLP markers (Robison et al. 1999, 2001). The F_1 fishes used in this study were obtained by crossing homozygous clonal lines of all-male (YY) rainbow trout from the Swanson River (Alaska, USA) and a domesticated all-female (XX) stock from Oregon State University. These two lines have been shown to differ in hatching times (Robison et al. 1999). The F_1 all-male progeny (XY) were used to produce two DH mapping families. Ultimately, 170 progeny were genotyped with 219 AFLPs, two SSRs (*One2ASC* and *One19ASC*), and an *Alu*I polymorphism in the 3′ UTR of the *P53* gene. The DH design is known to increase the power to detect QTLs in experimental populations (Martinez et al. 2001). The marker genotypes were used to construct a genetic linkage map with MAPMAKER/EXP version 3.1 and MAPMAKER version 2.0 (Lander et al. 1987). A standardized nomenclature for the linkage groups relative to the consolidated map was not fully realized because only seven markers shared with those of the map of

Nichols et al. (2003a). Therefore, the linkage groups were named using the letter R followed by a number, as in the original paper (Table 6; Robison et al. 2001).

CIM (Zeng 1993, 1994), implemented in QTL Cartographer software, was used to detect QTLs for embryonic development rate, embryonic length, and weight at swim-up. Three QTLs influencing time to hatch were detected on linkage groups R13 (OA-IX), R6, and R9, accounting for 14.7%, 5.3%, and 4.6% of the phenotypic variance for this trait, respectively. On the other hand, a QTL influencing embryonic length was detected on linkage group R13 (OA-IX) at the same position of a QTL for time to hatch. A second QTL was detected on linkage group R6, but 40 cM away from the QTL for time to hatch anchored at the same linkage group. The proportion of variance explained by these two QTLs was 13.1% and 9.5%, respectively. QTLs for embryonic weight were detected on linkage group R11 (accounting for 10.9% of phenotypic variance), and on linkage group R6 at the same position as the QTL for time to hatch (accounting for 15.3% of the trait variance). An interesting finding of this study was the probable detection of two QTLs on linkage groups R13 (OA-IX) and R6, with pleiotropic effects on time to hatch and embryonic length, and time to hatch and weight at swim-up, respectively (Table 6; Robison et al. 2001).

Further fine-mapping for time to hatch in rainbow trout was performed by the same laboratory using a congenic line carrying a major development rate QTL developed at Oregon State University through advanced backcross QTL analysis and marker-assisted selection (Sundin et al. 2005). With this approach, one QTL was identified for embryonic development rate on linkage group OA-VIII, linked to SSR loci *OmyFGT12TUF*, *Omy18INRA*, and *OMM1009* (Table 6), and explaining between 26% and 28% of variation in time to hatch in two different mapping backcross families (Sundin et al. 2005). This QTL may influence early sexual maturity (gamete production) as well as development rate and is interesting because the locus *OmyFGT12TUF* is also linked to the QTL for spawning time in this species (Table 6). In summary, four chromosomal regions influencing embryonic development rate (and other embry onic traits) have been identified in rainbow trout. Confirmation of these QTL in additional crosses is still needed.

1.6.4 Morphological and Meristic Traits

QTLs for several morphological traits, including fork length, body weight, absolute growth rate, and Fulton's condition factor (K, measured as $100 \times$ body weight $\cdot$ fork length^{-3}), and meristic traits (counts of fin rays, lateral line scales, vertebrae, and pyloric caeca) have been identified recently on salmonid genomes (Table 6).

In the study of Perry et al. (2005) on upper temperature tolerance in rainbow trout, morphological traits that showed phenotypic correlation with UTT were evaluated, such as fork length and Fulton's condition factor (Jackson et al. 1998). The QTL mapping population and strategies were similar to those used for UTT (Perry et al. 2005). A QTL affecting fork length was identified close to the sex-linked SSR locus *OmyFTG19TUF* on linkage group OA-I. Therefore, there is evidence for two QTLs close to *OmyFTG19TUF:* one for UTT and another for fork length, accounting for 9.7% and 9.6% of the variance in these traits, respectively. An alternative hypothesis is that there is only one QTL with pleiotropic effects on both these traits (Perry et al. 2005). This study did not detect any association with Fulton's condition factor in rainbow trout using 2,001 SSR markers.

In Atlantic salmon, four QTLs for condition factor and two QTLs for body weight have been detected in comparative studies with rainbow trout and Arctic charr (Table 7; Reid et al. 2005). In this case, several QTLs influencing these two traits altogether had been mapped using 91 SSR loci and 46 progenies from each of three full-sib families. The parental fishes were obtained from the Atlantic Salmon Broodstock Development Program, in Chamcook (New Brunswick, Canada) and from the St. John River. One strong QTL explaining 20.1% of variation in body weight was detected on linkage group AS-8 (locus *Ssa401UoS*). Another QTL with a relatively strong effect was found on group AS-11 (locus *Ssa417UoS*) and accounted for approximately 12% of phenotypic variation. A previous study by this research group in rainbow trout showed marginal evidence that several SSR loci previously associated with spawning time (for example *One2ASC, One19ASC, OmyRGT36TUF*) also showed association with body mass, but with modest phenotypic effects (variation explained between 0.7% and 11.3%). The strongest association for locus *OmyRGT1TUF* ex-

plained only 1.8% of the phenotypic variation (Martyniuk et al. 2003). Two other QTLs accounting for between 0.7% and 5.0% of total variance were found. One mapped close to the telomeric loci *OmyRTG51TUF* and *OmyRTG47TUF*, and the other mapped near *OmyRTG14TUF* on linkage group OA-XIII (Table 6).

Significant QTLs for condition factor have been detected on linkage groups AS-14 (locus *OtsG249UCD*), AS-11 (locus *BHMS211*), AS-5 (locus *Str58CNRS*), and AS-2 (locus *BHMS159*), accounting for 24.9%, 16.9%, 17.6%, and 17.6% of variation, respectively (Table 7). This result suggests that some portion of quantitative variation in body weight and condition factor in Atlantic salmon is under the control of a few QTLs with relatively large effect (Reid et al. 2005). Many of the morphological growth-related traits show phenotypic and genetic correlations, and there is evidence for QTLs with pleiotropic effects (Martyniuk et al. 2003; Reid et al. 2005). However, due to the low density of markers on existing salmonid linkage maps, it is still difficult to distinguish between single QTLs and overlapping QTLs (Reid et al. 2005). As has been usual for Danzmann's laboratory, QTLs mapping was performed using LINKMFEX software for single marker analysis (Danzmann 2005) and multiple interval mapping (MIM) implemented in MultiQTL version 2.1.2 (O'Malley et al. 2002).

QTLs for absolute growth rate have been mapped in lake whitefish in the same mapping population used for the construction of a recombination map based on AFLP markers (Rogers et al. 2001). Growth rate was estimated on the basis of monthly weight measurements performed for four months on a sample of 50 adult fish (more than 2 years old) belonging to a backcross family derived from mating between normal and dwarf parental ecotypes (Rogers and Bernatchez 2005). The objective of this study was to identify markers flanking potential QTLs among intervals rather than analyze the effect of single QTLs. This analysis revealed significant associations with growth on 11 linkage groups with LOD scores ranging from 3.4 to 8.7. In linkage groups Lg4-f, Lg5-f, Lg7-f, Lg8-f, Lg11-f, Lg13-m, Lg24-f, and Lg26-m, potential QTLs span over all 30 markers anchored in the map published by Rogers and colleagues (2001). The abundance of QTLs on a larger chromosomal interval seems consistent with the general approach used by these researchers, which could produce occasional type I error in the QTL identification. Only in the larger linkage group Lg1-m are there two more delimited regions carrying presumptive QTLs for absolute growth spanning 18.5 cM (between telomeric loci *GGTG199* and *GGT060*) and 6.7 cM (between more centromeric loci *GGTG104* and *GGTG107*; Rogers and Bernatchez 2005). Two other linkage groups also had relatively well-defined QTL regions, spanning 21.8 cM on Lg6-f (between loci *ACTA135* and *CTTC220*) and 28.3 cM on Lg12-m (between *AGAC126* and *AGAC175*). The map used in this study had relatively few markers, but if there is linkage between markers, such limited sampling should be sufficient to detect QTLs with strong effects. At this time, the chromosomal regions with potential QTLs are candidates for a subsequent fine-scan with codominant markers and marker types that are more transferable across species (Rogers and Bernatchez 2005). The QTL analyses in this study were performed using an algorithm of maximum likelihood interval analysis implemented in MAPMAKER/QTL version 1.0 (Lander and Botstein 1989).

Meristic or discontinuous quantitative traits in fish have in general higher heritability ($h^2 \geq 0.5$) and are used for fish identification and taxonomy (Kirpichnikov 1981). One interesting meristic trait with high heritability ($h^2 = 0.43$; Bergot et al. 1981), and for which QTLs have been identified, is the number of pyloric caeca in rainbow trout, a trait involved in nutrient absorption and therefore possibly indirectly involved in growth rate. Pyloric caeca are sac-shaped anatomical structures, distal to the pylorus in the upper zone of the small intestine, and histologically similar to the small intestine (Rust 2002). The inner lumens of pyloric caeca are extensively folded with large numbers of microvilli and secretory cells, producing a variety of digestive enzymes. These enzymes facilitate the enzymatic breakdown of proteins, lipid hydrolysis, and the absorption of amino acids, fats, water, sodium, carotenoids, and vitamins. In adult rainbow trout, the numbers of pyloric caeca range between 31 and 147, depending on the strain, and this number appears to become fixed in alevins when fish reach 4 cm in length (Zimmerman et al. 2005).

In the QTL mapping strategy, clonal lines of rainbow trout from an experimental population at Oregon State University (OSU) and from a cultured population from the state hatchery in Hot Creek (HC), California (USA) were used. The strains differ by approximately 34 pyloric caeca. An OSU female (XX)

fish was crossed with an HC male (YY) to produce a hybrid F_1 male, which was used to produce a doubled haploid population (OSU × HC) of 54 individuals by androgenesis. This population was genotyped with 330 AFLP and 39 SSR markers (Zimmerman et al. 2005). A genetic map was developed using MAPMAKER/EXP version 3.1 and MAPMAKER version 2.0 (Lander et al. 1987), and QTL mapping was performed by CIM (Zeng 1994) using model 6 of the QTL Cartographer software. Three QTLs for pyloric caeca number were identified (Table 6). The QTL explaining the highest percentage of phenotypic variance (19.2%) was located on linkage group OA-III in a chromosomal interval spanning 21.8 cM between AFLP markers *agcata.4.355.hc* and *aagacg.6.350.hc* and containing the SSR loci *Ogo1UW*. A second QTL accounting for 18.6% of trait variation was mapped on linkage group OA-VIII between markers AFLP *Eaccagt124o* and *accaca.12.62.osu*, spanning 38.2 cM. The SSR locus *OmyFGT12TUF* mapped in the same linkage group but was not associated with pyloric caeca number. The distance between the more proximal AFLP marker (*accaca.12.62.osu*) and *OmyFGT12TUF* was about 17.3 cM. The third QTL mapped in the linkage group OA-XIII in a region flanked by AFLP markers *agcaga.15.235.osu* and *accatc.12.249.hc*. The interval spanned 29.6 cM and explained 13.5% of phenotypic variance. No SSR locus mapped in the interval, but the locus *OMM1109* was 70.1 cM away from AFLP marker *agcaga.15.235.osu* (Table 6; Zimmerman et al. 2005). It is interesting that QTLs for spawning time and shorter time of hatch have been identified on linkage group OA-VIII, close to the loci *OmyFGT12TUF*, and on linkage group OA-III, near the locus *Ogo1UW*. This suggests a possible underlying basis for these three traits in rainbow trout, or as an alternative hypothesis, a bias toward the same chromosomal intervals in the QTL mapping in salmonids due to the reduced pool of markers for mapping.

QTLs have been reported for several other meristic traits, including the number of vertebrae, scales above the lateral line, and anal fin rays (Nichols et al. 2004). In this case, the QTL mapping was performed using 238 AFLP markers in DH progeny derived by androgenesis from an F_1 hybrid male. The parental fish were clonal lines from Oregon State University (OSU) and the Dworshak National Fish Hatchery on Clearwater River (CW) at Ashakha (Idaho, USA). Composite interval mapping (Zeng 1994), implemented in the QTL Cartographer software, was used for QTL detection, and MAPMAKER/EXP version 3.0 was used to assemble markers into linkage groups. Synteny with the consolidated rainbow trout map was determined using some shared AFLP markers. This analysis detected a QTL explaining 23% of the variation in vertebrae number within a 6 cM interval between markers *ACGATC1* and *ACGAAG15* on linkage group OA-VI. Two QTLs were detected for count of scales above the lateral line on linkage groups OA-VIII and OA-XIV, accounting for 27.5% and 15.9% of phenotypic variation, respectively (Table 6). One QTL was detected for anal fin ray counts on linkage group OA-XXII (linked marker *agcacg150o*), explaining 13.4% of variation in this trait.

In general, meristic variation has been negatively correlated with differences in embryonic developmental rate (time to hatch). Fish reared at higher temperatures show accelerated growth and fewer elements in meristic traits (Nichols et al. 2004). In this study, in spite of a strong negative association between time to hatch and counts of meristic traits, only one QTL (for scales above the lateral line) was mapped to the same linkage group (OA-VIII) bearing a QTL for developmental rate. The genetic distance between these QTLs is more than 50 cM, indicating independent segregation of these QTLs (Fig. 3) and suggesting that developmental rate and number of meristic traits are not tightly coupled in rainbow trout (Nichols et al. 2004). However, it is interesting to note that the two QTLs for meristic traits mapped close to the QTL controlling upper temperature tolerance. These QTL are number of scales above the lateral line on linkage group OA-XIV and vertebrae number on the OA-VI chromosome (Fig. 3), possibly indicating a common genetic mechanism for these traits.

1.6.5 Resistance to Disease and Immunologic Traits

Breeding for disease resistance (or tolerance) is an important area in livestock and aquaculture production, due to the multiple effects of epidemic events such as production loss, zoonotic problems, barriers for international trade, and animal welfare (Bishop 2002). Polymorphisms in major histocompatibility complex (MHC) or related disease response genes have been associated with disease resistance in several livestock

species. However, today it is clear that there is no gene, combination of genes, or level of genetic variation (heterozygosity) that confers a kind of "general resistance effect." Therefore, searching for individual QTLs affecting resistance/susceptibility to specific bacterial, viral, or parasitic diseases, along with identification of QTLs for immunological responses to pathogens, is a challenge that is relevant in salmonid fish. So far, QTLs for resistance to infectious pancreatic necrosis virus (IPNV), infectious hematopoietic necrosis virus (IHNV), and enzootic myxozoan parasite *Ceratomyxa shasta* have been identified (Table 6).

IPNV is a highly contagious and virulent disease with great impact in hatcheries. Differences in resistance have been found among cultivated varieties of rainbow trout (Hill 1982). This variation has allowed the selection of rainbow trout strains resistant and susceptible to IPNV at the Yoshida Research and Training Station of Tokyo (Okamoto et al. 1993). The average mortality evaluated in challenge tests in the period from 1979 to 1989 on the resistant and susceptible strains used for QTL mapping varied between 4.42% and 96.13%, respectively (Ozaki et al. 2001). These strains were crossed to produce an F_1 hybrid family, and an F_1 male was crossed with a susceptible female to produce a backcross family of 152 progeny. A linkage map using 51 SSR segregating loci was constructed. Interval mapping and calculation of likelihood ratio statistics was performed using Map Manager QT28 software (Manly and Olson 1999). Two putative QTLs were detected. One QTL was close to loci *OmyRGT41TUF* and *Ssa4DU* on linkage group OA-III. A second QTL was located close to *OmyOGT4TUF* and *OmyRGT6/iiTUF* on linkage group OA-XXII. Each QTL explained about 17% of phenotypic variance (Ozaki et al. 2001; Table 6).

Another viral disease for which QTLs have been mapped is IHNV, which affects almost all stages of salmonid production. Under intensive culture conditions, losses can approach 100% in alevins, principally in rainbow trout aquaculture (Palti et al. 1999). Several observations suggest that interspecific hybrids of rainbow trout and cutthroat trout (*Oncorhynchus clarki*), and interstrain hybrids of steelhead and rainbow trout, have significantly reduced susceptibility to IHNV relative to commercial strains of rainbow trout (Palti et al. 1999; Rodriguez et al. 2004). QTL mapping associated with resistance to IHNV was performed by genotyping 185 AFLP and 72 SSR markers in an interstrain hybrid. Crosses between steelhead (resistant) from the Idaho Department of Fish and Game (USA) and rainbow trout (susceptible) from Clear Spring Food (Buhl, Idaho, USA) were used to produce several hybrid F_1 families. Members of these families were backcrossed to rainbow trout. Challenge tests with the IHN virus were performed on approximately 200 individuals of the F_1 and BC generations, and mortalities were recorded during the three weeks after the challenge.

Sex-specific linkage maps were produced with MAPMAKER/EXP version 3.0 (Lander et al. 1987), and eight putative QTLs for IHN resistance were identified. Three were anchored on linkage groups of the male map, and five were anchored on the female map. Assignment of the QTLs to rainbow trout linkage groups was difficult, because several markers had not been mapped before. This was especially true for presumptive QTLs mapped on the female map. However, for the male map, there are syntenic markers that permit the identification of these QTL regions on the standard rainbow trout map (Fig. 3). The first QTL region on the male map was detected on linkage group OA-X (loci *Ssa7i/NVH* and *OtsG78*). A second QTL was mapped to linkage group OA-XXVII, where six AFLP markers (between *GGTCCC60* and *GATCC510*) and three SSR loci (*OmyFGT-1TUF*, *Ssa94NVH*, and *OtsG3*) showed significant association. A third QTL region was anchored in linkage group OA-III close to eight AFLP markers (from *GCTCTC178* to *GATCAG291*) and three SSR loci (*One8ASC*, *OMM1054,* and *Ogo2Ui/W*; Table 6). These three regions bearing presumptive QTLs on the male-based map have been confirmed in a separate analysis of three other families (Rodriguez et al. 2004). Interestingly, the locus *Ogo2Ui/W* has been mapped 1.8 cM from a gene of the major histocompatibility complex (Nichols et al. 2003; Fig. 3). *MHC1-TAP2B* encodes an ATP-binding transporter involved in peptide translocation for MHC class 1 molecules from the cytosol to the endoplasmic reticulum. *Ogo2Ui/W* maps close to locus *Ssa4DU,* which has been linked to a QTL for resistance to the IPN virus (Ozaki et al. 2001; Fig. 3). This is indirect evidence supporting a possible association of this chromosomal region with immunologic functions or resistance/susceptibility to disease. On the other hand, three loci linked with IHN resistance QTLs (one by each linkage group bearing QTLs) were detected to be duplicated on a previous map, i.e.,

OmyFGT-1TUF (Sakamoto et al. 2000), *Ssa7i/NVH*, and *Ogo2Ui/W* (Nichols et al. 2003a). Also, the QTL mapping was performed on the basis of a male map, which has a reduced recombination rate in comparison with a female map (Sakamoto et al. 2000). Therefore, it is not possible to eliminate the probability of pseudolinkage, an artifact in which markers appear to be linked to QTLs, when in reality they are on different chromosomes. Therefore, the evidence on resistance/susceptibility for IHN virus QTLs must be analyzed carefully and confirmed by other studies.

Only one immunological trait has been mapped on the salmonid genome, and it concerns the activity of nonspecific cytotoxic cells (NCC), analogous to mammalian natural killer cells (NK), in rainbow trout (Zimmerman et al. 2004). In fish, as in mammals, NK-like cells are involved in protection against bacterial and protozoan parasites and the elimination of tumor cells. The mapping strategy was to use 106 DH fish progeny of a cross between clonal lines of the OSU and HC strains of rainbow trout. The fish were screened with 330 AFLP and 39 SSR markers. NK-like activity was evaluated on NCC cells obtained from a blood sample and incubated with ^{51}Cr-label YAC-1 target cells to determine the percentage of cytotoxicity (Ristow et al. 2000). The QTL mapping was performed using CIM implemented in QTL Cartographer software (Zeng 1994). The genetic map was generated using MAPMAKER/EXP v3.0. This study revealed a unique chromosomal region affecting the NK-like cytotoxicity on linkage group OA-XXXI, spanning 25.7 cM between the AFLP markers *saagaag.17.120.osu* and *accagt.11.274.hc*, and accounting for 63.4% of phenotypic variation in the trait (Zimmerman et al. 2004). In this wide region, two SSR loci are also anchored, *Omy1006UW* and *OmyRGT1TUF* (Table 6; Fig. 3). All evidence shows that this QTL is unlinked with other QTL regions affecting disease resistance or immunological-related genes (Zimmerman et al. 2004), although the locus *OmyRGT1TUF* was previously associated with body mass (Martyniuk et al. 2003).

Evidence for multiple QTLs for resistance to a parasitic disease caused by *Ceratomyxa shasta* has been detected using clonal lines of rainbow trout (Nichols et al. 2003b). *Ceratomyxa shasta* is an enzootic myxozoan parasite that requires two hosts to complete its life cycle. Actinospores in the water column infect the digestive tract of salmon and cause intestinal inflammation, tissue degradation, and posterior mortality. Salmonids that migrate through or are reared where ceratomyxosis is endemic are more resistant or tolerant than fish outside these areas (Nichols et al. 2003b). Clonal hybrid fish were obtained by crossing females of OSU clonal rainbow trout susceptible to parasites with males of ARL or CW clonal lines, and 45 DH progeny were produced. After exposure to parasites, the mortality (live or dead) and days to death of every fish were recorded. Single marker associations for both traits were evaluated using a panel of 343 AFLP markers. Markers that showed evidence of association were tested in multiple regression models (linear and logistic) to determine the number of loci influencing these traits. A linkage map was constructed using MAPMAKER/EXP v3.0 to group and order these markers together (Lander et al. 1987). Interval mapping by regression was used to ascertain the position of binary trait QTLs (Haley and Knott 1992) implemented in r/QTL (Broman et al. 2003). For the metric trait (days to death), a Cox proportional hazard model was used to test differences between survival curves and AFLP genotypes in each marker. The analyses consistently identified three AFLP markers associated with QTL, explaining 41% of total variance. Two of these markers (*acgaga9* and *agcaga6*) were unlinked in the genetic map. The last marker (*acgact12*) was anchored in linkage group OC-17, but synteny with the published genetic map for rainbow trout has not been established (Table 6).

Infectious salmon anemia (ISA) is a viral disease of Atlantic salmon that causes severe losses in the aquaculture of this species (Thorud and Djupvik 1988). Challenge tests for ISA were carried out on two full-sib family and 79 progenies using intraperitoneal injection of infectious material. The mortality was monitored until 50% of the progenies were dead and the cause of death was verified by bacteriological test (Moen et al. 2004b). QTLs for resistance were identified using survival analysis and the transmission disequilibrium test (TDT), two statistically robust methods traditionally used in human genetics to detect associations between single nucleotide polymorphism (SNP) markers and disease (Lin et al. 2004). TDT is a nonparametric association-segregation-based test that compares the number of times that an allele is transmitted or not transmitted from a heterozygous parent to an affected progeny (Spielman et al. 1993).

This test is usually used only in cases where there is previous evidence of a close association between a marker and a candidate gene. However, the test has been successfully applied for the initial detection of associations between markers and QTLs. Survival analysis has been widely used in human medicine for testing medical treatments, and it is expected to be more powerful than TDT, since the variable used is continuous (time of survival of individuals), which is more informative than the binary variable (susceptible/resistant) used in TDT (Moen et al. 2004b). QTLs for resistance to ISA were mapped using 340 AFLP markers. Two AFLP markers (*aaccac74* and *agccta290*) were significant, producing a reduction of approximately 25% of mortality among fish inheriting the band-present allele in both markers. Both markers were anchored to the same linkage group 12f on the Moen et al. (2004a) map, tagging a presumptive QTL for ISA resistance on this chromosome spanning 7 cM. In this same linkage group, the SSR loci *BHMS394*, 17 cM away from *aaccac74,* was mapped (Moen et al. 2004a).

As it has been described in this section, QTL mapping in salmonid is an ongoing activity, and many putative QTLs must be confirmed with more extensive studies. It is noteworthy that in rainbow trout, QTLs for a number of different traits have been mapped to similar chromosomal positions. For example, the linkage group OA-VIII carries QTLs for scales above the lateral line, number of pyloric caeca, spawning date, and time to hatch. Linkage group OA-III carries QTLs for number of pyloric caeca, spawning date, and resistance to IPNV and IHNV (Table 6; Fig. 3). However, other large linkage groups with similar marker saturation do not carry any QTL, suggesting that there is no bias restricting QTL identification to larger or marker-saturated chromosomes. It is possible that these QTLs have pleiotropic effects on several traits, but this is unlikely due to the very different nature of the traits on each linkage group. An alternative scenario is that QTLs for different traits are located in a cluster, but with present map saturation, it is impossible to separate their individual effects. High-resolution QTL mapping, along with molecular marker mapping, physical mapping, positional cloning, and analysis of candidate genes and gene expression will be necessary for a precise identification of the genes determining the phenotypic expression of traits under study.

1.7 Association of Traits with Specific Molecular Markers

The low density of markers on many salmonid genetic maps, and the complete absence of maps for several species, has driven many researchers to look for associations of anonymous markers, generally RAPD or AFLP dominant markers, with monogenic and polygenic traits. The approach most often used is bulked segregant analysis (BSA; Michelmore et al. 1991) or selective genotyping (Darvasi and Soller 1992). Once a polymorphism segregating with the trait of interest is identified, the polymorphic fragment is cut from the gel (agarose or polyacrylamide), purified, cloned, and sequenced to design specific primers for PCR amplification. These primers are tested on genomic DNA, and the amplicons obtained tested for association with the trait of interest. To accelerate the search, the marker screening is usually performed on pools of DNA obtained from five to twenty individuals of the phenotypic extremes (Darvasi and Soller 1994).

1.7.1 Bulked Segregant Analysis

In BSA, the co-segregation of many markers is followed in a segregating population obtained by crossing a single pair of parental fish with different phenotypic expressions for the discrete trait under study. A number of segregating individuals are used in each pool, with the objective of minimizing variation not associated with the trait of interest (Michelmore et al. 1991). This approach was used to identify sex-specific sequence characterized amplified region (SCAR) markers *Omy*-163 and *Omy*P9 linked to the Y chromosome in rainbow trout using AFLP screening (Felip et al. 2005) or RAPD screening (Iturra et al. 2001a). In salmon aquaculture, the sex phenotype is an important commercial trait. Growers prefer to stock all-female fingerlings because they mature later than males (Table 8; Brunelli and Thorgaard 2004).

BSA was also used to identify markers associated with the dominant albino phenotype in a Japanese strain of rainbow trout (Table 8; Nakamura et al. 2001).

Table 8 Primers sequences and PCR conditions of molecular markers identified as associated with several traits in different salmon species

Marker	Type	Reference	Primers	Trait	Species
*Omy*P9	SCAR	Iturra et al. (2001a)	F 5′-TGCATTCCACTTCTTCTCGTT-3′ R 5′-GACGTCAAAGGTTGTCGGTT-3′	Sex	Rainbow trout
Omy-163	SCAR	Felip et al. 2005	F 5′-CTTCTGTCTACCAAAATC-3′ R 5′-CATCAAGTCACATGACTAAC-3′	Sex	Rainbow trout
OmyD-AlbnTUF	SSR	Nakamura et al. 2001	F 5′-GAATTCACGGGAGTGGAGTG-3′ R 5′-TCCATGATTGCCACGGGATTCT-3′	Albinism	Rainbow trout
GH1	RFLP(*Taq*I)-PCR	Gross and Nilsson 1999	F 5′-ATGGGACAAGGTAAGCCT-3′ R 5′-CTTTGAGGTCGCTGAGCTTC-3′	Body Weight	Atlantic salmon
GH2	RFLP(*Hinf*I)-PCR	Forbes et al. 1994	F 5′-ATCGTGACGCCAATCGACAAGCAC-3′ R 5′-GGGTACTCCCAGGATTCAATCGGA-3′	Sex	Coho salmon Chinook salmon
Oki206	SCAR	Araneda et al. 2005	F 5′-GAGGACGTCCTCCAACCAATCA-3′ R 5′-GAGGACGTCCTCACCTTACAATCC-3′	Flesh color	Coho salmon
OtY1	PCR based	Noakes and Phillips 2003	F 5′-GATCTGCTGGCTGGATTTGG-3 R 5′-CCAGCGATGGTTTGTTTGAG-3	Sex	Chinook salmon[1]
OtY2	SCAR	Devlin et al. 1994	F2 5′-CTGGTTCGAGCCTAAGTAG-3′ R2 5′-GATGCAGTAGGAGCAGATG-3′	Sex	Pacific Salmons[2]
Omy207	SSR	Heath et al. 2002	F 5′-ACCCTAGTCATTCAGTCAGG-3′ R 5′-GATCACTGTGATAGACATCG-3′	Reproductive female traits	Chinook salmon
GHRH/PACAP2	SNP A/G	Tao and Bouling 2003	F 5′-TTAATAAAGCCTACAGGAAAGCGC-3′ R 5′-GGCGTCCTTTGYTTCTATATCTCT-3′	Early juvenile growth	Arctic charr

NI Not informed.

[1] With exception of sockeye salmon (*O. nerka*) and rainbow trout.

[2] With exception of rainbow trout and not tested in pink salmon (*O. gorbuscha*).

In this case, the AFLP marker could be converted into a microsatellite marker *OmyD-AlbnTUF* due to the presence of pentanucleotide repeats (GGAGT) in the AFLP sequence. This discovery is not infrequent, and we have found variable number tandem repeats (VNTR) motifs, and micro- and minisatellites inside RAPD markers. For example, the dinucleotide microsatellite marker *OkiCAT229UCH* associated with spawning time (GenBank accession number AY926352) was isolated from a salmon coho RAPD marker during SCAR construction (C. Araneda et al. unpublished data). BSA has also been used to identify three RFLP markers associated with resistance to the IHN virus in hybrid fish between rainbow trout females and cutthroat trout males (*O. clarki*) from the facilities of the Department of Fish and Game at Hayspur and Henry's Lake (Idaho, USA), respectively (Palti et al. 1999). However, these three RFLP fragments associated with IHN resistance/susceptibility have not been characterized by cloning and sequencing.

1.7.2 Selective Genotyping

Selective genotyping is similar to BSA with the difference that the polymorphism is scored in only the most extreme phenotypes from the population. The hypothesis that underlies this approach is that the individuals on the ends of distribution for a quantitative trait have a higher probability of sharing more alleles that increase or diminish the trait value, and therefore they are more informative that the individuals in the middle of the distribution (Lander and Botstein 1989). Using a similar approach, but on the basis of breeding values instead of phenotypic values, a dominant SCAR marker (*Oki206*) was associated with variation in flesh color of coho salmon (Table 8; Araneda et al. 2005).

Markers such as *OmyD-AlbnTUF* and *Omy-163* have the advantage that they have been anchored in a recombination map (Fig. 3). The SCAR *OmyP9* has been assigned physically by means of fluorescent in situ hybridization (FISH) to a submetacentric chromosome pair in rainbow trout (Iturra et al. 2001b), but *Oki206* has not been assigned a chromosomal position, because there is not yet a genetic map for coho salmon.

1.7.3 Other Single Locus Markers

Several single markers have been developed for sex discrimination in salmonid fishes. The first step toward this objective was the identification of a growth hormone pseudogene (*GHψ*) present only in males of chinook salmon and other Pacific salmon (Du et al. 1993), with the exception of sockeye salmon (*O. nerka*) and rainbow trout (Devlin et al. 2001). This discovery allowed the development of a male-specific, PCR-based marker (*OtY1*) for chinook salmon (Devlin et al. 1994). A *Hinf*I RFLP in intron C of growth hormone gene *GH2* has also been described as sex linked in coho and chinook salmon (Table 8; Forbes et al. 1994). And, as mentioned previously, two allozymes (*bGLUA** and *sSOD-1**) are sex linked in rainbow trout (Allendorf et al. 1994).

Local liver expression of PGM-1 allozyme alleles coded by the *PGM-1r** regulatory locus are associated with an increase of embryonic developmental rate (time to hatch) and age at first reproduction in rainbow trout, possibly due to an accelerated metabolism on the glycolysis pathway in fish embryos (Allendorf et al. 1983). However, a syntenic locus in Atlantic salmon does not show a similar phenotypic effect (Verspoor and Moyes 2005).

Evidence of association of a PCR RFLP (*Taq*I) in the growth hormone (*GH1*) gene with body weight has been reported in Atlantic salmon. Different haplotypes at this marker show differences in size (Table 8; Groos and Nilsson 1999). An A/G polymorphism in the growth hormone-releasing hormone and pituitary adenylate cyclase-activating polypeptide genes (*GHRH/PACAP2*) accounts for 9.4% of the phenotypic variance in juvenile growth rate of Arctic charr. The heterozygous genotype A/G is associated with faster growth rate, and the homozygous genotype G/G shows suggestive association with early gonadal development, which may help to predict early maturation in males (Table 8; Tao and Boulding 2003).

Finally, a population analysis of correlations between heterozygosity and reproductive fitness in chinook salmon shows that one SSR locus (*Omy*207) is significantly associated with female reproductive traits, such as relative fecundity, egg size, and egg survival (Table 8; Heath et al. 2002). This locus has been proposed as a good candidate for further QTL analysis of reproductive traits in this species.

1.8 Physical Mapping

Physical maps can be constructed by a variety of different approaches and can be classified into two general types. Cytogenetic maps describe the order and spacing of markers on a DNA molecule based on microscopic analysis. Cytogenetic maps show the specific physical locations of genes and/or molecular markers on each chromosome. The second type of physical map consists of a set of cloned fragments of DNA that represent a complete chromosome or chromosomal segment, together with information about the order of the cloned sequences. Both types of physical maps are being developed in salmonids.

The complexity of salmonid genomes derives from the existence of duplicate loci arising from an ancestral tetraploid event. This introduces an additional difficulty in the construction of genetic maps in these species. Furthermore, it is known that differences in genetic recombination among the sexes are due to differences in the meiotic behavior of the chromosomes between males and females. Additional chromosome variation has arisen from Robertsonian rearrangements, inversions, and tandem translocations. So, the assembly of genetic and physical maps is of particular importance in these species.

1.8.1 Karyotypes

The cytological characteristics of salmon genomes have been widely described and are summarized in Table 9. The diploid number ($2n$) ranges from 52 to 84 chromosomes with a DNA content (C-value) ranging from 2.23 to 3.70 pg (Ojima et al. 1963; Johnson et al. 1987; Phillips and Ráb 2001; Hardie and Hebert 2004).

The chromosome evolution of salmonid species has been reviewed by Hartley (1987) and, more recently, by Phillips and Ráb (2001). These authors have proposed two categories of karyotypes in this species: Type A and Type B based on chromosome numbers, and two new subcategories, A′ and B′, according to the variation of the chromosome arm number (NF). As is shown in Table 9, cultured species of the genus *Salvelinus* and *S. trutta* belong to the Type A karyotype with diploid chromosome numbers between $2n = 78 - 84$ and NF = 98–102. All *Oncorhynchus* species show the Type B karyotype with $2n$ around 60 chromosomes. *S. salar* belongs to Type B′ karyotype with an NF = 72–74, lower than the typical salmonid NF, which is around 100 (Phillips and Ráb 2001).

Intraspecific polymorphism as a consequence of Robertsonian rearrangements is well documented in wild and farmed populations of rainbow trout, brown trout, and Atlantic salmon (Thorgaard 1983; Colihueque et al. 2001; Phillips and Ráb 2001). Different salmonid species also present structural chromosomal polymorphisms, including changes in the position of the nucleolar organizer region (NOR; Porto-Foresti et al. 2002), variation in the number and size of this chromosome region (Phillips and Hartley 1988), and in the distribution patterns of the constitutive heterochromatin (Phillips and Ráb 2001).

The application of fluorescence in situ hybridization (FISH) is one of the most powerful tools for the construction of cytogenetic maps in fishes. This method allows the localization of DNA sequences in the metaphase and meiotic chromosomes and in the nucleus. The DNA sequences used as probes can correspond to individual genes, specific regions of chromosomes, complete chromosomes, and even complete genomes (Phillips and Reed 1996). FISH probes are labeled with modified nucleotide precursors tagged with biotin or digoxigenin by enzymatic methods or PCR. They are then hybridized to denatured chromosomes and detected at the site of hybridization using proteins linked to fluorescent dyes. The chromosomes are observed by epifluorescence microscopy equipped with digital imaging devices, such as cooled charge-coupled device (CCD) cameras. The sensitivity of these cameras has permitted the development of FISH probes with modified nucleotides directly conjugated to a fluorescent dye.

During the last few years, bacterial artificial chromosomes (BAC) libraries (Katagiri et al. 2001; Palti et al. 2004) and a considerable database of expressed sequence tags (ESTs) (Rexroad et al. 2003, 2005) have become available for several species of salmonids. These resources represent important progress toward the creation of high-resolution genetic maps for these species. BAC and cDNA libraries for several salmonid species are also a valuable resource for preparing FISH probes. Identification of polymorphic genetic

Table 9 DNA content (pg), chromosome diploid number (2*n*), arm chromosome number (NF) and karyotype categories (follows Phillips and Rab 2001) of cultured salmonid species

Species	C-value (pg)	2*n*	NF	Type
Oncorhynchus				
O. mykiss	2.4[1]	58–64	104	B
O. kisutch	2.6[2]	60	100	B
O. masou	2.07–3.29[3]	66	100	B
O. tshawytscha	2.45[1]	68	100	B
O. gorbuscha	2.57[2]	52–54	100	B
O. nerka	3.04[3]	57♂–58♀	100	B
O. keta	2.97[3]	74	100	B
Salmo				
S. salar	3.27[1]	54–58	72–74	B′
S. trutta	2.94[3]	78–84	98–102	A
Salvelinus				
S. alpinus	3.70[1]	78	98	A
S. fontinalis	3.27[1]	84	100	A
S. namaycush	3.04[1]	84	100	A
Coregonus				
C. clupeaformis	2.44[1]	80	94	A

[1] Hardie and Hebert 2004
[2] Johnson et al.1987
[3] Ojima et al. 1963

markers in these clones provides an excellent tool for the integration of physical and linkage genetic maps. Moreover, it will enrich the comparative studies among the salmonid species and contribute to an understanding of the genomic architecture of these species.

1.8.2 Physical Mapping of Centromeric Repeats

Centromeric repeated sequences show very little evolutionary conservation among vertebrates, even among closely related species. This is also true of the centromeric sequences that have been isolated from the genome of some salmonid species. Three families of centromeric repeated sequences have been isolated in *Salvelinus* species by Hartley and Davidson (1994) and Reed et al. (1995). Physical mapping, using the sequences *Alu*I/*Rsa*I-Type A, *Alu*I/*Rsa*I-Type B, and *Dra*I/*Eco*RI as FISH probes on the chromosomes of *S. alpinus*, *S. fontinalis,* and *S. namaycush,* showed that these sequences are located mainly in the centromere of several metacentric chromosomes as well as in some acrocentric chromosomes in these species. Experiments using dual-FISH probes with combinations of these sequences show that the centromere of some chromosomes is characterized by more than one type of sequence. These studies have demonstrated that for each *Salvelinus* species, there is a unique pattern of hybridization of these centromeric sequences (Phillips et al. 2002). Recently, Viñas et al. (2004) have isolated a highly repeated *Hpa*I DNA family from the *Salmo salar* genome. This sequence, conserved in *S. trutta*, is absent in the genome of *Oncorhynchus* but shows certain similarity with *Alu*I/*Rsa*I in *Salvelinus*. FISH using *Hpa*I sequences shows hybridization in the centromere of three pairs of acrocentric chromosomes of the 29 pairs that constitute the karyotype of *S. salar*.

1.8.3 Physical Mapping of Telomeric Repeated Sequences

In vertebrates, telomeres constitute the physical end of the chromosomes, and the subjacent DNA corresponds to highly conserved sequences organized in tandemly repeated, G-rich sequences like $(TTAGGG)_n$. Probes of telomeric sequences constitute a valuable tool in the characterization of chromosomal rearrangements, like tandem fusions, that are implied in the intraspecific chromosomal variation in diverse species of salmonids (Hartley 1987; Phillips and Ráb 2001). Two strategies have been used for mapping the telomeric sequences using FISH. Perez et al. (1999) isolated and cloned a DNA sequence containing telomeric sequences of *S. salar*. They used a clone containing the sequence $(TTAGGG)_a$ as a FISH probe, and it showed hybridization only in the terminal ends of all the Atlantic salmon chromosomes. Abuin et al. (1996) described the distribution of telomeric sequences using $(TTAGCG)_n$ oligonucleotides as a probe to hybridize on chromosomes of *S. salar*, *S. trutta*, *O. kisutch,* and *O. mykiss.* They obtained similar results, but in addition detected interstitial hybridization in a long chromosome pair in *S. salar*. The detection of interstitial telomere sites in chromosomes of brook trout and androgenetic individuals of rainbow trout (*Oncorhynchus mykiss*) using primed in situ labeling (PRINS) has been reported by Ocalewicz et al. (2004a) and Ocalewicz et al. (2004b).

1.8.4 Physical Mapping of Multicopy Genes: Ribosomal Genes

Vertebrates have two units that code for ribosomal RNA genes (rDNA). The major unit, containing genes for 5.8S, 18S, and 28S rRNA, is repeated in tandem, separated by intergenic spacers, and located in one or several specific chromosomal regions called nucleolar organizer regions (NORs). In metaphase chromosomes, the NOR-bearing chromosomes are identified by means of AgNOR staining, because they have been transcriptionally active in the preceding interphase. In fishes, it is also possible to identify the NOR-bearing chromosomes using the fluorescent stain chromomycin A3. This fluorochrome has a great affinity to CG-rich regions of the 18S + 28S genes. However, FISH has been the most informative tool for mapping the ribosomal genes in salmonid karyotypes. It has been established that the coding sequences of the 18S + 28S rDNA genes in vertebrates are evolutionarily conserved, which makes it possible to map these genes using heterologous FISH probes.

The minor units contain the 5S rDNA genes and consist of tandem repeats of a highly conserved coding sequence of 120 bp, flanked by non-transcribed spacers (NTS). FISH studies of the 5S rDNA genes in salmonid genomes indicate that these genes are localized to one or several loci (Pendas et al. 1994; Moran et al. 1996; Fujiwara et al. 1998; Pardo et al. 2000; Iturra et al. 2001a; Stein et al. 2001).

FISH studies have revealed that in rainbow trout, the ribosomal 18S + 28S genes are located in a single medium size submetacentric chromosome pair, which agrees with the results obtained with silver staining and chromomycin A3 (Fujiwara et al. 1998; Porto-Foresti 2002). Nevertheless, variation in the position of 18S + 28S genes in the NOR-bearing chromosomes has been detected in Brazilian farmed stocks of rainbow trout as the result of pericentric inversions (Porto-Foresti et al. 2002). A single chromosome pair bearing the 18S + 28S genes has been demonstrated in *Oncorhynchus masou* (Fujiwara et al. 1998) and in *O. kisutch* (Fig. 5). Different results were observed in *Salmo* and *Salvelinus*. *S. trutta* presents brilliant signals of hybridization in one pair of chromosomes and dispersed weak signals in eight chromosomes, while in Atlantic salmon, the ribosomal genes map to a single chromosomal pair (Pendas et al. 1993a, b). In Arctic charr, lake trout, and brook trout, these genes are dispersed over several chromosomes (Reed and Phillips 1995; Philips 1996; Fujiwara et al. 1998).

The 5S rDNA probes show a diverse distribution pattern of these genes in the karyotypes of salmonids. In *Salvelinus*, the chromosomal distribution of the 5S rDNA sequences varies from a single metacentric pair in *S. fontinalis*, to three pairs in *S. namaycush,* and 3–4 acrocentric pairs in *S. alpinus* (Phillips et al. 2002). One locus of 5S rDNA genes in rainbow trout and Atlantic salmon is located adjacent to the cluster of 18S + 28S ribosomal genes (Moran et al. 1996; Fujiwara et al. 1998; Iturra et al. 2001a). This relationship among NORs and 5S rDNA genes is also observed in Brazilian rainbow trout stocks in which these clus-

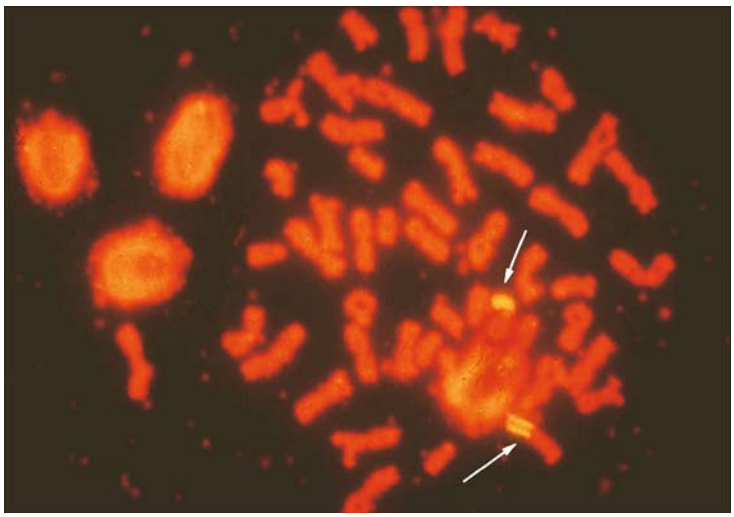


Fig. 5 Chromosomal mapping of 18S + 28S rDNA genes labeled with biotin on metaphase chromosomes of *O. kisutch*. Arrows show the NOR bearing pair with a bright hybridization signal along the long arm. (Source: Lam and Iturra unpublished)

ters are located in a different position in the chromosome (Porto-Foresti et al. 2002). In several rainbow trout strains, the second locus of the 5S ribosomal genes is located on the X chromosome (Moran et al. 1996; Iturra et al. 2001a; Ocalewicz 2002; Phillips et al. 2004). In chinook salmon, the 5S rDNA genes map on the Y chromosome, as well as on several other acrocentric chromosomes (Stein et al. 2001). Our results of physical mapping of 5S ribosomal genes in coho salmon indicate that these gene clusters are located in a medium size metacentric pair in males and females (N Lam et al. unpublished). Furthermore, we observed brilliant signals in the short arm of a single acrocentric chromosome in the males, which could correspond to a Y chromosome in this species (Iturra et al. 2001a; Lam 2002). The singular distribution of the 5S ribosomal genes in the genome of male and females of these species suggests the existence of differential regulation of gene expression among the sexes (Martins and Galleti 2001; Phillips et al. 2004).

1.8.5 Physical Mapping of Coding Genes

Physical maps of coding genes are at an early stage of development in salmonid species. Nevertheless, the currently ongoing genomics projects will make advances possible through the availability of resources, like BAC clones and ESTs, from which FISH probes can be prepared. An example is the elegant physical and genetic mapping of the major histocompatibility complex (MHC) in rainbow trout (Hansen et al. 1999; Phillips et al. 2003). Hansen et al. (1999) demonstrated by segregation analysis that rainbow trout MHC class I and II regions are located on different linkage groups. Recently, these authors used FISH probes of different BAC clones containing the MHC class II gene (*DAB*) class Ia and Ib loci and *ABCB2* gene respectively to demonstrate that the rainbow trout MHC regions are located on at least four different chromosomes, 17p, 3p, 18q, and 14q (Phillips et al. 2003).

Unique anonymous markers have been found within BAC clones, making it possible to link these probes to specific linkage groups in rainbow trout (Nichols et al. 2003a). A good example is the chromosome localization of the rainbow trout vitellogenin gene (*Vtg*) cluster. The *Vtg* genes form a large and complex family in the rainbow trout genome located on the short arm of an acrocentric pair, as demonstrated using FISH by Trichet et al. (2000). The multigene families for each of the five histone genes were physically mapped in rainbow trout, Atlantic salmon, and brown trout karyotypes (Pendas et al. 1994). They found hybridization signals of the histone DNA probes on a single submetacentric chromosome pair in the three species, suggesting that the histone cluster maps to a single locus (Pendas et al. 1994). Perez et al. (2000) isolated and studied the physical location of the methionine tDNA in the genome of Atlantic salmon and brown trout. They found evidence for a single locus in the chromosomes of both species.

1.8.6 Physical Mapping of Sex Markers

Fishes are characterized by the diversity of their sex determination systems. The most common genetic mechanism of sex determination among vertebrates is the existence of cytological differentiated sex chromosomes in one of the sexes. This situation is rather uncommon in the fish species studied until now. In many cases, the heterogametic sex has been identified by experimental crosses with sex reversal specimens (Yamazaki 1983). Male heterogamety has been identified in salmonid species, and this has been confirmed by cytogenetic evidence in lake trout, brook trout, rainbow trout, and sockeye salmon (Thorgaard 1977, 1978, 1983; Phillips and Ihssen 1985; Phillips and Hartley 1988). More recently, sex-linked molecular markers and FISH probes have given support to an XY sex determination system in salmonid species, but the sex chromosomes are morphologically indistinguishable or only slightly differentiated in the karyotype (Forbes et al. 1994; Devlin et al. 1994, 1998; Iturra et al. 2001a, b; Zhang et al. 2001; Stein et al. 2002; Phillips et al. 2001, 2002; Woram et al. 2003; Brunelli and Thorgaard 2004).

In *S. namaycush* and *S. fontinalis,* the sex chromosome pair corresponds to the largest submetacentric pair, which shows a heterochromatic band only on the short arm of the X chromosome (Phillips et al. 2001). Sex chromosome paint probes for FISH obtained by microdissection of the Yp of *S. namaycush* and a Yq-specific probe prepared from the largest acrocentric chromosome pair in rainbow trout have been used to investigate the sex chromosomes in North American charr species and the possible homology among sex chromosomes in rainbow trout and chinook salmon, and Atlantic salmon and brown trout (Phillips et al. 2001, 2002). The probes hybridized to the largest submetacentric chromosome pair in all of the studied charr species. However, the authors suggest that linkage studies are necessary to determine if this chromosome pair is conserved in all *Salvelinus* species (Phillips et al. 2002). On the other hand, the hybridization pattern with these probes in rainbow trout, chinook salmon, brown trout, and Atlantic salmon suggests that the sex chromosomes of these species would not show homology, but that they have evolved independently (Phillips et al. 2002).

Sequences related to the sex chromosomes have been mapped by FISH in chinook salmon, rainbow trout, and coho salmon. *Oty*1 is a male-specific repetitive DNA sequence isolated from the chinook salmon genome by Devlin et al. (1991) and used as a male sex marker to identify the genetic sex by PCR in this species (Table 8; Devlin et al. 1994). This Y-chromosome-specific sequence is located in a distal position of the short arm of a medium size acrocentric chromosome identified as the Y chromosome in this species by Stein et al. (2001). The sex pair in rainbow trout is characterized by small differences in the length of the short arm among subtelocentric X and Y chromosomes (Thorgaard 1977, 1983). The hypothesis that the X and Y chromosomes of rainbow trout appear to be in an initial stage of differentiation has been supported by the finding that some male specimens from different natural and cultured populations do not show heteromorphism between X and Y chromosomes (Thorgaard 1977, 1983; Colihueque et al. 2001). Three sex molecular markers have been physically located on the sex chromosomes of rainbow trout. *Omy*P9 (Table 8) is a polymorphic SCAR marker that shows FISH-hybridization to the sex chromosomes in rainbow trout (Iturra et al. 1998, 2001a, b; Lam et al. 2003). The *Omy*P9 probe hybridizes in the medial region of the long arm of the acrocentric/subtelocentric Y chromosome. These results, together with the *Omy*P9 pattern of inheritance in several Chilean-farmed rainbow trout strains, allows us to suggest the proximity of *Omy*P9 to the sex determination locus in this species (Iturra et al. 2001b).

Recently, two new sex linked markers, SCAR *Omy*-163 (Table 8) and *SNP-B4*, have been physically and genetically mapped on the Y chromosome of rainbow trout (Felip et al. 2004, 2005; Phillips et al. 2004). Both probes hybridize on the long arm of the sex chromosome pair but in different chromosomal positions. *SNP-B4* is localized close to the centromere, and SCAR *Omy*-163 hybridizes distal to the first one, in the same region on the long arm as *Omy*P9. The Y chromosome location of all of these three markers supports the hypothesis that the sex determination locus is localized on the long arm of the sex chromosomes in rainbow trout (Iturra et al. 1998, 2001a; Felip et al. 2004, 2005; Phillips et al. 2004). Other molecular markers related to sex in this species correspond to AFLPs and microsatellites that have been used in the

construction of genetic maps of the sex-linked group in several rainbow trout strains, including clonal lines (Young et al. 1998; Sakamoto et al. 2000; Nichols et al. 2003a; Woram et al. 2003; Palti et al. 2004; Felip et al. 2005). Iturra et al. (2001b) used *Omy*P9 to identify the sex chromosomes in coho salmon. This probe shows fluorescent signals in the medial region of the long arm of a subtelocentric pair with similar morphology as rainbow trout sex chromosomes, being identified as the sex pair in salmon coho (Lam 2002). Recently, Phillips et al. (2007) have identified the sex chromosomes of *O. gorbuscha* and *O. keta* using a probe to the male-specific *GH*-Y (growth hormone pseudogene).

Different approaches have been used to construct genetic linkage maps to identify syntenic markers which might help identify the primary sex-determining locus in salmonids. Recently, comparative maps of the sex-determining region among salmonid species, using mainly microsatellite and AFLP markers, have been published (Woram et al. 2003; Marshall et al. 2004; Felip et al. 2005). These studies confirm the sex linkage of markers among several species of salmonid. An example is the male-specific pseudogene *Ghψ*, which is linked to the Y chromosome in *Oncorhynchus*, but not in rainbow trout, sockeye salmon, or *Salmo salar* (Devlin et al. 2001). Also, the Y-linked marker *Oty*1 in chinook salmon is not sex-linked in rainbow trout (Noakes and Phillips 2003). A new Y-specific chromosome marker derived form an AFLP polymorphism has been described by Brunelli and Thorgaard (2004), which is useful in chinook and coho salmons, but not in rainbow trout, among other salmonid species. The availability of the cloned probes for FISH studies will be useful in the construction of sex chromosome maps for these species.

1.9 Integration of Genetic and Cytogenetic Maps

The full utilization of genetic maps requires knowledge of the correspondence between the genetic and cytogenetic maps. Several methods have been used to correlate genetic and cytogenetic maps (Stephens et al. 2004). FISH provides the most direct way of physically mapping DNA sequences on chromosomes. However, FISH mapping of fish genomes depends heavily on the development of recombinant DNA BAC libraries.

Recently, thanks to the information generated by genomics projects in rainbow trout, it has been possible to assign linkage groups to specific chromosomes of rainbow trout by means of FISH (Phillips at al. 2006). In this case, BAC clones containing markers previously identified from each linkage group were used as fluorescent probes.

The genetic and cytogenetic maps will be helpful for the QTL mapping in salmonids for breeding applications and for conducting genome comparisons between these species. Moreover, cytogenetic maps can quickly sort out duplicate loci, which help in building contigs, to determine linkage groups for duplicated loci from FISH experiments, and to determine which linkage groups are fused in other strains and species. SNPs mined from BACs could provide haplotypes of linked markers for population studies (Phillips at al. 2006).

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