Genetic Variation in Marine Molluscs

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Starch gel electrophoresis was carried out to survey genetic variation in 25 species of marine molluscs. The gel phenotypes for 19 enzymes and muscle proteins are described. Genetic variation measured as the proportion of polymorphic loci varied from 0.190 to 0.667 with a mean of 0.412 ± 0.030 , as observed heterozygosity from 0.059 to 0.216 with a mean of 0.129 ± 0.009 , and as expected heterozygosity from 0.060 to 0.250 with a mean of 0.147 ± 0.011 . Marine molluscs are generally more variable than marine teleosts.

An excess of homozygotes was observed at many loci in most species of molluscs. Such a phenomenon has not been observed in marine teleosts. Possible explanations for this observation are discussed.

The technique of gel electrophoresis has been used to study genetic variation in a wide range of organisms from *Drosophila* to man. A general finding has been that vertebrates have low levels of genetic variation and invertebrates high levels of genetic variation.¹⁾ This has been explained in terms of environmental grain or patchiness whereby the large mobile organisms, generally vertebrates, experience their environment as fine grained or homogeneous and smaller less mobile organisms, generally invertebrates, experience their environment as coarse grained or heterogeneous.²⁾ A heterogeneous environment would enhance and a homogeneous environment erode genetic variation.¹⁾

In a survey of genetic variation in marine teleosts heterozygosities varied from 0.000 to 0.174 with a mean of 0.059³⁾ a level typical of that in other vertebrates.¹⁾ There are fewer estimates of genetic variation in marine molluscs. Unlike marine teleosts the adult stages of marine molluscs are relatively immobile although the larvae are frequently pelagic promoting gene flow. In this paper we describe the gel phenotypes for 19 enzymes and estimate levels of genetic variation in 25 species of marine mollusc. In addition electrophoretic data can be used to estimate population structuring within a species by comparing the distribution of observed phenotypes with that expected under conditions of random mating (i.e. the Hardy-Weinberg test). Most enzyme loci tested in marine teleosts were in Hardy-Weinberg equilibrium⁴⁾ whereas an excess of homozygotes

has been observed at several loci in marine molluscs.^{4, 5)}

Materials and Methods

Twenty three species of bivalve and 2 species of gastropod were collected from coastal areas of Aomori, Iwate, Akita, Miyagi, Fukushima, Ibaragi, Hyogo, and Saga in Japan, from Wellington and Foveaux strait, New Zealand and from Sydney, Australia (Table 1). Two species, *Ostrea edulis* and *O. lurida* were collected from cultured stock originating from Europe and the U.S.A. (Table 1). All other species were collected from native stocks.

Samples were frozen at -20°C until tested. Extracts of adductor muscle or shell muscle and digestive caecum were subject to starch gel electrophoresis following the procedure of FUJIO (1977).⁶⁾ Nineteen enzymes, acid phosphatase (ACP), adenylate kinase (AK), alcohol dehydrogenase (ADH), alkaline phosphatase (ALP), aspartate aminotransferase (AAT), catalase (CAT), esterase (EST), glucosephosphate isomerase (GPI), α -glycerophosphate dehydrogenase (α GPD), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), octanol dehydrogenase (ODH), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), superoxide dismutase (SOD), xanthine dehydrogenase (XDH) and one muscle protein (PT) were examined.

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Table 1. Number of specimens and sample locations for the 25 species of marine mollusc collected for electrophoretic analysis.

Species	No. of samples	Location
Class Pelecypoda		
Family Arcidae		
<i>Anadara broughtonii</i>	32	Yuriage, Miyagi
Family Mytilidae		
<i>Septifer virgatus</i>	30	Shichigahama, Miyagi
<i>Mytilus edulis</i>	130	Yamada Bay, Iwate
<i>Mytilus coruscus</i>	29	Nakaminato, Ibaragi
<i>Perna canaliculus</i>	30	Wellington, New Zealand
Family Pectinidae		
<i>Chlamys nipponensis akazara</i>	5	Onagawa, Miyagi
<i>Patinopecten yessoensis</i>	30	Ogachi, Miyagi
<i>Pecten novaezelandiae</i>	31	Wellington, New Zealand
Family Ostreidae		
<i>Crassostrea gigas</i>	33	Ojika, Miyagi
<i>Crassostrea commercialis</i>	4	Sydney, Australia
<i>Crassostrea nippona</i>	25	Toga Bay, Akita
<i>Crassostrea rivulatis</i>	22	Ariakekai, Saga
<i>Ostrea edulis</i>	30	Origin from Netherland, maintained in culture
<i>Ostrea lurida</i>	60	Origin from U.S.A., maintained in culture
<i>Ostrea denselamellosa</i>	15	Harimanada, Hyogo
<i>Ostrea circumpecta</i>	13	Mutsu Bay, Aomori
<i>Tiostrea lutaria</i>	32	Foveaux Strait, New Zealand
Family Corbiculidae		
<i>Corbicula japonica</i>	32	Ishinomaki, Miyagi
Family Cardiidae		
<i>Clinocardium buellowi</i>	31	Haragama, Fukushima
Family Veneridae		
<i>Tapes japonica</i>	32	Haragama, Fukushima
Mactridae		
<i>Spisula sachalinensis</i>	29	Isobe, Fukushima
<i>Spisula voyi</i>	30	Haragama, Fukushima
Tellinidae		
<i>Peronidia venulosa</i>	32	Isobe, Fukushima
Glass Gastropoda		
Family Haliotidae		
<i>Haliotis discus hannai</i>	31	Kesenuma, Miyagi
Family Buccinidae		
<i>Neptunea arthritica</i>	32	Haragama, Fukushima

Results

Twenty proteins were studied in the 25 species of marine mollusc. In the absence of breeding studies the genetic basis of the gel phenotypes was assumed by comparison with homologous enzymes in many other species.⁷⁾ In addition repeat testing of a sample always produced the same result. Six enzymes, acid phosphatase, adenylate kinase, alcohol dehydrogenase, alkaline phosphatase, α -glycerophosphate dehydrogenase and octanol dehydrogenase did not always produce gel phenotypes expected from their

known quarternary structures in vertebrates and invertebrates.⁷⁾ Malic enzyme also exhibited a variable quarternary structure between loci, but this enzyme is known to have a variable structure between loci, but this enzyme is known to have a variable structure in other species.⁷⁾ We have tentatively scored these variable loci as polymorphic because of (a) the repeatable nature of the gel phenotypes, (b) the regular staining intensities of the bands which were not typical of storage artifacts and (c) constancy of phenotypes at a locus i.e. all individuals either 1 and 2 band types or 1 and 3 band types but never 1, 2 and 3

band types. A locus was scored as polymorphic if the frequency of the common allele was less than 0.95. The number of monomorphic and polymorphic loci for each species is given in Table 2.

Acid phosphatase (ACP) was detected with β -naphthyl acid phosphate in samples of digestive caecum. Some species produced a null activity result or diffuse gel patterns some of which were variable but could not be scored. One and 2 band phenotypes were observed at 10 loci in 8 species suggesting a monomeric structure for this enzyme in molluscs. In vertebrates and other invertebrates it has a dimeric structure⁷⁾ although a monomeric structure has been reported in human red cell acid phosphatase.⁸⁾

Adenylate kinase (AK) was tested in muscle tissue and resolved as 1, 2 or 3 zones. One and 2 band phenotypes were observed in *Anadara broughtonii*, *Septifer virgatus*, *Mytilus coruscus*, *Crassostrea nippona* and *Corbicula japonica*, indicating a monomeric structure as expected for this enzyme.⁷⁾ One and 3 band phenotypes were observed in *Clinocardium buellowi*, *Spisula voyi* and *Peronidia venulosa*. Six out of 16 species tested were polymorphic and rare variants were detected in 3 species.

Alcohol dehydrogenase (ADH) was tested in digestive caecum and produced diffuse gel phenotypes or a null activity result in most molluscs. It appeared as a single band in *Crassostrea nippona* and as 1 and 3 band phenotypes in *Tapes japonica* and *Spisula voyi* as expected for this dimeric enzyme.⁷⁾ *Peronidia venulosa* showed 1 and 2 band phenotypes typical of a monomeric enzyme.

Alkaline phosphatase (ALP) was detected with β -naphthyl acid phosphate in the digestive caecum. Some species produced a null activity result or diffuse gel patterns some of which were variable but could not be scored. One and 2 band phenotypes were observed at 7 loci in 6 species suggesting a monomeric structure for this enzyme in molluscs. In vertebrates and other invertebrates it has a dimeric structure.⁷⁾

Aspartate aminotransferase (AAT) was expressed in muscle tissue with 1 or 2 loci in most species. In oysters of the genus *Crassostrea* and *Ostrea* 3 loci were found. One and 3 band phenotypes were found in 14 of the 24 species tested indicating a dimeric structure as expected as expected for this enzyme.⁷⁾

Catalase (CAT) was examined in muscle samples of 23 species. In 18 species it appeared

as a single genetic locus and in 5 species as 2 loci (Table 2). It is highly variable with 1 and 2 band phenotypes in 18 of the 23 species.

Esterase (EST) was detected with α -naphthyl acetate in digestive caecum and showed activity in several zones. Some loci were clearly monomorphic, some polymorphic with 1 and 2 band phenotypes and others variable but not scoreable. Thirty-four polymorphic loci were found in 22 of the 24 species tested.

Glucosephosphate isomerase (GPI) was examined in muscle samples and resolved with the expected 1 and 3 band phenotypes for this enzyme. It was polymorphic in 16 species and 1 further species showed rare alleles.

α -Glycerophosphate dehydrogenase (α GPD) showed activity in either or both digestive caecum and muscle tissue. α GPD is typically a dimer⁷⁾ and 1 and 3 band phenotypes were found in digestive caecum of *Crassostrea gigas* and *Tapes japonica* and in muscle of *Perna canaliculus* and *Spisula voyi*. However clear 1 and 2 band phenotypes were found in digestive caecum of *Perna canaliculus* and *Corbicula japonica* and in muscle of *Patinoptecten yessoensis*. Seven of the 20 species examined produced diffuse gel phenotypes which could not be scored. Polymorphism was observed at 1 or 2 loci in 4 of the 13 species scored and rare variants at single loci in 2 species.

Isocitrate dehydrogenase (IDH) appeared as one or two zones of activity in most species. For the 14 species with 2 loci one locus was dominant in digestive caecum and the other locus dominant in muscle. Six species revealed 1 locus which stained strongly in digestive caecum but which was absent or stained weakly in muscle. Three species revealed 1 locus which stained strongly in muscle but which was absent or stained weakly in digestive caecum. Two species exhibited 2 loci in digestive caecum and a third locus in muscle tissue. Homozygous and heterozygous individuals showed 1 and 3 band phenotypes respectively as expected for this dimeric enzyme. Eleven species were polymorphic and rare alleles were found in a further 2 species (Table 2).

Lactate dehydrogenase (LDH) activity was detected in muscle tissue of 8 out of 25 species tested (Table 2). Staining intensities were generally lower than those in teleosts.⁸⁾ Monomorphic species produced a single band and polymorphic species 1 and 5 band phenotypes as expected for this tetrameric enzyme.⁷⁾ In *Haliotis discus hannai* 2 LDH zones were present suggesting

Table 2. Summary of the results of electrophoretic

Species	No. of tested	Glucose metabolizing enzyme								
		LDH	MDH	ME	IDH	α GPD	6PGD	PGM	AAT	GPI
<i>Anadara broughtonii</i>	32	P	2M	2P	P	S	P*	P*	M	2M
<i>Septifer virgatus</i>	30	NA	P+2M	M	M	NA	M	P	P+M	P
<i>Mytilus edulis</i>	130	NA	P*+2M	P	P	2M	P	3P	P+M	2P
<i>Mytilus coruscus</i>	29	NA	3M	2P	P+M	V	P	P	P	P
<i>Perna canaliculus</i>	30	NA	P+M	P	P+M	2P	2P	P	2P	P+M
<i>Chlamys nipponensis akazara</i>	5	NA	3M	—	2M	—	—	P+M	2M	P
<i>Patinopecten yessoensis</i>	30	NA	P*+M	2P	M	P	M	P	P	M
<i>Pecten novaezelandiae</i>	31	M	2M	P*+M	M	2M	P	P	P+M	P
<i>Crassostrea gigas</i>	33	NA	4M	P+M	P+M	P*	P	2P	2P+M	P
<i>Crassostrea commercialis</i>	4	NA	4M	M	2M	—	—	P+M	—	M
<i>Crassostrea nippona</i>	25	NA	P+4M	P+M	P*+M	M	2P+M	2P+P*	P+2M	P*+M
<i>Crassostrea rivularis</i>	22	NA	5M	M	2M	—	—	P	3M	M
<i>Ostrea edulis</i>	30	NA	P+4M	M	2M	S	P+M	P+M	3M	M
<i>Ostrea lurida</i>	60	NA	5M	M	P+M	—	—	P	3M	P+M
<i>Ostrea denselamellosa</i>	15	NA	P+4M	M	P+M	NA	V	M	P+2M	P+M
<i>Ostrea circumpicta</i>	13	NA	5M	M	2M	—	—	P	3M	M
<i>Tiostrea lutaria</i>	32	NA	P+M	3P	M	M	2P	P	P*	P
<i>Corbicula japonica</i>	32	P	P*	V	P	P*	P	2P+M	2M	P
<i>Clinocardium buellowi</i>	31	P	P+3M	2M	2P+M	M	P	P+2M	P+M	P+M
<i>Tapes japonica</i>	32	NA	P+3M	2P	P+M	P+M	P*	P	P+M	P
<i>Spisula sachalinensis</i>	29	M	P+M	P+M	3M	2M	P	2P+2M	M	M
<i>Spisula voyi</i>	30	M	2M	2P+2M	P*+M	P+M	M	3P	P	P
<i>Peronidia venulosa</i>	32	V	P+M	P+2M	P+M	V	P	P	P+M	P
<i>Haliotis discus hannai</i>	31	P+M	P*+2M	M	M	2M	P	2P	P+M	M
<i>Neptunea arthritica</i>	32	NA	3M	P+M	M	NA	M	P	P*+M	P
Total number of loci		8	81	40	43	20	24	43	49	32
Number of polymorphic loci		4	10	20	12	5	16	32	16	17
Proportion of polymorphic loci		.500	.123	.500	.279	.250	.667	.744	.327	.531

M = monomorphic; P = polymorphic; P* = variant allele less than 0.05; V = variable, no single genetic explanation; S = smear,

that there are 2 loci coding for LDH.

Leucine aminopeptidase (LAP) was tested in digestive caecum and muscle tissues and produced several zones of activity some of which could not be scored. Polymorphism was observed at 39 loci in 23 species and rare alleles in 2 species. Gel phenotypes resolved as 1 or 2 bands typical of this monomeric enzyme.⁷⁾

Malate dehydrogenase (MDH) resolved with a variable number of loci between species. One locus was found in *Corbicula japonica* and up to 5 loci in oysters of the genus *Crassostrea* and *Ostrea* (Table 2). Genetically variable loci showed 1 and 3 band phenotypes as expected for this dimeric enzyme.⁷⁾ Polymorphism was recorded in 10 species and rare alleles in a further 4 species.

Malic enzyme (ME) appeared as 1 or 2 zones in

most species indicating 1 or 2 loci. In *Tiostrea lutaria* and *Peronidia venulosa* there were 3 zones of activity and in *Spisula voyi* 4 zones suggesting 3 and 4 loci respectively. Presumed homozygotes appeared as a single band and heterozygotes as either 2, 3 or 5 band types. The number of bands in the heterozygous phenotype was constant for a given locus and only varied between loci or species. A variable quaternary structure has been reported for malic enzyme in both vertebrates and invertebrates.⁷⁾

A monomeric structure was exhibited in digestive caecum of *Anadara broughtonii*, *Mytilus coruscus*, *Tiostrea lutaria*, *Tapes japonica* and *Neptunea arthritica* and in muscle of *Patinopecten yessoensis*. A dimeric structure was exhibited in digestive caecum of *Crassostrea nippona*, *Spisula sachalinensis* and *S. voyi* and in muscle of *Anadara*

analysis of 25 species of marine mollusc.

Non-glucose metabolizing										PT
ADH	ODH	XDH	ACP	ALP	EST	SOD	CAT	LAP	AK	
NA	M	P	2M	2P	P+2M	P+P*	P	2P+M	P	2P+M
NA	NA	P	P	NA	3P+M	P*	P	P	P*+M	—
—	—	M	—	M	—	2M	M	2P+M	—	—
S	P*	M	S	M	2P+M	2M	P	3P	P+2M	P+M
S	NA	M	M	M	P+2M	P+M	P	2P+M	M	3P+M
—	—	—	5M	—	P+2M	2M	M	2P	—	—
NA	NA	NA	S	NA	P+M	P+M	P	P+M	2M	P+2M
NA	M	M	NA	M	2P*	P+M	P	2P	2M	2M
S	P+M	P	P+M	S	P+2M	M	P	P+M	S	P+M
—	—	—	2M	—	2P+M	2M	P+M	P	—	—
M	P	M	P+M	P	2P+2M	P	P+M	P	P+2M	P+P*
—	—	—	M	—	2P+2M	M	—	2P	—	—
NA	NA	M	P	M	P+3M	2M	M	2P+M	M	M
—	—	—	M	—	2M	M	—	P+2M	—	—
NA	NA	NA	M	V	P+M	P*	P+M	2P	M	M
—	—	—	M	—	P+2M	M	P+M	P*	—	—
NA	NA	P	NA	P	P	P+M	P	2P	M	P+2M
NA	P	P*	P+P*	V	P+P*	2M	P	2P+M	P+2M	2P+3M
NA	NA	M	2P	M	2P	M	P	2P	P+M	P+M
P	P	P*	P+M	P	2P+2M	P+M	P	P	M	M
S	2M	P	S	P	P	P*	P	2P	S	P+2M
P	P+P*	M	S	M	3P+2M	P+M	M	2P+P*	P*	2P+M
P	P	M	2P+M	P	2P+M	P+M	P	2P	P+P*	P+2M
NA	P	NA	M	S	P+2M	M	P+M	P+M	2M	P+2M
NA	S	NA	M	S	2P+M	2M	M	M	V	P
4	14	16	31	14	69	40	28	52	28	44
3	7	5	10	7	34	9	18	39	6	19
.750	.500	.313	.323	.500	.493	.225	.643	.750	.214	.432

not readable; NA=no activity detected; —=not tested.

broughtonii, *S. voyi* and *Peronidia venulosa*. A tetrameric structure was exhibited in digestive caecum of *Mytilus edulis*, *Tiostrea lutaria* and *Tapes japonica* and in muscle of *Mytilus coruscus*, *Perna canaliculus*, *Patinopecten yessoensis*, *Crassostrea gigas* and *Tiostrea lutaria*. Polymorphism was seen at 20 loci in 13 out of 24 species tested and rare variants at 1 locus in *Pecten novaezelandiae*. *Corbicula japonica* exhibited electrophoretic variation but this could not be scored.

Octanol dehydrogenase (ODH) was examined in muscle and activity was detected in 12 of the 19 species tested (Table 2). As for ADH, ODH appeared to exhibit either a monomeric or dimeric structure although this enzyme is typically a dimer.⁷⁾ Seven species were polymorphic, 3 were monomorphic one showed rare alleles and one diffuse activity.

Phosphoglucomutase (PGM) was active in both digestive caecum and muscle. Species showed 1, 2 or 3 loci and genetic variants appeared as 1 and 2 band phenotypes as expected for this monomeric enzyme.⁷⁾ PGM is highly variable with polymorphisms recorded in 23 species and rare alleles in a further 2 species (Table 2).

6-Phosphogluconate dehydrogenase (6PGD) was expressed mainly in muscle with the typical pattern of a dimer. It was polymorphic in 13 of the 20 species tested and a further 2 species showed rare alleles (Table 2).

Superoxide dismutase (SOD) was expressed in digestive caecum and muscle with 1 or 2 loci (Table 2). Polymorphism was revealed in 9 species with one and three band phenotypes as expected for this dimeric enzyme.⁷⁾ Rare alleles were found in a further 4 species.

Xanthine dehydrogenase (XDH) was examined in digestive caecum and revolved as 1 and 3 band phenotypes as expected for this dimeric enzyme.⁷⁾ Four out of 20 species tested showed no activity, 5 species were polymorphic and 2 species showed rare variants.

Protein (PT) appeared as many bands in muscle samples stained with amido black. Weakly staining bands were omitted from scoring. Nineteen loci were polymorphic in 14 out of 18 species tested.

Two estimates of genetic variation, the proportion of polymorphic loci and the mean individual heterozygosity are given for each species in Table 3. The observed heterozygosity (H_o) was calculated from direct count of the heterozygotes ($H_o = \sum h_j/M$ where h_j is the proportion of heterozygotes at the j th locus and N is the number of loci tested) and the expected heterozygosity (H_e) was calculated from the allele frequencies ($H_e = \sum h_i/N$ where $h_i = 1 - \sum q_i^2$ and q_i is the frequency of the i th allele).

The highest level of genetic variation is found in

the oyster *Tiostrea lutaria* and the lowest level in the oyster *Ostrea circumpecta*. The proportion of polymorphic loci varies from 0.143 to 0.667 with a mean of 0.412 ± 0.030 , the observed heterozygosity from 0.059 to 0.216 with a mean of 0.129 ± 0.009 and the expected heterozygosity from 0.060 to 0.250 with a mean of 0.147 ± 0.011 .

In most species tested the observed heterozygosity is lower than the expected heterozygosity (Table 3 columns 4 and 5). This is due to an excess of homozygotes at many loci. The excess of homozygotes from that expected under Hardy-Weinberg equilibrium was measured as $D = (H_o - H_e)/H_e$ where H_o is the observed heterozygosity and H_e is the expected heterozygosity. A negative value indicates an excess of homozygotes and a positive value an excess of heterozygotes. There is an overall excess of homozygotes in all but 3 of the 25 species of marine mollusc (Table 3 column 6).

An estimate of the influence of enzyme function on level of genetic variation was made by comparing glucose and non-glucose metabolising

Table 3. Genetic variation in 25 species of marine mollusc

Species	Proportion of polymorphic loci	Heterozygosity		D
		Observed	Expected	
<i>Anadara broughtonii</i>	0.500 (15/30)* ¹	0.164	0.171	-0.041 (8/15)* ²
<i>Septifer virgatus</i>	0.524 (11/21)	0.162	0.184	-0.120 (7/11)
<i>Mytilus edulis</i>	0.478 (11/23)	0.139	0.189	-0.265 (8/11)
<i>Mytilus coruscus</i>	0.536 (15/28)	0.122	0.144	-0.153 (12/15)
<i>Perna canaliculus</i>	0.613 (19/31)	0.172	0.208	-0.173 (13/19)
<i>Chlamys nipponensis akazara</i>	0.217 (5/23)	0.061	0.065	-0.062 (2/5)
<i>Patinopecten yessoensis</i>	0.455 (10/22)	0.160	0.182	-0.121 (5/10)
<i>Pecten novaezelandiae</i>	0.296 (8/27)	0.077	0.089	-0.135 (4/ 8)
<i>Crassostrea gigas</i>	0.500 (15/30)	0.186	0.205	-0.093 (10/15)
<i>Crassostrea commercialis</i>	0.250 (5/20)	0.100	0.092	+0.087 (0/ 5)
<i>Crassostrea nippona</i>	0.425 (17/40)	0.098	0.109	-0.101 (8/17)
<i>Crassostrea rivularis</i>	0.238 (5/12)	0.089	0.127	-0.299 (3/ 5)
<i>Ostrea edulis</i>	0.226 (7/31)	0.084	0.081	+0.037 (2/ 7)
<i>Ostrea lurida</i>	0.190 (4/21)	0.063	0.060	+0.050 (2/ 4)
<i>Ostrea denselamellosa</i>	0.333 (8/24)	0.078	0.085	-0.082 (4/ 8)
<i>Ostrea circumpecta</i>	0.143 (3/21)	0.059	0.065	-0.092 (2/ 3)
<i>Tiostrea lutaria</i>	0.667 (16/24)	0.216	0.250	-0.136 (10/16)
<i>Corbicula japonica</i>	0.484 (15/31)	0.146	0.174	-0.161 (10/15)
<i>Clinocardium buellowi</i>	0.515 (17/33)	0.170	0.181	-0.061 (10/17)
<i>Tapes japonica</i>	0.548 (17/31)	0.152	0.179	-0.151 (9/17)
<i>Spisula sachalinensis</i>	0.414 (12/29)	0.160	0.178	-0.101 (10/12)
<i>Spisula voyi</i>	0.486 (18/37)	0.143	0.160	-0.106 (11/18)
<i>Peronidia venulosa</i>	0.625 (20/32)	0.173	0.219	-0.210 (14/20)
<i>Haliotis discus hannai</i>	0.333 (10/30)	0.123	0.140	-0.121 (8/10)
<i>Neptunea arthritica</i>	0.300 (6/20)	0.119	0.129	-0.078 (4/ 6)
Average	0.412	0.129	0.147	-0.108

*¹ Number in parentheses presents number of polymorphic loci per total number of loci.

*² Number in parentheses presents number of the loci showing $-D$ per number of polymorphic loci.

enzymes. The average proportion of polymorphic loci was 0.436 ± 0.068 in glucose metabolising enzymes and 0.417 ± 0.063 in non-glucose metabolising enzymes.

Discussion

The estimates obtained in this study for the proportion of polymorphic loci and average heterozygosity in marine molluscs are in general agreement with those reported for marine invertebrates⁹⁾ but are higher than those in marine teleosts.¹⁰⁾

A comparison of 11 enzymes studied in both marine teleosts⁹⁾ and marine molluscs (this paper) for which more than 20 loci were tested shows that of the 5 most variable enzymes in teleosts and molluscs 4 of these aspartate aminotransferase, esterase, phosphoglucosmutase and 6-phosphogluconate dehydrogenase are common and that the 3 least variable enzymes adenylate kinase, malate dehydrogenase and superoxide dismutase are also common. There were no differences in levels of genetic variation between glucose and non-glucose metabolising enzymes in molluscs.

There are no obvious trends in heterozygosity with taxonomy or ecology. The highest and lowest levels of genetic variation are found in the oysters *Tiostrea lutaria* and *Ostrea circumpecta*. Two oysters *Ostrea edulis* and *O. lurida* which have low levels of genetic variation were sampled from Japanese cultured stock derived from the Netherlands and the USA and so may reflect a reduced gene pool. Low levels of genetic variation in another oyster *Crassostrea commercialis* may reflect small sample size as only 4 specimens were tested. Likewise for the scallop *Chlamys nipponensis akazara* for which only 5 specimens were tested.

Estimates of genetic variation are available for 4 species which we have studied. British populations of the mussel *Mytilus edulis*¹¹⁾ appear to be slightly less variable ($H_o = 0.095$) than the Japanese population ($H_o = 0.139$). Although it is not possible to establish locus homologies enzymes which are highly variable in British populations (glucosephosphate isomerase, leucine aminopeptidase and phosphoglucosmutase) are highly variable in the Japanese sample. Levels of genetic variation observed in *Crassostrea gigas* in this paper are similar to those previous studies (see summary in GOSLING¹²⁾). There is close agreement between the number of monomorphic and

polymorphic loci in *C. gigas* in this study and in a hatchery sample of Miyagi types derived from British Columbia.¹²⁾ Levels of genetic variation in *Crassostrea commercialis* ($H_o = 0.100$, $H_e = 0.092$) are lower than estimates ($H_o = 0.172-0.195$, $H_e = 0.184-0.196$) obtained by BUROKER *et al.*¹³⁾ but are based on only 4 specimens.

Our estimate for genetic variation in *Tiostrea lutaria* based on 32 specimens Foveaux Strait, New Zealand is not in agreement with an estimate made by BUROKER *et al.*¹⁴⁾ on 29 specimens from the same location. *T. lutaria* was the most variable species that we tested with an observed heterozygosity of 0.216 and an expected heterozygosity of 0.250. BUROKER *et al.*¹⁴⁾ found it to have a comparatively low observed heterozygosity of 0.067 and low expected heterozygosity of 0.062. Some of this discrepancy is due to the range of enzymes tested as we included 2 polymorphic loci (catalase and alkaline phosphatase) which were not tested by BUROKER *et al.*¹⁴⁾ However there are marked differences between some of the common enzymes. For malic enzyme we found 3 polymorphic loci, whereas BUROKER *et al.*¹⁴⁾ found 1 monomorphic locus, for 6-phosphogluconate dehydrogenase we found 2 polymorphic loci whereas BUROKER *et al.*¹⁴⁾ found 1 polymorphic locus, for superoxide dismutase we found 1 polymorphic and 1 monomorphic locus whereas BUROKER *et al.*¹⁴⁾ found 2 monomorphic loci and for xanthine dehydrogenase we found 1 polymorphic locus whereas BUROKER *et al.*¹⁴⁾ found 1 monomorphic locus. Clearly this species would warrant further study.

There is an excess of homozygotes at many loci in most species (Table 3). Similar findings have been made in other marine invertebrates⁵⁾ for example *Mytilus edulis*¹⁵⁻¹⁹⁾, *Mytilus californianus*¹⁹⁾, *Crassostrea virginica*²⁰⁾ and *Crassostrea gigas*.²¹⁾ Such a phenomenon has not been observed in marine teleosts (Fig. 1) and thus deserves an explanation. An excess of homozygotes could be produced by several mechanisms.

(a) Mis-scoring of gel phenotypes. If gel phenotypes were not interpreted correctly, an apparent excess of homozygotes could be reported. This is unlikely because we have not found an excess of homozygotes at homologous enzymes in marine teleosts when using similar electrophoretic techniques.³⁾

(b) Null alleles. The presence of a null, or no-activity, allele would give rise to scoring errors whereby heterozygotes were scored as homo-

Fig. 1. Distribution of the value of D averaged over loci for marine molluscs and teleosts.

The value of D for teleosts was calculated from the data of FUJIO and KATO.⁸⁾

zygotes. However we did not find specimens which showed no activity, i.e. null allele homozygotes, so that one has to postulate strong selection against null homozygotes. In addition null alleles are rare in insects and vertebrates and so it seems unlikely that they would occur in high frequency in molluscs.

(c) Inbreeding. Reproduction between closely related individuals or a small number of individuals could produce an excess of homozygotes. Such a mechanism is unlikely in marine teleosts where the adults are mobile and spawn in large schools. In molluscs where the adults are sessile animals which are close to each other have a much greater chance of reproductive success than spatially isolated individuals because of the rapid dilution of eggs and spermatozoa once released into the sea. However, unless batches of larvae remain together as discrete units until settlement time, this inbreeding effect will be reduced through pelagic dispersal.

(d) Selection against heterozygotes. An homozygous excess could be produced if homozygotes had a higher fitness than heterozygotes. This disruptive selection could ultimately lead to fixation of alleles and result in low levels of genetic

variation which is not in accord with our observations. There is little evidence to support selection against heterozygotes²²⁾ but more to support increased heterozygosity with size or age.^{19,20,28)} A selective mechanism which favoured different alleles or genotypes over a long spawning season or between life cycle stages could produce an excess of homozygotes.⁵⁾

(e) Two or more subpopulations. An excess of homozygotes would be observed in a sample which included individuals from 2 or more genetically distinct populations. This classical explanation, known as the Wahlund effect, gives no indication of the mechanisms maintaining the genetic distinction between populations. The differences could be due to local selection or drift. Populations need not represent discrete stocks as observed in marine teleosts²⁴⁾ but could be high and low shore distributions or age class cohorts.

Scoring errors and null alleles are unlikely explanations for the excess of homozygotes. Three explanations inbreeding, selection and subpopulations could contribute to the observed excess of homozygotes and are not necessarily mutually exclusive. In some species a greater excess of homozygotes has been observed in juveniles than in adults^{4,19)} suggesting that selection is an important component reducing the homozygous excess. The mechanism that produces the excess is unclear but must occur during the planktonic stages or during the spawning aggregation of adults. Clearly these stages of the life cycle require further genetic studies.

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