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# The genetic variation of allozyme in bloody clam from Qingdao coastal waters*

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**Abstract** The samples of bloody clam *Scapharca broughtonii* were from Qingdao coastal waters. 22 loci in 10enzymes were detected by using starch－gel electrophoresis and the percentage of polymorphic loci was 45.5%. The effective number of alleles per locus varied from 1.000 to 2.469 (mean value 1.415), and the observed heterozygosity per locus ranged from 0.000 to 0.595 (mean value 0.105 ± 0.023). The deficiency of heterozygous individuals obviously existed at 6 loci, and the reasons for it were discussed.

**Key words** *Scapharca broughtonii*, allozyme, genetic variation, starch－gel electrophoresis

## 1 Introduction

Studies on genetic variations of marine bivalves have considerably increased worldwide in last two decades due to their rapid development in aquaculture, and most studies were concentrated on oysters[8~10,12,13], mussels[4,5,14,15,20] and scallops[3,7,19,22,23] but a few on clam species. Bloody clam, *Scapharca broughtonii*, a widely distributing clam species in China, Korea and Japan coastal waters, has been of commercial importance in these countries with traditional fishery and aquaculture for the species. In recent years, however, it has suffered from overfishing which resulted in obvious decrease of its resources in China.

Electrophoresis has been used to estimate genetic variation and to detect population differentiation in bivalves. A few species have been studied in China: *Crassostrea*[17], *Pinctada fucata*, *P. chemnitzi*[18], *Argopecten irradians* and *Chlamys farreri*[24,25]. The initial result of allozymic variation in *S. broughtonii* using starch－gel electrophoresis is presented here.

## 2 Materials and methods

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Samples of *S. broughtonii* were from coastal water of Qingdao. Digestive gland tissues from each clam were frozen at −70℃ before analyzed or homogenized in phosphate buffer (pH 7.0) at ratio of 1 :2 (w/v) with ice−bath and centrifuged at $15\times10^3$ r/min for 15 min. Supernatant was used as the enzyme source and analyzed by electrophoresis and specific enzyme staining methods[1,16]. Electrophoresis was carried out on horizontal starch gels of 12% hydrolyzed potato starch (the product of Sigma Co.) at 8 v/cm (TC buffer) or 15 v/cm (EBT buffer) for approximately 5 or 6 h respectively at 4℃. Ten allozymes were assayed to assess allozyme variation (Table 1).

**Table 1 Enzymes assayed, buffer systems used, number of loci scored and locus−morphic.**

| enzyme | E.C.No | buffer | number of Loci | locus−morphic (P≤0.99) | |
|---|---|---|---|---|---|
| Esterase (EST) | 3.1.1.1 | EBT | 6 | 4M[1)], | 2P[2)] |
| Glucosephosphate isomerase (GPI) | 5.3.1.9 | TC | 1 | | 1P |
| Isocitrate dehydrogenase (IDH) | 1.1.1.42 | TC | 2 | 1M, | 1P |
| Lactate dehydrogenase (LDH) | 1.1.1.27 | EBT | 1 | | 1P |
| Malic enzyme (ME) | 1.1.1.40 | TC | 3 | 2M, | 1P |
| Malate dehydrogenase (MDH) | 1.1.1.37 | TC | 2 | 1M | 1P |
| Phosphoglucomutase (PGM) | 2.7.5.1 | TC | 1 | | 1P |
| Phosphogluconate dehydrogenase (PGD) | 1.1.1.44 | TC | 1 | | 1P |
| Sorbitol dehydrogenase (SDH) | 1.1.1.14 | TC | 2 | 1M, | 1P |
| Superoxide dismutase (SOD) | 1.1.1.15 | EBT | 3 | 3M, | |
| total | | | 22 | 12M | 10P |

1)M: monomorphic
2)P: polymorphic.

Alleles are notated by superscription which describes their mobility as a percentage of the mobility of the most common allozyme at their own loci. When enzymes are coded at more than one locus, each of the locus are numbered according to increasing anodal mobility. The raw electrophoretic data are then used to estimate the allelic frequency, observed heterozygosity (*Ho*), expected heterozygosity (*He*) under the assumption of Hardy−Weinberg equilibrium, the heterozygote deficiency index (*D*) and the effective number of alleles at a locus (*Ne*). These parameters are used as measures of allozyme variation.

## 3 Results

Table 2 lists the details of the 10 polymorphic loci which have been resolved in *S. broughtonii*. The loci of ME−1, ME−2, IDH−1, SDH−2, EST−1,2,3,6, SOD−1, SOD−2, SOD−3 and MDH−1 are monomorphic, and the proportion of polymorphic loci (P) is 45.5%, or 40.9% if a locus is considered polymorphic when the frequency of the most common allele ≤0.95. The numbers of alleles per locus varies from 1 (at the 22 monomorphic loci) to 8 (at the MDH−2 locus), and the observed heterozygosity per locus ranges from 0.000 to 0.595 at the IDH−2 locus. The average heterozygosity is 0.105±0.023 if the observed heterozygosities are used, and 0.201±0.038 if the expected heterozygosities are used. Value of *D* ranges from −1.000 to 0.063, in which positive *D* value means a heterozygote excess and negative *D* value indicates a deficit of heterozygotes on the assumption of Hardy−Weinberg equilibrium. Specifically, at the loci of ME−3, SDH−1, LDH and EST−

4, their negative $D$ values are rather low: −0.771, −1.000, −1.000, −1.000, respectively.

**Table 2** ***S. broughtonii*** **polymorphic loci in the samples from Qingdao**

| locus | *N* | *No* | allele | allelic frequency | *Ho* | *He* | *D* | *Ne* |
|---|---|---|---|---|---|---|---|---|
| EST−4 | 84 | 2 | 96 | 0.024 | 0.000 | 0.047 | −1.000 | 1.000 |
| | | | 100 | 0.976 | | | | |
| EST−5 | 84 | 3 | 88 | 0.250 | 0.452 | 0.442 | 0.023 | 1.825 |
| | | | 95 | 0.048 | | | | |
| | | | 100 | 0.702 | | | | |
| GPI | 84 | 2 | 82 | 0.214 | 0.357 | 0.336 | 0.063 | 1.555 |
| | | | 100 | 0.786 | | | | |
| IDH−2 | 84 | 5 | 85 | 0.262 | 0.595 | 0.569 | 0.046 | 2.469 |
| | | | 92 | 0.059 | | | | |
| | | | 100 | 0.596 | | | | |
| | | | 108 | 0.059 | | | | |
| | | | 132 | 0.024 | | | | |
| LDH | 72 | 6 | 87 | 0.028 | 0.000 | 0.678 | −1.000 | 1.000 |
| | | | 94 | 0.083 | | | | |
| | | | 98 | 0.083 | | | | |
| | | | 100 | 0.501 | | | | |
| | | | 103 | 0.083 | | | | |
| | | | 105 | 0.222 | | | | |
| ME−3 | 36 | 5 | 100 | 0.417 | 0.167 | 0.729 | −0.771 | 1.201 |
| | | | 103 | 0.167 | | | | |
| | | | 107 | 0.083 | | | | |
| | | | 110 | 0.222 | | | | |
| | | | 115 | 0.111 | | | | |
| MDH−2 | 60 | 8 | 88 | 0.283 | 0.333 | 0.758 | −0.561 | 1.499 |
| | | | 90 | 0.033 | | | | |
| | | | 94 | 0.083 | | | | |
| | | | 97 | 0.050 | | | | |
| | | | 100 | 0.367 | | | | |
| | | | 103 | 0.017 | | | | |
| | | | 109 | 0.117 | | | | |
| | | | 112 | 0.050 | | | | |
| PGM | 84 | 6 | 79 | 0.012 | 0.357 | 0.574 | −0.378 | 1.555 |
| | | | 88 | 0.238 | | | | |
| | | | 94 | 0.012 | | | | |
| | | | 100 | 0.595 | | | | |
| | | | 103 | 0.024 | | | | |
| | | | 106 | 0.119 | | | | |
| PGD | 46 | 2 | 70 | 0.022 | 0.044 | 0.043 | 0.023 | 1.046 |
| | | | 100 | 0.978 | | | | |
| SDH−1 | 84 | 3 | 88 | 0.048 | 0.000 | 0.216 | −1.000 | 1.000 |
| | | | 100 | 0.881 | | | | |
| | | | 111 | 0.071 | | | | |
| average | | | | | $\overline{Ho}=0.105\pm0.023$ | $\overline{He}=0.201\pm0.038$ | $\overline{D}=-0.456$ | $\overline{Ne}=1.415\pm0.043$ |

*N*: the number of genes sampled at each locus, *No*: number of alleles observed, *Ho*: the observed heterozygosity at each locus, *He*: the expected heterozygosity at each locus, *D*: the heterozygote deficiency index, *Ne*: the effective number of alleles.

## 4 Discussion

Generally speaking, invertebrate species have more genetic variation than vertebrate species. Ayala[2] reported that the average heterozygosity in vertebrate species is about 6.0%, and that in invertebrate species is about 13.4%. Among the invertebrate species, marine shellfish may be the group studied most. Like many other species of shellfish, the population of *S. broughtonii* that we sampled in Qingdao contains lots of genetic variation ($\overline{He}=0.201\pm0.038$, $\overline{Ho}=0.105\pm0.023$, $P=45.5\%$). The most variable locus observed in this study is IDH−2 with an effective number of alleles (*Ne*) 2.469, and an observed heterozygosity (*Ho*) 0.595. Although the loci of MDH−2 and PGM have the most and the second most observed alleles (8, 6) respectively, the values of their *Ne* and *Ho*

are obviously lower than those of locus IDH - 2 owing to deficiency of heterozygous individuals ($D$ = -0.561, -0.378). A noticeable point is the complete deficiency of heterozygous individuals at loci of LDH, SDH - 1 and EST - 4, where the $Ho$, $Ne$, $D$ are 0.000, 1.000, -1.000 respectively. $\overline{Ho}$ of *S. broughtonii* is higher than that of the average value 0.098 ± 0.022 given by 10 marine invertebrate species[21], lower than that of three clam species (0.50 for *Ruditapes aureus*, 0.259 for *R. decussatus*, 0.343 for *R. philippinarum*)[6] and close to the average of 0.151 ± 0.063 given by 51 marine shellfish[25].

Just as other shellfish, the deficiency of heterozygous individuals evidently exists in the studied samples, especially at the loci of ME - 3, SDH - 1, PGM, LDH, MDH - 2 and EST - 4. Several reasons may account for the observed deficiency of heterozygous individuals: (1) inbreeding, (2) natural selection against heterozygous individuals, (3) biased scoring against heterozygous individuals.

It is known that inbreeding would result in deficiency of heterozygous individuals, if $f$ is inbreeding coefficient per locus, $F$ is mean value of $f$ for all loci studied, $K$ is the proportion of self - fertilization, $f = 1 - (Ho/He)$, $F = \sum f/n$, and $K = 2F/(1 + F)$. The $F$ and $K$ will be 0.456 and 0.626 respectively. It is unlikely that the population has so much self - fertilization and such a high $F$ value if we do not have other believable evidences.

Although there are 3 and 2 alleles respectively at loci of SDH - 1 and EST - 4, we find no heterozygotes exist. It implies that the heterozygous individuals at these two loci are selected by nature without advantage, and one observation supporting this implication is that: EST - 4 locus is very poorly polymorphic. Actually the locus would be considered monomorphic if we set the polymorphic criterion as $P \leqslant 0.95$.

The third likely reason is that scoring of the genotype on gels is biased against heterozygous individuals. We find certain allozymes stained heavily and some others stained poorly on gels, so if two allozymes differ little in migration, the genotype of heterozygous individuals is often scored as that of homozygous individuals.

From above discussion, it is thought that natural selection against heterozygous individuals and biased scoring against heterozygous individuals are possible reasons for the deficiency of heterozygous individuals.

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# 青岛近海魁蚶群体等位基因酶遗传变异研究

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**摘 要** 采用淀粉凝胶电泳技术研究了青岛近海魁蚶(*Scapharca broughtonii*)自然群体的等位基因酶遗传变异。在10种等位基因酶中共检测到了22个基因座位,多态位点比例为45.5%,位点有效等位基因数分布为1.000~2.469(平均值1.415),位点杂合度观察值分布为0.000~0.595(平均杂合度0.105±0.023),在6个基因座位上存在相当明显的杂合子缺失现象。文中讨论了杂合子缺失的原因。

**关键词** 魁蚶,等位基因酶,遗传变异,淀粉凝胶电泳