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台灣大牡蠣族群之同功酶變異

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Allozyme Variation in the Pacific Oyster *Crassostrea gigas* along the Coast of Taiwan

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ABSTRACT

Nine populations of the oysters *Crassostrea gigas*, were assayed for allozyme variation by starch gel electrophoresis. Three of the seven enzyme loci investigated are polymorphic: *Lap*, *Mpi*, and *Pgi*. Significant heterozygote deficiency was found in all seven Taiwan populations but not in the two populations of the Penghu Islands. Low levels of genetic variability (p = 0.14 - 0.42; H = 0.044 - 0.084) and little genetic differentiation (Nei's D < 0.01) were found among populations. Individuals with *Lap* heterozygotes were smaller than those with homozygotes in one population (p = 0.03) but this difference was statistically insignificant when adjusted significance level for multiple tests was used. Our results suggest that environmental factors may be important in determining regional differences in oyster size and shape.

INTRODUCTION

The Pacific oyster *Crassostrea. gigas* (Thunberg) ranges from the coast of Japan and the Korean into peninsula to China and Southeast Asia (Ahmed 1975). It has also been introduced to Australia, France, New Zealand, and North America for commercial farming (Chew 1990). *C. gigas* is one of the most important cultured shellfish along the west coast of Taiwan and the adjacent Penghu Islands (Lin and Liang 1982).

In recent years, it has been reported that allozyme heterozygosity is positively correlated with growth rate in some marine bivalves, e.g., Pacific oyster *C. gigas* (Fujio 1982), American oyster *C. virginica* (Singh and Zouros 1978, Zouros et al. 1980), and blue mussel *Mytilus edulis* (Koehn and Gaffney 1984). There is little information about genetic variation and growth rate in *C. gigas* from Taiwan but regional differences in size and shape among aquaculture sites were reported by Soong et al. (1992). This study was undertaken to determine (1) whether genetic variation exists among oysters from various locations, and (2) whether there is any correlation between heterozygosity and size (expressed as shell

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length and wet meat weight) in *C. gigas* at collection sites in Taiwan.

MATERIALS AND METHODS

Oysters were collected along the west coast of Taiwan and Penghu Islands from September 1990 to January 1991, from seven aquaculture areas at Shunsun, Lukang, Dongshi, Budai, Chigu, Dongkang, and Tsaiyuan, and two wild sites at Yunan and Waton (Fig. 1). Three to five long lines with adult-sized oysters attached to artificial substrata were sampled at each aquaculture site. Oysters in wild sites were collected from natural substrata. Live oysters were stored at -70°C after transport back to the laboratory. After shell length and wet meat weight were measured, mantle tissues were homogenized in a Tekmar tissumizer with an equal volume of 10 mM Tris- HCl buffer (pH 7.0) containing 1 % Triton X-100. Homogenates were centrifuged at 5,000 g for 15 min and the supernatants were again stored at -70°C until electrophoretic analysis.

Seven loci were assayed: adenylate kinase (*Adk*, EC 2.7.4.3), arginine kinase (*Ark*, EC 2.7.3.3), isocitrate dehydrogenase (*Idh*, EC 1.1.1.42), leucine aminopeptidase (*Lap*, EC 3.4.11 or 13), malate dehydrogenase (*Mdh*, EC 1.1.1.37), mannose-6-phosphate isomerase (*Mpi*, EC 5.3.1.8), and phosphoglucoisomerase (*Pgi*, EC 5.3.1.9). Horizontal starch-gel electrophoresis with Tris-citrate pH 7.0 and 8.0 buffer systems were used. Enzyme-staining methods followed Murphy et al. (1990).

Alleles at each locus were scored by designating the most common allele as 100. All other alleles were numbered according to their relative anodal distance from the most common allele. Chi-square goodness-of-fit tests were computed at each locus to determine if there were significant deviations from Hardy-Weinberg equilibrium between observed and expected heterozygous genotype frequencies. The mean observed and expected heterozygosity in each population was also calculated. For each locus, the nine populations were tested for homogeneity using the G-contingency test (Sokal and Rohlf 1981) with pooled homozygotes and pooled heterozygotes. Nei's genetic distance coefficients (D) were also calculated. The Mann- Whitney U test was used to determine whether there was any difference between genotypes (homozygotes vs. heterozygotes) and size (expressed as shell length or wet meat weight).

RESULTS

When the commonest allele $\leqq$ 0.95 was used as the criterion for polymorphism, four of

Fig. 1. *Crassostrea gigas* sample locations in Taiwan and Penghu Islands.

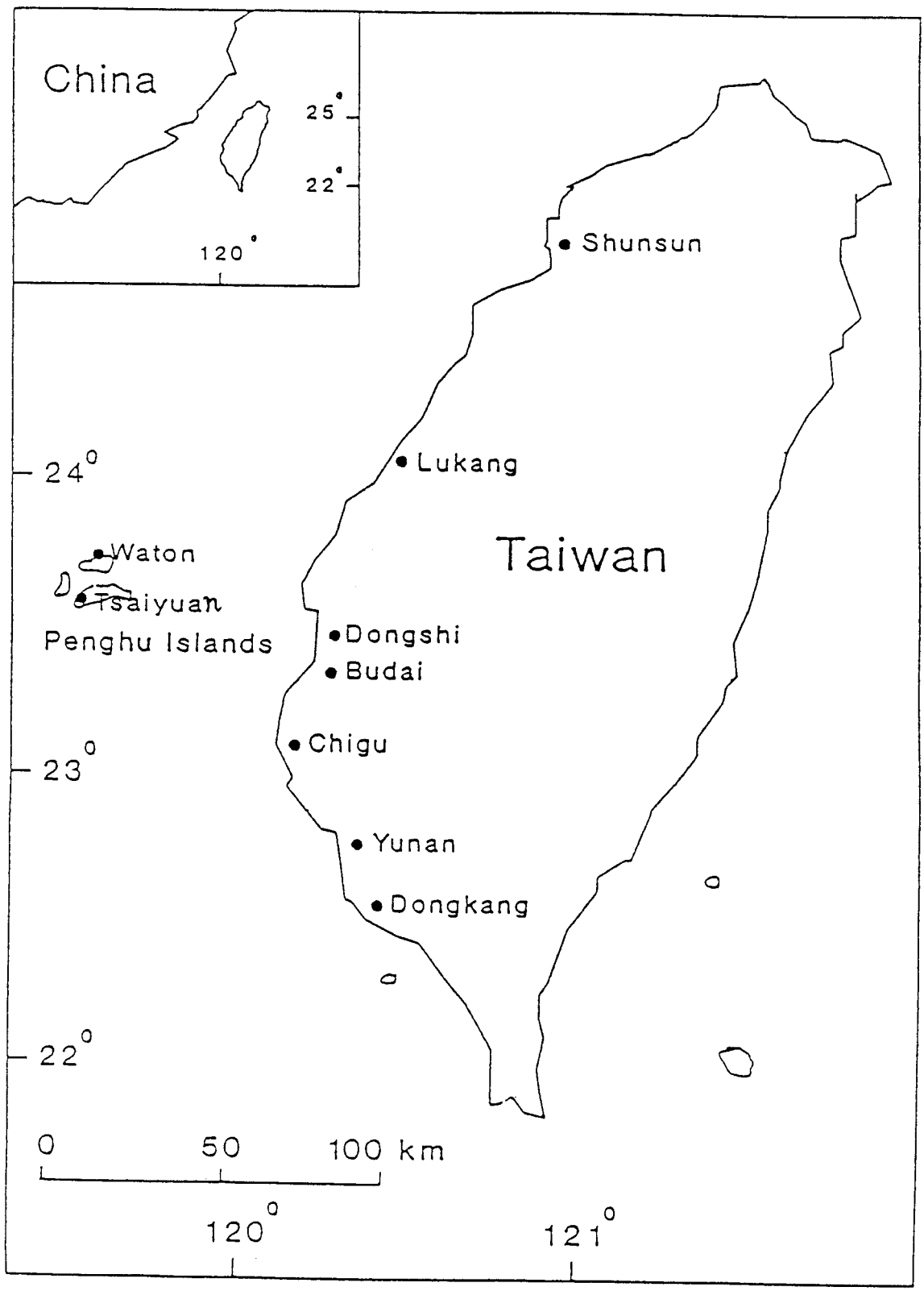

Table 1. Allelic frequencies for three polymorphic loci in nine populations of the oyster *Crassostrea gigas*.

Locus	Allele (RM)	Population								
		Cultured							Wild	
		Shun-sun	Lu-kang	Dong-shi	Bu-dai	Chi-gu	Dong-kang	Tsai-yuan	Yun-an	Wa-ton
Lap										
(N)		72	72	68	72	117	79	66	44	59
	125	.000	.000	.000	.000	.013	.000	.000	.034	.000
	120	.014	.000	.000	.000	.000	.000	.000	.000	.000
	113	.097	.042	.015	.028	.056	.051	.015	.034	.068
	100	.819	.868	.919	.924	.825	.823	.886	.795	.864
	90	.007	.000	.000	.000	.021	.006	.000	.000	.000
	88	.049	.090	.066	.049	.077	.114	.091	.136	.034.
	78	.014	.000	.000	.000	.009	.006	.008	.000	.034
	Ho	.292**	.181**	.132	.125	.282**	.203**	.197	.227**	.254
	He	.318	.238	.152	.145	.311	.309	.207	.350	.248
Mpi										
(N)		72	72	69	72	118	79	66	48	60
	113	.000	.021	.029	.014	.000	.000	.000	.010	.008
	100	.986	.917	.949	.944	.936	.937	.886	.906	.983
	90	.000	.000	.000	.000	.000	.000	.000	.010	.000
	75	.014	.056	.022	.042	.064	.063	.114	.063	.008
	67	.000	.007	.000	.000	.000	.000	.000	.010	.000
	Ho	.028	.125**	.072**	.083*	.093**	.101	.227	.104**	.033
	He	.028	.157	.098	.107	.120	.119	.203	.176	.033
Pgi										
(N)		72	72	69	72	118	76	66	44	60
	143	.090	.021	.022	.042	.064	.020	.053	.000	.000
	100	.889	.896	.957	.938	.886	.901	.924	.875	.975
	56	.021	.083	.022	.021	.051	.072	.023	.125	.017
	47	.000	.000	.000	.000	.000	.007	.000	.000	.008
	Ho	.083**	.181	.072**	.097*	.161**	.171**	.152	.068**	.050
	He	.203	.191	.085	.120	.210	.183	.144	.221	.049

(RM: relative allelic mobility; N: sample size; *Ho*, *He*: observed and expected heterozygosity from Hardy-Weinberg equilibrium; deviations from expected frequencies are tested by the X^2-test. **: $p<0.01$, *: p, 0.05).

the seven enzyme loci investigated were monomorphic: *Adk*, *Ark*, *Idh*, and *Mdh*. Allelic frequencies of polymorphic loci in the surveyed populations are shown in Table 1. Observed heterozygosity in the polymorphic *Lap*, *Mpi*, and *Pgi* loci among populations were 0.125 - 0.292, 0.028 - 0.227 and 0.050 - 0.181, respectively. Significant heterozygote deficiency was found at two to three polymorphic loci in all seven populations from Taiwan but not in the two populations from Penghu (Tsaiyuan and Waton). Mean heterozygosity of all loci in each population ranged from 0.044 to 0.084 and no significant difference was found between cultured and wild populations (Table 2).

Table 2. Summary of genetic variation in *Crassostrea gigas* in Taiwan

Population	Mean number alleles per locus ± S.E.	Percent polymorphic loci (95% criterion)	Mean heterozygosity ± S.E.	
			observed	expected
Cultured				
Shunsun	2.1 ± 0.7	28.6	0.058 ± 0.041	0.078 ± 0.049
Lukang	2.1 ± 0.5	42.9	0.071 ± 0.033	0.086 ± 0.040
Dongshi	2.1 ± 0.3	28.6	0.044 ± 0.019	0.052 ± 0.023
Budai	2.1 ± 0.4	42.9	0.050 ± 0.020	0.059 ± 0.024
Chigu	2.4 ± 0.6	42.9	0.079 ± 0.041	0.094 ± 0.047
Dongkang	2.3 ± 0.6	42.9	0.070 ± 0.033	0.093 ± 0.045
Tsaiyuan	2.0 ± 0.4	42.9	0.084 ± 0.039	0.081 ± 0.037
Wild				
Yunan	2.1 ± 0.6	42.9	0.057 ± 0.032	0.107 ± 0.054
Waton	2.0 ± 0.5	14.3	0.048 ± 0.035	0.047 ± 0.034

The nine populations were homogeneous at *Lap* and *Pgi* and heterogeneous at *Mpi* ($G_{(8)}$ =20.2, p<0.01). Nei's genetic distances among populations ranged from 0 to 0.004. In other words, very little genetic differentiation occurs at these seven loci among oyster populations in Taiwan and Penghu.

Due to low frequencies of heterozygotes found at the *Mpi* and *Pgi* loci, the relationship between heterozygosity and size was only assessed at the *Lap* locus. We found that, in theBudai population, individuals of *Lap* heterozygotes were significantly smaller than those of homozygotes (p = .003) (Table 3). However, by using adjusted significance level for multiple tests, this difference was statistically insignificant.

Table 3. Average shell length and meat weight of heterozygotes and homozygotes at *Lap* locus in *Crassostrea gigas*. Data are presented as mean ±std; N: sample size; significance of the difference is given (*: $p < 0.05$).

		Length (mm)			Weight (g)	
Population	*N*	Heterozygotes	*N*	Homozygotes	Heterozygotes	Heterozygotes
Shunsun	21	38.3±9.6	51	36.2±7.4	6.05±3.30	5.04±2.57
Lukang	13	34.6±7.9	58	37.6±9.8	5.14±3.57	6.00±3.50
Dongshi	9	45.4±17.7	57	43.6±10.7	6.88±4.11	7.74±5.59
Budai	9	24.4±14.9	63	36.2±14.4*	1.88±2.61	5.33±4.52*
Chigu	33	43.5±9.3	84	43.9±8.3	5.02±1.78	5.09±1.83
Dongkang	16	34.4±8.0	63	33.9±9.1	5.70±2.96	4.67±3.25
Tsaiyuan	13	59.2±10.6	53	59.4±12.5	12.68±5.70	11.34±6.12

DISCUSSION

Populations of *C. gigas* in Taiwan and Penghu have lower mean heterozygosity (0.044 - 0.084) than conspecific populations in other areas and other *Crassostrea* species. For instance, the mean heterozygosities were 0.186 (Fujio et al. 1983) and 0.234 (Ozaki and Fujio 1985) in *C. gigas* and *C. angulata*, respectively. In locus by locus comparisons, the observed heterozygosities of most loci in the surveyed populations were generally lower than other *C. gigas* populations, although the comparison was only based on six loci. For example, observed heterozygosities at *Lap* and *Pgi* were 0.125 - 0.292 and 0.050 - 0.181 , respectively, in the present study, but 0.256-0.571 and 0.217-0.474 in the study of Buroker et al. (1979) in Japan. Many factors might result in the phenomenon of low heterozygosity in the population in Taiwan and Penghu including the Wahlund effect, null alleles, inbreeding, small effective population size originally, bottleneck effect at Pleistocene due to sea level decrease (Emery et al. 1971), artificial selection, and others. However, it is not possible to elucidate the real cause at present.

The small genetic distances (0 to 0.004) among the nine populations of *C. gigas* indicate that little genetic differentiation occurs over a horizontal distance of up to 300 km. In Taiwan, oysters spawn throughout the year and the planktonic larval period ranges from one to two weeks (Lin and Liang 1982). Dongshi and Budai are the spat collection areas which supply spats for culturing in Taiwan and Penghu. The extence of planktonic larval stages and the procurement of spats from a single location has produced extensive gene flow which may have prevented genetic differentiation of these oyster populations. In addition, the practice by farmers of moving adult oysters between sites to increase their weight before

marketing (personal observation) may also prevent genetic differentiation among populations.

Significant heterozygote deficiency was found in all populations from Taiwan but not in populations from Penghu. In the Penghu Islands, both cultured population (Tsaiyuan) and wild population (Waton) exist, The spats for culturing at Tsaiyuan are imported from Taiwan. Although extensive gene flow might diminish population differences between Taiwan and Penghu, heterozygote deficiency only occurs in Taiwan. The presence of null alleles, the Wahlund effect, and inbreeding, among other factors, are important in generating the phenomenon of heterozygote deficiency (Singh and Green 1984). If the presence of null alleles is an important factor, heterozygote deficiency should be observed in both Taiwan and Penghu. Inbreeding can not explain the phenomenon either, as cultured oysters are all from the same geographic origin. In this case, differential mortality due to local environmental factors might play a role in causing the genetic differences between adult oysters of Taiwan and Penghu.

Only the Budai population exhibited a size difference between oysters carrying heterozygous *Lap* genotypes (smaller) and those carrying homozygous *Lap* genotypes (larger). But this difference was not significant when adjusted significance level for multiple tests was used (Hochberg, 1988). The relationship between heterozygosity and growth has been studied for various marine bivalves (Zouros and Pogson 1994). In the five-month-old blue mussel *Mytilus edulis*, a significant positive correlation was recorded between observed heterozygosity (total number of heterozygotes divided by sample size) and growth rate (samples separated into different sizecategories) at the octopine dehydrogenase locus (Gosling 1989). In the oyster *Ostrea edulis* (Alvarez et al. 1989), the relationship between heterozygosity and growth changed through time. Positive correlations were found at isocitrate dehydrogenase-2 and malate dehydrogenase-1 loci in the eighteen-month-old oyster and at isocitrate dehydrogenase-2 and esterase-3 loci in the thirty-month-old oyster. In the scallop *Placopecten magellanicus* (Volckaert and Zouros 1989), no significant difference was found between average shell height of heterozygotes and homozygotes at the 6-phosphogluconate dehydrogenase and glucosephosphate isomerase loci. The relationship between heterozygosity and growth clearly depends on enzyme the locus studied as well as the age of the organisms.

A study of American oyster *C. virginica* indicated that allozyme variation and shell morphology (i.e., shell shape and size)are not correlated related (Groue and Lester 1982). Others have suggested that site-specific growth rates in *C. gigas* (Brown 1988) and in mussel *M. edulis* (Incze et al. 1980) are mostly influenced by environmental factors such as food supply, temperature, salinity and physiological condition. Although age differences may also affect oyster size, the oysters at Tsaiyuan (Penghu) grow faster and larger than those cultured in Taiwan (Soong et al. 1992). With little genetic differentiation found among these oyster populations, regional differences in size and shape probably resulted from

environmental factors.

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台灣大牡蠣族群之同功酶變異

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摘　要

以蛋白質電泳法分析比較本省九個地區的大牡蠣其同功　在族群間之變異，七個同功酶中只有 Lap、Mpi 及Pgi 三個為多型性同功酶。異基因型個體缺少(heterozygote deficiency)的現象，只在本省之七個採樣點發現，澎湖之二採樣點則無此現象。本省牡蠣族群間基因平均異質性 (mean heterozygosity)，為 H =0.044～0.084， Nei's 遺傳距離為D <0.01，顯示台灣大牡蠣族群間遺傳變異很小。布袋族群中同功酶Lap 異基因型個體顯著小於同基因型個體 (p=0.03),但以多次測驗之調整顯著水準為標準時,則異基因型個體小於同基因型個體之現象不顯著。由本實驗結果推測，環境因子可能是造成本省養殖牡蠣族群間體型及大小差異之重要因素。

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