

Mitochondrial DNA variation in the scallop *Pecten maximus* (L.) assessed by a PCR-RFLP method

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Two PCR–RFLP mitochondrial DNA (mtDNA) markers were developed through the cloning and sequencing of mtDNA from the scallop *Pecten maximus*, and were used to study genetic differentiation of UK and Atlantic coast populations of this species. Although no distinct pattern of mtDNA haplotype frequencies was apparent and no diagnostic haplotypes were identified for any population, sequence divergence data provided convincing evidence that a *P. maximus* sample taken from Mulroy Bay, Eire, a semi-enclosed sea lough, was genetically differentiated from all other samples. However, this could not be unequivocally attributed to a restriction in gene flow, as the sample consisted of an ongrown single spatfall, which may not have been representative of the wild population. Despite the inability to separate populations on the basis of haplotype frequency, it was noteworthy that the frequency pattern of the commonest haplotype varied between sampling sites in a manner similar to that of allozyme allele frequencies in *Aequipecten opercularis*, a scallop species with a similar distribution and life history, for which there is evidence of population subdivision. *Pecten maximus* from St Brieuc Bay, reasoned to be a self-recruiting population from reproductive and physiological evidence, could not be separated from other populations using mtDNA markers. Further investigation of this population with alternative markers is warranted.

Keywords: length variation, mitochondrial DNA, *Pecten maximus*, PCR, population genetics, scallop.

Introduction

The scallop *Pecten maximus* (L.) has an extensive distribution throughout the coastal waters of northern Europe, ranging from Norway south to the Iberian Peninsula, and supports an important and valuable fishery (Ansell *et al.*, 1991; Brand, 1991). *Pecten maximus* are most common between 20 m and 45 m on bottoms of sand, fine gravel or sandy gravel (Brand, 1991) in areas of favourable bottom stress (Dare *et al.*, 1994). Such restrictions on suitable habitat result in a patchy distribution, with dense aggregations (beds) where scallops occur in sufficient densities for fishery exploitation (Sinclair *et al.*, 1985). Although *P. maximus* larvae and post-larvae have a considerable planktonic dispersal capability (Beaumont & Barnes, 1992), there is

evidence that larval movement between beds, and hence gene flow, may be constrained. The evidence for this claim ranges from differences between populations in growth rate (Mason, 1983) and colour (Minchin, 1991) to potential hydrographic restrictions to gene flow between exploited populations detected using the North Sea water parcel following (NORSWAP) model (Dare *et al.*, 1994). Scallops from a population in St Brieuc Bay, Brittany, present the clearest indication that gene flow between beds may be constrained, displaying a markedly different spawning cycle from neighbouring populations, the characteristics of which are maintained following reciprocal transplantation (Paulet *et al.*, 1988; Cochard & Devauchelle, 1993; Mackie & Ansell, 1993).

Past efforts to identify differences between *P. maximus* populations using allozyme frequency data have, however, proved unfruitful (Beaumont *et al.*, 1993). In contrast, for *Aequipecten opercularis*, a

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scallop species with a similar distribution and life history, there are significant allele frequency differences between populations, and separation of the St Brieuc population on the basis of allozyme data (Beaumont, 1982). As *A. opercularis* has an increased capacity for larval dispersal relative to *P. maximus* owing to its enhanced byssal drifting capability (Beaumont & Barnes, 1992), gene flow in *P. maximus* is *a priori* expected to be lower and, thus, genetic differentiation greater. If *P. maximus* is indeed genetically structured, then the inability to separate populations using allozyme methodologies may be explainable by the action of balancing selection at allozyme loci, which has been suggested as being capable of maintaining similar population allele frequencies in spite of negligible gene flow (Karl & Avise, 1992).

DNA-based methodologies targeted at essentially neutral loci are now being used to challenge some of the results based on allozyme data (Mitton, 1994). The marker of choice for the majority of population level comparisons has been the mitochondrial DNA (mtDNA), and restriction fragment polymorphism (RFLP) analysis of the mtDNA molecule has been widely applied to marine species (Ovenden, 1990). However, recent work has shown that in many scallops (Snyder *et al.*, 1987; Gjetvaj *et al.*, 1992; Repin & Brykov, 1992; Boulding *et al.*, 1993), including *P. maximus* (Rigaa *et al.*, 1993), mtDNA length differences between individuals and species are extensive. *Pecten maximus* mtDNA is variable in length as a result of varying numbers of a tandemly repeated 1.55 kb element (Rigaa *et al.*, 1993, 1995). This may complicate mtDNA RFLP studies, as the separation of fragment pattern differences because of restriction site losses and gains from those caused by length differences can prove difficult (Moritz *et al.*, 1987; Boulding *et al.*, 1993; Wilding, 1996). This problem can be avoided through examination of RFLPs in defined sections of mtDNA amplified by the polymerase chain reaction (PCR). PCR-RFLP has proved to be a useful technique for systematic studies and species identification (e.g. Chow & Inoue, 1993) and in population analysis (Cronin *et al.*, 1993, 1994; Bartlett & Davidson, 1995; Hall & Nawrocki, 1995).

To date, there has been no study examining population level DNA variability within *P. maximus*. Here, we demonstrate that specific primers designed for PCR-RFLP can be useful in the examination of population genetic structure and we apply the method to *P. maximus* from Britain, Eire and northern France.

Methods

Sampling

Twelve populations of *P. maximus* were sampled by dredge fishing or scuba diving (see Fig. 1). All were samples of adult animals. Mulroy Bay individuals had been collected as wild spat, ongrown to approximately 2 cm in Mulroy Bay, then transplanted to Kilkieran Bay. Mortality was negligible (Dr G. Burnell, University College, Cork, personal communication). Animals were received live and maintained in running seawater until dissection, or dissected and frozen (-70°C) until use. The Polperro and Lyme Bay samples were received frozen at -20°C and used after thawing of tissue.

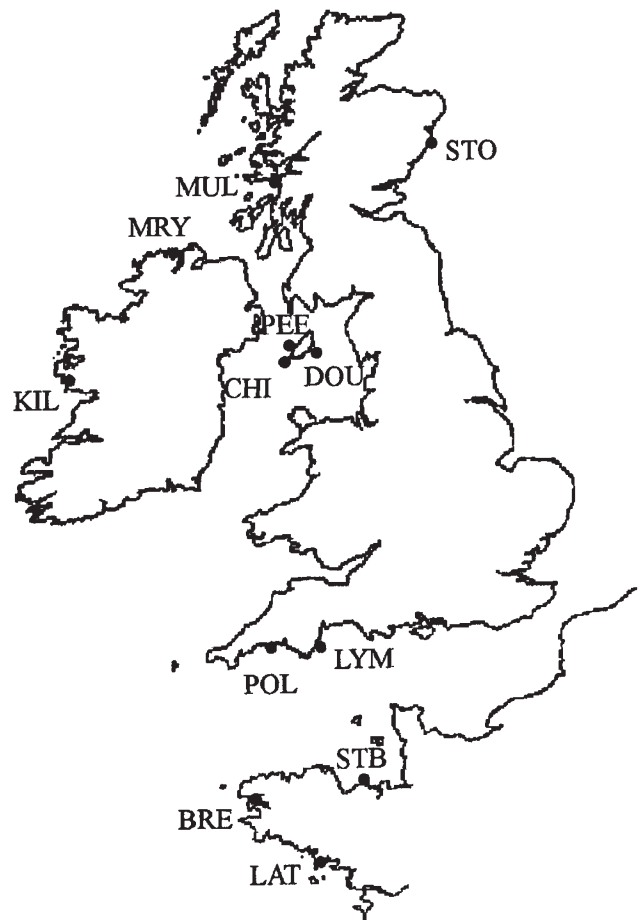


Fig. 1 Collection locations for *Pecten maximus* samples. DOU, Douglas; PEE, Peel; CHI, Chicken Rock; STO, Stonehaven; MUL, Mull; LYM, Lyme Bay; POL, Polperro; MRY, Mulroy Bay (transplanted to and ongrown in Kilkieran Bay); KIL, Kilkieran Bay; STB, Nerput, St Brieuc Bay; BRE, Tinduff, Daoulas Bay, Rade de Brest; LAT, La Trinité sur Mer.

DNA extraction

Mitochondrial DNA of individual animals was extracted from approximately 5 g of adductor muscle using the technique of Snyder *et al.* (1987) adapted to include the modifications of Boulding *et al.* (1993) and a CTAB (hexadecyltrimethyl ammonium bromide) incubation to remove polysaccharides (Fisher & Skibinski, 1990). MtDNA was resuspended in 50 μL of dH_2O .

Genomic DNA was isolated from approximately 50 mg of tissue, homogenized in 300 μL of CTAB buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2 per cent CTAB, 0.2 per cent 2-mercaptoethanol), then incubated at 65°C for 30–60 min after the addition of 15 μL of 10 mg mL^{-1} proteinase K. The lysate was then extracted once with chloroform-isoamyl alcohol (24:1), once with phenol-chloroform-isoamyl alcohol (24:24:1) and once with chloroform-isoamyl alcohol. DNA was precipitated with 0.1 volumes of 3 M NaOAc and 1 volume of isopropanol, air-dried and resuspended in 100 μL of dH_2O .

Cloning, sequencing and primer design

A number of restriction enzymes were applied to *P. maximus* mtDNA in order to identify fragments suitable for cloning, sequencing and primer design (Wilding, 1996). Two fragments of mtDNA, which showed no evidence of length variation after restriction digest screening of a number of individuals, were chosen for sequencing; a 2 kb *EcoRI*–*HindIII* fragment and a 3.85 kb *HindIII*–*HindIII* fragment. MtDNA for ligation was purified as above from a single animal except that two bouts of sucrose gradient centrifugation (Snyder *et al.*, 1987) were used to ensure complete separation of mitochondria from nuclei. Restriction enzyme-treated mtDNA was ligated into pBluescript KS⁺ (Stratagene) using standard protocols (Sambrook *et al.*, 1989) and used to transform competent XL1-blue *E. coli*. Recombinant colonies were identified using β -galactosidase complementation, and inserts of the correct size were detected via restriction enzyme digestion of mini-prepped DNA (Holmes & Quigley, 1981). Vector DNA containing the desired insert was further purified using Promega's Wizard mini-preps, and the ends were sequenced using Pharmacia's T7 sequencing kit following the manufacturer's specifications. PRIMER (The Lander Laboratory, Cambridge, MA, U.S.A.) was used to identify PCR primers from these sequences, accepting default conditions except primer length (20–25) and melting

temperature (60°C). Primers were synthesized by Cruachem, Glasgow. Primer sequences were as follows.

Primer pair Pma1, target 2 kb *EcoRI*–*HindIII* fragment (5'–3'):

TTTTAAGGAGGTAATCGCTATTCCG
CAGCAATCTGTATGGGTAGAACC

Primer pair Pma2, target 3.85 kb *HindIII*–*HindIII* fragment (5'–3'):

TTTTATGTGGTGGTATTGTTGAGG
ATAGGATAGTCTGGAATACGACGC

Amplification and restriction digestion of PCR products

Amplification using the Pma1 primer pair was undertaken in a 50 μL total volume of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM MgCl_2 , 0.1 per cent (v/v) Triton X-100, 200 μM each dNTP, 25 pmol of each primer and 1 U *Taq* (Promega). Cycle parameters of 3 min at 94°C, followed by 35 cycles of 45 s at 94°C, 30 s at 52°C and 2 min at 72°C with a final extension time of 7 min at 72°C were used. The Pma2 fragment was amplified in a 50 μL volume with 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 2.5 mM MgCl_2 , 0.1 per cent (v/v) Triton X-100, 200 μM each dNTP, 25 pmol of each primer, 1 U *Tbr* polymerase (NBL) and 1 U *Taq* extender (Stratagene). Cycle parameters of 3 min at 94°C and 40 cycles of 1 min at 94°C, 1 min at 56°C and 5 min at 72°C with a final extension time of 10 min at 72°C were used. DNA templates consisted of 1 μL of a 1:10–1:100 dilution of mtDNA or 1 μL (100–500 ng) of genomic DNA (gDNA). Restriction digestion was performed on 5 μL aliquots of successful amplifications in 20 μL total volume using 1–2 U of enzyme. The enzymes, *AluI*, *CfoI*, *DraI*, *HaeIII*, *HinfI*, *MspI*, *RsaI*, *Sau3AI* and *TaqI*, were used in trial screenings to assess variability. Digestion products were separated on 1.4 per cent agarose gels.

Statistical analysis

For each individual, a composite haplotype described the patterns generated by multiple restriction endonucleases and, for each RFLP pattern, the type of site change responsible could be ascertained from digestion patterns and attributed to one, or occasionally two, site changes. Restriction mapping was not performed. Using information on the number of composite haplotypes within each popu-

(AAAAAA) varies widely between sample sites, accounting for between 25 per cent and 53 per cent of all composite haplotypes, no significant differences in haplotype numbers could be detected

between populations using χ^2 analysis, either table-wise (37.3 per cent of pseudo χ^2 -values from the randomized data were higher than those generated from the real data), in pairwise comparisons of

Table 1 Pma1-amplified mitochondrial DNA haplotypes in 12 populations of *Pecten maximus*

Haplotype	LAT	BRE	STB	KIL	MRY	CHI	DOU	PEE	STO	MUL	LYM	POL
AAAAAA	8	8	17	14	15	8	13	11	9	12	16	8
AAAAAB	1											
AAAABA		2			2	1		1		3	1	1
AAAACA	2	2				2	3	1	1	1	1	4
AAAACB			1									
AAAADA		1										
AAAAEA			1					1	1			
AAAafa										1		
AAAAGA		1										
AAAABB									1			
AABAAB	8	4	5	7	2	6	3	5	4	7	9	8
AABAAA	3	4		1	2	4	3	2	2		1	1
AAABAA			1		1	1	1	2		1		1
AAABCB		1										
AAACHA							1	1				
AAACCA	1											
AAAGEA					1							
AAAGCA			1		2							
AAAGAA						1						
AAAGBA					1			1				
AAAFCC									1			
AAACAA								1				
AAACBA		1										
AABDCA			1									
AABABB		1				1	1		1			2
AABFAB												1
AABACB				1		1						1
AABAAD					1							
AADAAA	1	1	1	1	2	1	1	1	1			
AABBAB	1	1		1				2	1	1		2
AACGCA										1		
AABABA										1		
AABIAA												1
AABEAB				1								
AADBAB								1				
AABGAB	1											
AAEAAA								1				
AAFAAA				1								
AAFACA				1								
ABAAAA												1
ABBACA			1									
AFBAAA										1		
ACAAAA	1		1	1								
ACABEA	1											
ACBAAB			1									
ACAGCA									1			
ACACCA				1								
ADBAAB	1	2										
AEAAAA									1			
BAAAAA		1				1	1					1
BABAAA							1					
BABAAB						1	1					
BCAAAA			1									
BCAACA						1						
BDAACA						1						
CAAAAA					1					1		
CAAEAA											1	
CABAAA							1					
CABAAB												
N	29	30	32	30	30	30	30	31	24	30	1	32
Hap. Div	0.8522	0.9011	0.7056	0.7425	0.7471	0.8851	0.8	0.8538	0.8442	0.7931	0.6414	0.871
N.D. Frag	0.0148	0.0159	0.0132	0.0128	0.0107	0.0153	0.0109	0.0138	0.0168	0.0146	0.0092	0.0153
N.D. Site	0.0238	0.0256	0.021	0.02	0.0184	0.0243	0.0184	0.023	0.0265	0.0235	0.0145	0.0249

Population abbreviations as in Fig. 1. Order of restriction enzymes in composite haplotypes: *CfoI*, *HinfI*, *MspI*, *RsaI*, *Sau3AI* and *TaqI*.

populations or from single-enzyme haplotype data, when calculated values were adjusted via the Bonferroni procedure. Some haplotypes are confined to particular populations ('private haplo-

Table 2 Pma2-amplified mitochondrial DNA haplotypes in six populations of *Pecten maximus*

Haplotype	CHI	POL	PEE	KIL	STB	MUL
AAAAAA	3	6		4	6	3
AAAACA	2		1	1	6	4
AABAAA	1		1		1	
BFAABA	1			1		
ACABAA			1			
BBAABA	3	4	1	2	3	4
AAAEAC			1			
BEAABA		1			1	
AAAFA		1		1		
AAAGCA		1				
AAABAA		1				1
DAAACA					1	
AEAHAA					1	
AACAAA					1	
AABGAA					1	
AAAIAA					1	
AAAAEA						1
AAABCA						1
CBAABA				1		1
BBBADA						1
AACACA						1
AGAAAA						1
AAACBA						1
AAAFEA						1
BEAABC				1		
AAAACB				1		
AABACA	2			1	1	
AAADHA				1		
BBAAAA				1		
ABAACA	1			1		
BMBAAA	1					
ANEACA	1					
ADAACA	1					
BHAABA	1					
ADAAAA	1					
BBAADA			1			
N	18	14	6	16	23	20
Hap. Div.	0.9477	0.7692	1	0.9417	0.8696	0.9211
N.D.	0.01089	0.01348	0.01348	0.01118	0.00802	0.00992

Population abbreviations as in Fig. 1. Order of restriction enzymes in composite haplotypes: *Dra*I, *Hae*III, *Hin*fI, *Msp*I, *Rsa*I and *Sau*3AI.

types') but cannot be taken as evidence of population structure, only as an indication of considerable diversity. A UPGMA dendrogram of haplotype relatedness did not provide any evidence of geographical structure (Fig. 3).

Variation was extensive for the Pma1-amplified fragment with high values of haplotype diversity (the probability of encountering different haplotypes when two individuals are sampled from a popula-

tion) and extensive nucleotide diversity within populations (Tables 1 and 2). Nucleotide diversities (corrected for within-population diversity below the diagonal) are shown in Tables 3 and 4 for the two fragments. A number of (corrected) pairwise nucleotide divergence values are negative, indicating that within-population diversity is greater than between-population differences, and thus there is little separation of populations on the basis of sequence

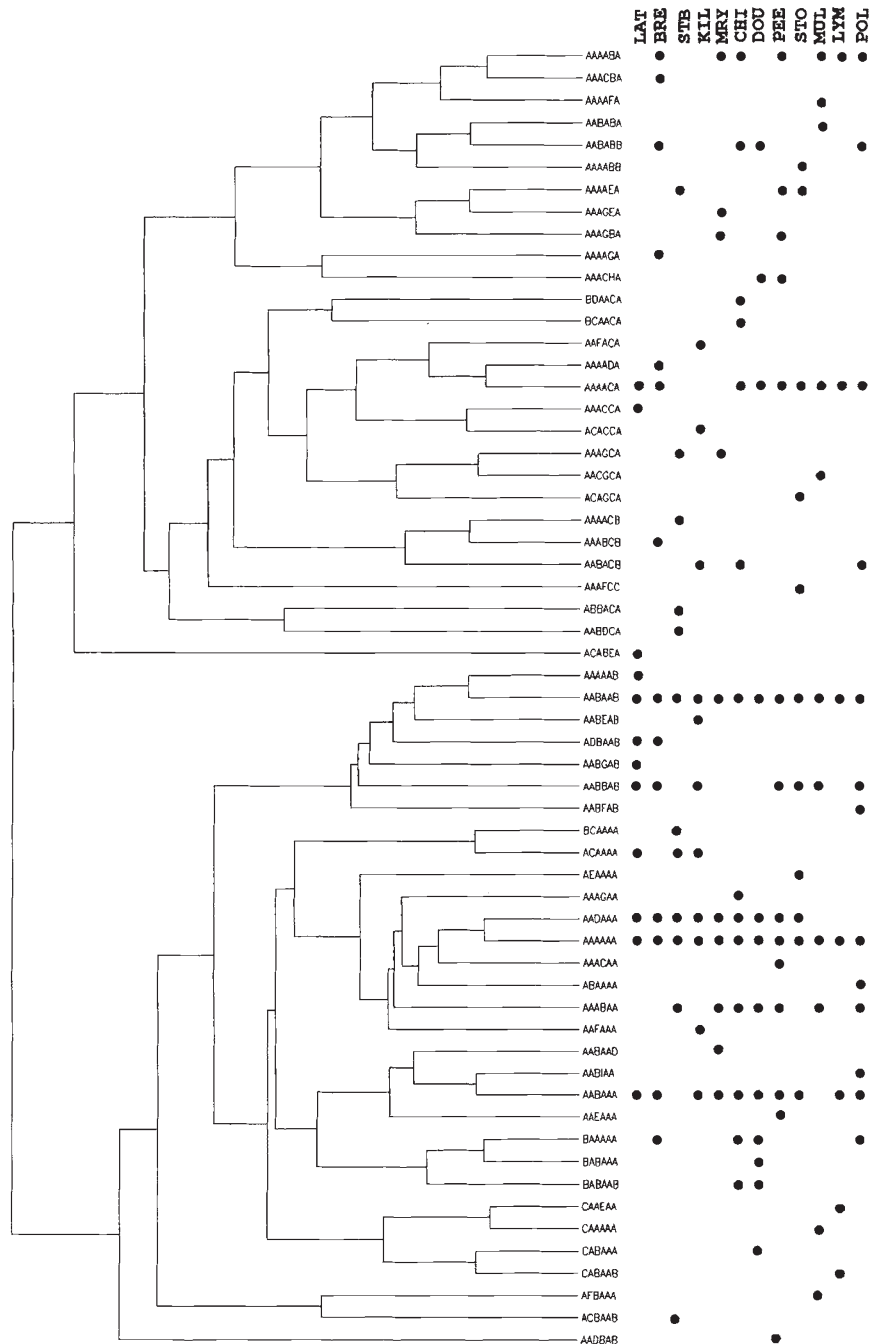


Fig. 3 UPGMA dendrogram generated from sequence divergence estimates for all *Pecten maximus* haplotypes. Presence of the haplotype in each population is indicated. For details of population abbreviations, see Fig. 1.

Table 3 Nucleotide divergence among *Pecten maximus* populations calculated by site methods from the Pma1-amplified fragment

	1	2	3	4	5	6	7	8	9	10	11	12
1 La Trinité		0.024546	0.022681	0.021438	0.023221	0.023701	0.021380	0.023395	0.024962	0.023691	0.018958	0.023848
2 Brest	-0.000125		0.023407	0.022913	0.022948	0.024422	0.021877	0.024083	0.025351	0.024082	0.020398	0.024974
3 St. Brieuc	0.000300	0.000106		0.020393	0.019886	0.022606	0.019406	0.021929	0.023319	0.022095	0.018009	0.023846
4 Kilkieran Bay	-0.000452	0.000103	-0.000128		0.020630	0.022052	0.019167	0.021410	0.023087	0.021765	0.016924	0.022641
5 Mulroy Bay	0.002167	0.000974	0.000202	0.001437		0.022517	0.018698	0.021236	0.022708	0.021320	0.017976	0.024371
6 Chicken Rock	-0.000327	-0.000526	-0.000053	-0.000115	0.001186		0.021057	0.023687	0.024866	0.023686	0.019571	0.024358
7 Douglas	0.000308	-0.000114	-0.000295	-0.000044	0.000324	-0.000292		0.020463	0.022310	0.020736	0.016554	0.022358
8 Peel	0.000006	-0.000226	-0.000090	-0.000119	0.000544	0.000021	-0.000246		0.024407	0.022886	0.018813	0.024355
9 Stonehaven	-0.000182	-0.000713	-0.000455	-0.000196	0.000261	-0.000554	-0.000334	-0.000374		0.024324	0.020577	0.025588
10 Mull	0.000049	-0.000480	-0.000177	-0.000016	0.000375	-0.000233	-0.000226	-0.000393	-0.000710		0.019065	0.024385
11 Lyme Bay	-0.000169	0.000351	0.000252	-0.000342	0.001546	0.000168	0.000107	0.000049	0.000058	0.000047		0.020058
12 Polperro	-0.000480	-0.000275	0.000887	0.000173	0.002740	-0.000247	0.000709	0.000389	-0.000133	0.000165	0.000165	

Uncorrected above diagonal (\hat{d}_{XY}) and below diagonal corrected for within-population diversity [$\hat{d}_{XY} - (\hat{d}_X + \hat{d}_Y)/2$].

divergence. However, figures generated from Pma1 data in pairwise comparisons involving Mulroy Bay are consistently positive, indicating that between-population differences are greater than within-population diversity. A UPGMA dendrogram was constructed using the Pma1-derived nucleotide divergence data, correcting negative (artefactual) values to zero but, as a consequence of the multiple zero values in the matrix, this produced a number of equally likely trees. The consensus tree (Fig. 4) indicates that only the Mulroy Bay sample is separable, but that this separation is clearcut. Trees constructed from Pma2-derived values do not add further resolution (figure not shown). From the Pma1-derived data, an estimate of $F_{ST}(\theta)$ of 0.005 was calculated.

Discussion

The presence of considerable length variation in *P. maximus* mtDNA causes substantial difficulties for the interpretation of RFLP patterns from whole mtDNA (Rigaa *et al.*, 1993; Wilding, 1996). The application of PCR-RFLP circumvents these difficulties and, in addition, potentially permits the analysis of DNA extracted from minute quantities of tissue, perhaps even larvae. Here, primers are presented that permit reliable amplification of a highly polymorphic section of mtDNA. MtDNA of *P. maximus* studied by this method proved to be considerably polymorphic, with variation within populations often as great as or greater than that between sampling sites and numerous private haplotypes (haplotypes confined to single populations). The high values of haplotype diversity reflect this variation and, although these figures may not be strictly comparable with values based on data from whole mtDNA digests, the diversity levels in this study are of a similar level and distribution to other marine species. The observed pattern of two common haplotypes dominating the distribution and multiple, rarer or private haplotypes has been recognized in both Pacific oysters (Reeb & Avise, 1990; Boom *et al.*, 1994) and pollock (Shields & Gust, 1995) and been attributed to a variety of possible causal mechanisms (Shields & Gust, 1995). No diagnostic haplotypes or high-frequency haplotypes confined to any particular location were seen, and χ^2 analysis failed to reveal any significant structure, nor was there any evidence of clustering of haplotypes with respect to area, suggestive of little phylogeographical structure. When allied to the low θ (F_{ST}) estimate, the mtDNA data appear to be consistent with the conclusion from allozyme data (Beaumont

Table 4 Nucleotide divergence among *Pecten maximus* populations calculated by site methods from the Pma2-amplified fragment

	1	2	3	4	5	6
1 Chicken Rock		0.009509	0.011609	0.010847	0.009500	0.010348
2 Polperro	0.000081		0.009907	0.009137	0.008002	0.008592
3 Peel	-0.000575	-0.000820		0.011543	0.010010	0.010833
4 Kilkieran Bay	-0.000186	-0.000438	-0.000789		0.009717	0.010177
5 St. Brieuc	0.000047	0.000005	-0.000743	0.000115		0.008885
6 Mull	-0.000053	-0.000352	-0.000867	-0.000372	-0.000085	

Uncorrected above diagonal (\hat{d}_{XY}) and below diagonal corrected for within-population diversity [$\hat{d}_{XY} - (\hat{d}_X + \hat{d}_Y)/2$].

et al., 1993) that *P. maximus* conform to panmixia. Indeed, the low F_{ST} estimate is typical of species with no observed genetic heterogeneity and far lower than figures at which restrictions to gene flow are detected, for example an F_{ST} value of 0.027 calculated from allozyme data in the limpet *Patella vulgata* was taken as evidence of genetic homogeneity, whereas θ of 0.427 from mtDNA was indicative of substantial population differentiation (Hurst & Skibinski, 1995). Thus, at the haplotype level, there is little evidence of genetic heterogeneity among *P. maximus* populations. However, when the data are analysed at the nucleotide level, then for the Mulroy Bay sample the corrected nucleotide divergence was consistently positive in all pairwise comparisons (Table 3). Such a finding is indicative of genetic divergence (Ovenden, 1990). The suggested differentiation of the Mulroy Bay population could be explained by reproductive isolation of

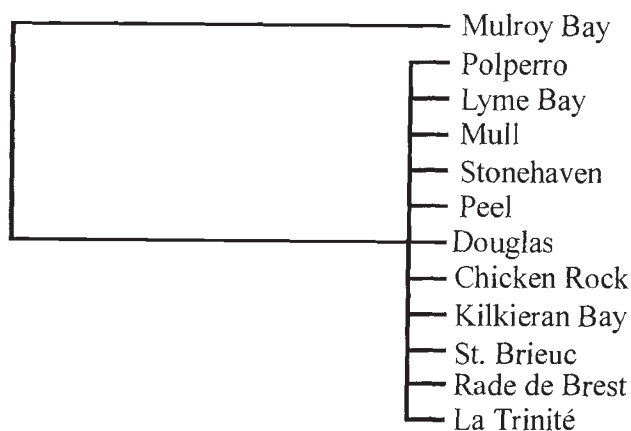


Fig. 4 Consensus dendrogram for *Pecten maximus* populations calculated from all equally likely UPGMA dendrograms using clustered nucleotide divergence values calculated from PCR-RFLP data on the Pma1-amplified fragment. The average sequence divergence of the Mulroy Bay sample from all other samples is 0.107 per cent.

the individuals within this population and genetic drift acting on haplotype frequencies. MtDNA has increased susceptibility to genetic drift as a result of the lowered effective population size (half that of nuclear genes for such a hermaphroditic species), which could explain why mtDNA reveals population structure not uncovered by allozyme (nuclear) loci. Mulroy Bay is a semi-enclosed sea lough with two entrance sills acting to reduce water movement in and out of the basin. This is probably an effective containment mechanism for larvae, which will promote genetic differentiation. The shell colour of *P. maximus* juveniles has previously been suggested as indicating that Mulroy Bay scallops may indeed be variant (Minchin, 1991). Although the mtDNA divergence values add support to this hypothesis, the Mulroy Bay sample in this study, which came from a single spatfall ongrown elsewhere, may not be representative of the population as a whole, whether because of temporal effects (variation in genetic composition of recruits between settlements) or postcollection mortality effects. Temporal variation in the composition of recruits has been documented in other marine species (Watts *et al.*, 1990; Hedgecock, 1994). Convincing evidence that Mulroy Bay scallops are self-recruiting will only be possible through the analysis of a sample of native adults. The possible self-recruiting nature of the Mulroy Bay population has implications for the management of the population because, although Mulroy Bay *P. maximus* are no longer heavily fished (Minchin, pers. comm., 1983), the population has been proposed as a suitable site for the collection of scallop spat for reseeded efforts (Slater, pers. comm., 1995).

The demonstration of substantial sequence divergence between populations of *P. maximus* represents the first genetic evidence that some populations may be, to some extent, reproductively isolated. Mulroy Bay *P. maximus* have not previously been analysed at the molecular level, but our own studies using allozyme electrophoresis (C. S. Wilding *et al.*, in

preparation) provide little evidence for the distinctness of this population on the basis of allozyme frequencies. Discrepancies between results based on protein electrophoresis and DNA-based markers, either in the ability to separate populations or in the magnitude of differentiation, have previously been recognized in a number of species, for example *Drosophila melanogaster* (Begun & Aquadro, 1993), mussels *Mytilus galloprovincialis* (Karakousis & Skibinski, 1992) and the American oyster *Crassostrea virginica*. In this species, Atlantic and Gulf of Mexico populations appear genetically homogeneous for allozyme markers but exhibit a pronounced genetic break for mtDNA and anonymous single-copy nuclear DNA markers (Karl & Avise, 1992). This was attributed to balancing selection maintaining similar allele frequencies in populations experiencing little gene flow. Such a mechanism may be an alternative explanation for why *P. maximus* appear panmictic on the basis of allozyme data, despite possible reproductive isolation or restrictions to gene flow.

The lack of significant differentiation of the St Brieuc scallops at the mtDNA level is surprising, if this population is indeed reproductively isolated as has previously been suggested (Paulet *et al.*, 1988; Cochard & Devauchelle, 1993; Mackie & Ansell, 1993). If population differentiation is present, then mtDNA is expected to reveal more differences than allozymes owing to high variability and lowered effective population size, and failure to establish that differences are present questions whether the population is indeed genetically different. If the reproductive data are truly an indication of genetic isolation, then the suitability of the marker is brought into question and analysis with alternative systems, e.g. microsatellites, may prove fruitful. It is noteworthy that variation in the frequency of haplotype AAAAAA for Pma1 is similar to that of PT-A allele frequencies in *A. opercularis*, in which substantial population subdivision, including separation of the St Brieuc population, is observed (Beaumont, 1982). Thus, the haplotype frequency at St Brieuc appears to be more similar to the Irish Sea populations than to the neighbouring Rade de Brest population, which is itself similar to the sample from La Trinité (for queen scallops, a population from nearby Belle Isle was studied). All Irish Sea samples are similar (although Chicken Rock appears to have a lower frequency of this haplotype). There are, however, no great differences between Irish Sea populations and those from the west coasts of Ireland and Scotland. This similarity in pattern is by no means convincing, but it may be indicative that

underlying differentiation does exist and that further study of St Brieuc *P. maximus* may prove productive.

Acknowledgements

We are grateful to Dr A. Brand (Port Erin Marine Laboratory), Dr G. Burnell (University College, Cork), Dr P. Dare (MAFF, Lowestoft), Dr P. G. Fleury (IFREMER, Brest) and T. Howell (SOAFD, Aberdeen) for provision of scallops. C.S.W. was supported by NERC grant GT4/92/275 A.

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