Genetic Diversity among Cultured Oysters (*Crassostrea* spp.) throughout Thailand

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ABSTRACT Nine samples of *Crassostrea belcheri* and seven samples of *Crassostrea lugubris* (=*C. iredalei*) were collected from up to seven provinces throughout Thailand and analysed for allozyme electrophoresis at six enzyme loci; Aat-B, Est-F2, Lap, Pep-A, Pgi and Pgm. No significant deviations of heterozygosities from Hardy-Weinberg expectations were observed at any locus. Genetic diversity between populations was quantified using the absolute variance of allele frequencies (σ^2) and the fixation index (Wright's F_{ST}) in a hierarchical analysis of genetic variation. In *C. belcheri* samples from both the Andaman Sea and the Gulf of Thailand, on either side of the Malay peninsula, F_{ST} was only 0.030. Levels of gene flow estimated as number of migrants per generation (Nm) were similar whether calculated for just Gulf samples (8.2) or for samples from both the Gulf and Andaman Sea (10.9). In *C. lugubris*, which was only found within the Gulf, F_{ST} was smaller at 0.007, and estimated gene flow was substantially higher with Nm = 33.5. For both species, most of genetic variance was found within a few populations in the main culture areas on the west coast of the Gulf of Thailand; 67 % for *C. belcheri* within four samples from Surat Thani province, and 83 % for *C. lugubris* within three samples from Chumphon province. Common practises of transplantation appear responsible for homogenisation of allele frequencies in both *C. belcheri* and *C. lugubris*.

KEYWORDS: *Crassostrea belcheri, Crassostrea lugubris,* genetic diversity, oyster.

INTRODUCTION

The oysters *Crassostrea belcheri* and *Crassostrea lugubris* have been cultured in Thailand for roughly fifty years.[1] Demand for these oysters greatly exceeds production, despite rapid increases in the area given over to oyster culture.[2] The main constraint on production has been a shortage of oyster spat.[3] Responses to this shortage have included both the production of hatchery seed and the practise of transplanting spat from "spat-rich" to "spat-poor" areas.[2] Hatcheries have been established in all the main areas of oyster culture. Facilities at Surat Thani, on the West coast of the Gulf of Thailand, produce *C. belcheri*, (K Youngvanitset, Thai Department of Fisheries, pers comm). Other hatcheries in Prachuab Khiri Khan on the north-west coast of the Gulf of Thailand and in Chon Buri on the east coast of the Gulf of Thailand produce both *Crassostrea* species .[4] Regardless of hatchery locality, most broodstock for spat production of *C. belcheri* are collected from Surat Thani.[2]

Transplantation whether as spat or adults has been widely practised for both *Crassostrea* species over the last 15 years. Such transplantation has been most frequent for *C. belcheri*, which dominates the market. For example, in 1987-88, the promotion of oyster culture in the Andaman Sea province of Ranong led to the importation of *C. belcheri* spat from Phangnga, also bordering the Andaman Sea, as well as from the Gulf of Thailand (Prachuab Khiri Khan). Oysters have been transplanted not only to bolster production, but also to improve marketability. Environmental conditions in the Andaman Sea result in brown meat that is difficult to market. Therefore, *C. belcheri* spat are routinely transplanted from Phangnga for on-growing at Surat Thani in the Gulf of Thailand.

Transplantation can have a number of adverse consequences. Disease transmission is an obvious hazard, as is the unintended introduction of undesirable species. For example, transplantation within the Gulf of Thailand has resulted in the introduction of *C. lugubris* from Chumphon and Prachuab Khiri Khan to Ban Don Bay in Surat Thani, in the mistaken belief that transplanted oysters were *C. belcheri*.[2] More insidious, are the effects of transplantation on the inter-population component of genetic diversity.

Oviparous oysters have the potential for successful gene flow between widely separated populations. Stenzel[5] suggests that given the right

hydrographic conditions, free-swimming larvae, which endure in the plankton for 6 days or more, can be transported up to 1300 km. Nevertheless, phenotypic and genetic divergence of oysters from different regions has been observed for natural populations of a number of *Crassostrea* species.[6,7,8] Widespread transplantation can obliterate such intra-specific diversity. Examples where this has occurred include the loss of some physiological "races" of *C. gigas* in Japan,[9] and the homogenisation of allele frequencies in Atlantic-coast populations of *Ostrea edulis* in Europe.[10,11] Such loss of diversity can have economic consequences both by reducing the number of unique genotypes for use as potential broodstock, and by preventing local adaptation possibly resulting in increased mortalities and decreased production.

Arising from these concerns, the aim of this project was to compare levels of genetic diversity remaining within and between populations of the cultured oysters *C. belcheri* and *C. lugubris* from throughout Thailand.

Material and Methods

Sampling regime

In September 1995 and April 1996, nine samples of *C. belcheri* and seven samples of *C. lugubris* were collected from up to seven provinces, throughout Thailand, including three in the Andaman Sea and four in the Gulf of Thailand. Sample site locations and sample numbers are given in Table 1. *Crassostrea belcheri* were only available from oyster culture in the provinces of Surat Thani, Phangnga and Krabi, as was *C. lugubris* in Surat Thani. Samples from other provinces came from natural populations. Seven of the eight samples of *C. belcheri* that came from oyster culture were also found to include *C. lugubris*, the one monospecific sample being from site 13 in the Andaman Sea. However, except for one sample from site 6 in the western Gulf of Thailand, there were not sufficient *C. lugubris* in those mixed samples for use in studying population genetics.

Electrophoresis

Adductor muscles were excised from oysters in the field, and frozen immediately in liquid nitrogen. Muscle samples were homogenised in 75 µl of 0.05M tris-Cl pH 7.0 plus 20% dimethyl sulphoxide (DMSO), and stored at -70°C prior to starch-gel electrophoresis. Six enzyme loci were resolved using a tris/citrate/edta buffer pH 7.0 (TCE of Buroker et al.[12] Stains for leucine aminopeptidase, phospho-glucose isomerase and phospho-glucose mutase each revealed only one locus; *Lap, Pgi* and *Pgm*, respectively. Two zones of enzyme activity, which migrated towards the anode, were seen with the stain for aspartate aminotransferase, but only the most anodal, *Aat-B*, could be reliably scored. Four zones of enzyme activity were observed with a fluorescent substrate for esterase (4-methylumbelliferyl acetate, Sigma-aldrich Co Ltd). One zone of activity migrated

Table 1. Numbers of *Crassostrea* species sampled at different sites throughout Thailand (See Fig 1) in 1995 and 1996.

Sample Group	Site No	Province	Site Location	*Crassostrea belcheri*	*Crassostrea lugubris*
East Gulf	1	Trat	Ko Chang	12	30
East Gulf	2	Chon Buri	Ban Si Racha	-	6
North-West Gulf	3	Chumphon	Ban Saphan	-	35
North-West Gulf	4	Chumphon	Ban Pak Nam	-	29
North-West Gulf	5	Chumphon	Panang Bay	-	21
West Gulf	6	Surat Thani	Phom Roeng	40	41
West Gulf	7	Surat Thani	Ban Don Bay 1.	49	(2)
West Gulf	8	Surat Thani	Ban Don Bay 2.	49	(3)
West Gulf	9	Surat Thani	Ban Don Bay 3.	48	(1)
West Gulf	10	Surat Thani	Ko Prab	39	(1)
South Gulf	11	Pattani	Loem Pho	-	46
Andaman Sea	12	Ranong	Kapoe	33	(2)
Andaman Sea	13	Phangnga	Thap Put	36	-
Andaman Sea	14	Krabi	Aoluk	36	(4)

( ) *C. lugubris* present at "contaminant" levels in *C. belcheri* culture systems. Insufficient numbers for population genetics.

cathodally while the other three zones migrated towards the anode. Only the slowest of the three anodal zones could be consistently scored, which we designate *Est-F2*. Different peptidase loci are observed according to the substrate used. We stained gels with gly-leu, which we were then able to score for *Pep-A*.[13] We do not follow the numbering system for loci or alleles that was used for *Crassostrea* species by Buroker *et al*.[14] This study used different buffer systems, including different substrates and tissues for certain loci. Therefore, we cannot be certain of homology of alleles or loci with Buroker *et al*.[14] Alleles were numbered in order of mobility with the highest numbers being the most anodal. Samples of *C. belcheri* and *C. lugubris* were run alongside one another and a unified labelling system applied. Thus for a given locus, an allele designated "100" for *C. belcheri* had the same mobility as the "100" allele for *C. lugubris*. The shell morphology of *C. lugubris* is similar to that of *C. iredalei* cultured elsewhere in Southeast Asia. Therefore, a reference sample of *C. iredalei* from Penang in Malaysia (supplied by BL Bayne) was run to resolve uncertainties over the identification of Thai species.

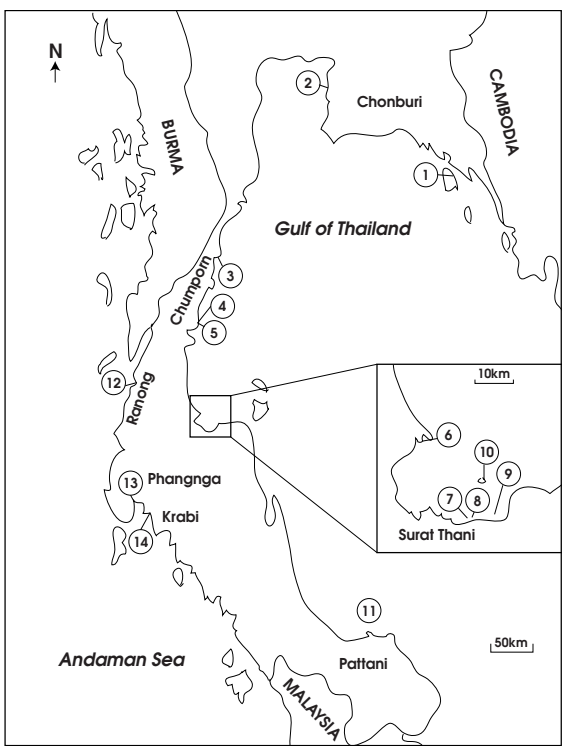

Fig 1. Locations of site nos 1-14 (see table 1) sampled throughout Thailand in 1995 and 1996 for *Crassostrea belcheri* and *Crassostrea lugubris* (=*C. iredalei*)

Geographic variation in allele frequencies

For both species, a hierarchical analysis of allelic variation was carried out using the absolute variance of allele frequencies, σ^2 and Wright's F_{ST} (Wright, 1978). These statistics were calculated for up to three groups of samples that were separated by distances that varied by orders of magnitude. Scale 1, for *C. lugubris* included three North-west Gulf samples from sites 3-5 separated by up to 100 km, and for *C. belcheri* included four West Gulf samples from sites 7-10 separated by up to 10 km. Scale 2 samples, for both species, came from throughout the Gulf of Thailand. Samples for *C. belcheri* included six samples from sites 1 and 6-10 which were separated by up to 1000 km, and for *C. lugubris* seven samples from sites 1-6 and 11 which were separated by up to 1500 km. Scale 3 included samples from both the Andaman Sea and the Gulf of Thailand. Only a few individuals of *C. lugubris* were obtained from the Andaman Sea. Therefore, Scale 3 statistics were calculated for *C. belcheri* alone.

At each geographic scale, the absolute variance for each allele (σ^2) was summed to give the absolute variance per locus $\Sigma\sigma^2$. The sum of the variances for all loci gave the total variance at each scale ($\sigma^2 t$). Results are expressed as a percentage of the total genetic variance in all samples, which for *C. lugubris* came from the Gulf of Thailand only, and for *C. belcheri* came from both the Gulf and the Andaman Sea. Wright's F_{ST} was estimated as Weir and Cockerham's[16] θ, and was calculated using a modified version of Weir's program.[17,18] Weir and Cockerham's estimator has the advantage that it is relatively unaffected either by numbers of populations sampled or by sample size.

Individual tests for deviation of heterozygosities from Hardy-Weinberg expectations[19] were carried out for all loci in all samples where the sample size was more than twenty, and where the frequency of the most common allele was less than 0.95. Twenty-nine and thirty-five tests were carried out for *C. belcheri* and *C. lugubris*, respectively. Therefore, significance levels were adjusted for the number of tests using the sequential Bonferroni method of Hochberg.[20]

Results

Variability within Populations

Allele frequencies and observed heterozygosities in individual samples of *C. belcheri* and *C. lugubris* are given in Tables 2 and 3. Allelic mobilities in *C. lugubris* from Thailand were identical to allelic mobilities in our reference sample of *C. iredalei* from

Table 2. Allele frequencies in samples of *Crassostrea belcheri* collected in 1995 and 1996 from different sites throughout Thailand.

Locus	Allele	East Gulf Site 1	West Gulf Site 6	West Gulf Site 7	West Gulf Site 8	West Gulf Site 9	West Gulf Site 10	Andaman Sea Site 12	Andaman Sea Site 13	Andaman Sea Site 14
Aat-B	100	0.833	1.000	1.000	0.974	1.000	1.000	1.000	0.986	0.946
	110	0.167	0	0	0.026	0	0	0	0.014	0.054
	H_o	0.333	0	0	0.053	0	0	0	0.028	0.115
	(N)	(12)	(40)	(45)	(38)	(49)	(10)	(33)	(36)	(38)
Est-F2	100	1.000	1.000	1.000	1.000	1.000	1.000	0.919	0.958	0.986
	110	0	0	0	0	0	0	0.081	0.042	0.014
	H_o	0	0	0	0	0	0	0.194	0.083	0.057
	(N)	(12)	(40)	(40)	(49)	(49)	(50)	(31)	(36)	(35)
Lap	80	0	0	0.012	0	0	0.026	0.054	0.145	0.015
	90	0.125	0.092	0.155	0.178	0.149	0.171	0.071	0.129	0.121
	100	0.875	0.908	0.833	0.822	0.851	0.803	0.875	0.726	0.864
	H_o	0.083	0.184	0.167	0.244	0.216	0.316	0.250	0.548	0.212
	(N)	(12)	(38)	(42)	(45)	(37)	(38)	(28)	(31)	(33)
Pep-A	70	0	0.081	0.122	0.163	0.087	0.231	0	0	0.100
	90	0	0.162	0.122	0.093	0.100	0.115	0	0	0.050
	100	1.000	0.757	0.756	0.744	0.813	0.654	1.000	1.000	0.850
	H_o	0	0.487	0.317	0.419	0.325	0.487	0	0	0.2667
	(N)	(8)	(37)	(41)	(43)	(40)	(39)	(26)	(11)	(30)
Pgi	90	0.042	0	0	0	0	0	0.018	0.065	0.029
	100	0.916	1.000	1.000	1.000	0.990	1.000	0.893	0.903	0.956
	110	0.042	0	0	0	0.010	0	0.089	0.032	0.015
	H_o	0.167	0	0	0	0.020	0	0.214	0.129	0.088
	(N)	(12)	(40)	(40)	(49)	(49)	(50)	(28)	(31)	(34)
Pgm	80	0.042	0.050	0.028	0.014	0	0.014	0.037	0.069	0.139
	90	0.042	0.100	0.139	0.056	0.250	0.028	0.055	0.167	0.097
	100	0.916	0.850	0.833	0.930	0.750	0.958	0.908	0.764	0.764
	H_o	0.167	0.300	0.333	0.139	0.400	0.083	0.185	0.361	0.333
	(N)	(12)	(40)	(40)	(36)	(36)	(36)	(27)	(36)	(36)
	Mean H_d	0.125	0.162	0.140	0.142	0.194	0.154	0.141	0.192	0.179

N = number of individuals.

Penang, Malaysia (Table 3). The name of *Crassostrea lugubris* has precedence (Sowerby, 1871).[21] Therefore, we retain use of *C. lugubris* as the name for this widespread tropical oyster.

For *C. lugubris*, all loci were polymorphic at all sites and observed heterozygosity averaged over all six loci was more than double that for *C. belcheri* (0.445 *cf* 0.158). No comparisons of expected and observed heterozygosities within individual samples were significantly different in either species.

Geographic variation in allele frequencies

For *C. belcheri*, which is distributed throughout Thailand, 67.0 % of the total variance was found within four West Gulf samples that came from sites separated by a maximum of 10 km (Scale 1). Addition of two further samples, including one from the East Gulf (site 1), added 8.5% to the total variance and average value of F_{ST} increased from 0.017 for Scale 1 to 0.020 for Scale 2 (Table 4). East Gulf site 1 was separated by more than 1,000 km

Table 3. Allele frequencies in samples of *Crassostrea lugubris* (=*C. iredalei*) collected in 1995 and 1996 from different sites throughout Thailand.

Locus	Allele	East Gulf Site 1	East Gulf Site 2	NW Gulf Site 3	NW Gulf Site 4	NW Gulf Site 5	West Gulf Site 6	South Gulf Site 11	Malaysia *C. iredalei*
Aat-B	100	0.917	0.875	0.814	0.904	0.952	0.921	0.861	1.000
	110	0.083	0.125	0.186	0.096	0.048	0.079	0.139	0
	H_o	0.167	ND	0.400	0.192	0.143	0.158	0.302	ND
	(N)	(18)	(4)	(35)	(26)	(21)	(38)	(43)	(8)
Est-F2	90	0.225	0.083	0.152	0.312	0.262	0.137	0.233	ND
	100	0.675	0.584	0.727	0.667	0.690	0.688	0.589	ND
	110	0.100	0.333	0.121	0.021	0.048	0.175	0.178	ND
	H_o	0.650	ND	0.485	0.583	0.571	0.350	0.578	ND
	(N)	(20)	(6)	(33)	(24)	(21)	(40)	(45)	ND
Lap	90	0	0.100	0	0	0.050	0	0	0
	100	0	0.100	0.044	0.121	0.025	0.050	0.037	0
	110	0	0	0.059	0	0	0.025	0	0
	120	0.950	0.700	0.867	0.845	0.850	0.863	0.939	1.000
	130	0.033	0	0.015	0.034	0	0.050	0.012	0
	140	0.017	0.100	0.015	0	0.075	0.012	0.012	0
	H_o	0.100	ND	0.265	0.241	0.200	0.200	0.073	ND
	(N)	(30)	(5)	(34)	(29)	(20)	(40)	(41)	(8)
Pep-A	60	0.143	0.100	0.132	0.148	0.175	0.200	0.196	0.250
	70	0.036	0	0.015	0.074	0.025	0.025	0.087	0
	80	0.732	0.800	0.735	0.611	0.700	0.600	0.641	0.563
	100	0.089	0.100	0.118	0.167	0.100	0.175	0.076	0.187
	H_o	0.464	ND	0.412	0.630	0.300	0.575	0.348	ND
	(N)	(28)	(5)	(34)	(27)	(20)	(40)	(46)	(8)
Pgi	90	0.121	0.300	0.363	0.380	0.143	0.295	0.288	0.376
	100	0.466	0.100	0.167	0.086	0.190	0.180	0.200	0.062
	110	0.034	0.300	0.182	0.172	0.262	0.180	0.250	0.062
	120	0.379	0.300	0.288	0.362	0.405	0.345	0.262	0.500
	H_o	0.586	ND	0.697	0.690	0.667	0.795	0.700	ND
	(N)	(29)	(5)	(33)	(29)	(21)	(39)	(40)	(8)
Pgm	100	0.083	0.200	0.194	0.115	0.191	0.158	0.136	0.125
	110	0.521	0.300	0.532	0.577	0.476	0.463	0.466	0.563
	120	0.042	0.100	0.048	0.135	0.095	0.098	0.080	0.062
	130	0.354	0.400	0.226	0.173	0.238	0.281	0.318	0.250
	H_o	0.583	ND	0.645	0.615	0.619	0.561	0.455	ND
	(N)	(24)	(5)	(31)	(26)	(21)	(41)	(44)	(8)
	Mean H_o	0.425	ND	0.484	0.492	0.417	0.440	0.409	ND

N = number of individuals.

Table 4. *Crassostrea belcheri*. The absolute variance ($\Sigma\sigma^2$) and Wright's F_{ST} calculated per locus for three groups of samples from different geographic scales in Thailand. Both seas included samples from the Andaman Sea and the Gulf of Thailand.

	Scale 1 (<10 km) West coast sites 7-10 N=4		Scale 2 (<1000 km) Gulf of Thailand N=6		Scale 3 (Both Seas) All Thailand N=9	
	$\Sigma\sigma^2$	F_{ST}	$\Sigma\sigma^2$	F_{ST}	$\Sigma\sigma^2$	F_{ST}
Aat-B	0.001	0.012	0.003	0.079	0.002	0.055
Est-F2	0.008	0.000	0.007	-0.000	0.006	0.027
Lap	0.001	-0.012	0.003	-0.006	0.006	0.005
Pep-A	0.007	0.008	0.010	0.015	0.018	0.042
Pgi	0.000	0.001	0.001	0.070	0.002	0.037
Pgm	0.015	0.068	0.012	0.046	0.012	0.035
$\sigma^2 t = \Sigma\Sigma\sigma^2$	0.031		0.035		0.046	
% total variance	67.0		75.5		100	
Mean F_{ST}		0.017		0.020		0.030
Nm		14.6		12.3		8.2

from the other sites, and two loci *Aat-B* and *Pgi* that were monomorphic in West coast samples, (with frequencies of the common allele at each locus being more than 0.95), were polymorphic in the East Gulf sample (Table 2). Consequently, for samples at Scale 2, the highest F_{ST} values were 0.079 for *Aat-B* and 0.070 for *Pgi*. Scale 3 included all samples from both the Andaman Sea and the Gulf of Thailand. Some slight genetic differentiation between these bodies of water was evidenced by an increase in F_{ST} from 0.020 at Scale 2 to 0.030 at Scale 3 and the addition of 24.5% to the absolute variance. These increases were chiefly due to variation at the *Pep-A* locus, which had the highest value for the absolute variance (of 0.018) when calculated for all nine samples. This locus was fixed for the common allele in two out of three Andaman Sea samples, whereas in five out of six Gulf samples the frequency of the common allele was <0.813. F_{ST} indicates the extent to which differentiation between populations has proceeded to fixation as opposed to the total amount of variation, and the highest value of F_{ST} for all *C. belcheri* samples was for the virtually monomorphic locus *Aat-B* with 0.055. Samples from the West Gulf and from the Andaman Sea were monomorphic or nearly so for this locus, with the frequency of the common allele exceeding 0.946 in all cases. Therefore, as already noted for Scale 2 samples, the high F_{ST} value for this locus was solely due to differentiation of the sample from East Gulf site 1.

The fixation index for *C. lugubris* was lower than for *C. belcheri*, with F_{ST} equal to 0.007 for all (Gulf) samples. Within the Gulf of Thailand, 83.0 % of the total variance was found in Chumphon, despite only three out of seven samples having come from this province, and other samples coming from populations that were separated by up to 1500 km (Table 5). Heterogeneity only increased with geographic scale at the *Pgi* locus, for which F_{ST} increased from 0.009 to 0.028 between Scales 1 and 2 (Table 5). This was chiefly due to an increase in the frequency of the Pgi^{100} allele in the sample from East Gulf site 1, that was 0.466 compared to <0.200 in all other samples (Table 5).

Table 5 *Crassostrea lugubris* (=*C. iredalei*). The absolute variance ($\Sigma\sigma^2$) and Wright's F_{ST} calculated per locus for two groups of samples from different geographic scales in Thailand.

	Scale 1 (<100 km) North-West Gulf N=3		Scale 2 (<1500 km) Gulf of Thailand N=7	
	$\Sigma\sigma^2$	F_{ST}	$\Sigma\sigma^2$	F_{ST}
Aat-B	0.003	0.025	0.005	0.007
Est-F2	0.015	0.010	0.009	0.011
Lap	0.004	0.012	0.004	0.008
Pep-A	0.009	-0.003	0.007	-0.004
Pgi	0.013	0.009	0.029	0.028
Pgm	0.006	-0.011	0.007	-0.010
$\sigma^2 t = \Sigma\Sigma\sigma^2$	0.050		0.060	
% total variance	83.0		100	
Mean F_{ST}		0.004		0.007
Nm		60.7		33.5

The chief value of F_{ST} is that it permits estimation of gene flow (22). Assuming an island model of population structure, number of migrants per generation (*Nm*) can be calculated as:

$Nm = [(1/F_{ST})-1] / 4.$

Nm calculated for each geographic Scale for each species is given in Tables 4 and 5. For populations within the Gulf of Thailand, values of *Nm* were substantially higher in *C. lugubris* (33.5) than in *C. belcheri* (8.2). However, in *C. belcheri*, gene flow between the Andaman Sea and the Gulf of Thailand was similar to gene flow within the Gulf alone (8.2 versus 10.9, respectively).

Discussion

Widespread transplantation of the cultured oysters' *C. belcheri* and *C. lugubris* has been occurring in Thailand for the last fifteen years (refer Introduction). Consequently, although *C. lugubris* samples came from sites that were separated by up to 1,500 km, and *C. belcheri* samples came from two separate seas, F_{ST} values were only 0.007 and 0.030 respectively. In *C. belcheri*, transplantation between the Andaman Sea and the Gulf of Thailand has largely negated the effect of the Malay peninsula as a natural barrier to gene flow. Consistent slight differences in allele frequencies between the Gulf of Thailand and the Andaman Sea were only evident for the *Pep-A* locus. Therefore, estimates of gene flow between the two Seas and within the Gulf of Thailand alone were similar, as evidenced by values for *Nm* of 8.2 and 10.9, respectively. Within the Gulf of Thailand, the only samples of either species that showed any divergence in allele frequencies came from a natural population in a province with no record of any transplants: East Gulf site 1 in Trat province was separated by more than 1,000 km from all other sites, with allelic differentiation at the *Aat-B* and *Pgi* loci in *C. belcheri*, and at the *Pgi* locus in *C. lugubris*.

Historically the west and north-west regions of the Gulf of Thailand have dominated oyster production, and have been the main source of broodstock for hatcheries (refer Introduction). Within the provinces of Surat Thani and Chumphon located in those regions, we found 67% and 83% of the total genetic variance for *C. belcheri* and *C. lugubris*, respectively. Yet within one of those provinces catastrophic mortalities of cultured oysters have occurred. During the monsoons of November 1996, subsequent to our sampling, salinity fell to zero for a prolonged period leading to almost universal mortality of *C. belcheri* in Ban Don Bay (K. Youngvanitset, Thai Department of Fisheries; pers comm). Although occurring relatively infrequently, local extinctions such as the one above, emphasize the importance of maintaining genetic diversity in several populations.

Conclusion

Transplantation in Thailand is traditionally carried out to improve marketability, restock depleted populations, and bolster production where shortage of spat has been a problem.[2] However, these practises have had a number of unwanted side effects. These include the homogenisation of allele frequencies in both *C. belcheri* and *C. lugubris*, and the contamination of nearly all *C. belcheri* cultures with *C. lugubris*. Recurrent transplantation effectively prevents genetic adaptation to local conditions, and could result in sub-optimal growth rates and increased mortalities. The resulting loss in production is difficult to quantify but may be economically significant.

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References

1. Brohmanonda P, Mutarasint K, Chongpeepien T and Amornjaruchit S (1988) Oyster culture in Thailand. In: McCoy, EW and Chongpeeepien, T (Eds), Bivalve mollusc culture research in Thailand. ICLARM Technical Report **19**, 31-9.
2. Tandavanitj S (1995) Experiences from oyster transplantation in Thailand. *Phuket Mar Biol Cent Spec Pub* **15**, 61-3.
3. Jarayabhand P and Thavornyutikarn M (1995) Realized heritability estimation on growth rate of oyster, *Saccostrea commercialis* Born, 1778. *Aquaculture* **138**, 111-8.
4. Wong TM (1994) Hatchery production of bivalve seed in South east Asia: state of the art and future research directions. *Phuket Mar Biol Cent Spec Pub* **13**, 1-80.

5. Stenzel HB (1971) Treatise on invertebrate paleontology. Part N Vol 3 Mollusca 6 Bivalvia Kansas: The geological society of America, Inc and The University of Kansas 1224 pp.
6. Buroker NE, Hershberger WK and Chew KK. 1979a. Population genetics of the family Ostreidae. 1. Intraspecific studies of *Crassostrea gigas* and *Saccostrea commercialis*. *Mar Biol* **54**, 157-69.
7. Hedgecock D and Okasaki NB (1984) Genetic diversity within and between populations of American oysters (*Crassostrea*). *Malacologia* **25**, 535-49.
8. Hare MP and Avise JC (1996) Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). *Evolution* **50**, 2305-15.
9. Osaki H and Fujio Y (1985) Genetic differentiation in geographical populations of the Pacific oyster (*Crassostrea gigas*) around Japan. *Tohuku J Agric Res* **36**, 49-61.
10. Johannesson K, Rödström EM and Aase H (1989) Low genetic variability in Scandinavian populations of *Ostrea edulis* L. - possible causes and implications. *J Exp Mar Biol Ecol* **128**, 177-90.
11. Saavedra C, Zapata C, Guerra A and Alvarez G (1993) Allozyme variation in European populations of the oyster *Ostrea edulis Mar Biol* **115**, 85-95.
12. Buroker NE, Hershberger WK and Chew KK (1975) Genetic variation in the Pacific oyster, *Crassostrea gigas*. *J Fish Res Board Can* **32**, 2472-7.
13. Harris H and Hopkinson DA (1978) Handbook of enzyme electrophoresis in human genetics. North-Holland publishing company.
14. Buroker NE, Hershberger WK and Chew KK. 1979b. Population genetics of the family Ostreidae. 11. Interspecific studies of the genera *Crassostrea* and *Saccostrea*. *Mar Biol* **54**, 171-84.
15. Wright S (1978) Evolution and the Genetics of Populations. Vol 4. Variability within and among natural populations. Chicago and London: University of Chicago Press.
16. Weir BS and Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-70.
17. Weir BS (1990) Genetic Data Analysis. Sinauer Associates, Sunderland, Massachusetts.
18. Goudet J, De Meeus T, Day AJ and Gliddon CJ (1994) The different Scales of population structuring of the dogwhelk , *Nucella lapillus*, along the South Devon coast. In: Beaumont, AR (Ed.), Genetics and Evolution of Aquatic organisms. London: Chapman and Hall, pp 81-95.
19. Pamilo P and Varvio-Aho S (1984) Testing genotype frequencies and heterozygosities. *Mar Biol* **79**, 99-100.
20. Hochberg Y (1988) A sharper Bonferroni proceedure for multiple tests of significance. *Biometrika* **75**, 800-2
21. Sowerby GB (1871) Monograph of the genus Ostrea. In L A Reeve. Conchologia Iconica; or illustrations of the shells of molluscous animals Vol 18. London: L Reeve & Co.
22. Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* **236**, 787-92.