

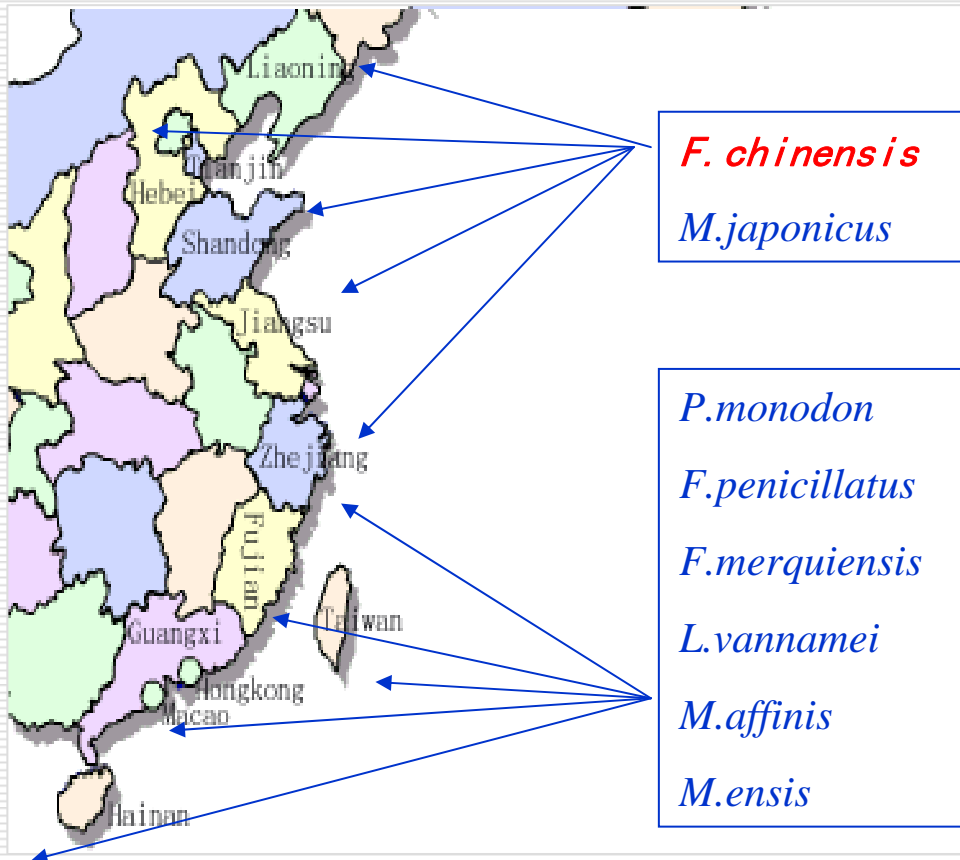
Exploitation and Application of  
DNA Markers in the Breeding  
Program of Chinese Fleishy Shrimp,  
*Fenneropenaeus chinensis*

*Kong Jie*

*Yellow Sea Fisheries Research Institute, CAFS*

# Distribution of Farming Species

---



Aproximately 10 species are cultured in China. There are mainly 2 species cultured in northern China. There are more than 6 species cultured in southern China because of the warm climate.

# Chinese fleshy shrimp?

---

*Peneaus orientalis* 东方对虾

(Osbeck, 1765)

*Peneaus chinensis* 中国对虾

(Liu, 1986)

*Fenneropeneaus chinensis* 中国明对虾

(Perez Farfante and Kensley, 1997)

---

# *What* has happened?

---



- ❑ Fishery, the landings reached 40 000 MT in an autumn fishing season from the Bohai Sea and reduced to a few hundred tons today.  
海捕产量由4万吨减少到百吨。
  - ❑ Farming, annual harvest has been of some 150 000 MT. Because of the WSSV, *F. chinensis* farming production has been reduced to 50 000MT since 1993.  
养殖产量由15万吨减少到5万吨。
-

# Genetic Breeding– fast growth

- ❑ Duration 1997-2003,
- ❑ Mass selection: The best for growth were selected from one stock generation after generation
- ❑ New variety, **Huanghai-No.1.**
- ❑ The body weight was increased by 26% at harvest.



经历1997–2003年，采用群体选育，选择生长表现优秀的留种。新品种命名“黄海1号”，收获体重提高26%。

# Genetic Breeding– WSSV Resistance

---

- ❑ Duration 1998-2009,
- ❑ Mass selection+BLUP: The best for growth and surviving the WSSV infection were selected
- ❑ New variety, **Huanghai-No.2.**
- ❑ The survival was increased by 15% in the challenging test.

经历1998–2009年，采用群体选育+多性状复合育种技术，选择生长+抗病表现优秀的留种。新品种命名“黄海2号”，感染存活率提高15%。

---

# DNA Markers for ???

---

- For base population construction, to evaluate the genetic variation of different stocks, populations, etc. 为构建基础群体，评估遗传结构。
  - For tagging, to differentiate different groups, families, individual, etc. 群组、家系、个体识别。
  - For traits' marking, to find trait(s), e.g. disease resistance, genetically linkaged markers. 与性状连锁的遗传标记。
-

# Methods

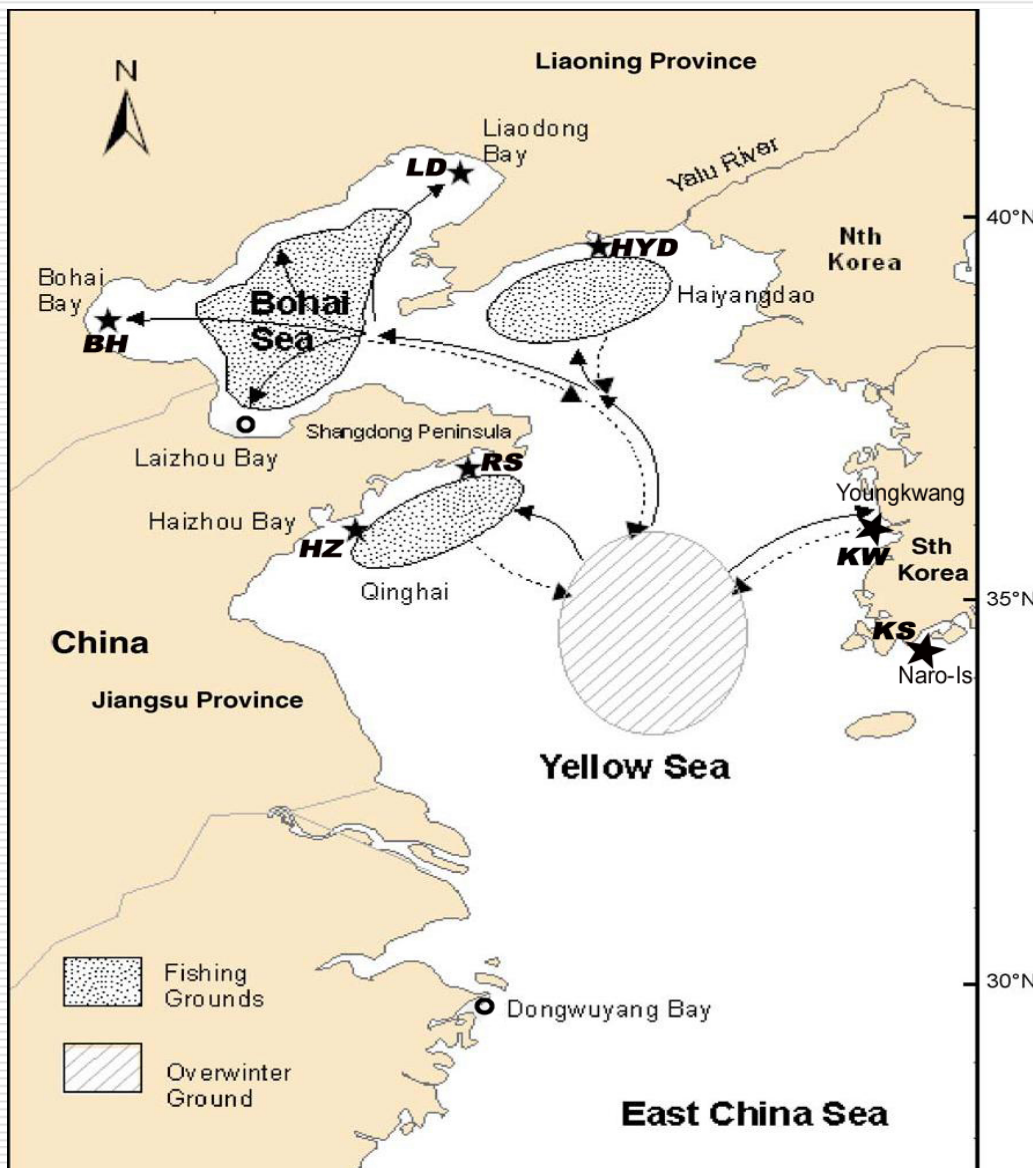
---

- ❑ Random Amplified Polymorphism of DNA (RAPD)
  - ❑ Simple Sequence Repeats (SSR)
  - ❑ Amplified Fragment Length Polymorphism (AFLP)
-

---

Genetic variation of *F. chinensis*  
based on RAPD  
遗传结构分析

---



## Designation:

各群体命名

**LD:** Liaodong Bay

**BH:** Bohai Bay

**HZ:** Haizhou Bay

**RS:** Rushan Bay

**HYD:** Haiyangdao Bay

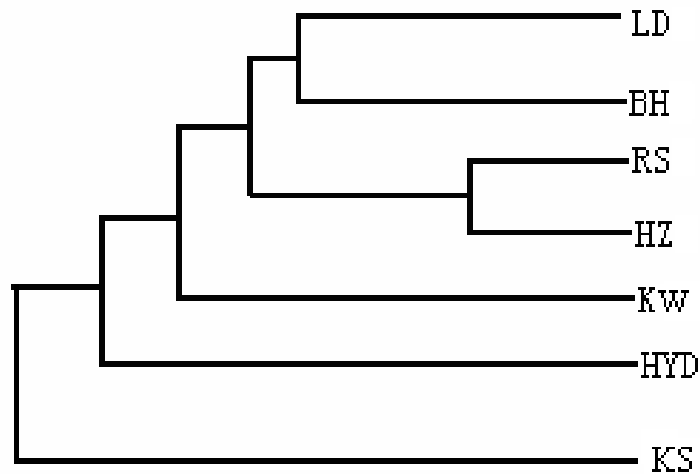
**KW:** West coast of  
Korean Peninsula

**KS:** South coast of  
Korean Peninsula

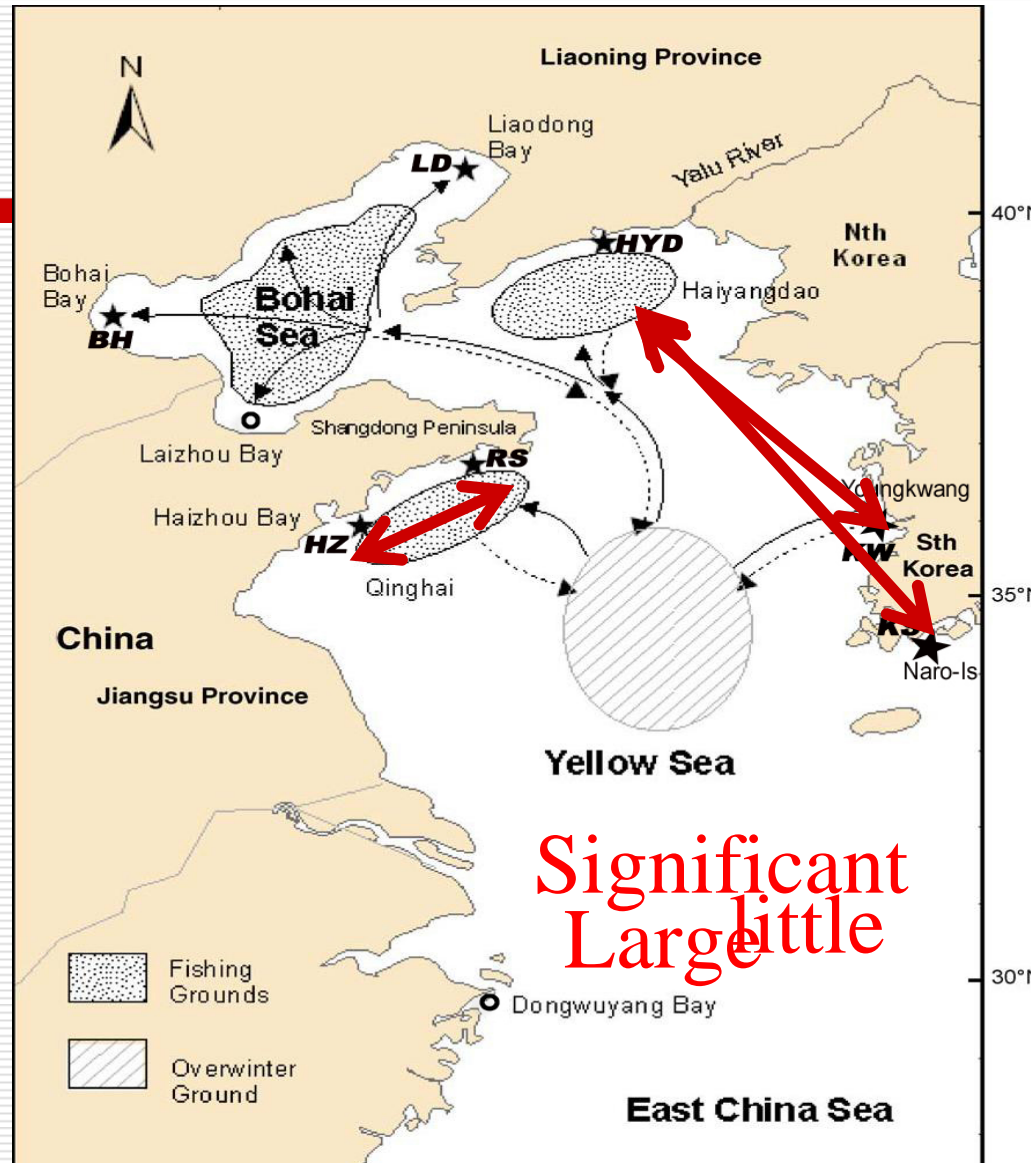
# Genetic differentiation based on $G_{st}$

Populations	LD	BH	HZ	RS	HYD	KW	KS
LD	0.000						
BH	0.064	0.000					
HZ	0.083	0.120	0.000				
RS	0.108	0.135	0.032	0.000			
HYD	0.185	0.199	0.164	0.159	0.000		
KW	0.153	0.196	0.128	0.123	0.233	0.000	
KS	0.209	0.207	0.199	0.184	0.271	0.145	0.000

(Note: Differentiation based on  $G_{st}$ : 0-0.05, **little**; 0.05-0.15, moderate; 0.15-0.25, **large**; more than 0.25, **significant**(Wright, 1978; Buso, 1998))



Cluster analysis by UPGMA of the seven *F. chinensis* stocks



# Conclusion

---

-multiple populations of *F. chinensis*

- Stocks along the China coast
- Haiyangdao stock
- West coast of Korea peninsula
- South coast of Korea peninsula



More informative than that of tagging-recapture results (Deng et al., 1990)

---

# Composite (“Synthetic”) Base Population

---

- domesticated stocks
  - selected for growth
  - selected for WSSV-resistance

- wild stocks
  - Korea South Sea
  - China (4 stocks)



---

Pedigree tracing  
based on SSR

系谱识别

---

# Triplex PCR (三重PCR):

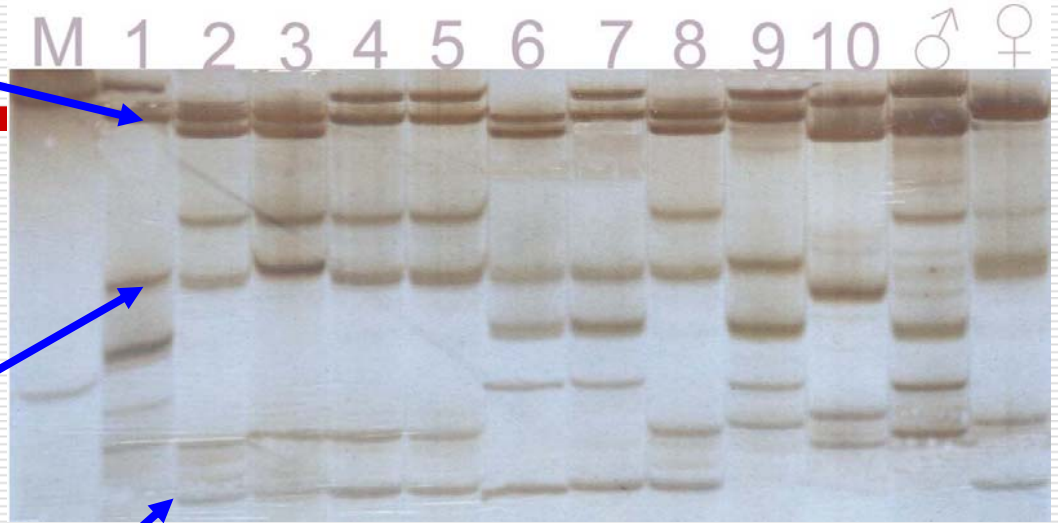
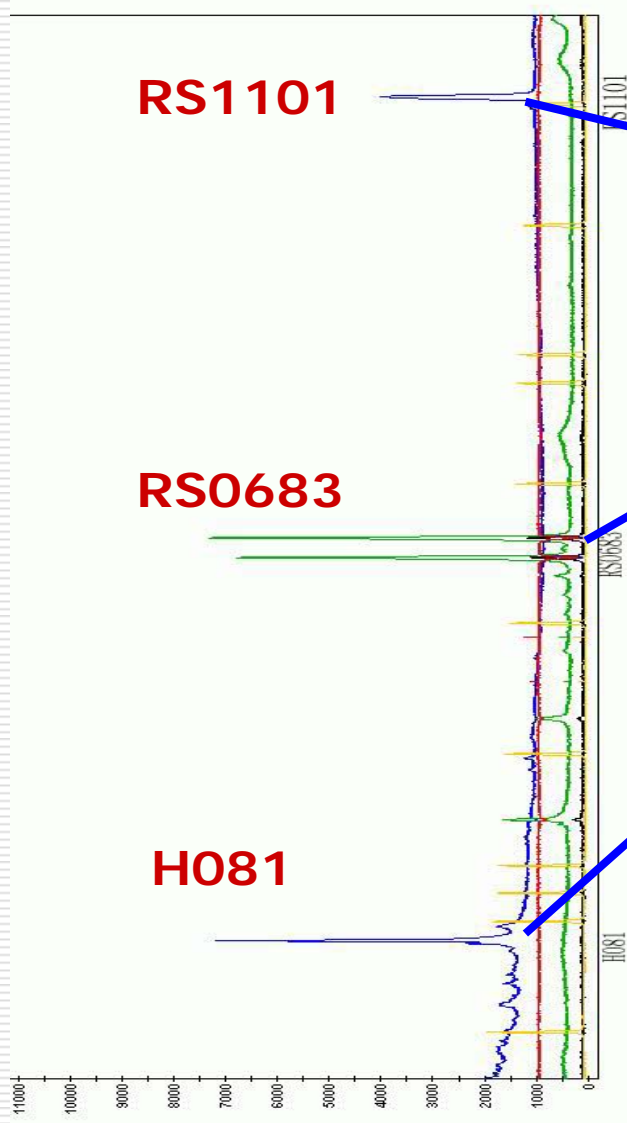


Fig. amplification results of the triplex PCR in the parentage 35191

Genescan results of the triplex PCR

## Data of triplex PCR基本数据

---

SSR locus	<i>H<sub>o</sub></i>	<i>H<sub>e</sub></i>	<i>PIC</i>	<i>PPE</i>	<i>CPPE</i>
RS1101	0.767	0.747	0.8356	0.8224	0.5221
RS0683	0.80	0.8417	0.8010	0.9255	0.7127
H081	0.933	0.9203	0.8637	0.9491	0.7664

**Note:**

***H<sub>o</sub>*:** observed heterozygosity

***H<sub>e</sub>*:** expected heterozygosity

***PPE*:** Probability of paternity exclusion (Odelberg and White, 1990)

***CPPE*:** Cumulate probabilities of paternity exclusion (Wang, You et al., 2004)

---

The PPE of this triplex PCR is 0.967 9,  
and the discrimination power is 0.  
999327.

# Families to be traced

Six female were mated by 6 male. Only two of them were artificially mated. 145 offspring, their 12 parents and grand parents were tested. 两个家系的父母混合养殖、交配产生混合后代。

Grandparents	Parents		F <sub>1</sub>	F <sub>2</sub>
	♀	♂		
G02007(♀, ♂)	P03003	P03??	03003	
	P03019	P03??	03019	
	P03014	P03??	03014	
G02001 (♀)	P03034	P03034	03034	
	P03027	P03??	03027	
	P03157	P03157	03157	

Question:

1. which offspring is F<sub>1</sub> or F<sub>2</sub>?
2. Who is the father of the offspring?
3. From which grandparents were the parent (♀, ♂) derived?

Three  $F_1$  and three  $F_2$  were determined by matching the genotypes at each locus.

---

Grandparent	Parents		F1	F2
	♀	♂		
G02007	P03003 P03019 P03014 P03034	P03014	03003 03019 03034	03014
G02001	P03027 P03157	P03003 P03019 P03027 P03157 P03034		03027 03157

---

# Physical tagging is still used 还是物理标记好

---



Family coding using elastomers, ID using eyestalk ring.

---

**Genetic linkage DNA marker**

**遗传连锁标记**

---

# 1. SSR markers related to WSSV survival 抗性标记

---

- ❑ The individuals from one F<sub>2</sub> family were challenged with WSSV by oral infection in indoor tank. Nest-PCR was used to confirm the infection of WSSV.
  - ❑ Eighteen loci were selected to genotype the individuals in the family.
  - ❑ Associations of individual marker (allele) with resistance to WSSV were analysed by ANOVA.
  - ❑ The results indicated that one genotype of RS0622 was associated with resistance to WSSV.
-

# Basic data of 18 loci in the F<sub>2</sub>.

<i>Locus</i>	<i>N</i>	<i>He</i>	<i>PIC</i>	<i>F</i>	<i>P</i>
<i>EN0033</i>	2	0.426	0.334	0.292	0.748
<i>RS1101</i>	2	0.500	0.374	0.577	0.564
<i>RS0676</i>	3	0.637	0.562	0.536	0.659
<i>FC012</i>	3	0.615	0.542	0.742	0.530
<i>EN0018</i>	2	0.500	0.374	1.182	0.312
<i>RS0622</i>	2	0.502	0.375	5.233**	0.007**
<i>RS0683</i>	2	0.502	0.375	0.074	0.929
<i>FC029</i>	3	0.667	0.589	0.510	0.677
<i>FC015</i>	2	0.358	0.293	0.214	0.645
<i>FC030</i>	3	0.638	0.562	0.511	0.676
<i>FC031</i>	2	0.395	0.316	0.013	0.908
<i>FC008</i>	4	0.752	0.700	0.535	0.660
<i>FC021</i>	2	0.389	0.312	1.159	0.319
<i>FC027</i>	3	0.608	0.537	0.103	0.958
<i>IOPC04</i>	3	0.658	0.580	0.283	0.754
<i>FC028</i>	2	0.502	0.374	0.600	0.551
<i>FC019</i>	3	0.575	0.509	0.740	0.480
<i>FC002</i>	2	0.417	0.328	0.204	0.653
<i>Mean</i>	2.50	0.536	0.446		

The effect of locus *RS0622* was significant ( $P < 0.01$ )

# The effect of *RS0622* locus on the survival days

---

Genotypes	Number	Mean/d
<i>BB</i>	19	6.84a
<i>AB</i>	44	8.61a
<i>AA</i>	17	10.31b**

---

Mean survival days (d) were significantly different in the same group with different letter (a, b). *BB* and *AB* genotypes had similar effects on survival days, however *AA* genotype was significantly associated to a larger number of days ( $P < 0.01$ ).

---



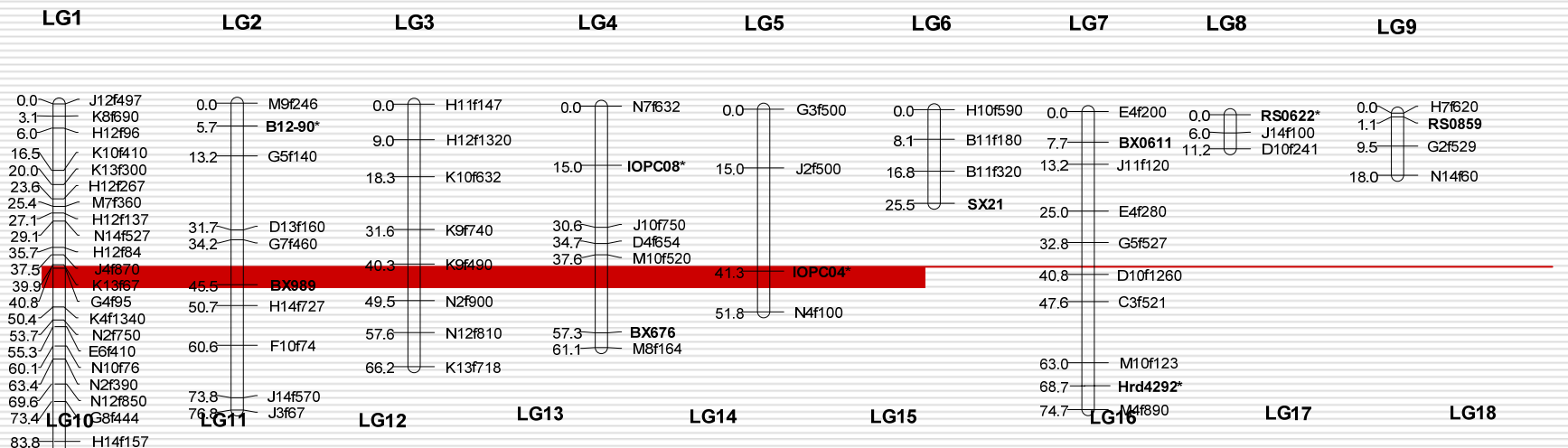
### Demonstration of RS0622 microsatellite loci

**M: DL2000 marker; 4, 9, 13, 14, 16:AA genotypes; 1, 3, 5, 7, 10, 12, 15, 19: AB genotypes; 2, 6, 8, 11, 17, 18: BB genotypes**

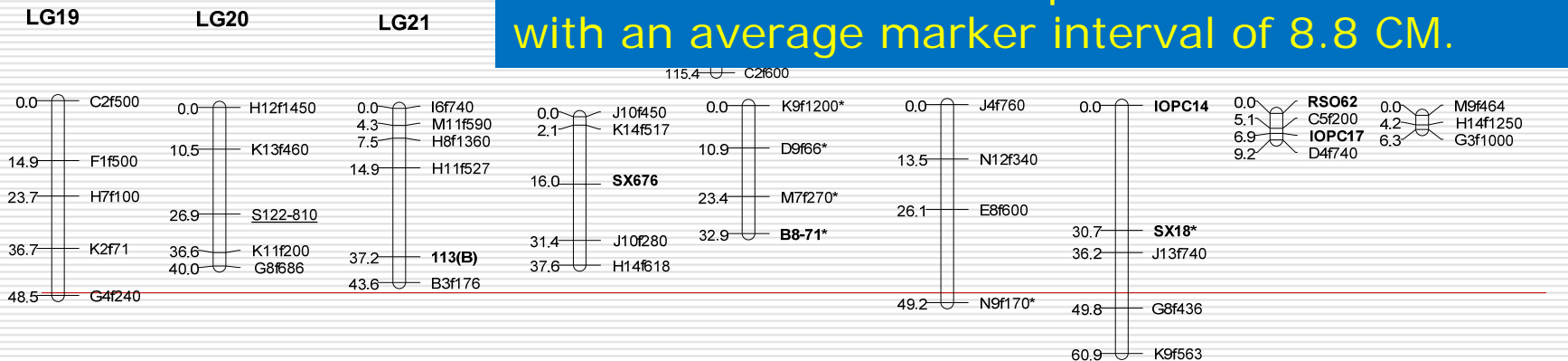
## 2. Genetic linkage map by multi-markers

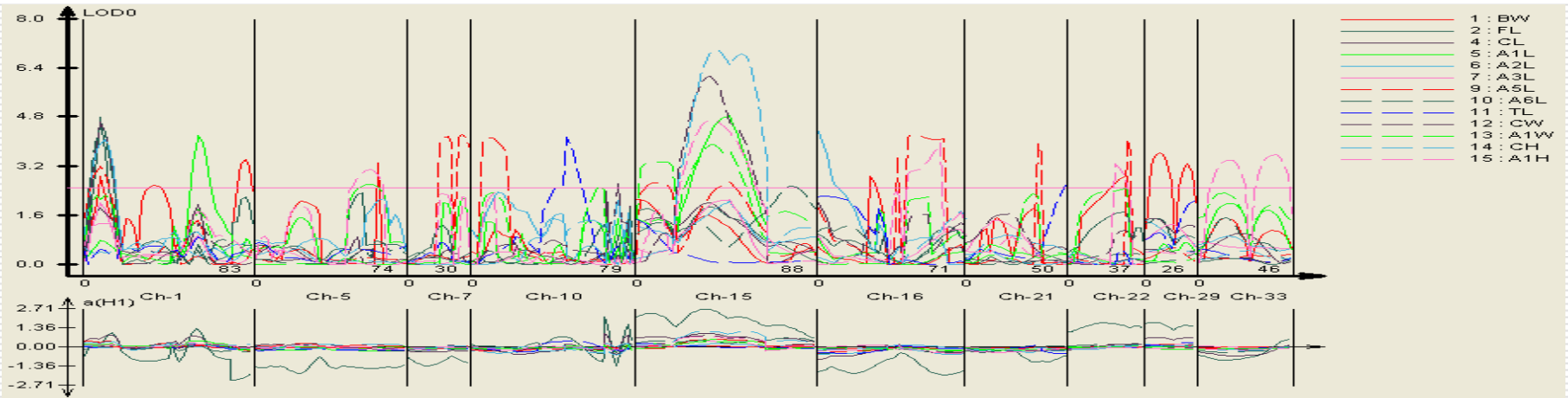
---

- ❑ One F<sub>2</sub> family including parents and 100 offsprings
  - ❑ Totally 52 SSR, 94 RAPD and 453 AFLP were genotyped
  - ❑ The female framework map was composed of 231 markers and male framework map of 204 markers in 44 linkage groups.
-

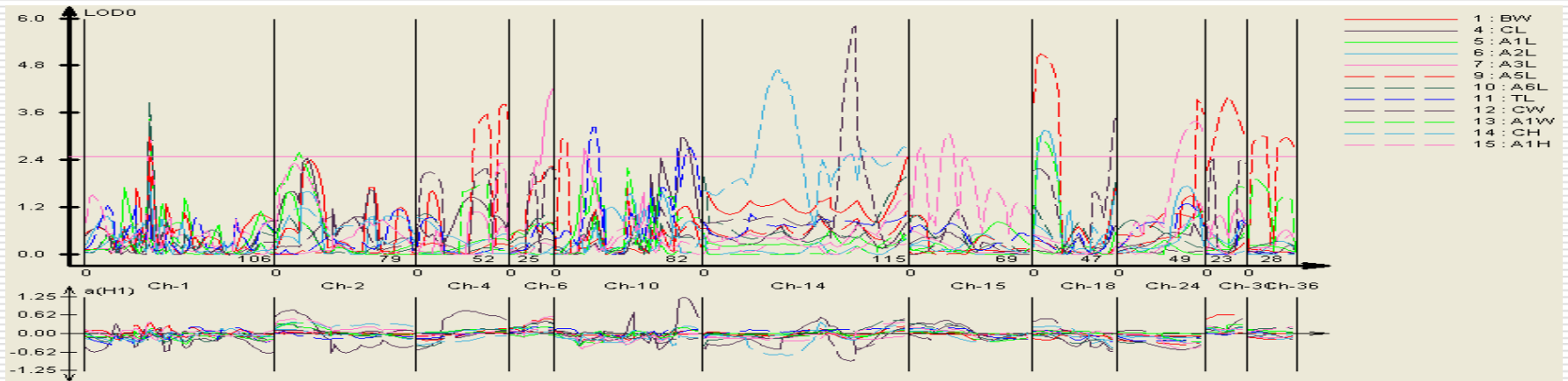


The female framework map covers 2397 CM with an average interval of 7.8 CM.  
 The male framework map covers 2422.5 CM with an average marker interval of 8.8 CM.





The growth-related QTL detected on the 1, 2, 4, 6, 10, 14, 15, 18, 24, 31, 36 linkage group of female



The growth-related QTL detected on the 1, 5, 7, 10, 15, 16, 21, 22, 29, 33 linkage group of male

# Summarization

---

DNA marker, can be successively used to evaluate the genetic background of stocks. 遗传背景评价。

DNA marker are powerful tool for tagging, pedgree tracing, etc., but practical application is still to be developed. 家系、个体识别。

Quatitative trait loci can be located in DNA markers' linkage map, but the 'true' makers need lots of works and MAS is beyong our patient. 真正的标记与应用离我们很远。

---



持續 가능한 水産養殖을 위한 韓.中 심포지엄 및 워크숍

# The UNDP/GEF Yellow Sea Project Korea-China Symposium & Workshop on the Sustainable Mariculture



■ June 18-20, 2007  
2007. 6. 18-20

■ Hosted by West Sea Mariculture Research Center, NFRDI  
주최 국립수산과학원 서해수산연구소 서해특성화연구센터

■ Sponsored by MOMAF and UNDP/GEF  
후원 해양수산부, UNDP/GEF 황해광역생태계사업

