Genetic Differentiation in Seven Geographic Populations of Fleshy Shrimp (*Fenneropenaeus chinensis*) Based on Microsatellite DNA

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INTRODUCTION

- Fleshy shrimp distributed along Bohai and the Yellow Sea and migrate long distance for over-wintering

- High economical value and large amount of resources(1989-1992 200 000 MT / Y)

- Commercially important in northern China and Korea both for fishery and mariculture

INTRODUCTION

- **Two Problems**
  - Fishery yield: Decreased dramatically due to overfishing and environment destruction during the last decade
  - Farming industry: Has been in a state of collapse when the shrimp virus disease (WSSV) played havoc since 1993.

- **Two Questions**
  - Hatchery release: Since 1986, about 10-30 billion seeds have been released in Bohai and Yellow Sea to recover the natural resource. If hatchery juveniles releasing produce negative effects on natural populations?
  - Genetic differentiation: With broad distribution (from East coast 118° E in China to South coast 127° 30'E in Korean peninsula) and the long migration characteristics, is there any genetic differentiation?

MATERIALS AND METHODS

Sample Details of Seven Populations of *F. chinensis*

Populations	Locations	Capture date	Sample No.
Liaodong Bay (LD)	42° 20' N, 121° 30' E	Sep. 2001	30
Bohai Bay (BH)	38° 50' N, 118° E	Sep. 2001	30
Haizhou Bay (HZ)	35° N, 120° E	Sep. 2001	30
Rushan Bay (RS)	36° 40' N, 121° 30' E	Aug. 2001	30
Haiyangdao Bay (HYD)	39° 50' N, 123° E	Aug. 2001	30
West coast of Korean Peninsula (KW)	35° 34' N, 126° E	Apr.1997	30
South coast of Korean Peninsula (KS)	35° 30' N, 127° 30'E	Sep. 2001	30

Distribution and Migration Rout of *F. chinensis*
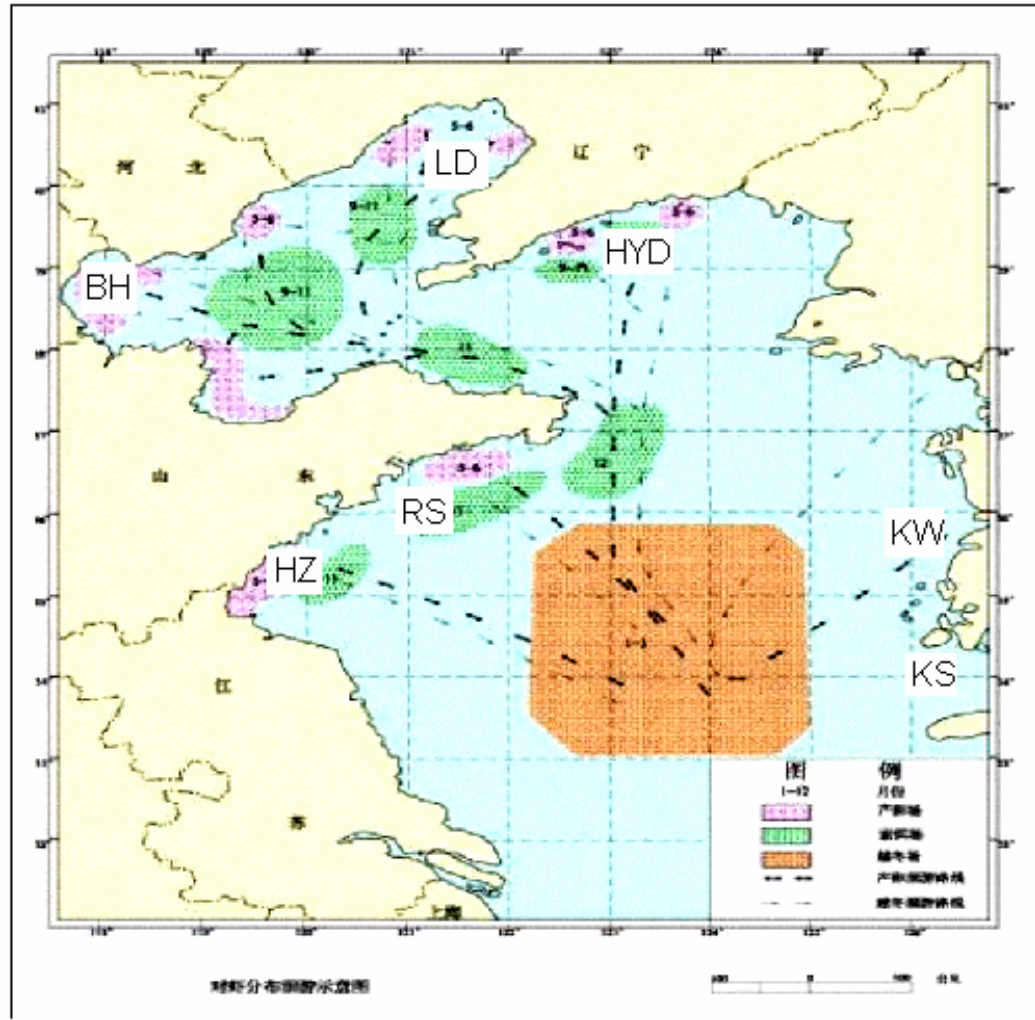
Sequences of Seven Microsatellite Primers and their Annealing Temperatures of PCR Amplification

Locus	Accession NO.	Primer sequence（5'-3'）	Anneal(℃)
EN0033	*AY132813l*	Patent No. 02135714.5	64
HD2545	/	F: TTA CGG ACC AGG AGA CAA TAC AC R: AGA GAC CCG CAG ATT TCA CC	64
72A64	*AY566113*	F: ATGCTCAGATGCTTTGGAR: TTGATGTTGCTTCGCTGG	45
RS1101	*AY132811*	F: CGA GTG GCA GCG AGT CCT R: TAT TCC CAC GCT CTT GTC	52
RS0683	*AY132823*	Patent No. 02135713.7	66
BX613	*AY132778*	F:TCA GTC CGT AGT TCA TAC TTG G R: CAC ATG CCT TTG TGT GAA AAC G	66
RS062	*AY132778*	F: TGC TGA AGC TAC ACT ACC TTC G R: TGA TGA AAC GCA AGC AAA GGC	66

Reaction Mixture（25 uL）

- 50 m mol/L KCl
- 10 m mol/L Tris-HCl, pH 9.0
- Triton-X 100 0.1 %
- 2.0 m mol/L MgCl$_2$
- 0.1 m mol/L d NTP
- 0.2 u mol/L primer each
- 100 ng Genomic DNA
- 0.6 U *Taq* DNA Polymerase

Condition of PCR

- 94℃ pre-denaturation for 5 min
- 94 ℃ denaturation for 1 min
- *** ℃ annealing for1 min
- 72 ℃ extension for 2 min

30 cycles

- 72 ℃ final extension for 10 min
- Hold at 4℃

  ✓ *** primer-specific temperature

  ✓ 4ul PCR product: PAGE(8%) and silver-staining

DATA ANALYSIS

- Popgene 32(Version 1.31)

    - Allele frequencies & mean number of alleles

    - observed heterozygosity (*Ho*) & expected heterozygosity (*He*)

- AMOVA in ARLEQUIN software package

    - Exact tests for Hardy-Weinberg Equilibrium (*HWE*)

    - Population pairwise F_{st} values

- Polymorphism Information Content (*PIC*) value

$$PIC = 1 - \sum_{i=1}^{n} Pi^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2Pi^2 Pj^2$$ (Botstein & White 1980)
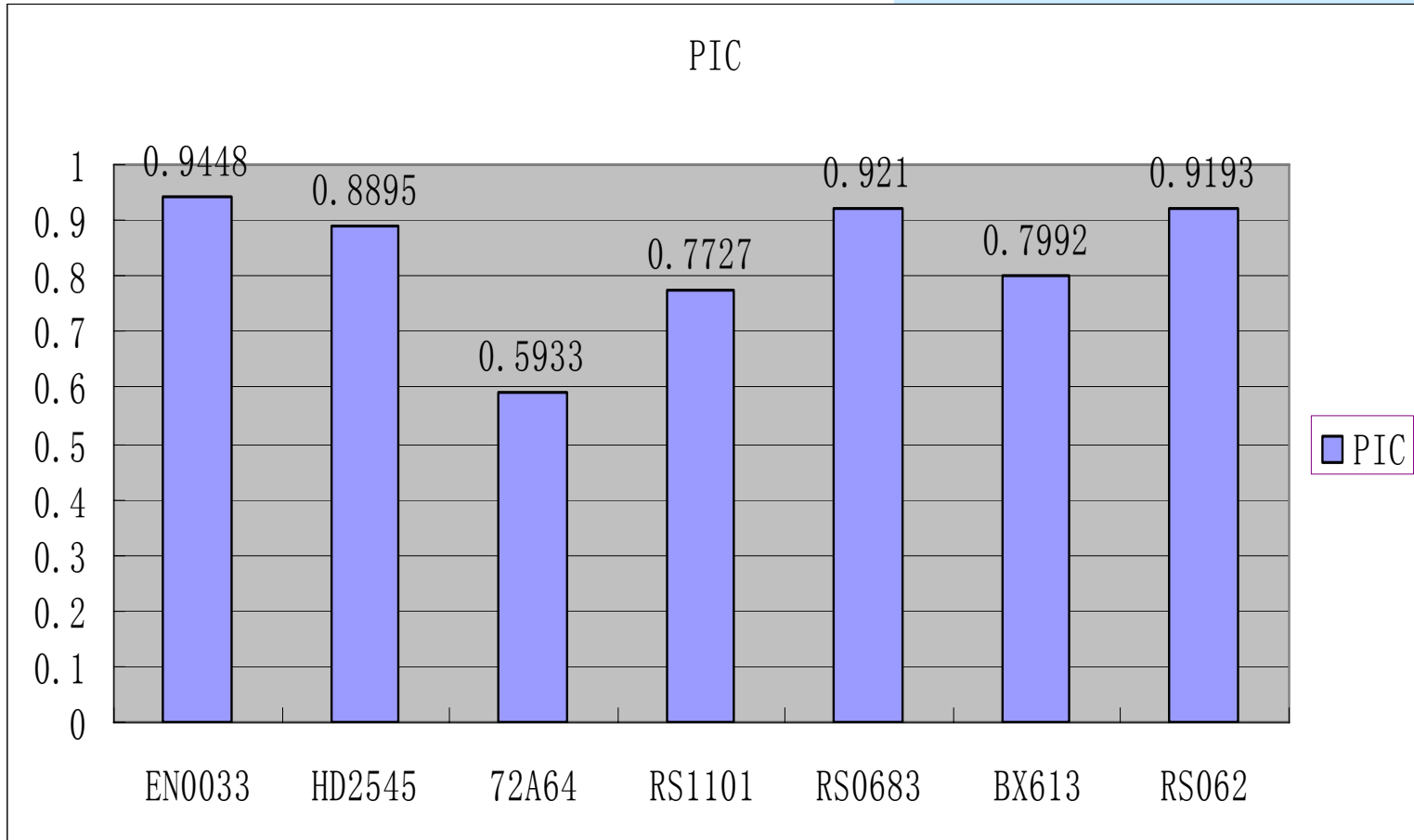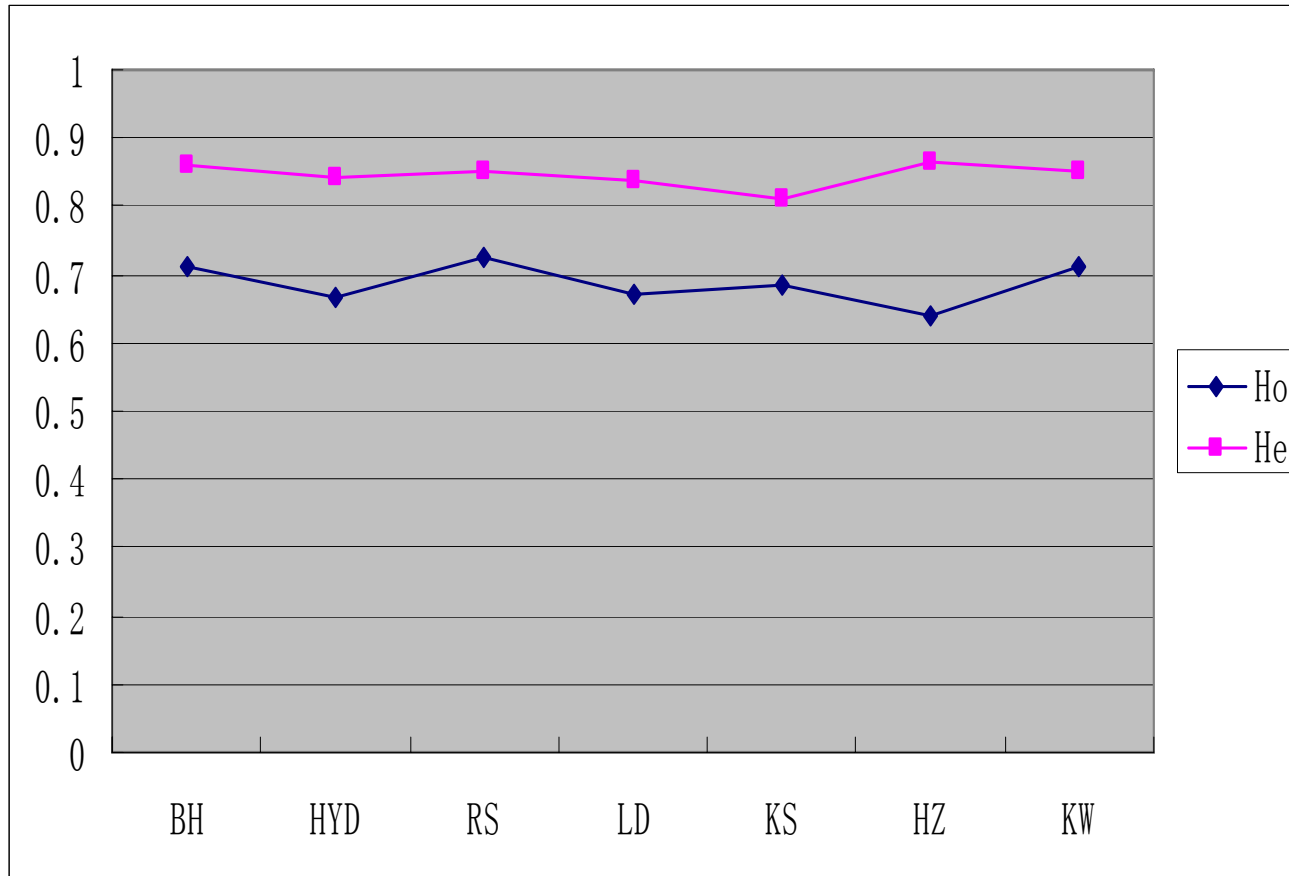
RESULTS

Alleles per locus
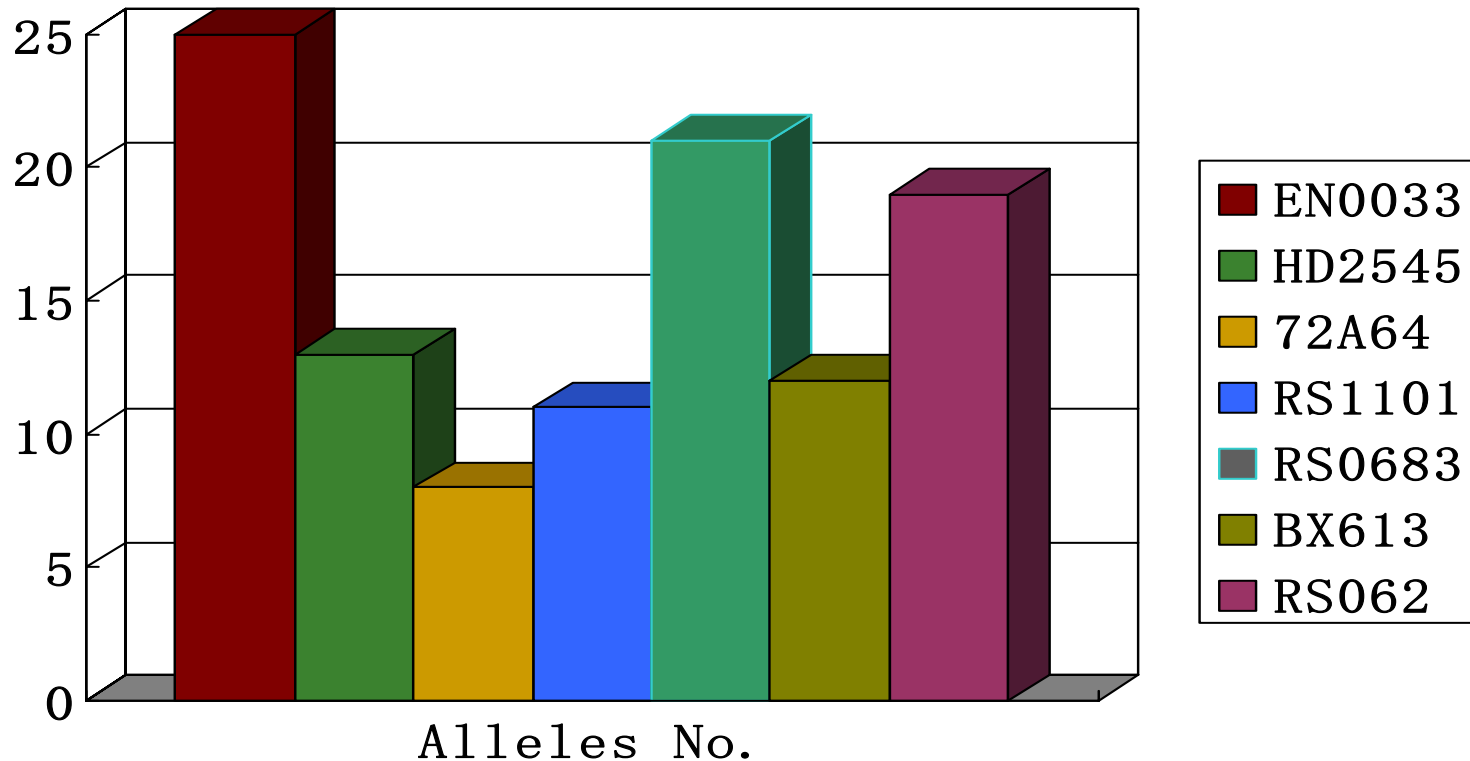
Total of 109 alleles were obtained from seven loci in 210 shrimps and the mean number of alleles per locus was 15.6.

Polymorphism Information Content (*PIC*) per Locus

PIC

Locus	PIC
EN0033	0.9448
HD2545	0.8895
72A64	0.5933
RS1101	0.7727
RS0683	0.921
BX613	0.7992
RS062	0.9193

Mean *PIC*: 0.8343

Average Observed Heterozygosity (Ho) and Expected Heterozygosity (He)

Ho ranged from 0.638 (HZ) to 0.725 (RS),
He varied from 0.810 (KS) to 0.864 (HZ)

EXACT TEST FOR HARDY-WEINBERG EQUILIBRIUM

Locus	B H	HYD	R S	L D	K S	H Z	K W
EN0033	0.238	0.000*	0.300	0.067	0.877	0.000*	0.880
HD2545	0.003	0.002	0.264	0.147	0.020	0.428	0.137
7 2 A 6 4	0.001	0.000*	0.021	0.000*	0.069	0.003	0.380
RS1101	0.000*	0.000*	0.002	0.003	0.326	0.018	0.000*
RS0683	0.003	0.003	0.004	0.415	0.000*	0.011	0.000*
B X 6 1 3	0.007	0.595	0.266	0.004	0.017	0.000*	0.002
R S 0 6 2	0.009	0.000*	0.011	0.000*	0.000*	0.000*	0.000*

* Out of 49 population-locus cases, 15 showed a significant departure from the Hardy-Weinberg Equilibrium (BH, 1; HYD, 4; LD, 2; KS, 2; HZ, 3; KW, 3)

GENETIC DISTANCE (D) AND GENETIC IDENTITY (I)

	B H	HYD	R S	L D	K S	H Z	K W
B H	****	0.8492	0.8044	0.8657	0.8227	0.8149	0.8044
HYD	0.1634	****	0.7929	0.8420	0.8080	0.8371	0.8387
R S	0.2177	0.2321	****	0.8351	0.7912	0.8513	0.7812
L D	0.1442	0.1719	0.1802	****	0.7770	0.8380	0.7927
K S	0.1951	0.2133	0.2342	0.2523	****	0.7876	0.7880
H Z	0.2047	0.1778	0.1610	0.1768	0.2387	****	0.8448
K W	0.2176	0.1760	0.2469	0.2323	0.2382	0.1687	****

I values are above the diagonal and G values are below the diagonal

Dendrogram based on Nei's (1978) Genetic Distance Method = UPGMA
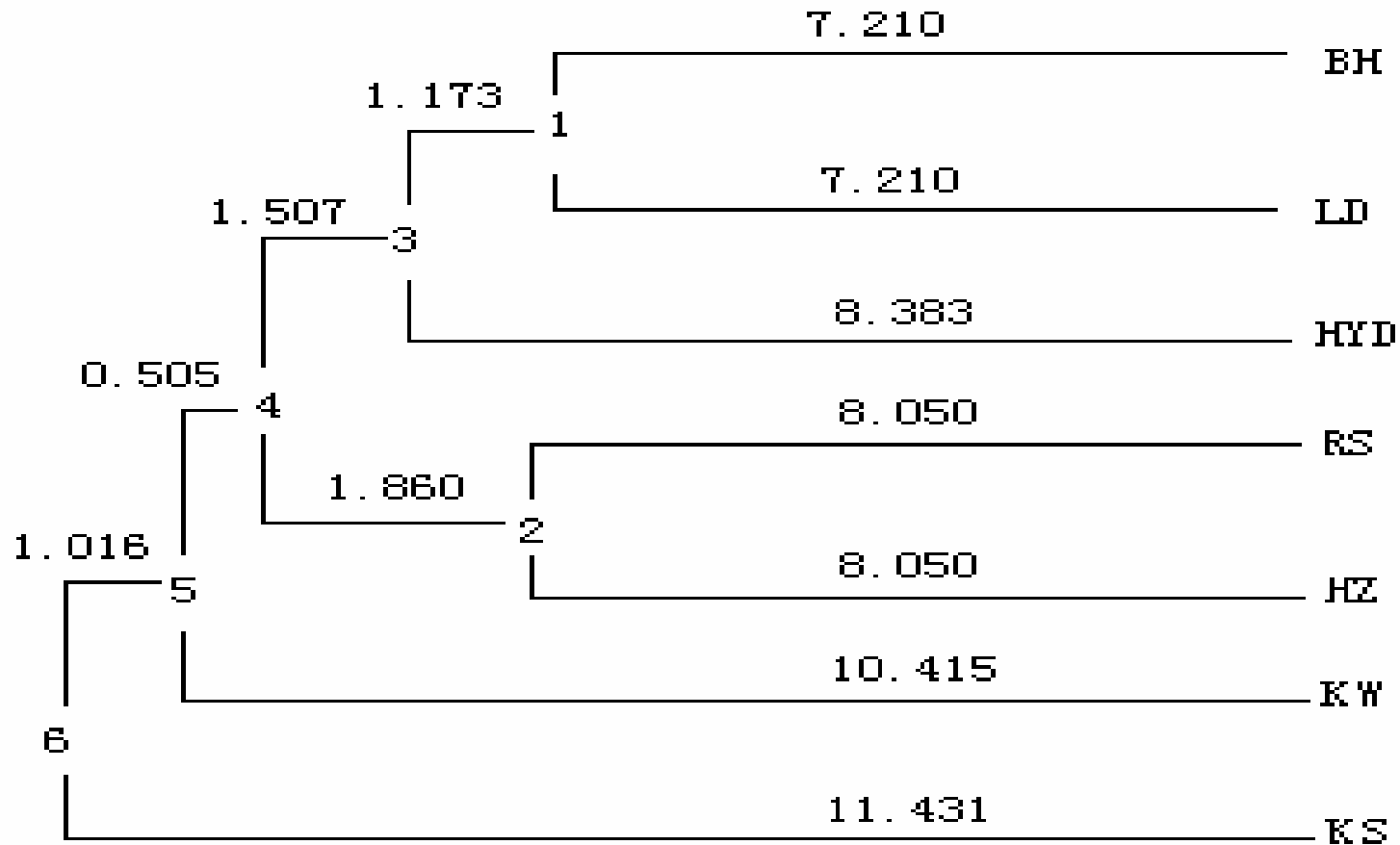
Genetic Differentiations in Seven Populations of *F. chinensis*

	B H	HYD	R S	L D	K S	H Z	K W
B H	****	0.02703	0.00000	0.06306	0.00901	0.00000	0.00000
HYD	0.01268	****	0.00901	0.00000	0.00000	0.00901	0.01802
R S	0.02042	0.02323	****	0.00000	0.00000	0.05405	0.00000
L D	0.01045	0.01604	0.01762	****	0.00000	0.02703	0.00000
K S	0.02334	0.02697	0.02946	0.03569	****	0.00000	0.00000
H Z	0.01800	0.01399	0.01116	0.01562	0.02943	****	0.01802
K W	0.01938	0.01361	0.02412	0.02491	0.02941	0.01161	****

F_{st} values are below the diagonal and *P* values are above the diagonal

Migration rout of *F. chinensis*

Bohai Sea population including BH, LD and HYD, etc.
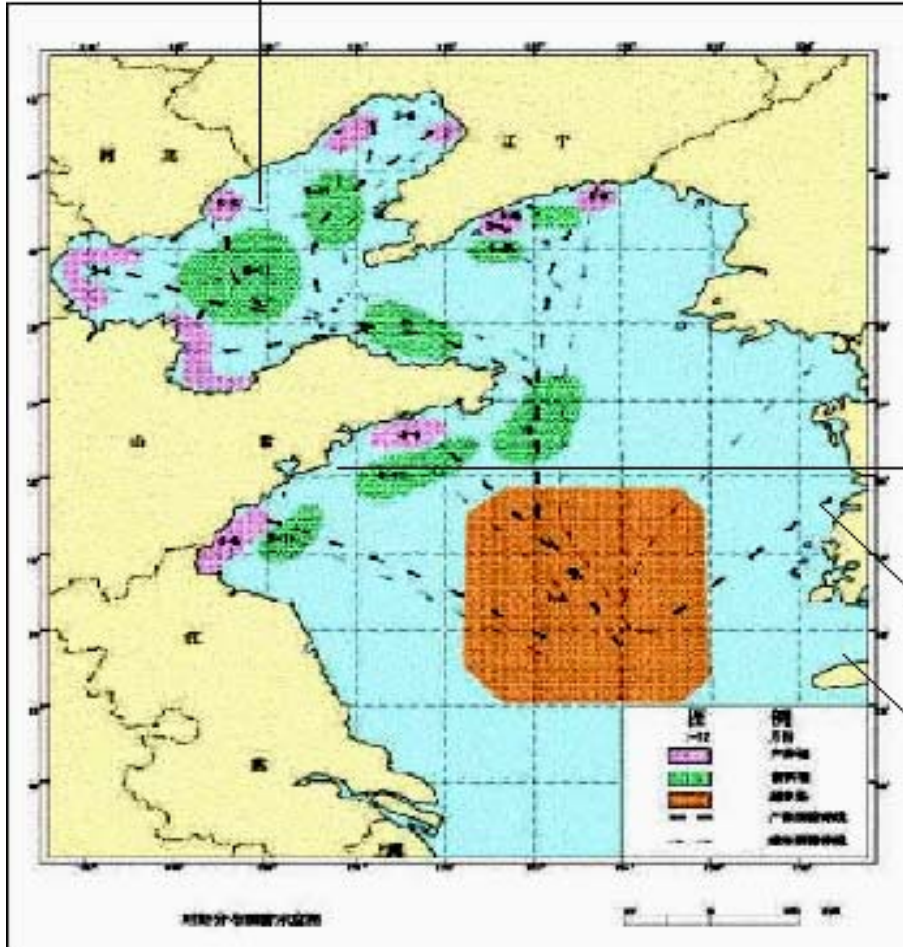
Yellow Sea population including RS, HZ, etc.

West coast of Korean Peninsula population

South coast of Korean Peninsula population

AMOVA Analysis (Average over Seven loci)

Source of variation	Sum of squares	Variance components	Percentage variation（%）	Significance test (*P*)
Among groups	24.387	0.03019	1.00343	0.00000**
Among populations within groups	15.747	0.02978	0.98989	0.00000**
Among individuals within populations	695.489	0.54318	18.05231	0.00000**
Within individuals	496.500	2.40576	79.95437*	0.01564
Total	1232.123	3.00891	100	

Percentage variation (%)

1.00343
0.98989
18.05231
79.95437

□ Among groups

■ Among populations within groups

□ Among individuals within populations

□ Within individuals
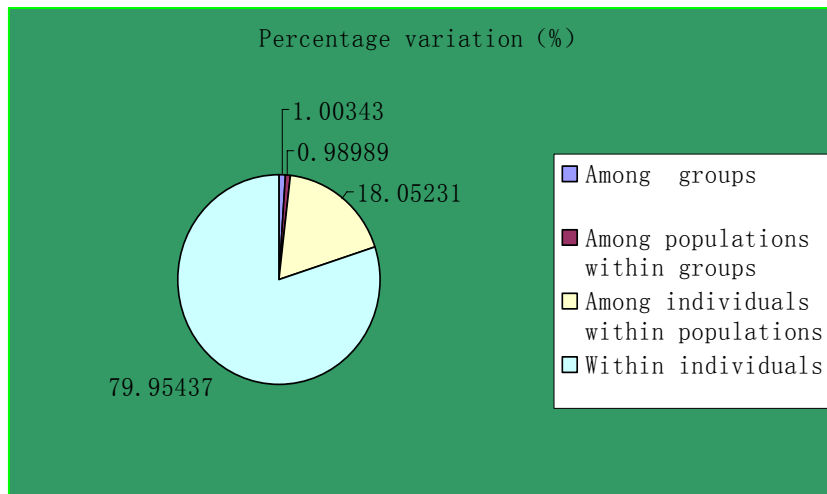
* About 80% of the variation occurred within individuals

** Played most of the important role in the genetic differentiation

DISCUSSION

■ Genetic Diversity

- Low genetic diverstiy in Isozyme, RAPD, AFLP, mtDNA

- Higher genetic diversity than the previous studies, but significant departure from the Hardy-Weinberg equilibrium

- Artificial interference: over exploitation, unexpected escape individuals of farmed shrimps, large scale hatchery release (Since 1986, ca.10-30 billion seeds have been released in Bohai and Yellow Sea without considering quality of shrimp juveniles and genetic diversity).

DISCUSSION

■ **Genetic differentiation**

- Before 1990's, according to phenotypic characters, tagging and recapture rate: Bohai & Yellow Seas, West coast of Korean Peninsular

- After 1990's, molecular biology technique was used: Concept from low genetic diversity to genetic differentiation

- Diverse physical and nourishing environment among each feeding and spawning habitat

- Migration charachter of procreation and over- wintering: entirely different migration routes, mating and migration time, which led to persistent mating barrier

SUGGESTION

Policy of fishery management for sustainable utilization must be established by Chinese and Korean governments.

- Avoid over-fishing and environment destruction

- Strengthen monitoring and evaluation of genetic diversity

- Develop rational enhancement strategy based on genetic structure and background, and

- Release HHS (high health shrimp) juveniles into sea

INFORMATION OF CRUSTACEANS IN GENBANK

http://www.ncbi.nlm.nih.gov/

- PROTEIN & NUCLEOTIDES

  - Shrimp: 31

  - Crab: 28

  - Shellfish: 60

Information on Shrimp and Crab Compared with Shellfish in GenBank

Items		shrimp	crab	shellfish
Protein	**All**	**2 476**	**3 918**	**8 865**
	Bacteri	83	129	368
	RefSeq	216	545	635
	Related Structures	1 945	2 950	6 357
	Others	232	294	1 505
Nucleotide	**All**	**38 953**	**247 555**	**388 257**
	Bacteri	97	185	6 242
	mRNA	35 297	192 719	299 151
	RefSeq	73	221	73
	Others	3 486	54 430	82 791
	CoreNucleotide — **All**	**4 240**	**9 140**	**11 473**
	Bacteria	97	185	493
	mRNA	642	875	1 313
	RefSeq	73	221	73
	Others	3 423	7 859	9 594
	EST — **All**	**34 655**	**191 844**	**297 838**
	Bacteria	0	0	0
	mRNA	34 655	191 844	297 838
	GSS — **All**	**58**	**46 571**	**78 946**
	Bacteria	0	0	5 749
	mRNA	0	0	0
	Others	58	46 571	73 197

Information on Shrimp and Crab Compared with Shellfish in GenBank

Protein
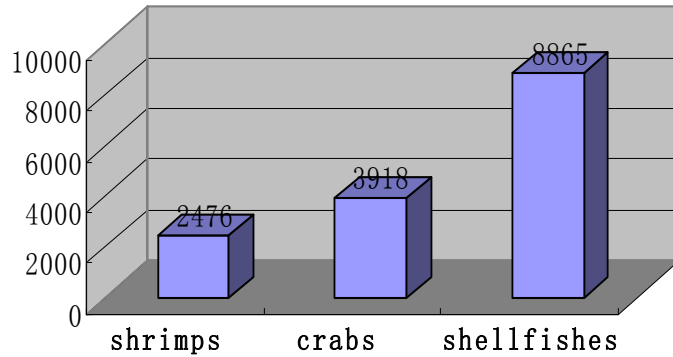
- shrimps: 2476
- crabs: 3918
- shellfishes: 8865

CoreNucleotide

- shrimps: 38953
- crabs: 24755
- shellfishes: 388257

EST

- shrimps: 4240
- crabs: 9140
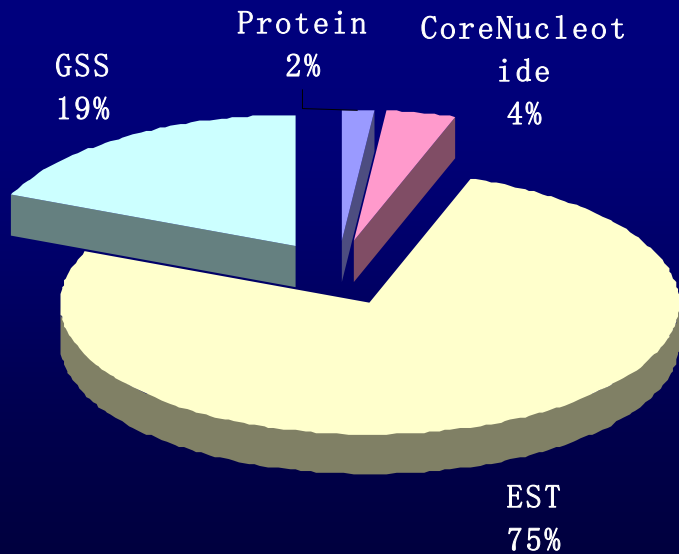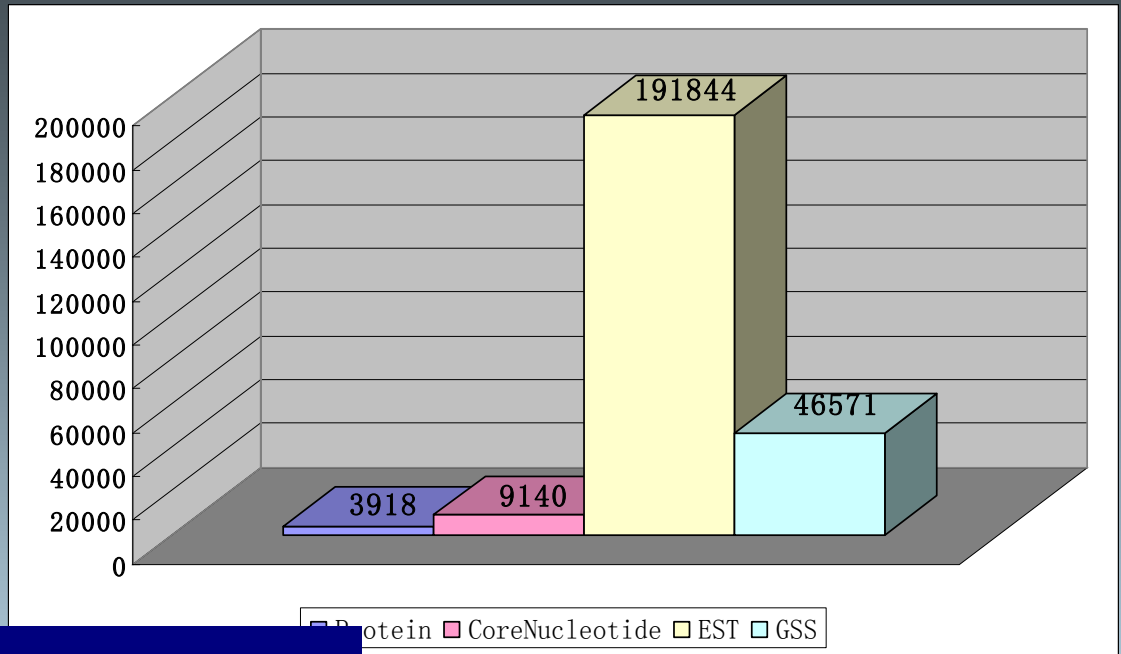- shellfishes: 11473

GSS
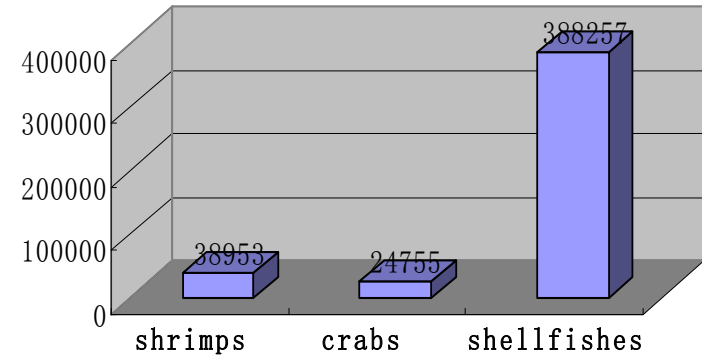
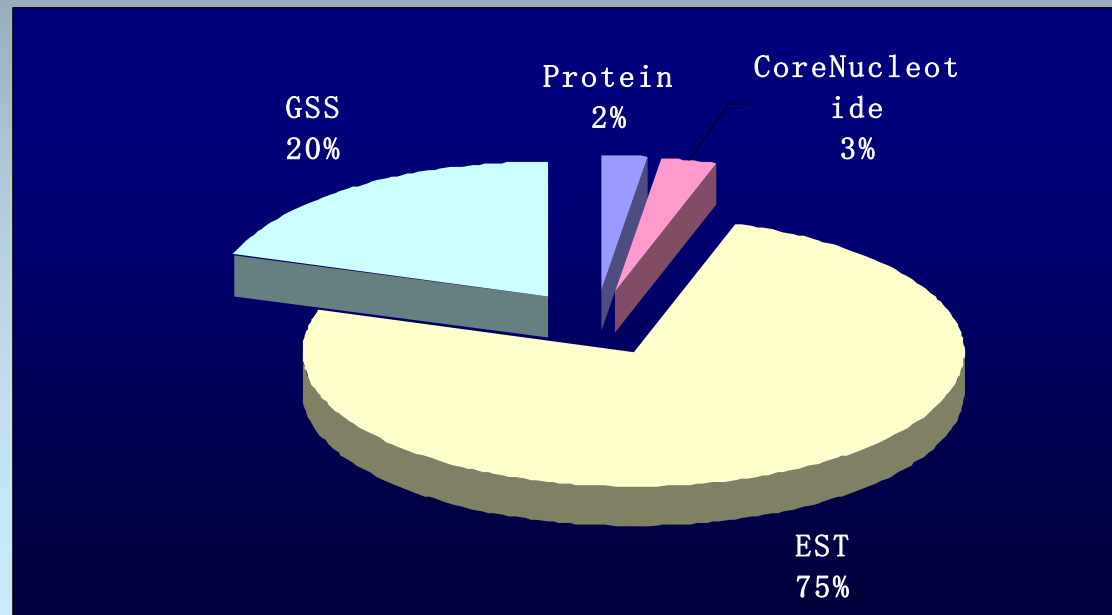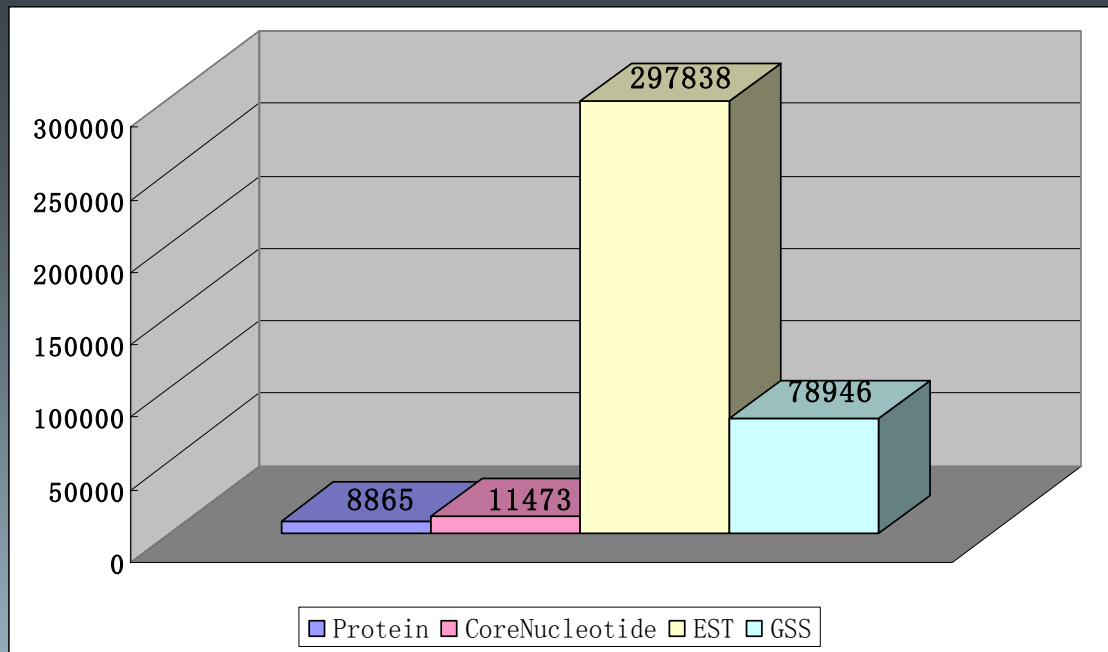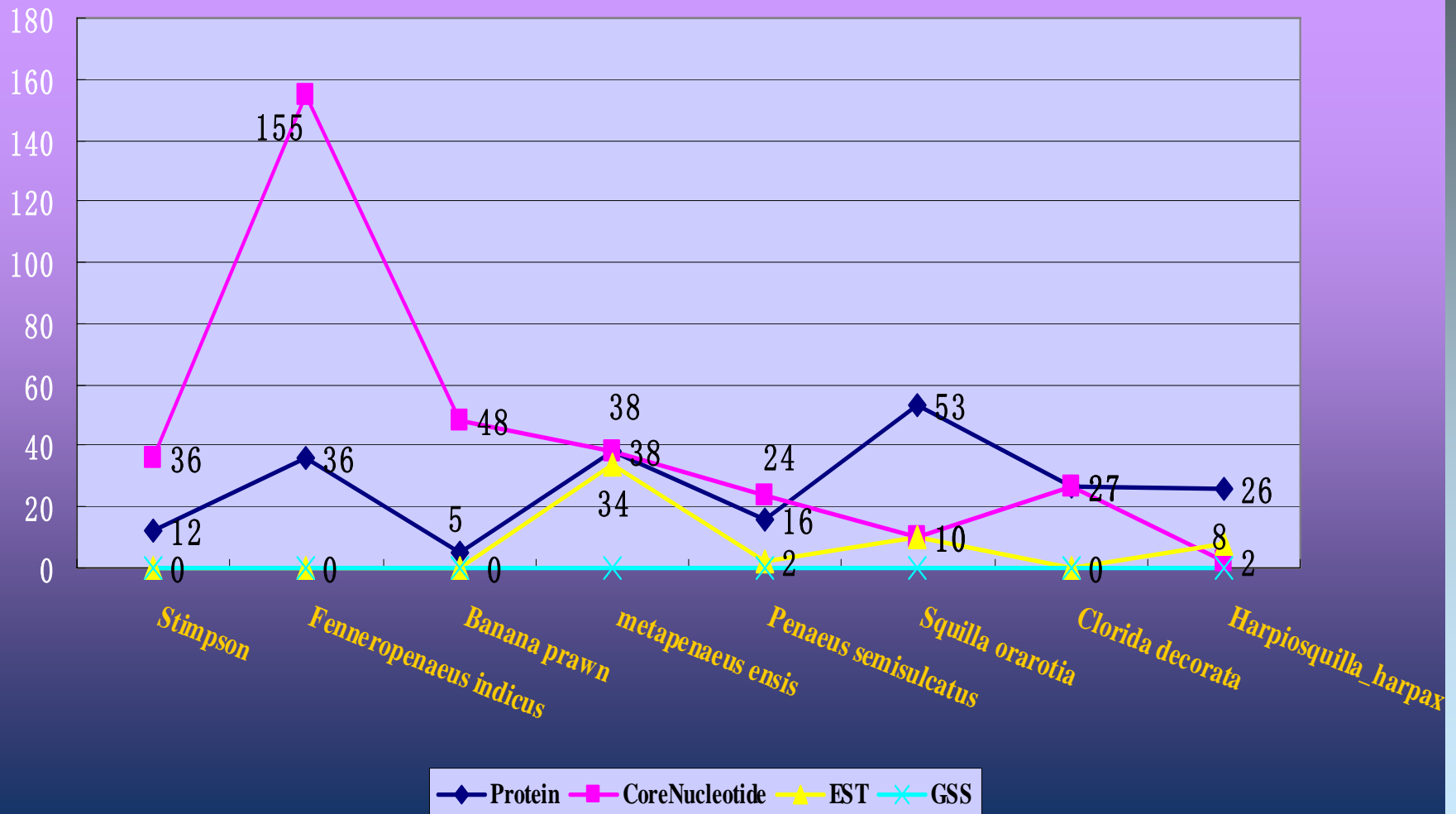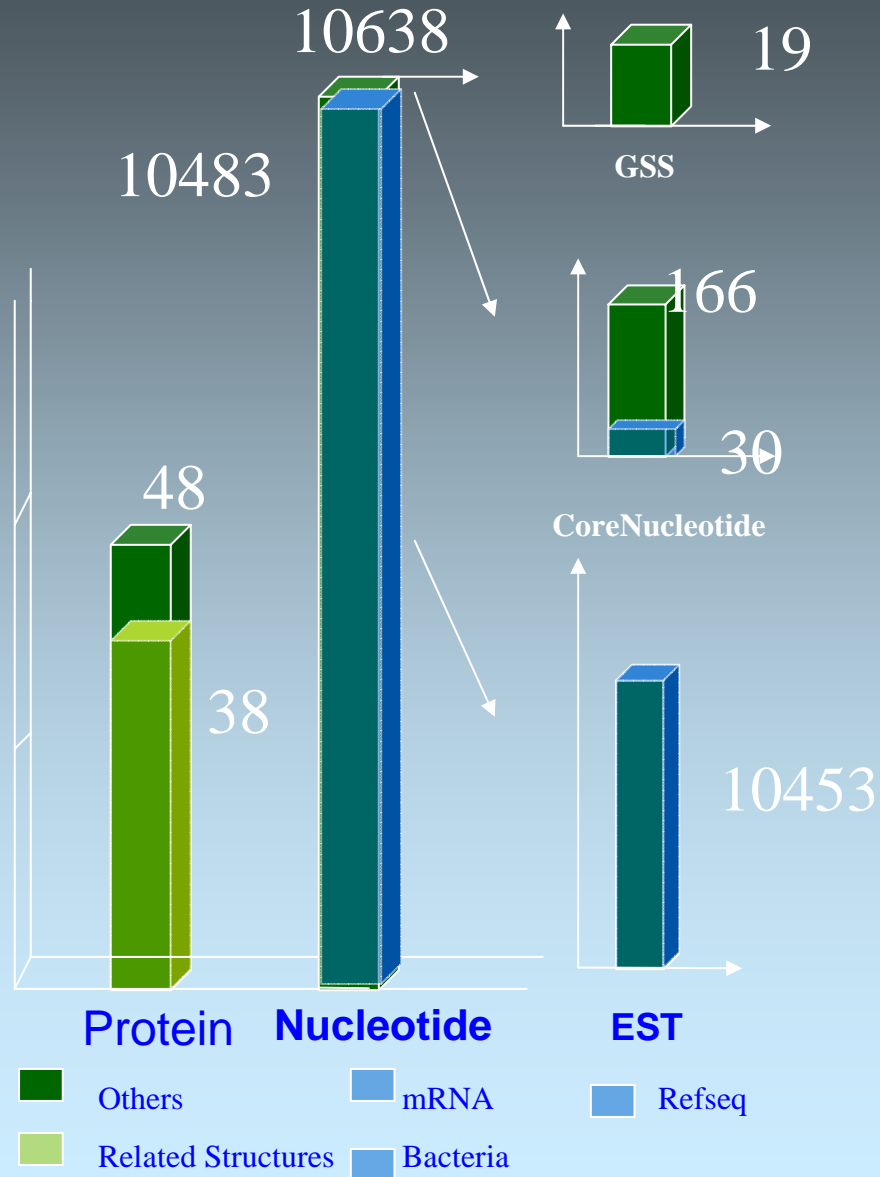- shrimps: 58
- crabs: 46571
- shellfishes: 78946
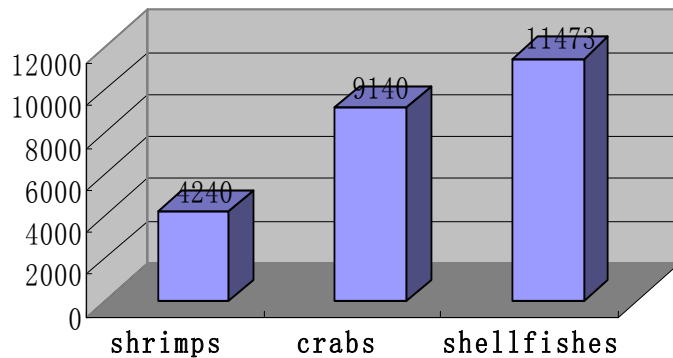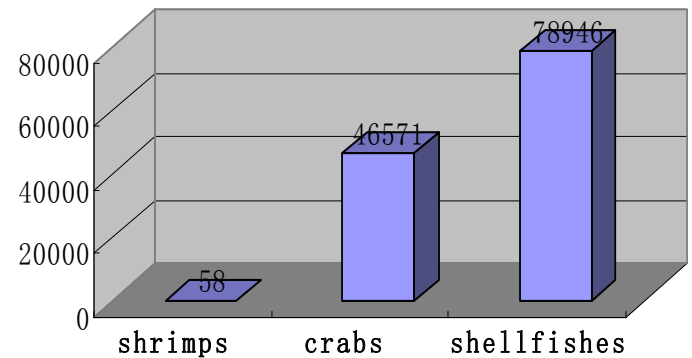
Protein, CoreNucleotide, EST and GSS in shrimp
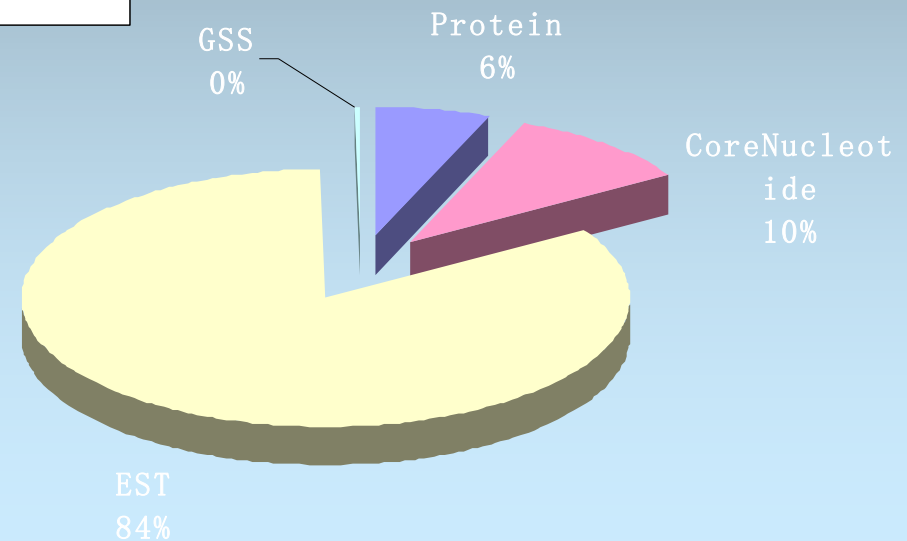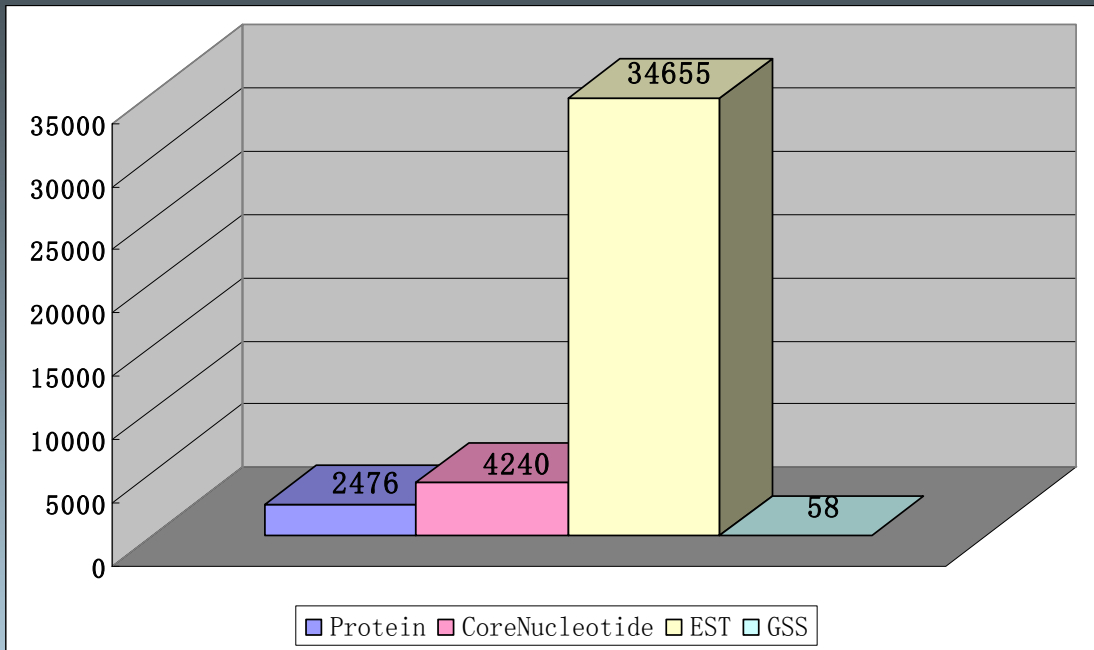
Protein, CoreNucleotide, EST and GSS in Crab
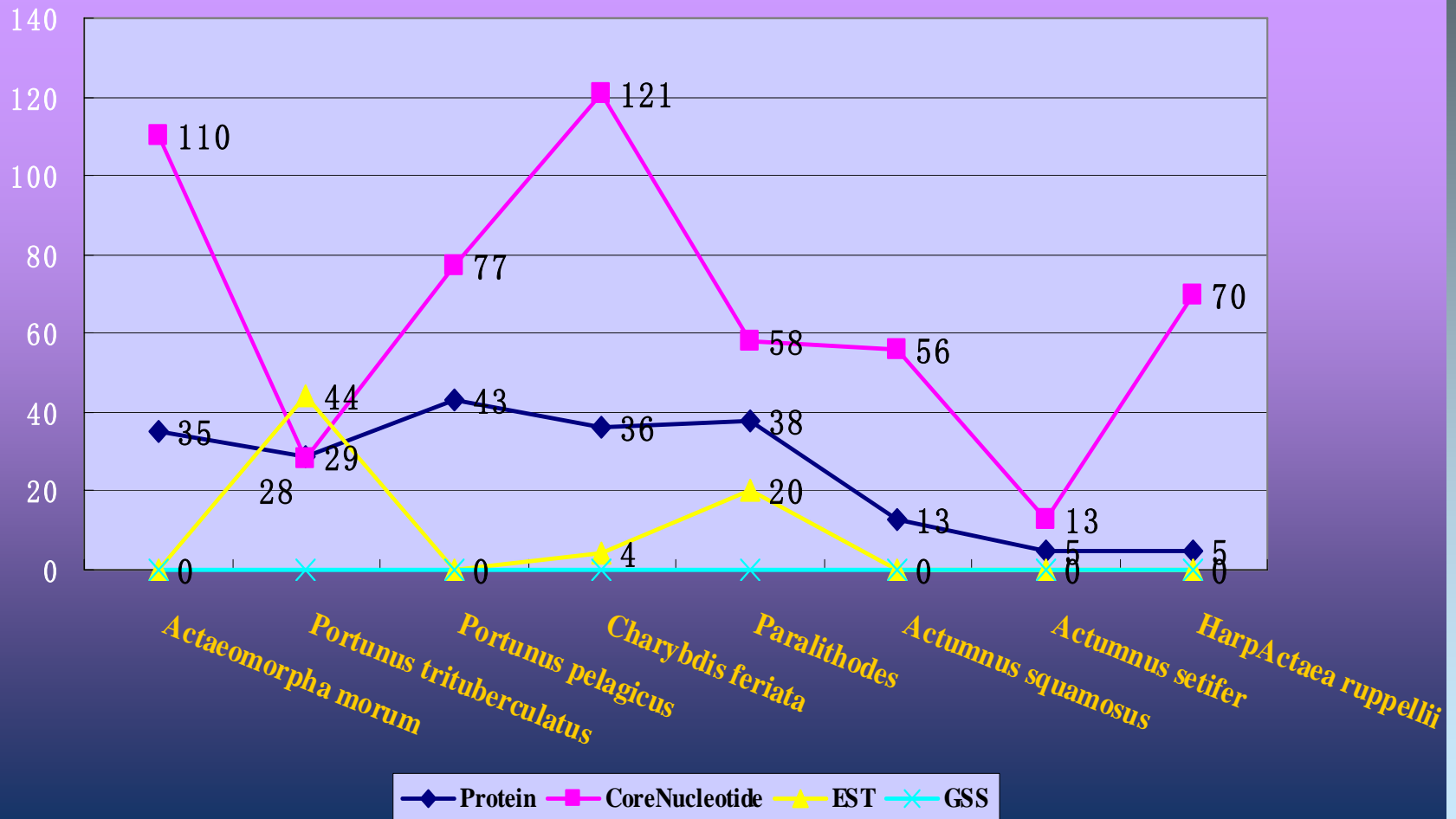
Protein, CoreNucleotide, EST and GSS in Shellfish

Protein, CoreNucleotide, EST and GSS in Different Shrimp Species

Protein, CoreNucleotide, EST and GSS in *F. chinensis*

Protein, CoreNucleotide, EST and GSS in Different Crab Species

Conclusion

No matter shrimp or crab, the information of protein and nucleotide was much less than that of shellfish.

Let's look forward to the marine species genome project like Human Genome Project (HGP)
Let's hope science make the world
clean, healthy, equal, safer and richer!

Thank you