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AN INVESTIGATION OF POSSIBLE STOCK STRUCTURE IN *PECTEN MAXIMUS* (L.) USING MULTIVARIATE MORPHOMETRICS, ALLOZYME ELECTROPHORESIS AND MITOCHONDRIAL DNA POLYMERASE CHAIN REACTION–RESTRICTION FRAGMENT LENGTH POLYMORPHISM

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ABSTRACT Population heterogeneity in the scallop *Pecten maximus* (L.) has been studied by multivariate morphometrics and allozyme electrophoresis and compared with data from a mitochondrial DNA polymerase chain reaction–restriction fragment length polymorphism method. Principal component analysis applied to shell measurements revealed some variation in shape, with significant differences in aspects of morphology detectable among populations. Trends suggestive of morphological distinctness of a population or populations were difficult to uncover; however, animals from Brest and La Trinité (Brittany, France) were consistently different from other *P. maximus* populations on the basis of principal component 1, largely attributable to hinge length. St. Brieuc Bay *P. maximus,* which are known to exhibit differences in reproductive cycle from neighboring populations and thus are thought to be reproductively isolated, could not be separated on the basis of shell shape, although limited differences in the number of ribs in comparison to other populations are evident. Allele frequencies at seven loci assessed by allozyme electrophoresis were essentially homogeneous throughout the sample range in accord with previous studies and provided little evidence for population subdivision, although allele frequencies at the *Odh* locus provided some evidence that two Scottish populations were genetically differentiated. This contrasted with both the morphological differences detected for two Brittany populations and with data from the mitochondrial DNA, which indicated that the *P. maximus* population from the semienclosed sea lough Mulroy Bay, Eire, was genetically differentiated from any other population sampled on the basis of sequence divergence values.

KEY WORDS: allozyme electrophoresis, genetic differentiation, mitochondrial DNA, morphology, *Pecten maximus,* population structure, scallop

INTRODUCTION

Typically, conclusions from allozyme data have conformed to the idea that the partitioning of genetic variation within marine species is linked to life history such that broadcast spawning species with pelagic drifting larvae are essentially genetically homogeneous across continuous ranges (Palumbi 1992), in contrast to directly developing species (e.g., Berger 1973), which often display abrupt allele frequency differences over short geographic distances. However, as DNA level studies accumulate, the theory that species with pelagic larvae have little or no population structure is being challenged by the observation of population subdivision in species for which allozymes suggest homogeneity (Mitton 1994). This is exemplified by the study of Karl and Avise (1992) on *Crassostrea virginica,* where a sharp genetic break was detectable between populations on the East Coast of the United States with mitochondrial (mt) DNA and single-copy nuclear DNA, whereas allozyme frequencies were invariant (Buroker 1983). This suggests that balancing selection may be maintaining similar allozyme frequencies across oyster populations, in the face of genetic subdivision (Karl and Avise 1992). Therefore, barriers to gene flow do exist even where species are continuously distributed, and dispersal by pelagic larvae does not necessarily produce panmixis.

Despite evidence of noticeable differences in reproductive cycle between populations of the scallop *Pecten maximus* from northwest Brittany, which suggest genetic divergence (Paulet et al. 1988, Cochard and Devauchelle 1993, Mackie and Ansell 1993), allozyme studies (Huelvan 1985, Beaumont et al. 1993) have been unable to detect significant differences in population structure. DNA technologies have only recently been applied in a population genetic context to this species (Wilding 1996, Rigaa et al. 1997, Wilding et al. 1997), and genetic differences between scallop populations might be revealed by DNA data where allozyme data have suggested homogeneity. Nevertheless, the use of both allozyme and DNA techniques in parallel may be particularly informative because consistency among outcomes will fortify conclusions arrived at concerning population subdivision and perhaps permit further insights into the evolutionary mechanisms (drift, selection, etc.) acting on the populations to create the observed pattern. For comparison, morphological data, which might also be useful for stock discrimination (e.g., Karakousis and Skibinski 1992, Krause et al. 1994), can be gathered from the same samples.

These three approaches (morphology, allozymes, and mtDNA) present a gradient of cost but not necessarily cost effectiveness, and comparisons will provide information on the most appropriate method for studying population structure in this species. Here, we compare and contrast results from multivariate morphometrics with data from allozyme analyses and with mtDNA PCR (polymerase chain reaction)–restriction fragment length polymorphism (RFLP) variation (Wilding 1996, Wilding et al. 1997) to assess population structure in *P. maximus* from the United Kingdom and France.

MATERIALS AND METHODS

Sampling

Scallops were taken by dredge fishing or SCUBA diving from 15 populations around the United Kingdom, Eire, and northern

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France (Fig. 1). Morphometric data were collected from all populations, but because some populations were geographically very close to each other, allozyme data (9 populations) and mtDNA (12 populations) were not analyzed from all sites. Sample sizes for the morphometric analysis are given in Table 1 and for the allozyme analysis in Table 5. Sample sizes for the mtDNA study are given in Wilding et al. (1997).

Morphology

Four shell dimensions—height, width, length, and hinge length (Fig. 2)—were measured to the nearest 0.1 mm with vernier calipers. Weight of both shell valves was taken to the nearest 0.1 g, and the number of ribs was counted. Meristic data (number of ribs) were tested for a fit to normality using the nscore command of MINITAB followed by correlation. Populations for which rib numbers fitted the normal distribution were subsequently compared by one-way analysis of variance (ANOVA), and where significant, pairwise differences were analyzed by Scheffé's test. The remaining measurements were tested for allometry with $\log_{10}$ transformed data by performing standard linear regression between length (as an indicator of overall size) and each other variable (height, depth, width, weight, and hinge length) to gain a measure of the slope (b). If growth is isometric, the expected value of the slope (β) is 1 for the linear comparisons, or 3 for weight. One sample *t*-tests were used to compare b and β. The continuous data (after taking $\sqrt[3]{\text{shell mass}}$) were then subjected to a principal component analysis (PCA) using the covariance matrix in the

TABLE 1.

Rib counts in 15 populations of *P. maximus*.

Population	n	Mean	SE
Port St. Mary 1	63	12.909	0.080
Port St. Mary 2	53	12.745	0.098
Douglas	47	12.796	0.092
Peel	42	13.160	0.096
Chicken Rock	75	12.948	0.069
Anglesey	31	12.892	0.101
Mull	28	13.067	0.095
Stonehaven	23	12.958	0.127
Lyme Bay	60	12.950	0.090
Polperro	54	13.033	0.096
Kilkieran Bay	30	13.100	0.121
Mulroy Bay	30	13.000	0.083
Brest	30	12.733	0.095
La Trinité	28	12.483	0.128
St. Brieuc	49	12.380	0.090

Figure 1. Collection sites of *P. maximus* samples. ANG, 27 m northwest of Puffin Island, Anglesey; BRE, Rade de Brest, Brittany; CHI, Chicken Rock, Isle of Man; DOU, Douglas, Isle of Man; Kil, Kilkieran Bay, Eire; LAT, La Trinité sur Mer, Brittany; LYM, Lyme Bay; MUL, Mull; MRY, Mulroy Bay, Eire; PEE, Peel, Isle of Man; POL, Polperro; PS1, Port St. Mary (nearshore), Isle of Man; PS2, Port St. Mary (offshore), Isle of Man; STB, St. Brieuc Bay, Brittany; STO, Stonehaven. Samples used in (1) morphometric analysis, (2) allozyme electrophoresis, (3) MtDNA PCR-RFLP.

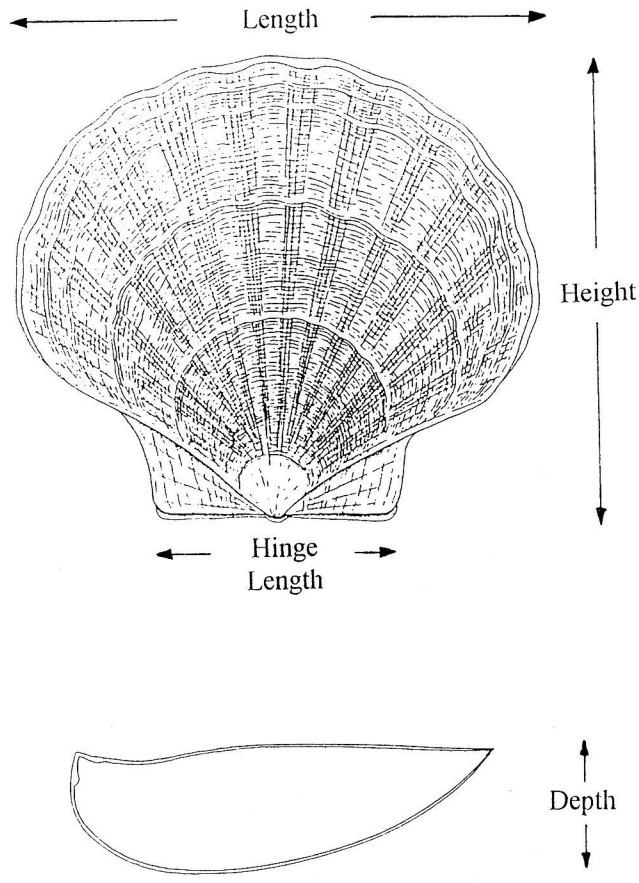

Figure 2. Shell dimensions measured for morphological analysis of *P. maximus*.

MINITAB package. Unless specimen size is rigorously controlled or some size transformation is used, it is typically the case that the majority of variation is due to size alone (Shea 1985, Krause et al. 1994). These shell sizes varied considerably (52.2-154.3 mm shell length), and we adopted two approaches to this problem. First, we divided each measurement by the geometric mean of all continuous variables and used $\log_{10}$ (transformed data) as input to the PCA. This serves to transform the linear measurements for each shell to an apparent absolute size, at which the variation in shape would occur (Reist 1985). Second, we used nontransformed data as input for the PCA and disregarded the first principal component, which is often largely attributable to size variation. After PCA, the resultant scores were tested for homogeneity of variance with Levene's test and for normality with the Anderson-Darling test. Where data satisfied these tests, they were further analyzed with ANOVA, followed by Scheffé's test to detect significant differences in scores between populations.

Allozyme Electrophoresis

Electrophoresis was undertaken on 12.5% gels essentially according to the methods described by Beaumont and Beveridge (1984) with either Tris-citrate (pH 8) (Saavedra et al. 1993) or Tris-maleic acid-EDTA (pH 7.4) buffers. Preliminary screening (Wilding 1996) identified two polymorphic enzyme systems not previously screened in *P. maximus*—NADH diaphorase (*Dia,* E.C. 1.6.2.2) and glutathione reductase (*Gr,* E.C. 1.6.4.2)—and these along with the polymorphic esterase-D (*Est-D,* E.C. 3.1.1.1), glucose phosphate isomerase (*Gpi,* E.C. 5.3.1.9), octopine dehydrogenase (*Odh,* E.C. 1.5.1.11), phosphogluconate dehydrogenase (*Pgd,* E.C. 1.1.1.44), and phosphoglucomutase (*Pgm,* E.C. 2.7.5.1) systems were screened across nine populations. Banding patterns for *Pgd* were only resolved from adductor muscle tissue; for all other loci, adductor muscle and digestive gland tissues were combined. Gel slices were stained with 2% agar overlays following the recipes of Harris and Hopkinson (1976) with meldolas blue in place of PMS (Turner and Hopkinson 1979), with the exception of Balakirev and Zaykin (1990) for *Gr,* and a preincubation in 0.1 M Tris-maleate (pH 5.3) for 10 min before stain application for *Est-D.* Statistical analyses of allozyme data were undertaken with BIOSYS-1.7 (Swofford and Selander 1981) to calculate allele frequencies, the effective number of alleles ($N_e = 1/\sum x_i^2$ where x_i is the frequency of the ith allele), and observed and expected heterozygosities. Genetic distances between populations were calculated as Nei's D with either BIOSYS or GD (Ritland 1989) and clustered via UWPGMA in GD. Manual calculation of gene diversity statistics was performed following Nei (1987). Gene diversity measures serve to effectively partition the variation encountered into practical divisions of H_T (the total gene diversity), H_S (the sample gene diversity), D_{ST} (the gene diversity among populations), $\overline{D}_m$ (the absolute measure of gene differentiation as $\overline{D}_m$ but excluding comparisons of populations with themselves), and G_{ST} (the coefficient of gene differentiation among populations). A Monte Carlo simulation method (Roff and Bentzen 1989) was also used to analyze differences in allele frequencies between populations. The MONTE option of REAP (McElroy et al. 1992) was used with 1,000 randomizations of the data (keeping row and column totals equal) to calculate a probability of obtaining by chance a χ^2 value higher than that obtained from the real data. Because of multiple tests, significance levels for tests at each locus were adjusted (Hochberg 1988). Agreement to the Hardy-Weinberg model was investigated through the calculation of Wright's fixation index (F_{IS}) (the average deviation from H-W proportions within populations), which was tested for significance by the χ^2 method of Waples (1987) as $\chi^2 = F_{IS}^2 N(k-1)$ with $[k(k-1)]/2$ *df,* where k is the number of alleles and N is the sample size. Significance levels were adjusted according to Hochberg (1988) to correct for multiple tests of the same hypothesis. *F* statistics for *Gpi* were calculated after pooling to five artificial allelic categories.

RESULTS

Morphology

Meristic Data

The numbers of ribs were seen to vary between sites (Table 1), and rib counts were detectably different among sites by ANOVA ($F = 7.22$, $p < 0.001$). Three pairwise comparisons, all involving St. Brieuc Bay scallops, which usually had fewer ribs, were identified as significantly different by Scheffé's test (STB-CHI, STB-PEE, STB-KIL). The comparison between St. Brieuc and Polperro also was significantly different, but the Polperro sample was not normally distributed.

Continuous Data

Each character was seen to exhibit evidence of negative allometry relative to length (Table 2). There was also extensive size variation both within and between samples. Two approaches were taken to account for this in the PCA. Removal of PC1, which from the heavy loadings of all measurements was taken as reflecting size variation (data not shown; see Wilding 1996), caused two main difficulties. First, only 4% of the remaining variation was unaccounted for by the first PC, and second, the resultant scores were largely inappropriate for our further analyses because of considerable heterogeneity of variance. Our second method of controlling size, using a geometric mean transform, did not suffer from these problems. From PCA undertaken on the five metric variables, the first three principal components (PCs 1–3) explained 96% of the total variation in continuous characters (Table 3). These PCs were accountable to particular measurements (Table 3); for instance, PC1 is attributable largely to hinge length, as indicated by the strongly positive eigenvector for hinge length, with relatively weak (negative) eigenvectors associated with other metric variables. PC2 is strongly associated with the depth contrasted with length and height of the animals, and PC3 is associated with the shell mass contrasted with length and depth. Heterogeneity of variance was problematical for further ANOVA, particularly for

TABLE 2.

Regression analysis of allometry in populations of *P. maximus*.

Character	b	SE of b	β	n	*t*
Depth	0.85139	4.63×10^{-4}	1	699	321***
Height	0.97145	2.15×10^{-4}	1	700	132.7***
Hinge length	0.84002	6.13×10^{-4}	1	667	261.169***
Weight	2.5011	9.45×10^{-4}	3	699	52.8***

b, observed slope; SE, standard error; β, expected slope if growth is isometric; n, sample size; *t*, *t* value.
***significant at 0.001 level.

TABLE 3.

Summary of PCA performed on *P. maximus* morphometric dataset using the covariance matrix.

	Eigenvector				
Character	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Length	−0.204	0.498	−0.314	0.641	−0.447
Depth	−0.271	−0.676	−0.505	−0.120	−0.447
Height	−0.270	0.462	0.092	−0.711	−0.447
Weight	−0.145	−0.285	0.796	0.254	−0.447
Hinge length	0.890	0.001	−0.068	−0.064	−0.447
Eigenvalue	0.000753	0.000338	0.000162	0.000057	0.00000
% Proportion	57.4	25.8	12.4	4.3	0.000
% Cumulative	57.4	83.3	95.7	100	100

Eigenvectors describe the rotation of the original axes necessary to define the new principal components, thus indicating the correlation of the measured variable with that PC. Eigenvalues express how much variability is explained by each of the PCs, and the proportion reflects the percentage of the total variation explained by each eigenvalue.

PC1 scores, where the ANG, STO, and MUL populations had to be disregarded. Similarly, there was significant heterogeneity of variance for the ANG population when PC3 scores were tested. Application of the Anderson-Darling test suggested that non-normality was not a problem. Only CHI for PC1, STO for PC2, and CHI for PC3 were non-normal. Non-normality does not affect the application of ANOVA unless no more than 50% of the data is non-normally distributed. Principal component scores for PCs 1–3 were then subjected to ANOVA. All three tests were highly significant, suggesting that differences between some populations exist for PCs 1–3 (PC1: $F = 42.07$, $p < 0.0001$; PC2: $F = 15.56$, $p < 0.0001$; PC3: $F = 22.32$, $p = 0.0006$). Scheffé's test was used to detect where these differences occurred (see Table 4). There are many significant outcomes; however, the most obvious differences occur in PC1 for LAT and BRE, which are significantly different from all other populations but are not detectably different from each other. This was also noted (on PC2) from the PCA, where size was controlled by removal of PC1 (Wilding 1996). For PC2, LYM, POL, and LAT are significantly different in most comparisons from other populations but are not different from each other, and for PC3, KIL and LYM are significantly different in 9 and 8 comparisons, respectively, of 13 total comparisons. It is striking from Table 4 that geographically proximate samples rarely show differences, e.g., note the lack of differences between the six Irish Sea populations, between the two Irish samples, and between the two southern coast populations.

Allozymes

The loci screened in this study had been chosen either on the basis of high polymorphism (*Est-D, Gpi, Odh, Pgd, Pgm*) or, for *Dia* and *Gr,* because they had not been screened in this species before and were polymorphic; thus, the high level of variation encountered is neither surprising nor meaningful in its own right. Such high levels of variation (N_{eff} and h in Table 5) coupled with relatively low sample sizes may, however, cause high sampling variances that could affect other analyses of variation. Up to 15 alleles were found for *Gpi,* and no locus exhibited fewer than 4 alleles (Table 5). Allele frequencies are shown in Table 5. No alleles with a frequency greater than 0.067 (Pgd^{52}) were confined to any population. *Dia* and *Est-D* were not resolvable from Lyme Bay scallops—probably as a result of postcollection storage, which

TABLE 4.

Matrix displaying significant outcomes of Scheffé's pairwise comparisons after ANOVA on principal components 1, 2, and 3. Population abbreviations are as in Figure 1 legend. Populations 6, 7, and 8 were not analyzed by Scheffé's test for PC1 data (see text).

	PS2	DOU	PEE	CHI	PS1	ANG	STO	MUL	LYM	POL	MRY	KIL	STB	LAT	BRE
PS2	–														
DOU	1	–													
PEE			–												
CHI				–											
PS1		1			–										
ANG						–									
STO							–								
MUL			3	3			3	–							
LYM	1,2,3	2,3	1	1,2	1,2,3	2		2,3	–						
POL	1,2,3	2		2	1,2,3	2		2,3		–					
MRY	1		1,3	1,3	1		3		2,3	3	–				
KIL	1,3	3	3	3	3		3		2,3	2,3		–			
STB	1		1	1	1			3	2	2	3	3	–		
LAT	1,2	1,2	1	1,2	1,2			2	1,3	1	1	1,2	1,2	–	
BRE	1	1	1	1	1				1,3	1,3	1	1,2	1		–

TABLE 5.

Allele frequencies at seven loci for nine populations of *P. maximus*.

Locus	R.M.	BRE	STB	KIL	MRY	LAT	PEE	STO	MUL	LYM
Dia	94	0.000	0.033	0.000	0.017	0.087	0.016	0.021	0.017	
	100	0.638	0.750	0.667	0.700	0.609	0.742	0.729	0.683	
	105	0.310	0.200	0.300	0.233	0.283	0.242	0.229	0.217	
	110	0.034	0.017	0.033	0.050	0.022	0.000	0.021	0.083	
	116	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
	n	29	30	30	30	23	31	24	30	
	h	0.379	0.3	0.433	0.533	0.435	0.323	0.417	0.467	
	Neff	1.98	1.66	1.87	1.83	2.18	1.64	1.71	1.92	
Est-D	64	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	
	75	0.017	0.000	0.000	0.000	0.000	0.017	0.000	0.000	
	84	0.000	0.017	0.017	0.017	0.017	0.050	0.000	0.000	
	100	0.569	0.550	0.500	0.533	0.586	0.500	0.667	0.595	
	108	0.069	0.067	0.052	0.000	0.052	0.017	0.000	0.000	
	119	0.328	0.317	0.431	0.433	0.328	0.367	0.292	0.310	
	125	0.017	0.033	0.000	0.017	0.017	0.033	0.042	0.071	
	140	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.024	
	n	29	30	29	30	29	30	24	21	
	h	0.379	0.467	0.414	0.3	0.586	0.5	0.417	0.619	
	Neff	2.29	2.44	2.28	2.12	2.2	2.57	1.88	2.19	
Gpi	38	0.033	0.000	0.017	0.017	0.000	0.000	0.042	0.000	0.017
	50	0.000	0.000	0.017	0.000	0.017	0.000	0.000	0.033	0.000
	64	0.083	0.033	0.100	0.017	0.017	0.065	0.083	0.050	0.083
	74	0.067	0.083	0.050	0.217	0.086	0.081	0.104	0.067	0.100
	78	0.033	0.017	0.017	0.000	0.017	0.000	0.000	0.017	0.017
	87	0.217	0.183	0.200	0.217	0.190	0.210	0.167	0.167	0.183
	93	0.033	0.033	0.017	0.017	0.000	0.000	0.021	0.033	0.000
	100	0.200	0.250	0.167	0.366	0.207	0.306	0.313	0.367	0.067
	105	0.000	0.033	0.017	0.000	0.000	0.000	0.000	0.017	0.000
	112	0.217	0.233	0.150	0.050	0.103	0.145	0.083	0.117	0.317
	114	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	115	0.017	0.000	0.050	0.017	0.017	0.000	0.042	0.017	0.033
	119	0.033	0.000	0.017	0.017	0.069	0.081	0.021	0.067	0.017
	124	0.033	0.100	0.167	0.067	0.190	0.097	0.104	0.017	0.150
	130	0.000	0.017	0.016	0.000	0.017	0.000	0.000	0.033	0.000
	136	0.033	0.017	0.000	0.000	0.069	0.016	0.021	0.000	0.017
	n	30	30	30	30	29	31	24	30	30
	h	0.833	0.8	0.767	0.6	0.828	0.677	0.875	0.867	0.833
	Neff	6.56	5.84	7.39	4.23	6.94	5.38	6.02	5.2	5.55
Gr	62	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
	75	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
	85	0.100	0.067	0.017	0.067	0.077	0.016	0.021	0.050	0.000
	96	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019
	100	0.500	0.500	0.500	0.567	0.692	0.500	0.500	0.500	0.519
	110	0.033	0.000	0.033	0.017	0.019	0.016	0.021	0.017	0.019
	119	0.300	0.417	0.450	0.300	0.212	0.419	0.438	0.417	0.423
	125	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.017	0.000
	130	0.017	0.000	0.000	0.017	0.000	0.000	0.021	0.000	0.019
	140	0.000	0.000	0.000	0.000	0.000	0.048	0.000	0.000	0.000
	n	30	30	30	30	26	31	24	30	26
	h	0.733	0.667	0.567	0.6	0.384	0.613	0.667	0.667	0.5
	Neff	2.83	2.33	2.2	2.4	1.89	2.33	2.26	2.34	2.23
Odh	78	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
	87	0.000	0.000	0.033	0.017	0.017	0.016	0.000	0.000	0.017
	90	0.333	0.283	0.217	0.133	0.259	0.226	0.417	0.433	0.217
	100	0.633	0.683	0.733	0.833	0.707	0.726	0.583	0.567	0.733
	108	0.033	0.033	0.000	0.017	0.017	0.032	0.000	0.000	0.033
	n	30	30	30	30	29	31	24	30	30
	h	0.4	0.5	0.4	0.333	0.448	0.419	0.667	0.4	0.3
	Neff	1.95	1.83	1.71	1.4	1.76	1.73	1.95	1.97	1.71

TABLE 5.

continued

Locus	R.M.	BRE	STB	KIL	MRY	LAT	PEE	STO	MUL	LYM
Pgd	52	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000
	75	0.133	0.067	0.100	0.100	0.069	0.065	0.188	0.067	0.121
	100	0.867	0.917	0.867	0.817	0.914	0.919	0.813	0.933	0.879
	125	0.000	0.017	0.033	0.017	0.017	0.016	0.000	0.000	0.000
	n	30	30	30	30	29	31	24	30	29
	h	0.266	0.1	0.2	0.267	0.103	0.161	0.375	0.133	0.241
	Neff	1.3	1.18	1.31	1.47	1.19	1.78	1.44	1.14	1.27
Pgm	71	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
	77	0.017	0.000	0.000	0.000	0.017	0.032	0.021	0.033	0.033
	89	0.050	0.017	0.050	0.000	0.034	0.065	0.042	0.033	0.067
	100	0.883	0.933	0.917	0.967	0.914	0.839	0.896	0.833	0.850
	114	0.050	0.050	0.033	0.033	0.034	0.065	0.021	0.083	0.050
	124	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000
	n	30	30	30	30	29	31	24	30	30
	h	0.233	0.133	0.133	0.067	0.172	0.29	0.208	0.3	0.3
	Neff	1.27	1.15	1.18	1.07	1.19	1.4	1.24	1.42	1.37

h, observed heterozygosity; Neff, effective number of alleles; R.M., relative mobility. Population abbreviations are as in Figure 1 legend.

may have involved several accidental, partial freeze-thaw cycles. Table 6 details the results from *F*-statistic analysis. F_{IT} was positive for all loci except *Gr* and *Pgm,* suggesting a general deficiency of heterozygotes, although no F_{IT} estimate was significant. Deviations within samples (F_{IS}) contributed most to this trend, although within individual populations, there was no tendency for deficiency or excesses of heterozygotes (sign test, $Z = -0.6114$; not significant). The genetic diversity within populations was analyzed by Nei's *G*-statistics (Table 7). Most variability (98%) is within populations (H_S; Table 7), only 2% being partitioned among populations (G_{ST}; Table 7). Low G_{ST} figures suggest little partitioning of variation between populations, and only G_{ST} figures for *Gpi* and *Odh* are significant at the 0.001 level. However, as mentioned above, *Gpi* is so variable that we consider it inappropriate to consider this as evidence of population structure because of the likely high sampling variance for such sample sizes. Using Monte Carlo–based χ^2 tests, we found significant differences only between LYM-MUL/MRY for *Gpi* and MRY-MUL for *Odh,* once correction for multiple tests had been performed. Both BIOSYS and GD were used for calculation of Nei's *D* and subsequent dendrogram construction. GD is advantageous in that it computes standard errors of branch lengths for the dendrogram to allow significant clusters to be made obvious; however, GD cannot be used where there are missing data or where there are more than 10 alleles at a locus. We therefore omitted Lyme Bay and reduced *Gpi* to 10 alleles (pooling Gpi^{38} with Gpi^{50}, Gpi^{74} with Gpi^{78}, Gpi^{100} with Gpi^{105}, Gpi^{115} with Gpi^{119}, and Gpi^{130} with Gpi^{136}). This did not affect the tree topology compared with unpooled data treated with the GDD program (Ritland 1989), where error bars are not possible, or with the tree produced from BIOSYS after more severe pooling (see Fig. 3.3 of Wilding 1996). The dendrogram constructed from values of Nei's genetic distance (*D*) suggests some, limited evidence for geographically close populations to cluster together (Fig. 3), as may be expected if the data conform to an isolation by distance model, but a Mantel test performed in NTSYS comparing Nei's *D* with approximate geographic distance (shortest route by sea) was insignificant ($p[\text{random } Z \geqslant \text{observed } Z] = 0.1531$). It is also evident that no single population is apparently exceptionally distant from any other population (Fig. 3), although the error bars allow this to be further scrutinized. In order to consider a branch length to be significantly greater than zero, the standard error (shaded) bars should be smaller than half the branch

TABLE 6.

***F*-statistics for seven loci in nine populations of *P. maximus*. Calculations for the *Gpi* locus were performed on pooled data (five alleles).**

Population	*Dia*	*Est-D*	*Gpi*	*Gr*	*Odh*	*Pgd*	*Pgm*
Rade de Brest	0.234	0.327	0.065	−0.135	0.178	−0.154	−0.088
St. Brieuc	0.243	0.211	−0.028	−0.166	−0.107	0.355	−0.057
Kilkieran Bay	0.067	0.263	0.083	−0.038	0.034	0.159	0.146
Mulroy Bay	−0.178	0.431	0.181	−0.029	−0.161	0.162	−0.034
La Trinite	0.197	−0.074	0.013	0.05	−0.036	0.353	−0.062
Peel	0.174	0.182	0.176	−0.073	0.004	−0.073	−0.011
Stonehaven	−0.004	0.111	−0.08	−0.196	−0.371	−0.231	−0.071
Mull	0.026	−0.137	−0.114	−0.163	0.186	−0.071	−0.013
Lyme Bay	–	–	−0.054	0.091	0.275	−0.137	−0.113
Mean F_{is}	0.05	0.179*	0.046	−0.083	−0.01	0.018	−0.044
F_{it}	0.073	0.2	0.075	−0.061	0.029	0.036	−0.03

*$p < 0.05$.

TABLE 7.

Gene diversity (Nei 1987) analysis of nine populations of *P. maximus*.

Locus	H_T	H_S	D_{ST}	$\overline{D}_m$	G_{ST}	mN_e
Dia	0.4592	0.4543	0.0049	0.0056	0.0107 NS	23.11
Est-D	0.5584	0.5515	0.0069	0.0080	0.0124 NS	19.91
Gpi	0.8496	0.8260	0.0236	0.0265	0.0277***	8.68
Gr	0.5748	0.5632	0.0116	0.0131	0.0202 NS	12.13
Odh	0.4470	0.4318	0.0152	0.0171	0.0340***	7.10
Pgd	0.2140	0.2102	0.0038	0.0043	0.0178 NS	13.80
Pgm	0.1995	0.1967	0.0028	0.0032	0.0142 NS	17.36
Mean over all loci	0.4718	0.4620	0.0098	0.0111	0.0196	12.51

Calculations for *Dia* and *Est-D* were based on only eight populations (excluding Lyme Bay, see Text). Gene diversities: H_T, total diversity; H_S, within populations; D_{ST}, among populations; $\overline{D}_m$, absolute differentiation among populations. Coefficient of gene differentiation: G_{ST}, among populations. ***$p < 0.001$. NS, not significant. N_em, effective number of migrants per generation.

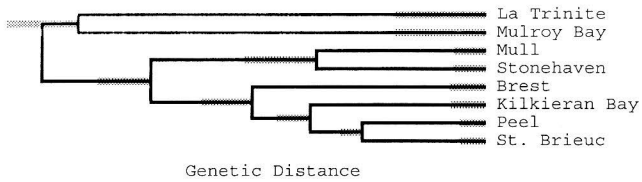

Figure 3. UWPGMA dendrogram based on Nei's genetic distance for nine populations of *P. maximus*. Shaded bars represent standard errors of branch lengths. The length of the dendrogram is 0.024 units of genetic distance.

length (Ritland 1989). This can only really be considered so for the Scottish grouping of Mull and Stonehaven and the Peel–St. Brieuc grouping. However, we consider these significances to be tenuous for two reasons. First, because the standard error calculation used in the construction of the dendrogram uses variances of distances among loci, more loci are really needed than used here for the method to be valid, with a suggested number of 12 (from GD manual), and for this reason, the standard error bars may be a poor estimate. Second, it is difficult to extract causes of these apparent significances from the actual data because a χ^2 test suggested no differences involving these populations, with the exception of Mull vs. Mulroy Bay for *Odh* (which did have a significant G_{ST} estimate). Although a limited number of other tests involving these samples were initially significant at the 0.05 level, they were deemed nonsignificant after correction for multiple comparisons. From inspection of allele frequencies, there seems no obvious reason for considering Peel and St. Brieuc to be different, and indeed no χ^2 test suggests this. We cannot therefore consider this clustering in the dendrogram to be truly significant. It would seem unsurprising that the two most northerly populations be significantly different, and although we do not disregard these results, we warn against overinterpretation in light of the limited quantity of loci studied.

DISCUSSION

The data given here represent a multiapproach effort at uncovering the nature of population structure of the valuable fishery resource, *P. maximus.* Other recent studies have been limited to comparing allozymes and DNA results, where DNA data usually provide similar or greater resolution than allozymes but occasionally reveal structure in species with apparently homogeneous allozyme patterns (e.g., DeSalle et al. 1987, Karl and Avise 1992). Here, morphology, allozymes, and mtDNA (Wilding et al. 1997) are compared and we have not found total concordance across methodologies for *P. maximus.*

For shape, although numerous significant differences between populations are evident, the only obvious, interpretable patterns within the data suggest distinctness of La Trinité and Brest scallops from all others on the basis of PC1 (attributable largely to hinge length), and to a lesser extent, Lyme Bay from most others on the basis of PC2 and PC3. However, the differences noted for PCs 2 and 3 are not as clear as those of PC1 because the pattern is less consistent. Although hinge length is negatively allometric (i.e., gets relatively smaller as the animal grows), the shells of La Trinité and Brest animals were not unusually large, and therefore, the smaller hinge length seems not to be an allometry-related artefact of sampling larger animals. The heretibility of morphological traits in *P. maximus* is unknown, but although morphology is known to be plastic in some bivalves (Seed 1968, Galtsoff 1964), Wilbur and Gaffney (1997) have suggested that many morphological characters in *Argopecten irradians* have an underlying genetic basis. These differences may therefore may be evidence of genetic differentiation of these populations. Although *P. maximus* shells have a dearth of useful landmarks for taking measurements, a more in-depth study using different measurements from the gross shell dimensions used here, perhaps using techniques such as Fourier analysis (Kenchington and Full 1994), may be of value in the future for detecting population differences.

Although the PCA did not pick out St. Brieuc scallops as different, they were found to have significantly fewer ribs than certain populations. Unusual colors of St. Brieuc Bay *P. maximus* shells were also noted and have been recognized previously in *P. maximus* from Brittany (Minchin 1991). The genetic basis of this color polymorphism has not been investigated. If color is under predominantly genetic control as in *A. irradians* (Adamkewicz and Castagna 1988), then this may be a further indication of genetic differentiation of this population.

Although there were these limited differences detectable in shell morphology, these were not mirrored by the allozyme dataset, which appears typical of free spawning bivalves with few obvious differences between populations despite high allozyme variation. The high allozyme variation is reflected in the high within-population diversity (H_S) values, which explain the majority of the total variance, and the G_{ST} figures given in this study are comparable to estimates for other bivalve populations (Buroker et al. 1979, Skibinski et al. 1983, Hedgecock and Okazaki 1984). High variability and low heterogeneity are typical of species with high gene flow and large population sizes (Palumbi 1992) and are often shown in bivalve populations (e.g., Buroker 1983, Skibinski et al. 1983, Borsa et al. 1994). Although levels of variability (as measured by the effective number of alleles and observed heterozygosity) do vary considerably between sites, there seems to be no consistent pattern to this. This may therefore reflect sampling variance as opposed to information on stock structure, and only a study using much larger sampling sizes would detect this. Allele frequencies also vary between sites but again without recognizable pattern, and no population is obviously distinct. Only the *Odh* locus shows possible significant differences between populations,

with these data implicating genetic differentiation of the two Scottish populations; we suggest that further loci would be needed to further investigate the limited differences suggested by this study. However, we do not advocate this because other allozyme studies (Huelvan 1985, Beaumont et al. 1993) have consistently failed to detect differences. Instead, we suggest that this effort be targeted into further DNA-based studies (see below).

The allozyme G_{ST} values calculated from allozymes are also concordant with the mtDNA θ (F_{ST} analogue) value of 0.005 (Wilding et al. 1997), which indicates little variation between populations. Despite this, there are differences between conclusions from the two genetic datasets. The most significant contradiction concerns the apparent genetic isolation of Mulroy Bay *P. maximus* on the basis of mtDNA sequence divergence data (Wilding et al. 1997), which is not borne out by either allozymes or morphology.

There may be three reasons for detecting separation with mtDNA but not with allozymes. First, there is a lower effective population size for the mtDNA molecule as a result of its maternal inheritance (½ that of allozymes in the case of hermaphroditic species), and this will produce more rapid evolutionary change due to random genetic drift. Second, it could be that balancing selection on allozyme loci is maintaining similar allele frequencies despite negligible or curtailed gene flow. Finally, the mode of analysis could have an effect on the amount of separation that is detectable: the difference between allozymes and mtDNA in the ability to separate Mulroy Bay scallops from other populations may be because the molecular changes necessary to interconvert mtDNA haplotypes can be inferred, whereas the evolutionary history of allozymes (protein variants) is not easily discernible (Mitton 1994). Thus, mtDNA haplotype analysis need not be confined solely to frequencies, as do allozyme data, because DNA sequence divergence levels can be reliably estimated. It is the sequence divergence estimates, not haplotype frequencies (which are also the input for the nonsignificant θ analysis; see above), that distinguish Mulroy Bay. This in itself is somewhat surprising, because recently separated populations are expected to show haplotype frequency differences before sequence divergence (Cronin 1993). There is some evidence that frequency differences, particularly for the commonest haplotype (AAAAAA), exist, but these are not at levels that produce statistical significance (Wilding et al. 1997).

DNA data also suggest that further population structure remains to be uncovered, and it is possible that Lyme Bay scallops may be to some extent an isolated population. This sample exhibits reduced mitochondrial haplotype diversity (Wilding et al. 1997) but similar allozyme heterozygosity relative to other populations, suggesting that this population may have been through a partial bottleneck. Bottlenecks affect mtDNA more severely than allozyme loci because of the reduced effective population size of the mtDNA compared with nuclear DNA (Wilson et al. 1985). Although a few private haplotypes do occur, the two common haplotypes (AAAAAA and AABAAB) predominate, accounting for 83% of all of the Lyme Bay individuals. Lower than average mtDNA diversity could imply reduced gene flow into Lyme Bay and suggests either that the population is undergoing some level of inbreeding, which will reduce diversity, or that a bottleneck has occurred and haplotype frequencies have not yet returned to prebottleneck levels. Either way, there must be an element of self-recruitment such that gene flow does not equilibrate diversity. In the light of this, it is particularly noteworthy here that Lyme Bay scallops show some evidence of morphological discrimination on the basis of PC2 and PC3.

Our *a priori* expectations were that if population differentiation was detectable, then the observed pattern would be not unlike that displayed by the queen scallop *A. opercularis,* which has a similar larval and byssal drifting dispersal potential and a similar adult habitat (Beaumont 1982, Beaumont 1991, Beaumont and Barnes 1992). Variation in frequency of the common mtDNA haplotype (AAAAAA) is concordant with this idea. The morphological discreteness of the Brest and La Trinité samples also fits with this hypothesis because they would fall into a Celtic Sea stock (Beaumont 1982) and would therefore be expected to be similar to each other but variant from other samples. The Mull population, which along with scallops from Stonehaven exhibited evidence of genetic differentiation, would also fall into a separate province, North and West Scotland, as denoted by Beaumont (1982). Our data in conjunction with reproductive cycle data (Paulet et al. 1988, Cochard and Devauchelle 1993, Mackie and Ansell 1993) and evidence for stock structure in *A. opercularis* (Beaumont 1982) suggest that *P. maximus* may indeed have genetically structured populations, but that high-resolution techniques will be necessary to fully elucidate these (Wilding et al. 1997) because although shape, allozymes, and mtDNA all provide useful information, their resolution is not sufficient, and the patterns they reveal are neither concordant nor conclusive. Inability to fully differentiate populations with selected genetic markers between populations should not be taken as evidence of lack of real differentiation (Carvalho and Hauser 1995), and the underlying nonsignificant structure to mtDNA haplotype frequencies suggests that further work on high-resolution technologies such as microsatellite DNA, which are now considered more appropriate for investigating population differentiation (Wright and Bentzen 1995), may be worthwhile.

The three approaches used here do represent a gradient of increasing cost in terms of both development and execution, but not cost effectiveness. It is evident that the most expensive (mtDNA analysis) has provided the most interpretable evidence of genetic differentiation. The high cost of development of a microsatellite protocol may well prove justifiable in terms of elucidation of population structure for this valuable resource.

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