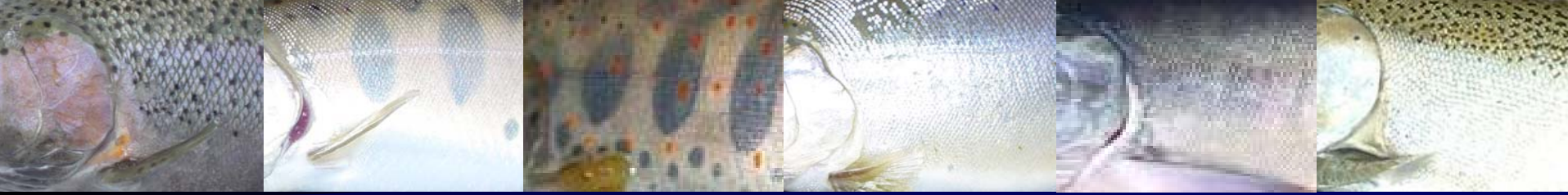

Quantitative Trait Loci (QTL) analysis and marker-assisted breeding for economical important trait in aquaculture

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National Research Institute of Aquaculture,
Fisheries Research Agency, Mie, Japan,

Target of genetic breeding

- **Disease resistance,**
- **Stress (situation) resistance**
- **Growth,**
- **Fecundity,**

Phenotype was dramatically changed in the history of selective breeding

Wild boar ⇒ Swine

（ Number of vertebra have changed to obtain quantity of meat ）

Wild rice ⇒ Domesticated rice

(high-yield, content of nutrient)

Marker-assisted selection (MAS) will change genetic breeding

- Control the phenotypes in genetic breeding using molecular markers associated with particular economic characters.
- A systematic method using the molecular landmark of genomic DNA.

Particular economical important trait in aquaculture

- Disease resistance,
- Stress (situation) resistance
- Growth,
- Fecundity,

The advantage of genetic breeding in aquaculture

- Number of progeny (fecundity) to do linkage analysis
- Variety of phenotype, most of the strain is not very long isolated from wild species

The information available to do the DNA marker assisted selection

- Analysis of phenome （ statistical genetics)
  The method to evaluate phenotype,
  Find the remarkable phenotype

- Analysis of Genome (molecular genetics)
  Marker and gene mapping,
  Construct the huge library,
  Construct database about genome information

Several species had constructed linkage map in aquaculture

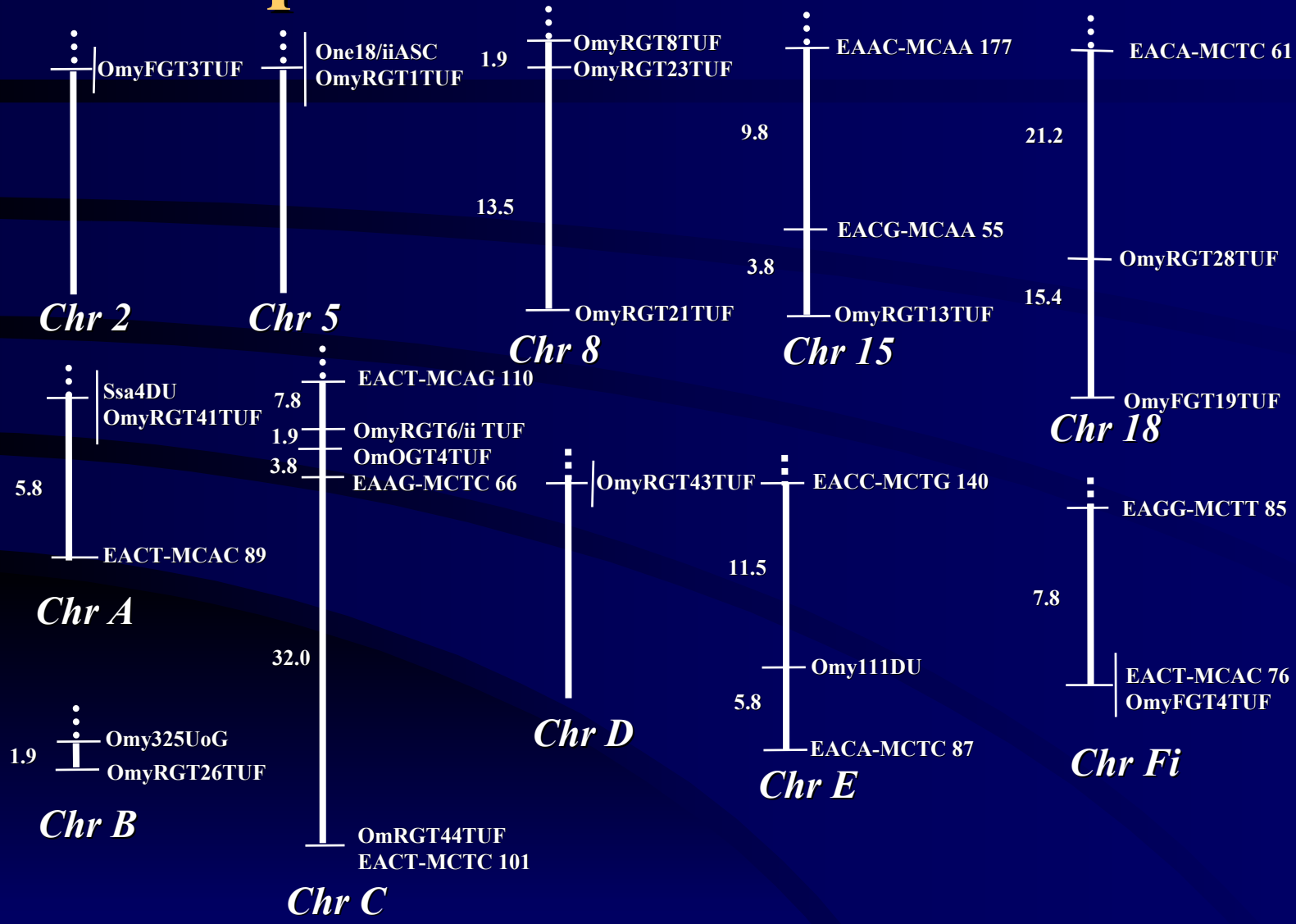
- **Rainbow trout (*Oncorhynchus mykiss*)**
- **Atlantic salmon (*Salmo salar*)**
- **Brown trout (*Salmo trutta*)**
- **Amago salmon*(Oncorhynchus masou ishikawae)***
- **Channel catfish *(Ictalurus punctatus)***
- **Thilapia (*Oreochromis niloticus*)(*Oreochromis aureus*)**
- **Common carp *(Cyprinus carpio L.)***
- **Japanese flounder (*Paralichthys olivaceus*)**
- **Yellowtail, Gold-striped amberjack*(Seriola quinqueradiata*)(*Seriola lalandi*)**
- **Tiger pufferfish *(Takifugu rubripes)***
- **Kuruma prawn (*Penaeus japonicus*)**
- **Black tiger shrimp(*Penaeus monodon*)**

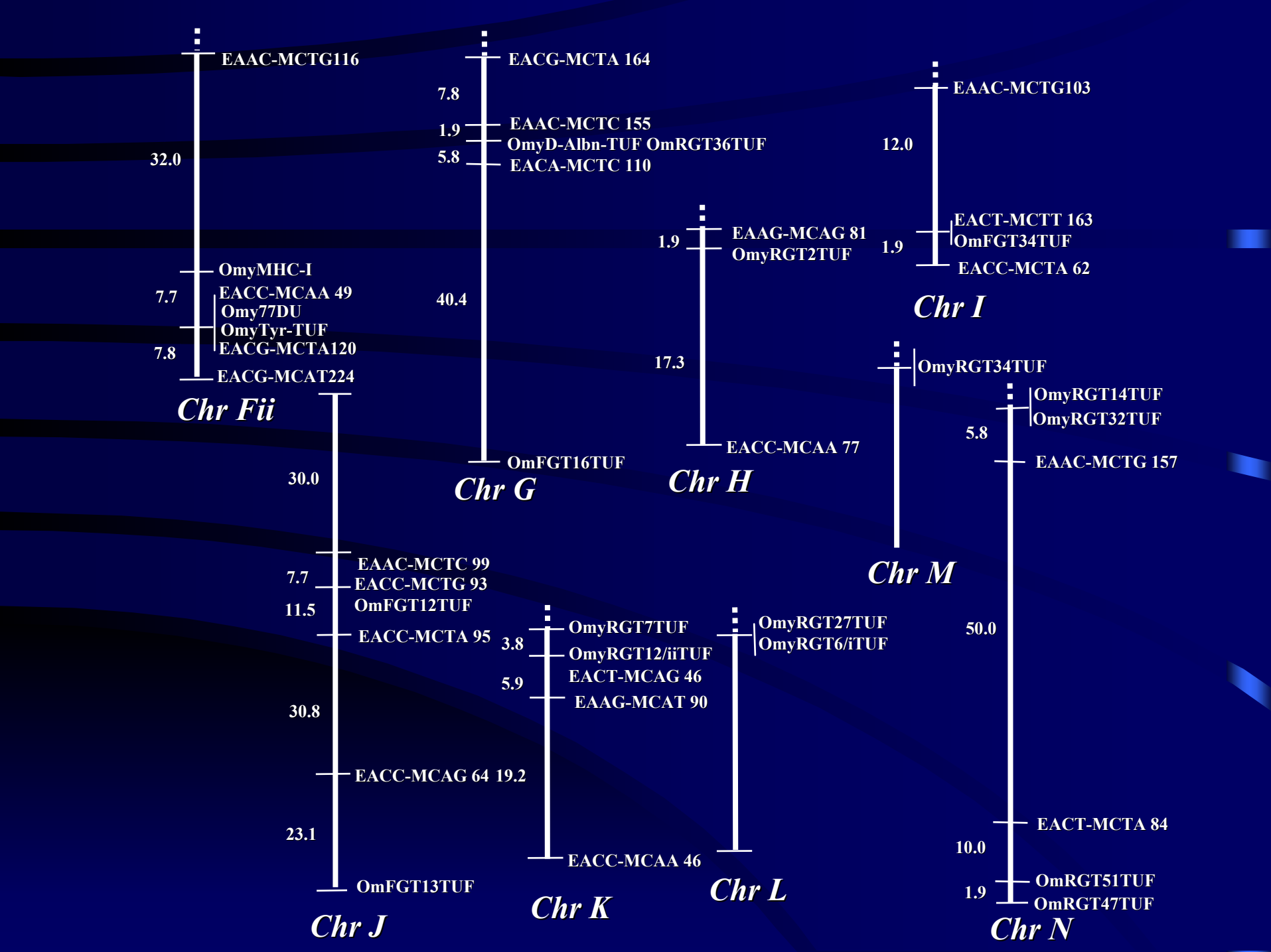
Genetics linkage map and DNA marker in salmonid species

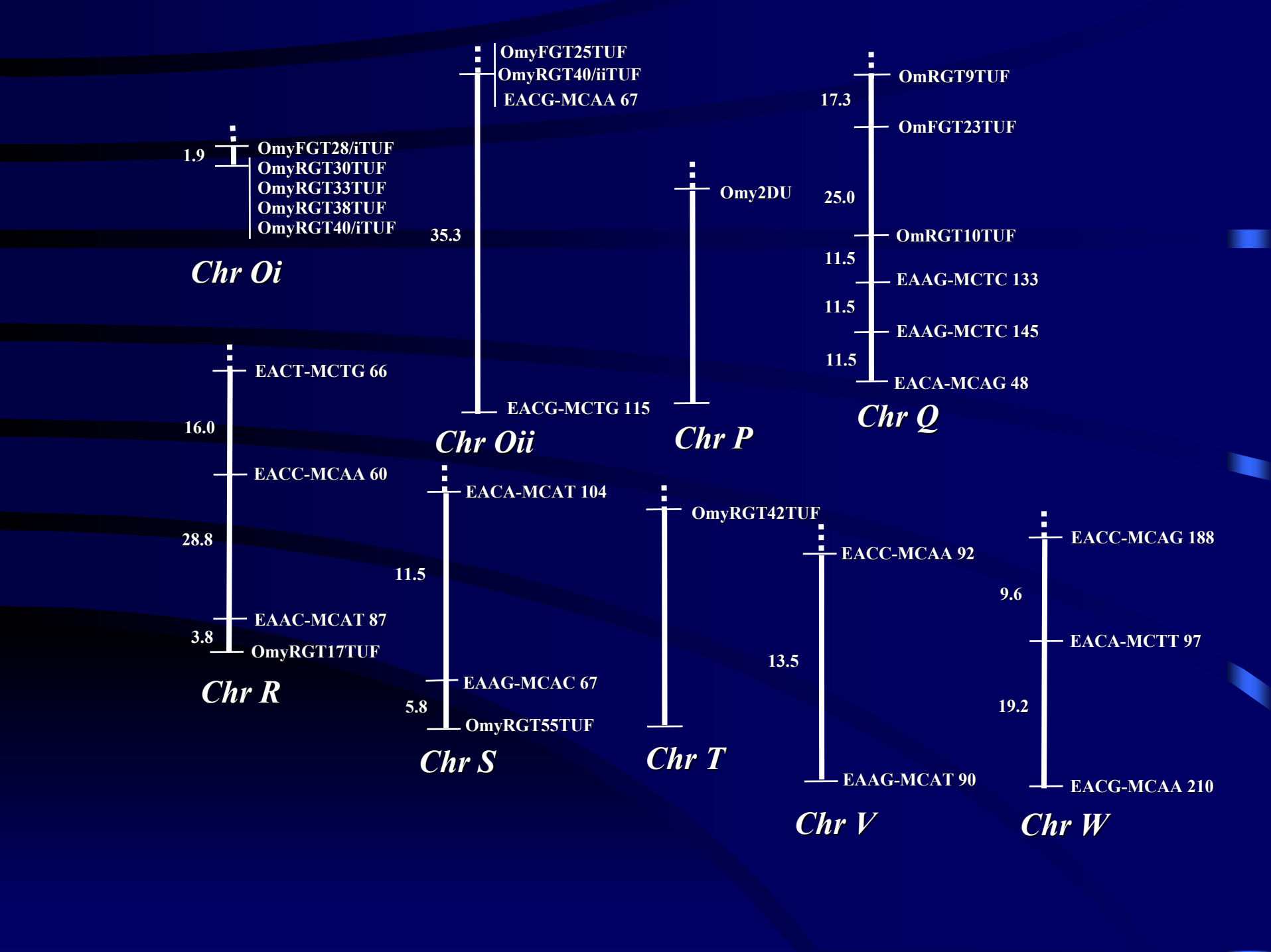
- **Available over one thousand microsatellite marker in salmonid species**

- **Linkage map is constructed in five species**
  Rainbow trout (*Onchorhynchus mykiss*)
  Atlantic salmon (*Salmo salar*)
  Brown trout (*Salmo trutta*)
  Arctic charr (*Salvelinus alpinus*)
  Amago salmon (*Onchorhynchus masou*)

Example of genetic linkage map in Rainbow trout




EAAC-MCTG116
32.0
OmyMHC-I
7.7
EACC-MCAA 49
Omy77DU
OmyTyr-TUF
7.8
EACG-MCTA120
EACG-MCAT224
Chr Fii
EACG-MCTA 164
7.8
EAAC-MCTC 155
1.9
OmyD-Albn-TUF OmRGT36TUF
5.8
EACA-MCTC 110
40.4
OmFGT16TUF
Chr G
EAAG-MCAG 81
1.9
OmyRGT2TUF
17.3
EACC-MCAA 77
Chr H
EAAC-MCTG103
12.0
EACT-MCTT 163
1.9
OmFGT34TUF
EACC-MCTA 62
Chr I
OmyRGT34TUF
Chr M
OmyRGT14TUF
OmyRGT32TUF
5.8
EAAC-MCTG 157
50.0
EACT-MCTA 84
10.0
OmRGT51TUF
1.9
OmRGT47TUF
Chr N
30.0
EAAC-MCTC 99
7.7
EACC-MCTG 93
11.5
OmFGT12TUF
EACC-MCTA 95
30.8
EACC-MCAG 64
19.2
23.1
OmFGT13TUF
Chr J
OmyRGT7TUF
3.8
OmyRGT12/iiTUF
EACT-MCAG 46
5.9
EAAG-MCAT 90
EACC-MCAA 46
Chr K
OmyRGT27TUF
OmyRGT6/iTUF
Chr L


OmyFGT25TUF
OmyRGT40/iiTUF
EACG-MCAA 67
1.9
OmyFGT28/iTUF
OmyRGT30TUF
OmyRGT33TUF
OmyRGT38TUF
OmyRGT40/iTUF
35.3
Chr Oi
EACG-MCTG 115
Chr Oii
Omy2DU
Chr P
OmRGT9TUF
17.3
OmFGT23TUF
25.0
OmRGT10TUF
11.5
EAAG-MCTC 133
11.5
EAAG-MCTC 145
11.5
EACA-MCAG 48
Chr Q
EACT-MCTG 66
16.0
EACC-MCAA 60
28.8
EAAC-MCAT 87
3.8
OmyRGT17TUF
Chr R
EACA-MCAT 104
11.5
EAAG-MCAC 67
5.8
OmyRGT55TUF
Chr S
OmyRGT42TUF
Chr T
EACC-MCAA 92
13.5
EAAG-MCAT 90
Chr V
EACC-MCAG 188
9.6
EACA-MCTT 97
19.2
EACG-MCAA 210
Chr W

Candidate loci associated with various phenotype in aquaculture

- **Rainbow trout** (***Oncorhynchus mykiss***)
  - resistance to Infectious Pancreatic Necrosis [IPN]
  - resistance to Infectious haematopoietic necrosis [IHN]
  - resistance to Ceratomyxa shasta
  - upper temperature tolerance
  - spawning time
  - embryonic development rate
  - dominant albino locus
- **Amago salmon** (***Oncorhynchus masou ishikawae***)
  - Osmotic regulation
- **Atlantic salmon** (***Salmo salar***)
  - resistance to Cryptobia salmositica
- **Arctic char** (***Salvelinus alpinus***)
  - upper temperature tolerance
- **Thilapia** (***Oreochromis niloticus***)
  - lower temperature tolerance
- **Japanese flounder** (***Paralichthys olivaceus***)
  - resistance to lymphocystis disease

Subject

Study 1.

Quantitative Trait Loci (QTL) associated with resistance to Infectious Pancreatic Necrosis [IPN] in Rainbow trout (*Oncorhynchis mykiss*)

Study 2.

Quantitative Trait Loci (QTL) associated with resistance to Infectious Haematopoietic Necrosis [IHN] in Rainbow trout (*Oncorhynchis mykiss*)

Study3.

Quantitative Trait Loci (QTL) associated with resistance to *Cryptobia Salmositica* disease in Atlantic salmon (*Salmo salar*)

Study4.

Pilot examination of Marker-Assisted Selection (MAS) in IPN resistance family

Study 1.
Quantitative Trait Loci (QTL) associated with resistance to Infectious Pancreatic Necrosis [IPN] in rainbow trout (*Oncorhynchis mykiss*)

Rainbow trout infected with IPN

Affected fish showed IPN-typical signs: abnormal behavior, color changing black, abdominal bulge, and exophthalmos

Target trait and strain

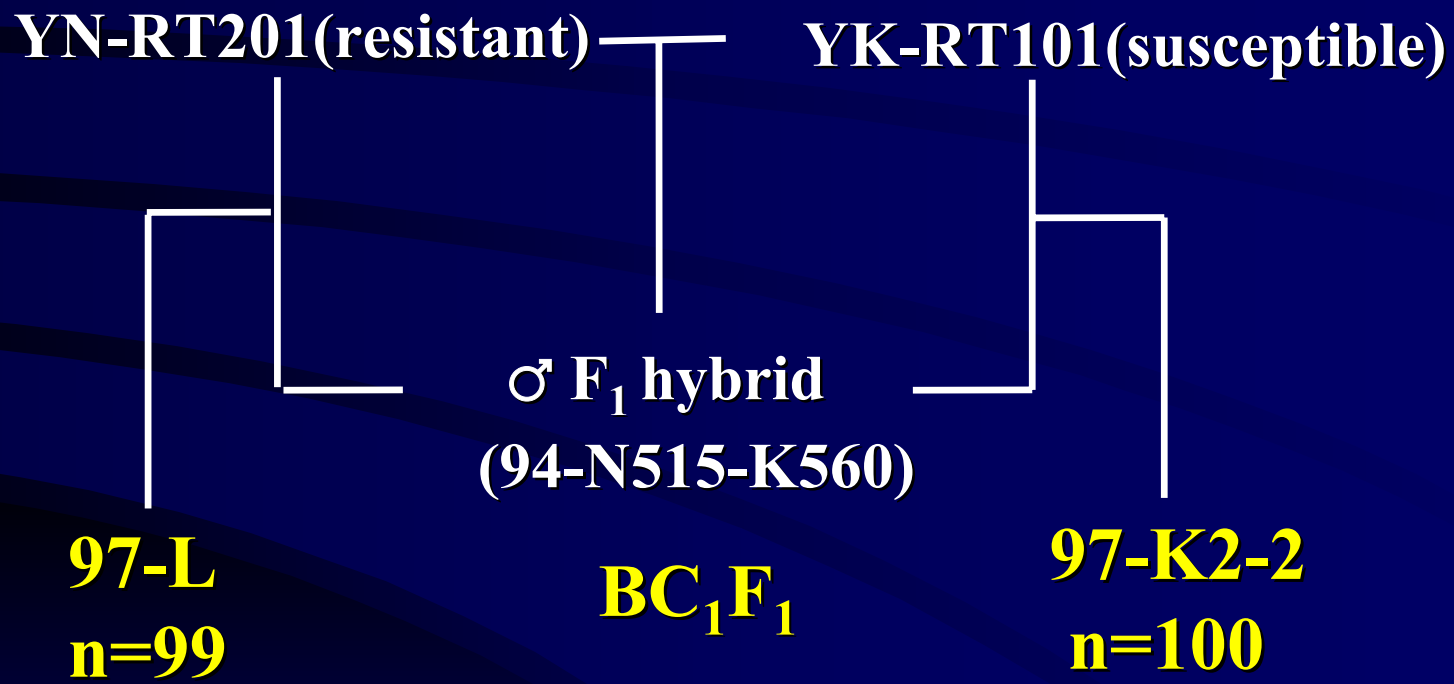
Resistance to Infectious Pancreatic Necrosis (IPN) in rainbow trout

IPN resistant strain (YN-RT201)
IPN high susceptible strain (YK-RT101)

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

lot(year/month)	YN-RT201		YK-RT101	
1979, 6	10.0%		95.0%	
12	1.7		95.0	
1980, 6	3.3		86.7	
9	3.3		98.3	
12	3.3		100	
1981, 6	23.3		100	
9	3.3		100	
12	1.7		95.0	
1982, 6	6.7		85.0	
9	1.7		100	
12	3.3		100	
1983, 6	0		98.3	
9	1.7		100	
12	15.0		100	
1984, 6	0		100	
12	0		100	
1986, 6	5.0		85.0	
9	0		100	
12	0	mean	98.3	mean
1989, 2	5.0	4.4%	86.0	96.1%

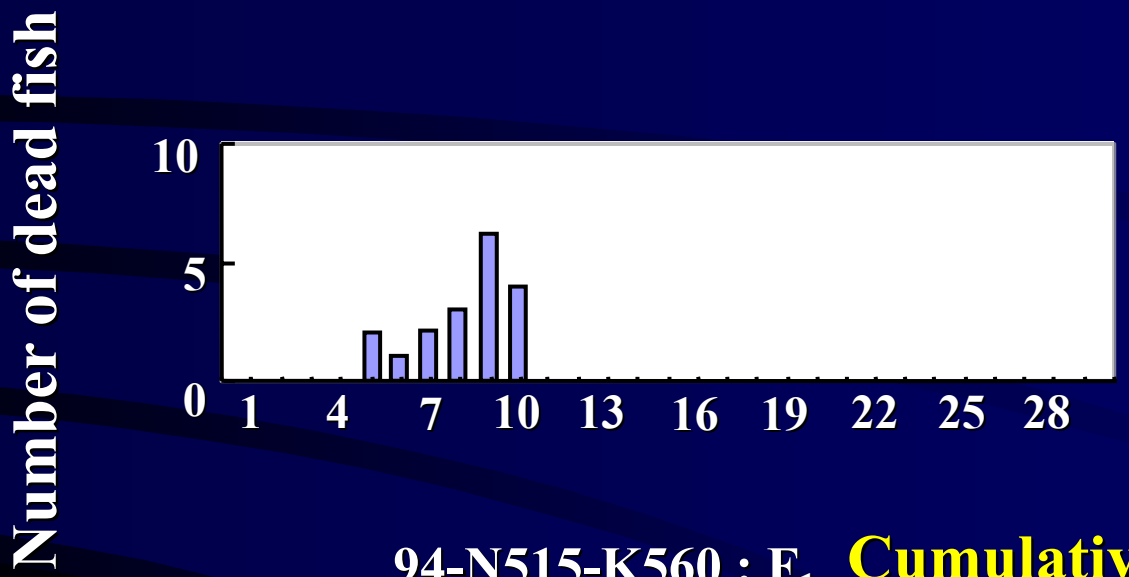
Schematic representation of the rainbow trout population used in this study



Condition of IPN infection experiment

- Virus strain IPNV. Buhl, Passage20 times
- Virus titer $10^{5.0}$ $TCID_{50}$/ml
- Fish weight average 0.15g
- Temperature 15±1℃
- Experiment term 30 days

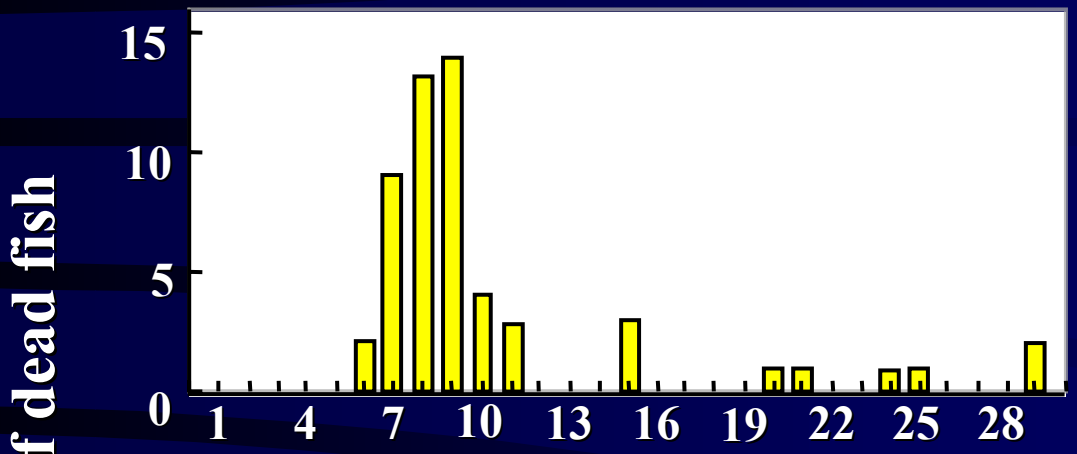
Frequency distribution of infection experiment



Dead : n=18
Survivors : n=32
Total n=50

94-N515-K560 : F_1 **Cumulative mortality: 36%**

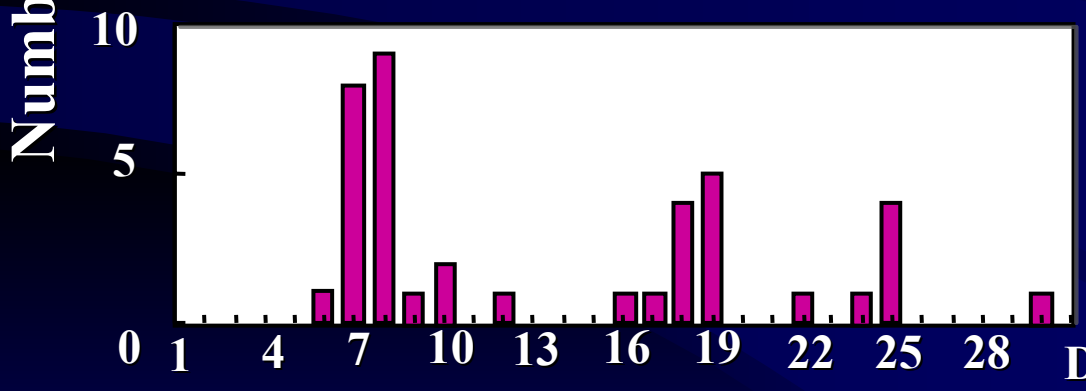
Frequency distribution of infection experiment



Dead : n=54
Survivors : n=46
Total n=100

97-K2-2 : Backcross family

Cumulative mortality: 54%



Dead : n=40
Survivors : n=59
Total n=99

97-L : Backcross family

Cumulative mortality: 40.4%

Estimated effects of suggestive and significant QTL with IPN disease susceptibility

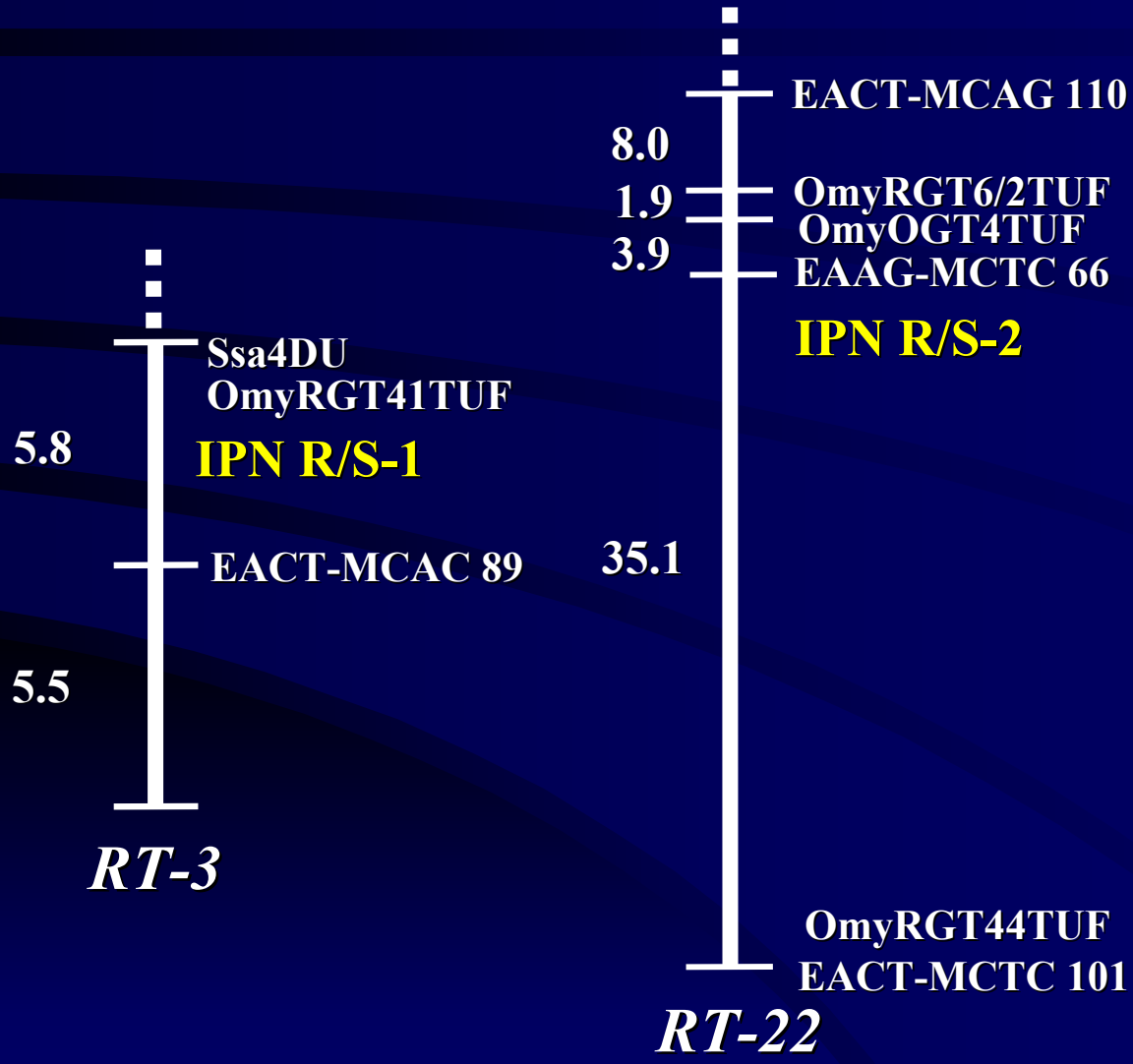
markers {chr}	QTL	family	LOD	χ^2 (1 d.f.)	R^2(%)	*P* value
EACA-MCTC58 {31}		97-K2-2	2.2*	10.0	9	0.00155
EACT-MCAC89 {3}	IPN R/S-1	97-K2-2	2.7*	12.4	11	0.00043
OmyRGT41TUF {3}		97-K2-2	3.6**	16.7	15	0.000043
Ssa4 DU{3}		97-K2-2	3.6**	16.7	15	0.000043
EACT-MCAG110 {22}	IPN R/S-2	97-K2-2	3.1*	14.2	13	0.00016
OmyRGT6TUF {22}		97-K2-2	2.8*	13.1	11	0.00029
OmyOGT4TUF {22}		97-K2-2	3.3**	14.8	13	0.00012
EAAG-MCTC66 {22}		97-K2-2	3.2*	14.8	13	0.00012
Omy2DU {11}		97-K2-2	1.1	5.3	8	0.02128
OmyFGT3TUF {29}		97-L	1.1	5.2	8	0.02253
EACC-MCTG66 {29}		97-L	1.5	6.7	10	0.00947
EAAC-MCTT122 {29}		97-L	2.6*	10.4	11	0.00125
EAAC-MCTC115 {11}		97-L	1.3	6.2	8	0.01258
Omy111DU {11}		97-L	1.2	5.5	8	0.01925
EAAG-MCAG231{12}	IPN R/S-3	97-L	3.6**	16.7	15	0.000045
OmyRGT27TUF {17}		97-L	1.1	5.2	8	0.02253
EAAC-MCAA199 {23}		97-L	1.4	6.6	10	0.00993

* = Suggestive linkage : $P < 3.3\times10^{-3}$, LOD score>1.9

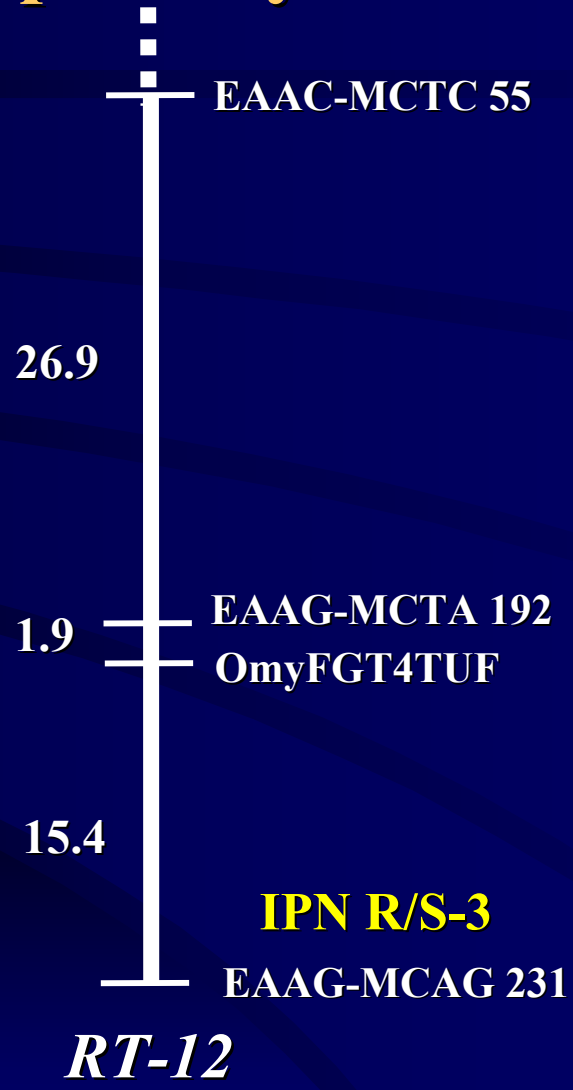
** = Significant linkage : $P < 1.0\times10^{-4}$, LOD score>3.3

(E. Lander and L. Kruglyak, *Nat Genet* 11, 241 ,1995) QTL mapping in backcross family

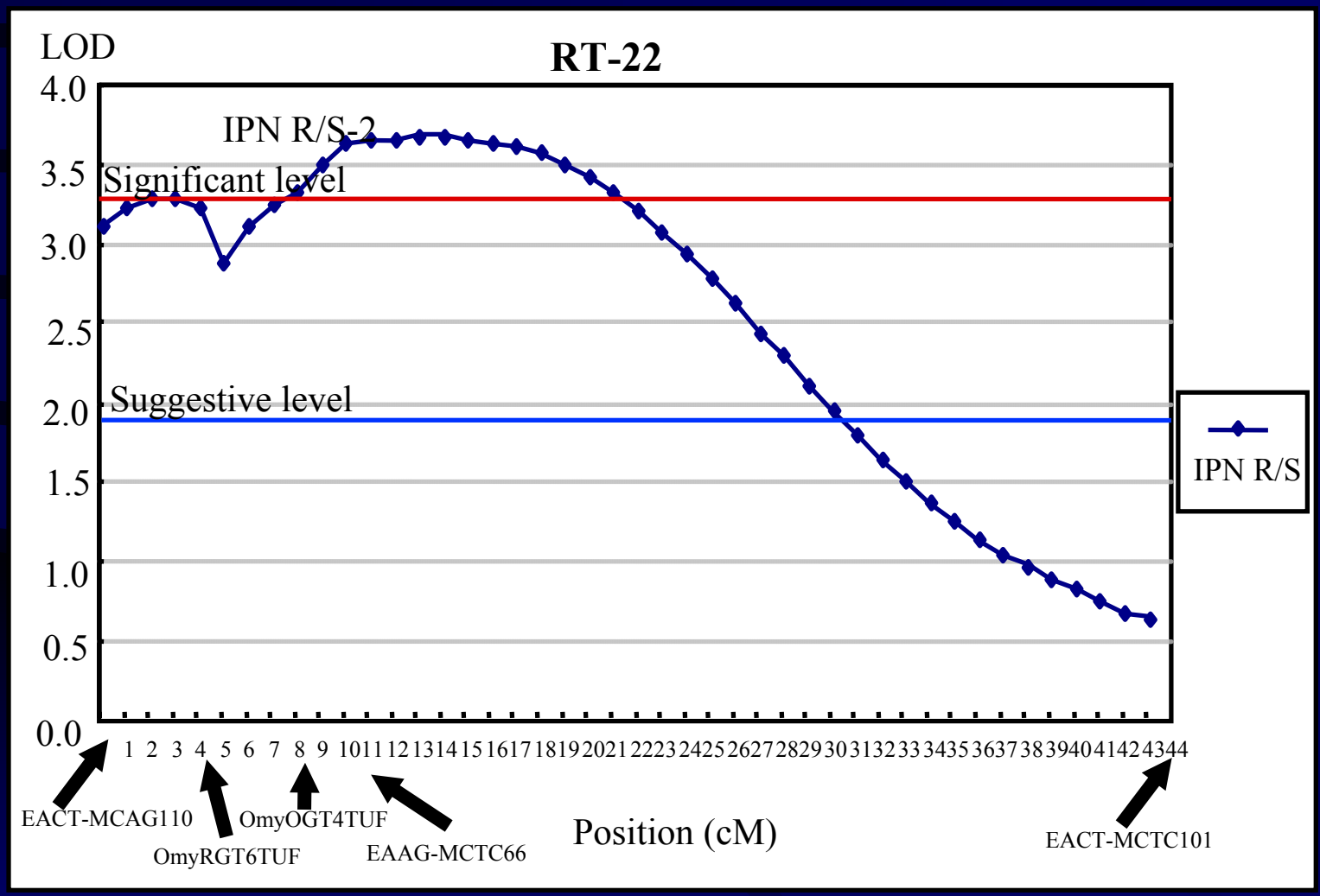
Mapping of QTL associated with IPN resistance/susceptibility in 97-K2-2 family



Mapping of QTL associated with IPN resistance/susceptibility in 97-L family



Interval mapping in QTL regions (*RT-22*)



Linkage analysis of QTL associated with IPN resistance in rainbow trout

- **Identified three QTL associated with IPN resistance**
  - **Significant loci**

    IPN R/S-1: OmyRGT41TUF and Ssa4DU,

    located on *RT-3*

    IPN R/S-2: OmyOGT4TUF and OmyRGT6/iiTUF,

    located on *RT-22*

    IPN R/S-3: EAAG-MCAG231;

    located on *RT-12*
  - **Suggestive loci**

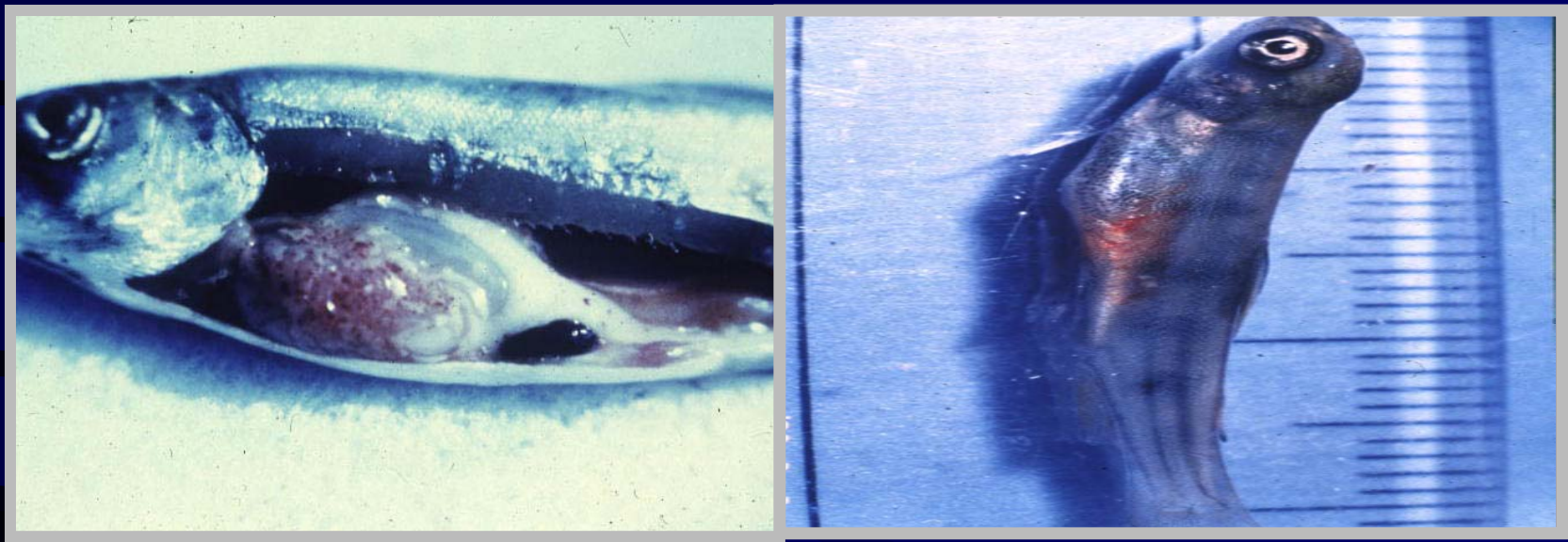
    EACA-MCTC 58, located on *RT-31*

    EAAC-MCTC 115, located on *RT-29*

(A. Ozaki *et al.*, 2001 *Mol Genet Genomics*)

Study 2.
Quantitative Trait Loci (QTL) associated with resistance to Infectious Haematopoietic Necrosis [IHN] in rainbow trout (*Oncorhynchis mykiss*)

Salmonid infected with IHN



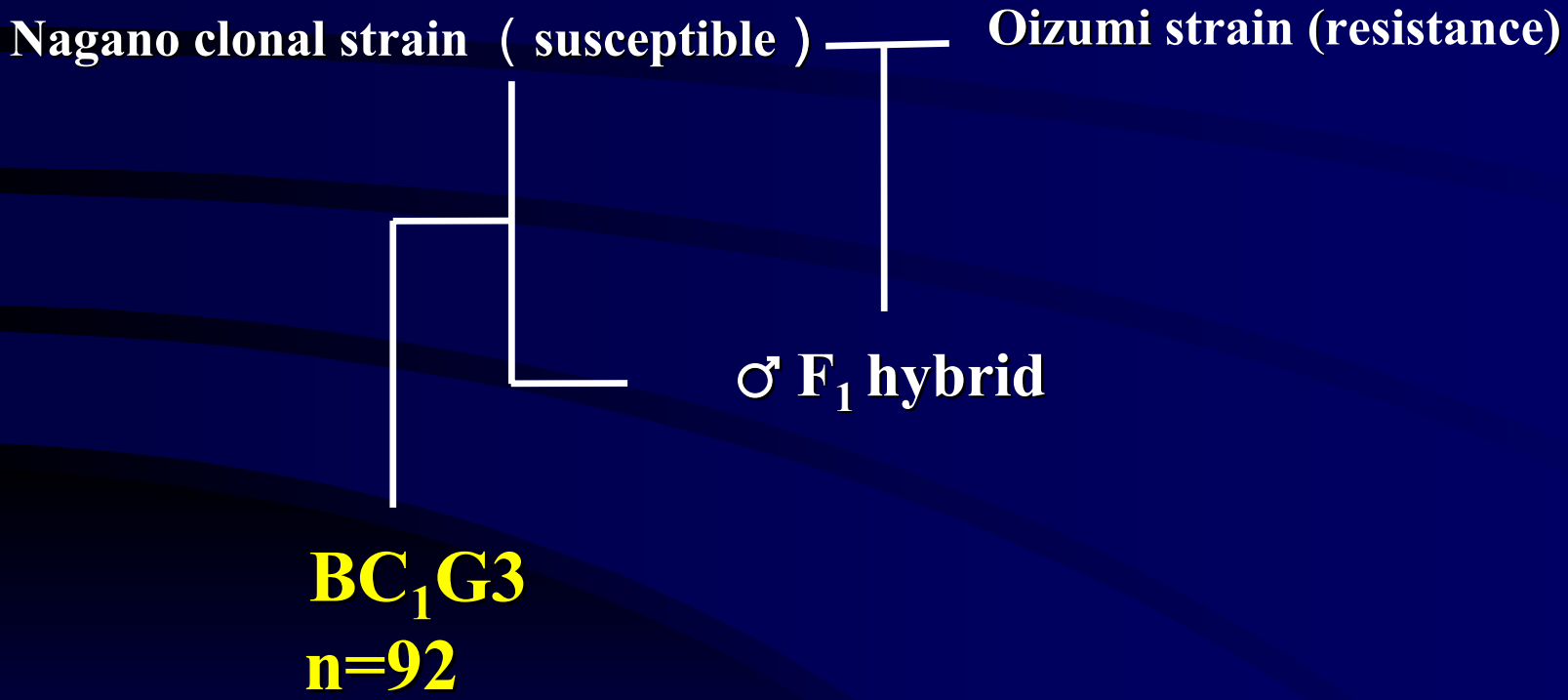
Affected fish showed IHN-typical signs: abnormal behavior, bleeding body side, abdominal bulge, and exophthalmos

Target trait

Resistance to Infectious Haematopoietic Necrosis (IHN) in rainbow trout

IHN relatively high resistance strain (Oizumi)
IHN high susceptible strain (Nagano clonal line)

Schematic representation of the rainbow trout population used in this study



Condition of IHN infection experiment

- Virus strain IHNV.
  Passage17 times
- Virus titer $10^{4.0}$ $TCID_{50}$/ml
- Fish weight average 2.5±0.5 g
- Temperature 12±1℃
- Experiment term 30 days

Linkage analysis of QTL associated with IHN resistance in rainbow trout

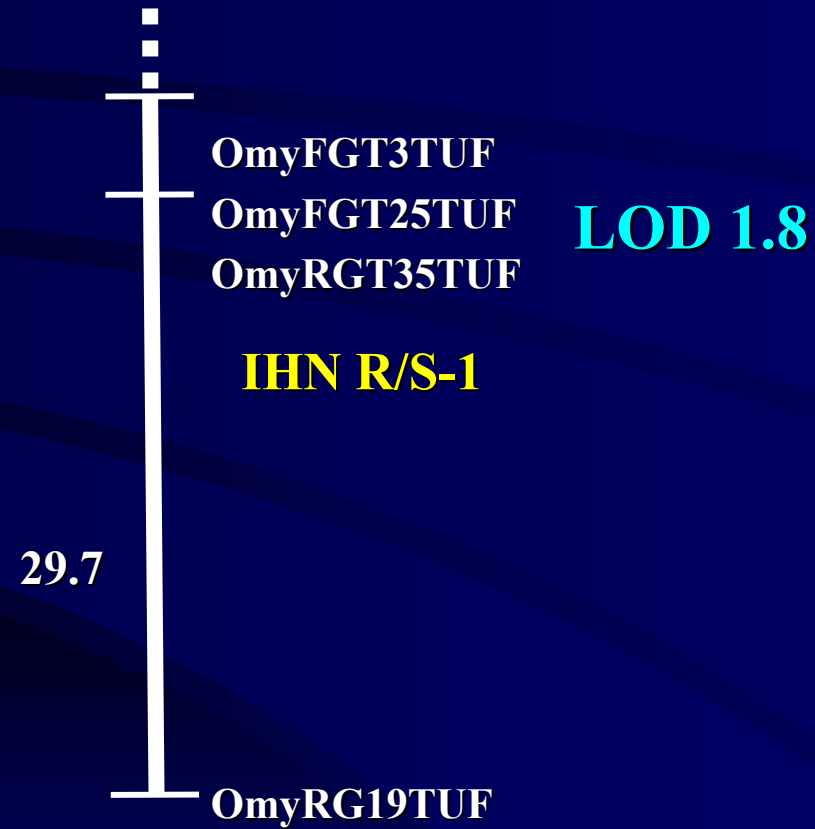
- **Identified QTL associated with IHN resistance**
  - **Suggestive loci**

OmyFGT3TUF, OmyFGT25TUF, OmyRGT35TUF

IHN R/S-1 located on *RT-29*

(Sok kean Khoo *et al.*, 2004 *Fish Pathology*)

Mapping of QTLs associated with IHN resistance/susceptibility in BC_1G3 family



Study 4.
Pilot examination of marker-assisted selection in IPN resistance family

Next step strategy for genetic breeding, after estimated location of candidate loci associated with phenotype

- Importance of applied genetics and genetic breeding . Using information of candidate QTL region which control the phenotype. perform selective breeding to improve breeding value.

- Using molecular marker, easy to distinguish each allele associated with candidate loci in those strain. To check the marker loci genotype , we can know the phenotypic character in those offspring

Idea of Marker-Assisted Selection [MAS] has proposed before the development of DNA marker

- **Crow JF (1957)**

  In Drosophila, DDT resistance/susceptibility had changed depend on combination of chromosome

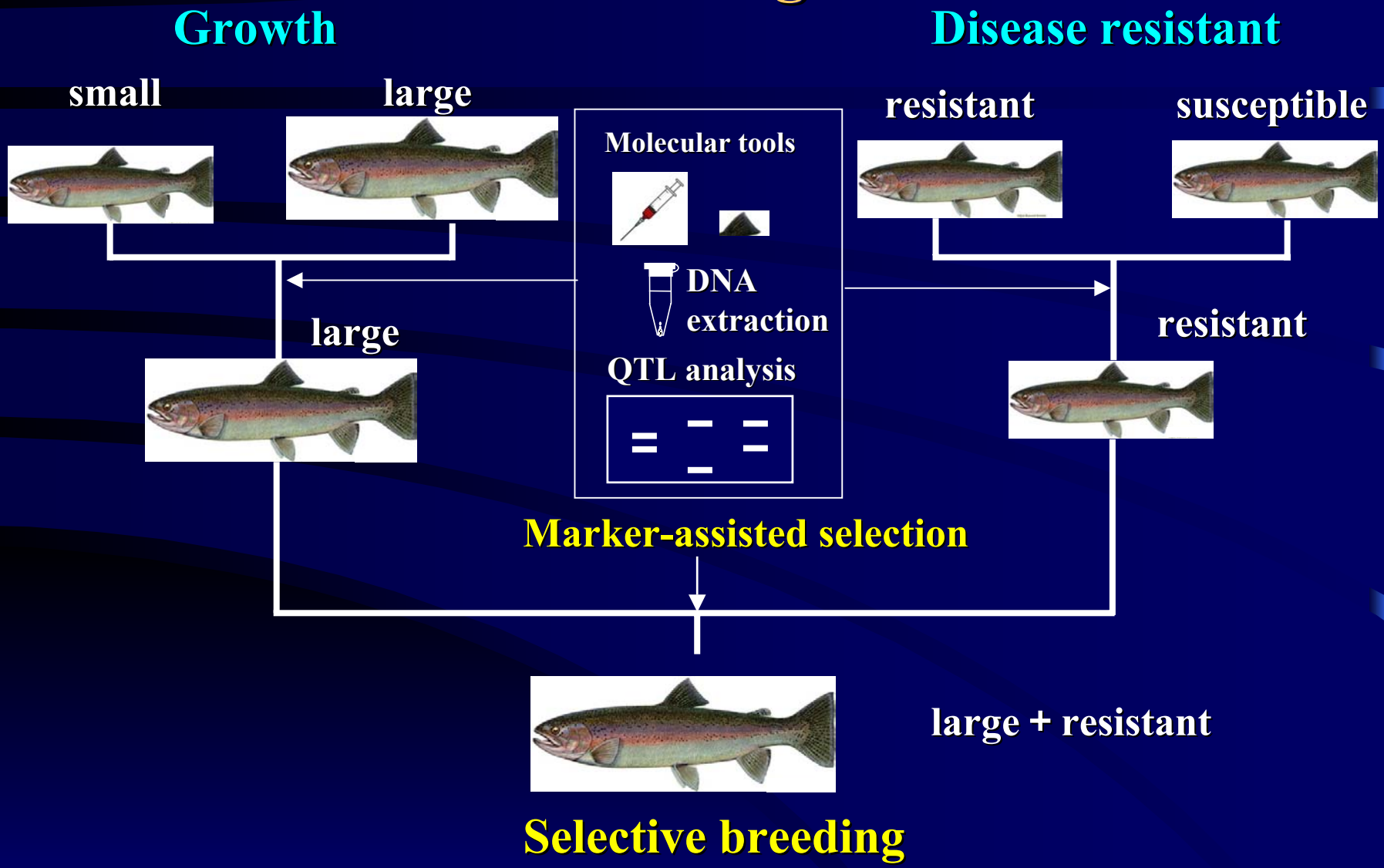
- **Neimann-Sorensen A, Robertson A (1961)**

  In milk cow, try to improve phenotype about amount of milk .Using difference of blood type.

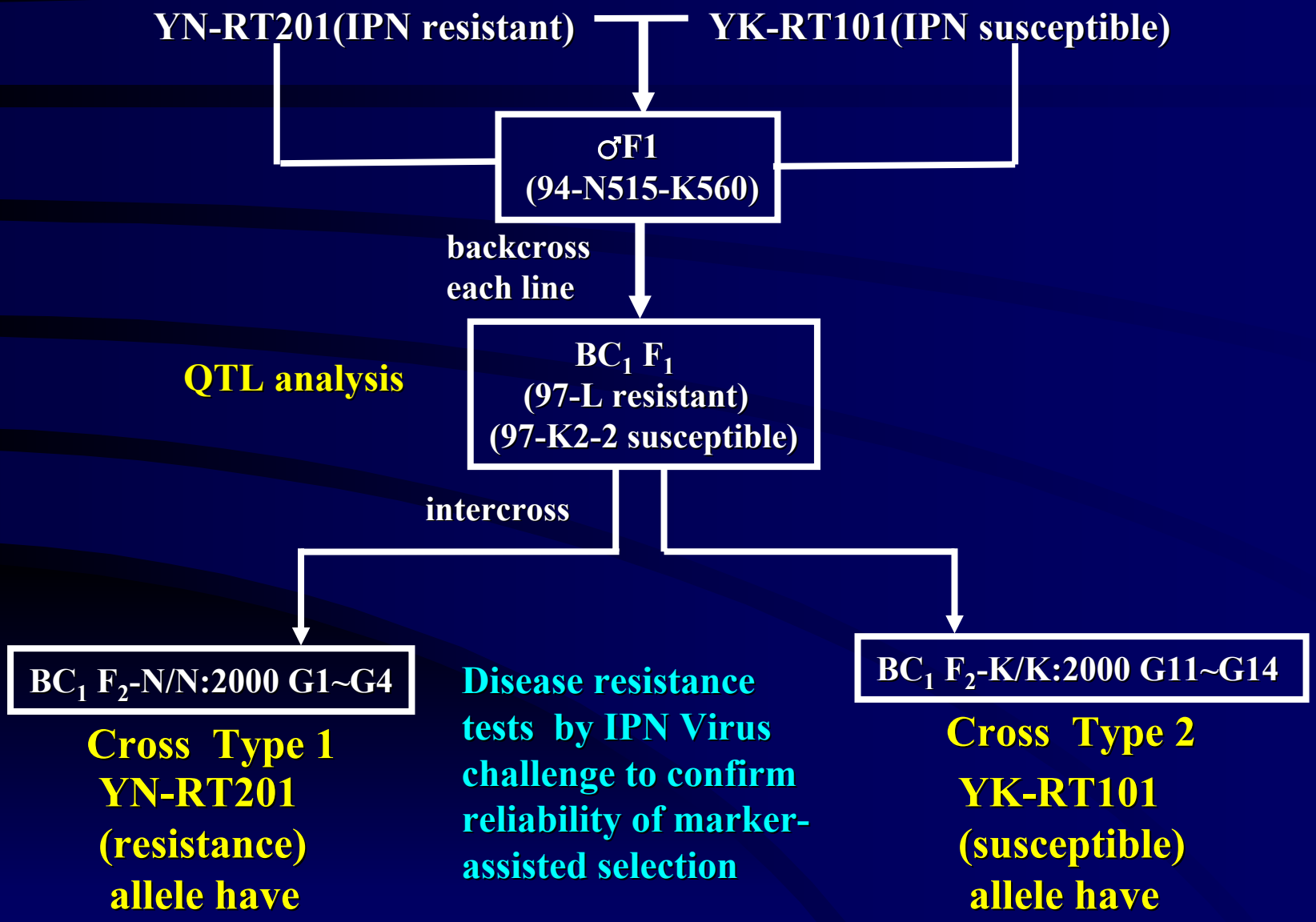
- **Lande R, Thompson R (1990)**

  Advance a suggestion to use DNA markers, MAS becomes general idea.

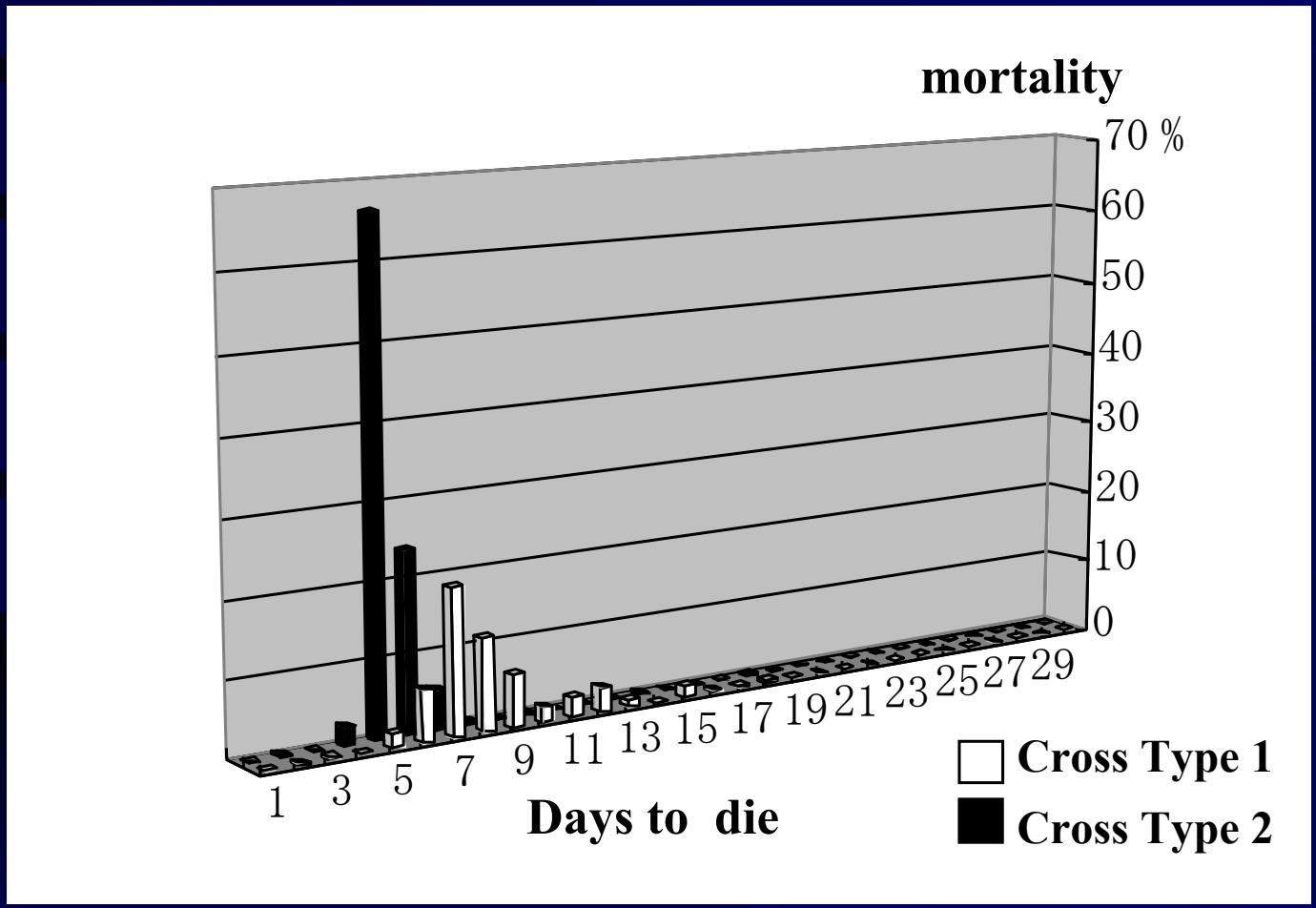
Marker-assisted selection for genetic breeding



Pilot examination of marker-assisted selection due to IPN R/S locus



Frequency distribution of infection experiment



Marker-assisted selection due to IPN R/S

Left : Cross Type 1 Right : Cross Type 2

5 days post-artificially induced infection

Marker-assisted selection in IPN resistance BC_1 F_2 family

IPN R/S-1	Family name of BC_1F_2		Cumulative mortality
Cross Type 1 YN-RT201 (resistance) allele have	BC_1 F_2-N/K:2000		
	G1,	n=100	64%
	G2,	n=102	53%
	G3,	n=98	58%
	G4,	n=98	60%
	total,	n= 398	Ave. 58.7%
Cross Type 2 YK-RT101 (susceptible) allele have	BC_1 F_2-K/K:2000		
	G11,	n=101	100%
	G12,	n=101	99%
	G13,	n=100	98%
	G14,	n=100	98%
	toal,	n=404	Ave. 98.7%

CONCLUDING REMARKS

- **Study 1**

  Identified three significant loci and two suggestive loci associated with resistance to IPN in Rainbow trout

- **Study 2**

  Identified one suggestive loci associated with resistance to IHN in Rainbow trout

- **Study 3**

  Identified three suggestive loci associated with resistance to *Cryptobia Salmositica* disease in Atlantic salmon

- **Study 4**

  confirmed reliability of Marker-Assisted Selection (MAS) in IPN resistance family

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