

Isozyme variation in four populations of *Penaeus chinensis* shrimp

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Abstract : Horizontal starch gel electrophoresis was used to investigate the isozyme genetic variation in *Penaeus chinensis* shrimp. Each 50 individuals from two wild geographic populations distributed in the China Coast of the Yellow Sea (YP) and the western coast of Korean Peninsula (KP), and from two cultivated populations (CP₁ and CP₂) were surveyed. Of 20 loci encoding 12 enzymes, four are polymorphic. The mean proportions of polymorphic loci ($P_{0.99}$) of YP, KP, CP₁ and CP₂ are 15%, 20%, 10% and 20% with the average heterozygosities (H_o) of 0.014 ± 0.007 , 0.020 ± 0.010 , 0.010 ± 0.007 and 0.033 ± 0.017 , respectively. The mean effective allele number (N_e) of these four populations are 1.015 ± 0.008 , 1.023 ± 0.011 , 1.011 ± 0.007 and 1.042 ± 0.022 while the heterozygous divergent indexes (D) of them value $+0.037$, -0.030 , -0.098 and -0.030 , respectively. The genetic similarity index (I) and genetic distance (D_{nei}) between two wild geographic populations are 0.99998 and 0.00002, respectively.

Key words : *Penaeus chinensis*, genetic diversity, isozyme, horizontal starch gel electrophoresis, conservation biology

Introduction

Penaeus chinensis shrimp has been playing an economically important role in the fishing and farming industries in northern China. The maximum catches in an autumn fishing season used to reach 40 000 MT in 1970's (Deng *et al.*, 1990). During the major onset of shrimp mariculture from 1989 to 1992, the shrimp farming scale summed up to 150 000 hm² with an annual harvest of some 200 000 MT, which made China a leading producer of cultured shrimp in the world (Cen, 1993). Fatally, due to overfishing and shrimp diseases, since 1993, the shrimp landings in autumn fishing season was declining to less than 1000 ton (Deng & Zhuang, 2001), while the shrimp cultivation harvest has dramatically dropped to a yearly harvest about 25% of that before 1992.

In the last two decades, up to one billion shrimp (*P. chinensis*) larvae have been annually released into the Yellow Sea and Bohai Sea to replenish the decreasing stock. From the genetic point of view, the genetic

structure of natural shrimp population may be affected by those genetically unsafe larvae released or escaped from shrimp farms. The smaller spawning stock and the deteriorated spawning environment can affect the natural population genetic structure as well. To protect this valuable marine resource and supply with genetic background for a select breeding program, it is vitally necessary to investigate and evaluate the genetic diversity in the natural and cultivated shrimp populations.

The isozyme technology has been widely applied to investigate and assess the genetic variation within or among populations (Yu *et al.*, 1997). This study primarily reported the genetic variation of *P. chinensis* by means of isozyme analysis.

Materials and Methods

Two wild geographic populations and two cultivated populations of *P. chinensis* were investigated. The sampling information is listed in Table 1. Fifty live shrimp of each population were collected and kept in dry ice on the way to the lab. The muscle and liver tissues

were dissected from each shrimp and preserved in deep freezer at -86°C as the sub-sample.

0.2 ~ 0.3 g sub-sample of tissue was homogenized in 5 volume 0.1 mol/L Tris-HCl (pH 7.0) with ice-bath. Homogenates were centrifuged and 15 000 rpm for 15 min at 4°C to obtain supernatants. Electrophoresis was performed using a 12% ~ 14% starch gel consisting of potato and soluble starch , which was pre-

pared according to Wang (1996). The particulars of buffers and running conditions were as follows : TC 6.9 , Tris-citrate , pH 6.9 , 8v/cm 12 h ; TC8.0 , Tris-citrate , pH 8.0 , 12 v/cm 10 h and TMME , Tris-Maleicacid-MgCl₂-EDTA , pH 8.2 , 15 v/cm 15 h (Wang , 1996 ; Wang *et al.* , 1996). The gels were then sliced and the related histochemical stain used to each slice (Table 2). Stain recipes were applied

Table 1 Details of shrimp sampled

Population	Sampling location	Sampling date	Number of individuals	Body length of samples (cm)
Population in the western coast of Korean Peninsula (KP)	The western coast of Korea Peninsula ($35^{\circ}34'N$, $126^{\circ}E$)	May. 1998	50	12 ~ 15
Population in the China coast of the Yellow Sea (YP)	The Haizhou Bay of the Yellow Sea	Aug. 1998	50	11 ~ 12
Cultivated population I (CP ₁)	A shrimp farm in Jimo , Shandong	Aug. 1998	50	9 ~ 12
Cultivated population II (CP ₂)	Health Shrimp Farming Demonstration Base in Jimo , Shandong	Aug. 1998	50	9 ~ 11

Table 2 Enzymes assayed , buffer systems used and number of loci scored

Enzyme	E. C. No.	Tissue	Buffer system	No. of loci scored
Lactate Dehydrogenase LDH	E. C. 1. 1. 1. 27	Muscle	TC6.9	1
Malate Dehydrogenase MDH	E. C. 1. 1. 1. 37	Muscle	TC8.0	2
Malic Dehydrogenase ME	E. C. 1. 1. 1. 30	Muscle	TC6.9	1
Isocitrate Dehydrogenase IDH	E. C. 1. 1. 1. 42	Muscle	TC6.9	1
Acid Phosphatase ACP	E. C. 3. 1. 3. 2	Liver	TC8.0	4
Alkaline Phosphatase ALP	E. C. 3. 1. 3. 1	Liver	TC8.0	3
Phosphoglucomutase PGM	E. C. 2. 7. 5. 1	Muscle	TC8.0	3
Glucose Phosphate Isomerase GPI	E. C. 5. 3. 1. 9	Muscle	TC8.0	1
Adenylate Kinase AK	E. C. 2. 7. 4. 3	Muscle	TC8.0	1
Glucose-6-Phosphate Dehydrogenase G6PDH	E. C. 1. 1. 1. 44	Muscle	TMME	1
Glutamate Dehydrogenase GDH	E. C. 1. 4. 1. 4	Liver	TC8.0	1
Sorbitol Dehydrogenase SORD	E. C. 1. 1. 1. 14	Liver	TC8.0	1
Esterase EST	E. C. 3. 1. 1. 1	Liver	TC8.0	4

subject to the protocols (Wu & Lin , 1983 ; Harris & Hopkinson , 1976 ; Wang , 1996). The nomination of loci and description of alleles were conducted according to Shaklee *et al.* (1989). Loci are nominated according to the relative abbreviation of enzyme encoded. If an enzyme is encoded by multiple loci , allelic variants are designated according to their relative mobilities. The most common allele is designated 100 and the others are given numbers according to their mobilities correspondingly to that of the common allele. To assess the genetic structures of these four populations , proportion of polymorphic loci (*P*) , effective number of alleles per locus (*Ne*) , allelic frequency , observed heterozygosity (*Ho*) , expected heterozygosity (*He*) and

divergent index (*D*) at Hardy-Weinberg equilibrium were calculated (Wang , 1996). The genetic similarity (*I*) and genetic distance (*d*) were also applied to estimate the enzyme variation between the YP and the KP (Wang , 1996).

Results

The experiment totally investigated 13 enzymes. Twenty loci encoding 12 enzymes were scored and used for genetic analysis except for Esterase due to its complex banding pattern (Table 3). As Table 3 listed , the frequencies of the most common alleles in YP , KP and CP₁ were above 0.92 , but those in CP₂ could range from 0.80 to 1.00.

Variant alleles were detected at the *sMdh* , *Pgm-a* , *Pgm-c* and *Gpi* loci (Figs. 1 ~ 3). A variant allele at *Pgm-c* locus (*Pgm-c89*) was detected among the samples of YP , KP and CP₂ , but can not be detected in CP₁. The values of the observed and expected heterozygosities (*Ho* & *He*) , divergent index (*D*) and effective number of alleles (*Ne*) for each polymorphic locus are given in Table 4. Among these 4 polymorphic loci , *Pgm-a* showed relatively high variability consistently in all four populations and the variability of the others varied with different populations , e. g. the higher variability of *sMdh* was found in KP and that of *Gpi* in CP₂. The minus *D* values were found at *Gpi* locus of KY and CP₁ as well as CP₂.

Across these 20 loci (Table 3) , considering a locus polymorphic if the frequencies of the most common alleles were less than 0.99 (*P*_{0.99}) , the mean proportions of polymorphic loci were 20% for both KP and CP₂ , 15% for YP and 10% for CP₁. Table 5 summarizes the estimates of genetic variation in these four populations of *P. chinensis*.

Table 3 Allele frequencies in four populations of *P. chinensis*

Locus	Allele	Population			
		YP	KP	CP ₁	CP ₂
<i>Ldh</i>	100	1.00	1.00	1.00	1.00
<i>sMdh</i>	100	0.99	0.95	0.99	0.92
	133	0.01	0.05	0.01	0.08
<i>mMdh</i>	100	1.00	1.00	1.00	1.00
<i>Me</i>	100	1.00	1.00	1.00	1.00
<i>Idh</i>	100	1.00	1.00	1.00	1.00
<i>Acp-a</i>	100	1.00	1.00	1.00	1.00
<i>Acp-b</i>	100	1.00	1.00	1.00	1.00
<i>Acp-c</i>	100	1.00	1.00	1.00	1.00
<i>Acp-d</i>	100	1.00	1.00	1.00	1.00
<i>Alp-a</i>	100	1.00	1.00	1.00	1.00
<i>Alp-b</i>	100	1.00	1.00	1.00	1.00
<i>Alp-c</i>	100	1.00	1.00	1.00	1.00
<i>Pgm-a</i>	100	0.94	0.92	0.94	0.8
	10	0.06	0.08	0.0	0.13
<i>Pgm-b</i>	100	1.00	1.00	1.00	1.00
<i>Pgm-c</i>	100	0.97	0.9	1.00	0.98
	89	0.03	0.03	0.00	0.02
<i>Gpi</i>	100	0.9	0.94	0.95	0.80
	8	0.04	0.0	0.05	0.20
<i>Ak</i>	100	1.00	1.00	1.00	1.00
<i>G pdh</i>	100	1.00	1.00	1.00	1.00
<i>Gdh</i>	100	1.00	1.00	1.00	1.00
<i>Sord</i>	100	1.00	1.00	1.00	1.00

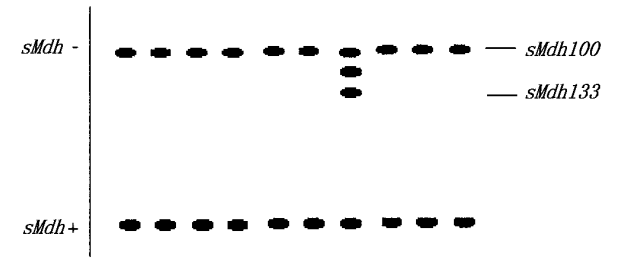


Fig. 1 Zymogram and its interpretation of MDH in YP of *P. chinensis*

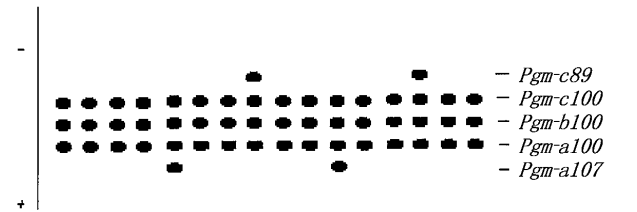


Fig. 2 Gymogram and its interpretation of PG7 in YP of *P. chinensis*

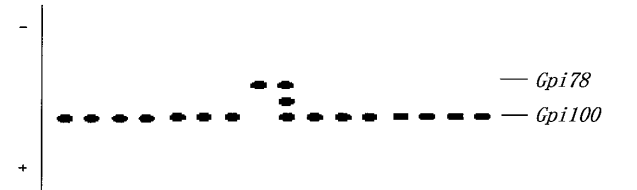


Fig. 3 Gymogram and its interpretation of DPI in CP₁ of *P. chinensis*

Discussion

This study primarily investigated the genetic diversity at 20 enzyme loci in four populations of *Penaeus chinensis* with the average proportions of polymorphic loci ranging from 10% to 20% , which are quite similar to previous electrophoresis studies on Penaeid shrimp with a polymorphism range from 11% to 33% (Marris *et al.* , 1990 ; Medgecock *et al.* , 1982 ; Lester , 1983 ; 7 ulley & Later , 1980 ; Sbordoni *et al.* , 198 ; Iunden & Zavis , 1991 ; 6huang *et al.* , 2000). The relevant results through the starch gel electrophoresis examination of isozyme variability support a conclusion that Penaeid shrimps exhibit relatively low polymorphism level due to fewer polymorphic loci that could be detected. In this study , the average heterozygosities observed in the four populations of *P. chinensis* varied from 0.010 to 0.033 , which were within the limits of the heterozygosities (0.00 ~ 0.09) observed in other Penaeid shrimps , but much less than 0.03 in crustaceans and

Table 4 The observed and expected heterozygosities (*Ho* & *He*) , divergent index (*D*) and effective number of alleles (*Ne*) at each polymorphic locus

Loci	Estimates	YP	KP	CP ₁	CP ₂
<i>sMdh</i>	<i>Ho</i>	0.02	0.10	0.02	0.16
	<i>He</i>	0.0198	0.095	0.0198	0.1472
	<i>D</i>	+0.0101	+0.0526	+0.0101	+0.0870
	<i>Ne</i>	1.0204	1.1111	1.0204	1.1905
<i>Pgm-a</i>	<i>Ho</i>	0.12	0.16	0.12	0.26
	<i>He</i>	0.1128	0.1472	0.1128	0.2262
	<i>D</i>	+0.0638	+0.0087	+0.0638	+0.1494
	<i>Ne</i>	1.1364	1.1905	1.1364	1.3514
<i>Pgm-c</i>	<i>Ho</i>	0.06	0.06	0	0.04
	<i>He</i>	0.0582	0.0582	0	0.0392
	<i>D</i>	+0.0309	+0.0309	/	+0.0204
	<i>Ne</i>	1.0638	1.0638	/	1.0417
<i>Gpi</i>	<i>Ho</i>	0.08	0.08	0.06	0.20
	<i>He</i>	0.0768	0.1128	0.095	0.32
	<i>D</i>	+0.0417	-0.2908	-0.3684	-0.375
	<i>Ne</i>	1.0870	1.0870	1.0638	1.25

Table 5 Estimates of genetic variation in four populations of *Penaeu chinenshi*

	YP	KP	CP ₁	CP ₂
<i>P</i> _{0.99}	15%	20%	10%	20%
<i>Ho</i>	0.014 ± 0.007	0.020 ± 0.010	0.010 ± 0.007	0.033 ± 0.017
<i>He</i>	0.013 ± 0.007	0.021 ± 0.010	0.011 ± 0.007	0.037 ± 0.020
<i>D</i>	+0.037	-0.030	-0.098	-0.030
<i>Ne</i>	1.015 ± 0.008	1.023 ± 0.011	1.011 ± 0.007	1.042 ± 0.022

0.051 in Penaeoidea and Caridea (Hedgecock *et al.* , 1982).

As listed in Table 5 , the average values of *Ho* and *Ne* present a declining gradient of CP₂→KP→YP→CP₁. But there exists a significant difference of *D* values among these four populations (Table 4 , 5). Due to no rare homozygous genotype was detected in YP , the rare allele (*Gpi*78) was inherited by heterozygotes. Being of lower genetic variation in *Penaeus chinensis* shrimp , the function of those heterozygotes performing inheritance of rare alleles might be over-estimated. As a result , the *D* value denotes positive. Besides , minus *D* values occurred in CP₂ and CP₁ as well as KP , suggesting a critical deficit of heterozygotes on the assumption of Hardy-Weinberg equilibrium in these three populations. The most likely explanation to this result obtained in this study may be : a) the occurrence of rare homozygous genotypes at *Gpi* locus resulted in the heterozygote deficiency in CP₂ and CP₁ as well as KP and b) the performance of rare homozygous genotype inheriting rare allele , to some extent , dominates that of heterozygous genotype. Having analyzed a species of deep-sea shrimp (*Rimicaris exoculata*) , Crea-

sey *et al.* (1996) revealed that heterozygote deficiency could be caused by the occurrence of rare homozygous genotypes.

Unfortunately , very little research work was conducted to reveal the genetic background of *P. chinensis* before its resource collapsed and the hatchery release conducted. According to Deng *et al.* (1990) , there exists two geographic populations of *P. chinensis* in the Yellow Sea and Bohai Sea , one is distributed in the Yellow Sea and the Bohai Sea (YP) , and the other in the western coast of the Korean Peninsula (KP). These two populations share the same over-wintering ground in the central to southern part of the Yellow Sea but their spawning and feeding grounds are completely different. Based on the biological characters , these two populations were reproductively isolated from each other. The genetic similarity index (*I*) and genetic distance (*D*_{nei}) between YP and KP are 0.99998 and 0.00002 , respectively , which means less differentiation between two populations. But the genetic diversity in YP is , to some extent , poorer than that in KP. This may be correlated with human activities like hatchery release on large scale and shrimp farming industry.

At present, there has been no artificially selected breed shrimp in mariculture. Most of the domesticated brood stocks belong to the wild strains. Bottleneck effect from the genetically unwarranted brood stocks, including quality and quantity, may be the main causes of the loss of progeny genetic diversity. Furthermore, most of the shrimp farms adopt the closed or semi-closed farming models currently, inbreeding may be another reason why the shrimp genetic variation loses (Sbordoni *et al.*, 1986, Qiu, 1998). As indicated by the mean heterozygosity observed value of 0.010 ± 0.007 and the mean proportion of polymorphic loci of 10%, CP₁ possesses lower genetic variation level than that of YP.

Since the natural *P. chinensis* resource has been destroyed and its genetic variation has been threatened, it is of great significance to restore the genetic diversity, at the beginning of which, to select beneficially genetic markers for high yield and disease-resistant strains is the priority to shrimp farm industry. CP₂ is regarded as a high-health cultivated stock by selective breeding. The values of *Ho* and *P*_{0.99} in CP₂ are 0.033 and 20%, respectively. The higher genetic diversity level in CP₂ encourages the further work on selective breeding. Moreover, *Pgm-a* and *Gpi* loci possess higher genetic variation in *P. chinensis*, which is accordant with the previous reports on other shrimp species (Villaescusa *et al.*, 1984; Nevo *et al.*, 1981). From this point of view, PGM and GPI can be used as the special protein markers of *P. chinensis* for genetic diversity assessment and selective breeding program.

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中国对虾(*Penaeus chinensis*)4 个种群的同工酶遗传变异

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摘要 : 采用水平淀粉凝胶电泳技术分析了中国对虾(*Penaeus chinensis*)黄渤海沿岸种群(F?)、朝鲜半岛西海岸种群(J?)和 ! 个养殖种群(N?₀ 和 N?₁)的同工酶遗传变异水平。每个种群随机选取 [C 尾中国对虾进行同工酶检测。在所分析的 Q! 种同工酶编码的 !C 个基因位点中 , 有 " 个是多态位点。" 个种群的多态位点比例(*P*_{C.%})分别为 Q[a、!Ca、QCa 和 !Ca。种群平均杂合度(观测值 χ *H*_o)分别为 C5CQ" b C5CCE、C5C!C b C5CQC、C5CQC b C5CCE 和 C5C' ' b C5CQE。" 个种群的位点有效等位基因数(*N_e*)分别为 Q5CQ[b C5CCc、Q5C! ' b C5CQQ、Q5CQQ b C5CCE 和 Q5C"! b C5C!。杂合子平衡偏离指数(*D*)分别为 dC5C'E、eC5C'C、eC5C%c 和 eC5C'C。! 个地理种群(F? 和 J?)的遗传相似性系数(*I*)和遗传距离(*D_{nei}*)分别为 C5%%%c 和 C5CCCC!。

关键词 : 中国对虾(*Penaeus chinensis*) , 遗传多样性 , 同工酶 , 水平淀粉凝胶电泳 , 保护生物学

中图分类号 : P'Q , PQ# , P%[% ! ! ' ^d5#'\$ \$ \$ 文献标识码 : R\$ \$ \$ 文章编号 : QCC[eCC%"(!CCQ)C' eC! "Q eC#

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