J. Mar. Biol. Ass. U.K. (1999), **79**, 687–695
Printed in the United Kingdom

Mitochondrial DNA variation among open-sea and enclosed populations of the scallop *Pecten maximus* in western Britain

D.A. Heipel*‡, J.D.D. Bishop† and A.R. Brand*

*Port Erin Marine Laboratory (School of Biological Sciences, University of Liverpool), Port Erin, Isle of Man, IM9 6JA. †Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, PL1 2PB. ‡Present address: CEFAS Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB

The great scallop *Pecten maximus* (Bivalvia: Pectinidae) supports a substantial European fishery with a recent history of declining catches caused by over-exploitation. The sustainable exploitation of shellfish populations requires knowledge of the extent to which commercial grounds are reproductively self-sustaining or supplied with larvae originating from further afield. The degree of genetic differentiation between locations can provide important indirect evidence, reflecting the pattern and scale of effective larval dispersal. *Pecten maximus* were sampled from five locations around the Isle of Man, from Mulroy Bay (Ireland) and from Plymouth. Restriction-site variation was investigated in two PCR-amplified mitochondrial DNA fragments of 2 and 3 kb, respectively. Haplotype and nucleotide diversity within populations, and nucleotide divergence between populations, were calculated. Mean nucleotide sequence divergence was corrected for within-population polymorphisms and visualized by UPGMA cluster diagrams. Molecular analysis of variance (AMOVA) was carried out. Results showed low levels of population differentiation. Slight but significant differentiation between Isle of Man populations was revealed, with East Douglas appearing distinct from the remaining Manx locations. The analyses also indicated the distinction of Mulroy Bay from the other populations, and the lowest genetic variability was recorded from this enclosed habitat. This probably reflects the relative isolation of Mulroy Bay, whereas dynamic hydrographic conditions in the Irish Sea and the Channel may generally ensure extensive mixing of the planktonic larvae.

INTRODUCTION

The great scallop *Pecten maximus* (Bivalvia: Pectinidae) is a very valuable shellfish, supporting a fishery in European waters with a first-sale value of more than 40 million ecu in 1997. However, the total European capture fishery declined from 48,571 tonnes in 1973 to 14,931 tonnes in 1990, and the overall trend is still downwards, although there has been a short-term increase in the landings in the eastern English Channel (23,000 tonnes in 1993; Brand & Prudden, 1997). Landings of *P. maximus* on the Isle of Man rose steadily until 1985 (2100 tonnes live weight), followed by a decline to less than 650 tonnes in 1993. Constantly increasing effort is now necessary to maintain catches, at the same time further reducing scallop abundance and the profitability of fishing. Depletion of the older year-classes endangers the breeding stock, since the replenishment of overfished beds becomes dependent on the strength of the recruiting year-class (Brand et al., 1991; Allison, 1993). Failure of recruitment in one year could thus result in the collapse of the fishery. To prevent this happening, attempts have been made to conserve the exploited beds almost from the beginning of the Manx scallop fishery. The introduction of the closed season (1 June–31 October), minimum legal landing size (110 mm shell length), and restrictions on the size of dredges and fishing (Brand et al., 1991) are measures which have ensured continued exploitation up to the present, although they have failed to prevent a steady decline in the stocks.

Characterization of population structure is an essential element in the management of fisheries. Localized overfishing of a panmictic species might not seriously affect future fishing, since replenishment by recruitment from neighbouring areas can take place. However, in the case of self-sustaining populations, overfishing can lead to the extinction of the breeding stock and collapse of the fishery in that area. To ensure continued exploitation, the management of a scallop fishery therefore requires knowledge of the extent to which the stocks on particular fished areas are reproductively self-sustaining or reliant on larvae originating from elsewhere. This will depend on the scale and pattern of larval dispersal, which, however, is very difficult to monitor directly. Indirect techniques are therefore generally used to estimate widespread larval movement and the relationship between potential and realized dispersal. The degree of genetic divergence between natural stocks on different fishing grounds provides valuable evidence, reflecting the balance between diversifying processes such as genetic drift, mutation, and local selection vs the homogenizing effect of gene flow (Slatkin, 1985). The long period of time for which many marine larvae can drift in the water column apparently gives them an extensive dispersal capability,

especially when originating from open-water populations. The gene flow arising from wide-scale larval dispersal would be expected to suppress population differentiation. Indeed, population genetic studies have shown that extensive panmictic units exist in many aquatic species (Palumbi, 1992). However, there are also exceptions, where populations show genetic differentiation on a small geographical scale (Palumbi, 1995). Hydrographic features such as fronts, gyres and tidal circulation systems, and other, more subtle, factors like differences in light, food availability and water temperature, can potentially restrict distribution and lead to differences in size, age, and genetic structure between different fishing grounds.

With the aim of providing a sound genetic understanding of scallop stock differentiation for scallop fishery management, previous workers have investigated allozyme variation and, more recently, have begun to employ DNA-based molecular techniques. The few allozyme studies carried out on population structure of *P. maximus* have so far failed to reveal differentiation between various populations from the British Isles and France (Beaumont et al., 1993). This would imply that the populations of *P. maximus* within the Irish Sea represent a panmictic unit. In contrast, parallel studies of the pectinids *Aequipecten opercularis, Argopecten irradians* and *Chlamys islandica* have revealed differentiation on relatively small geographical scales (Beaumont, 1982; Lewis & Thorpe, 1994; Fevolden, 1992; Blake & Graves, 1995). The failure to detect small-scale differentiation between *P. maximus* populations could be a consequence of the limitation of the allozyme methodology in which only a few loci were available for analysis. As a single locus of those studied revealed population structure in *A. opercularis* (Beaumont, 1982), it can not be ruled out that the inclusion of data from other loci, or the use of high-resolution DNA-based methods, would do the same for *P. maximus*. A recent study using RAPD (predominantly nuclear DNA) markers has indicated a low but significant degree of differentiation between open-sea populations, and more pronounced differences between *P. maximus* from Mulroy Bay and from the Irish Sea (Heipel et al., 1998). This confirmed earlier findings by Wilding et al. (1997), who also used PCR–RFLP of mitochondrial DNA to investigate population structure of *P. maximus* from the UK, Ireland and Brittany. No evidence of differentiation within and between the UK and Brittany populations was found by Wilding et al. (1997), but the population from Mulroy Bay was distinct from the others.

In this study, we further investigate mitochondrial DNA variation in *P. maximus* from a number of different fishing grounds. The mitochondrial DNA of *P. maximus* ranges in size from 20.0 to 25.8 kb due to variable numbers of a repeated element, a (presumably) non-coding stretch of DNA, which occurs in the control region (Gjetvaj et al., 1992; Rigaa et al., 1993, 1995). The existence of an unknown number of repeated elements within the mtDNA of every individual can considerably complicate the interpretation of restriction patterns derived from the whole mitochondrial molecule. Therefore, after initial confirmation of variability by restriction analysis of whole mtDNA (not detailed here), only non-size-variable parts of the mitochondrial genome were analysed for sequence differences. This was accomplished by PCR-amplification of mtDNA fragments not containing repeated elements, followed by digestion of these fragments with restriction enzymes (PCR–RFLP).

MATERIALS AND METHODS

Three-year old *Pecten maximus* (spawned and settled in 1992) were obtained using the RV 'Roagan' during October 1995 from the Chickens Rock, Bradda Inshore, Targets, Ramsey and East Douglas fishing grounds (Figure 1); these are more or less distinct areas around the Isle of Man fished regularly by commercial vessels. *Pecten maximus* from Bigbury Bay, Plymouth, south-west England, were dredged by the RV 'Squilla' in summer 1996; the animals sampled were 3 and 4-y old (the 1993 and 1992 year-class). Scallops originating as spat from Mulroy Bay (Co. Donegal, Eire) were obtained as 2-y old (1994 year-class) in 1996 after ongrowing at a scallop farm in Strangford Lough (Northern Ireland).

Amplification of fragments

Total DNA was extracted according to Heipel et al. (1998). Based on the sequences of parts of the 12S and 16S rRNA genes of the mitochondrial genome of *P. maximus*, placed by D.Y. Sellos in the EMBL/GenBank/DDBJ databases in 1995 (acc. no. X82501 and X67246), a primer pair was designed for amplification of these genes. The PCR product is referred to below as DPM. Primers were checked for their suitability with the program, PRIMER (The Whitehead Institute for Biomedical Research, 1991), and had the following sequences:

primer DPM-F 5′ AGA TCG ACC CGA GGA AGT AGG G

primer DPM-R 5′ CAC AGT ACA GGC CAT CAC CTG G

The DPM primer pair amplified a fragment of ~3 kb under the following PCR-conditions: total genomic DNA 1 μl (the optimal concentration of template DNA was determined in preliminary trials, and sample dilutions adjusted after comparison on agarose gels), 0.25 μmol of each primer (synthesized by Perkin Elmer, Warrington, UK), 0.25 mM of each deoxynucleotide triphosphate (dATP, dCTP, dTTP, dGTP; Sigma, Poole, UK), 5 μl 10×PCR-buffer (100 mM Tris-HCl, pH 9.0; 500 mM KCl; 1.0% Triton X-100; Promega Ltd, Southampton, UK), 3.75 mM $MgCl_2$ (Promega), and water (molecular biology grade, BDH, Poole, UK) to make up a total volume of 50 μl. The reaction mixture was overlaid with mineral oil (Sigma). After a hot start of 3 min at 94°C, 1.5 units Taq-polymerase (Promega) was added and the samples were subjected to 25 temperature cycles of 30 s 94°C, 1 min 60°C, 3 min 72°C in a Perkin Elmer DNA thermal cycler (model TCl).

The primer pair referred to below as PMA amplified a 2 kb fragment. The primer sequences were obtained from Wilding (1996; PMA=Pma1) and amplified a HindIII/EcoRI fragment of the mitochondrial genome. According to the restriction mapping carried out by Rigaa et al.

https://doi.org/10.1017/S0025315498000848 Published online by Cambridge University Press

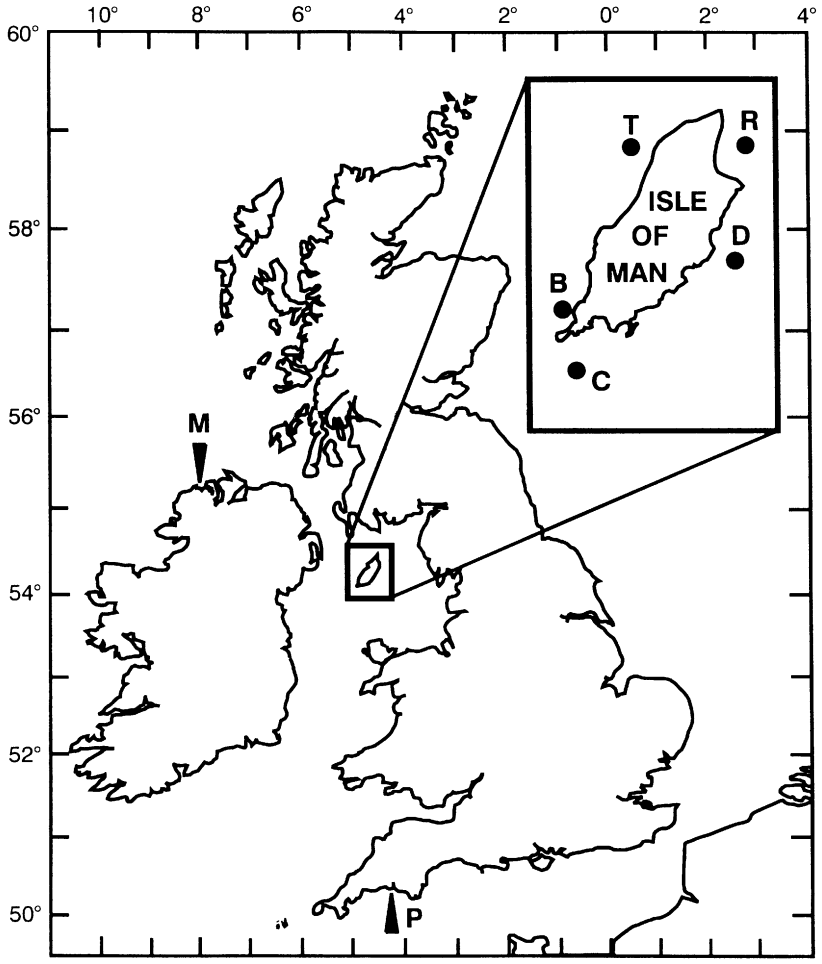

Figure 1. Sampling locations for *Pecten maximus* in the British Isles. B, Bradda Inshore; C, Chickens Rock; D, East Douglas; R, Ramsey; T, Targets; M, Mulroy Bay; P, Plymouth.

(1993), the 2 kb HindIII/EcoRI fragment does not contain repeated elements; there is some indication of sequence similarity to the cytochrome b gene (Wilding, 1996). The sequences were as follows:

PMA-F 5′TTT TAA GGA GGT AAT CGC TAT TCG

PMA-R 5′CAG CAA TCT GTA TGG GTA GAA CC

Best results with the PMA primer pair (synthesized by Cruachem, Glasgow, UK) were obtained under the same amplification conditions as for the DPM pair, except that annealing during the 25 cycles was carried out at 55°C and extension time was reduced to 2 min.

Restriction digests

In a preliminary experiment, both the 3 kb (DPM) and the 2 kb (PMA) PCR fragments were digested with a range of restriction enzymes (AluI, RsaI, MvaI, DraI, PvuII, AvaI, BamHI, HindIII, HpaI, EcoRV, ClaI, EcoRI, CfoI, HaeIII, HinfI, TaqI, Tru9I) and combinations of these. The following restriction enzymes produced polymorphic bands in 6–12 individuals and were chosen for population analysis (the other enzymes produced monomorphic bands or bands of low polymorphism): HaeIII, MspI, RsaI, TaqI, and the combination DraI/PvuI. The amplified DPM and PMA fragments of 24 individuals from each of the locations were subjected to restriction analysis. The digest was carried out in 0.5 ml microfuge tubes, containing 4–6 μl PCR-product, 3 units restriction enzyme, 2 μl 10 × buffer and water to make up a total volume of 20 μl. The samples were incubated for 3 h at the appropriate temperature in a Perkin Elmer TCl thermal cycler. The restriction digest products were electrophoresed through 1.4% agarose gels (MP-agarose, Boehringer-Mannheim, Lewes, UK), containing ethidium bromide (BDH), in 1x tris-borate-EDTA-buffer (pH 8.3, Sigma), for 12–14 h at 60 V. After de-staining the gel for several hours in distilled water, it was photographed with a polaroid camera (DS34, Polaroid, Herts, UK) and monochrome negative film type 665 (Polaroid).

Data analysis

Restriction sites were scored as present or absent, and haplotypes for each restriction enzyme were designated A, B, C, etc., whereby A was the commonest. A composite haplotype then described the haplotype composition of each individual for all enzymes employed. The two fragments (DPM and PMA) were analysed separately as well as in combination.

https://doi.org/10.1017/S0025315498000848 Published online by Cambridge University Press

Table 1. *Numbers of each composite haplotype observed in DPM; order of restriction enzymes: MspI, RsaI, DraI/PvuII, HaeIII, TaqI.*

	B	C	D	R	T	M	P
AAAAA	8	8	14	5	11	17	10
AABBA	9	7	3	9	6	3	8
ADBBC	1						
AABBB	2	2		2	1		
CAAAA	1						
AADAA	1		2		1		1
ABBBA	1	1					
AAABA		1			1		2
AABBD		1					
AAACA		1					
AACAA		1	1	1			
ACAAA		1					1
DAAAA			1				
AEAAA			1				
AAAAB			1	1			1
AAAAD			1				
BAABA				1			
ABAAA				1			
EAAAA				1			1
CABBA				1			
BABBA					1		
ADBBB						1	
AABCB						1	
BAAAA						1	
AABAA						1	

B, Bradda Inshore; C, Chickens; D, East Douglas; R, Ramsey; T, Targets; M, Mulroy Bay; P, Plymouth.

Table 2. *Numbers of each composite haplotype observed in PMA; order of restriction enzymes: TaqI, MspI, RsaI, DraI/PvuII, HaeIII.*

	B	C	D	R	T	M	P
AAAAA	10	10	14	9	10	14	8
BBAAB	5	5		7	5	1	3
BBBBB	1	3	1	1			
ABAAC	2						
ADAAB	1	1		1	1	2	2
ACAAB	1			1	1		
BBABB	1	1					
ABAAB	1			1			1
AFAAB	1						
BAAAA	1		1			1	
AABAA		2	1				
BBBAB		1		1	1		
BBBCB		1					
BBIBB			1				
AAAAB			2		1		2
AAIAA			1			1	
AAGAA			1	1		1	
BAAAB			1				1
ABAAA			1		1	2	3
BACAA				1			
AAICA				1			
AAADA					1		
BBAAC					1		
ACAAA						1	
DBAAB						1	
ABBAA							1
BBAAA							2
ABBAB							1

B, Bradda Inshore; C, Chickens; D, East Douglas; R, Ramsey; T, Targets; M, Mulroy Bay; P, Plymouth.

Table 3. *Summary statistics for DPM.*

	B	C	D	R	T	M	P
No. individuals	23	23	24	22	21	24	24
No. haplotypes	7	9	8	9	6	6	7
Haplotype diversity	0.74	0.80	0.66	0.80	0.67	0.50	0.73
% mean nucleotide sequence diversity	0.83	0.84	0.54	0.89	0.67	0.55	0.67

Table 4. *Summary statistics for PMA.*

	B	C	D	R	T	M	P
No. individuals	24	24	24	24	22	24	24
No. haplotypes	10	8	10	10	9	9	10
Haplotype diversity	0.80	0.79	0.67	0.69	0.76	0.66	0.87
% mean nucleotide sequence diversity	2.16	2.62	1.41	2.41	1.97	1.33	1.74

Only individuals for which complete banding information was available were included in the analysis. An overall weighted estimate of divergence ('*d*') between each pair of composite haplotypes was generated from a binary character state matrix of restriction site data according to Nei & Tajima (1981) and Nei & Miller (1990, eqn 4) (D program in the REAP package (McElroy et al., 1992)). Weighting followed Nei & Tajima (1983) and was based on the proportion of sites generated by each class of enzyme. Based on the dissimilarity matrix of *d* values, and a frequency distribution matrix (number of each haplotype in each population), haplotype and nucleotide diversity within populations, and nucleotide divergence between populations, were calculated following Nei & Tajima (1981) and Nei (1987) (DA program in REAP). Total nucleotide sequence diversity between two populations was corrected for within-population polymorphisms by subtracting the average of within-sample diversities (Nei, 1987). Cluster diagrams (UPGMA) were constructed for nucleotide divergence with negative values set to zero (NTSYS-pc; Rohlf, 1993). This resulted in a number of equally probable trees (ties), which were combined in a majority-rule consensus tree (NTSYS-pc). Monte Carlo simulation (Roff & Bentzen, 1989) was carried out to assess the significance of haplotype frequency variation between all locations and between Manx locations only (MONTE in REAP). A mean χ^2 value was determined by randomizing the matrix of haplotype frequency data ten thousand times, and the probability of encountering a χ^2 value as large as that calculated for the original matrix was thereby determined. Based on a matrix of squared Euclidian distances (Nei & Tajima, 1981) among haplotypes (NTSYS-pc), molecular analysis of variance (AMOVA; Excoffier et al.,

https://doi.org/10.1017/S0025315498000848 Published online by Cambridge University Press

Table 5. *Comparative statistics of the separate analyses of both fragments (DPM and PMA) and the combined analyses (DPM+PMA).*

	DPM	PMA	DPM+PMA
No. individuals	161	166	161
No. populations	7	7	7
No. haplotypes	25	28	63
Main haplotypes	AAAAA	AAAAA	AAAAAAAAAA
	73, 43.98%,	62, 37.96%	52, 34.16%
	AABBA	BBAAB	AABBABBAAB
	45, 27.11%	26, 15.66%	20, 12.42%
No. of unique haplotypes	15, 9.30%	11, 6.63%	43, 26.70%
χ^2 all populations	144.66	172.34	374.46
Probability value	0.462	0.181	0.472
χ^2 Manx populations only	76.76	85.91	170.65
Probability value	0.701	0.608	0.934

Table 6. *Summary statistics for DPM+PMA.*

	B	C	D	R	T	M	P
No. individuals	23	23	24	22	21	24	24
No. haplotypes	14	14	16	15	12	11	16
Haplotype diversity	0.89	0.90	0.89	0.92	0.85	0.71	0.93
% mean nucleotide sequence diversity	1.27	1.41	0.85	1.36	1.03	0.80	1.03

B, Bradda Inshore; C, Chickens; D, East Douglas; R, Ramsey; T, Targets; M, Mulroy Bay; P, Plymouth.

1992) was used to partition variance between and within populations and regions (three regions: the Manx populations, Mulroy and Plymouth), and to provide pairwise estimates of population differentiation in the form of phi_{ST} values. Phi_{ST} is an F_{ST}-analog, developed for haplotype data (Excoffier et al., 1992). One thousand permutations of the original data matrix were performed to test for significance of the variance components and phi_{ST} values, avoiding the assumption of normality. Data were also subjected to a Bartlett's test for heteroscedasticity (Stewart & Excoffier, 1996) (AMOVA package) to test for unequal genetic variability between populations.

Assessment was made of possible correlation of mtDNA nucleotide divergence between populations and Euclidean distances between the same samples based on fragment frequencies in randomly amplified polymorphic DNA (RAPD) fingerprints (Heipel et al., 1998). A Mantel test was used, with 2000 permutations of the RAPD distance matrix to assess the significance of the test statistic.

RESULTS

Amplified fragments did not differ in size between individuals. All PCRs gave a high yield of product. Test-amplifications carried out with total DNA and purified mitochondrial DNA gave the same results, excluding the possibility of amplification of nuclear copies (Zhang & Hewitt, 1996). The positions of restriction sites within the fragments could be deduced by comparing the different banding patterns obtained. The combined length of the two fragments was ~5.0 kb, thus 25% of the total mtDNA was screened, assuming an average mitochondrial genome size of 20.0 kb (including two repeated elements). The recognition sequences of the six enzymes accounted for approximately 1.12% of nucleotides within the amplified parts of the mitochondrial genome.

Data for mitochondrial fragments DPM and PMA were analysed both separately and in combination. Both fragments showed similar frequencies of the commonest haplotypes, although PMA generated a few more haplotypes (28) than DPM (25) (Tables 1 & 2). Whereas the haplotype diversity was of similar magnitude in both fragments, the values for nucleotide sequence diversity were up to three times higher in PMA than in DPM (Tables 3 & 4). χ^2 values for frequencies of haplotypes were non-significant for both fragments (Table 5). In general, the results of both the separate and the combined analyses agreed with each other. In the following, the

Table 7. *Nucleotide divergence among populations for DPM+PMA.*

	Bradda	Chickens	East Douglas	Ramsey	Targets	Mulroy
Bradda	0.000000					
Chickens	−0.000176	0.000000				
East Douglas	0.002107	0.001898	0.000000			
Ramsey	−0.000438	−0.000358	0.001817	0.000000		
Targets	0.000167	0.000419	0.000394	0.000078	0.000000	
Mulroy	0.001578	0.001691	−0.000087	0.001388	0.000059	0.000000
Plymouth	−0.000098	0.000063	0.000869	−0.000138	−0.000298	0.000487

https://doi.org/10.1017/S0025315498000848 Published online by Cambridge University Press

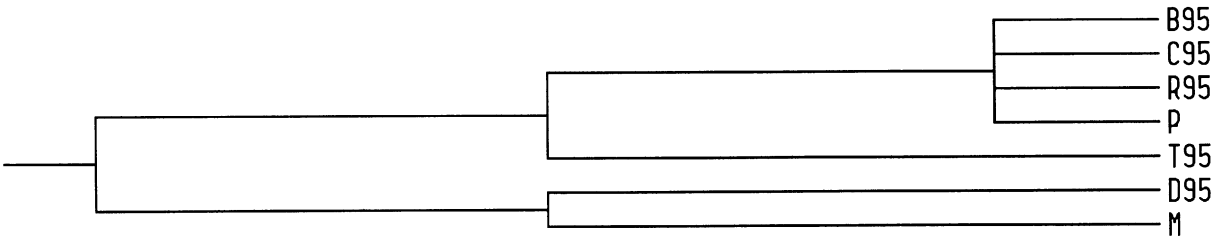

Figure 2. Consensus tree of UPGMA dendrograms based on nucleotide divergence, with negative values set to zero, for DPM+PMA.

Table 8. *Analysis of molecular variance for DPM+PMA.*

Source of variation	df	SSD	MSD	Variance component	% of total variance	*P*-value
All regions, nested analysis						
Among regions	2	6.13	3.07	−0.01	−0.71	0.56
Among locations within regions (Isle of Man region only)	4	13.71	3.43	0.08	4.59	0.03
Among individuals within locations	154	253.82	1.65	1.65	96.12	0.01

df=degrees of freedom; SSD=sum of squared deviations; MSD=mean of squared deviations; *P*-value=probability of more extreme random variance component, estimated by permutational analysis of data matrix.

Table 9. *Above diagonal: pairwise Phi_{ST} values for DPM+PMA; below diagonal: pairwise Bartlett's test values for DPM+PMA; no significant values after Bonferroni correction.*

	B	C	D	R	T	M	P
B	—	−0.0124	0.1643	−0.0336	−0.0049	0.1308**	−0.0124
C	0.7011	—	0.1133*	−0.0258	−0.0076	0.1035*	−0.0083
D	6.2440*	4.9651*	—	−0.1422**	0.0600	−0.0113	0.0886*
R	0.3035	0.4390	5.9562*	—	−0.0116	0.1129**	−0.0118
T	1.0280	1.0325	2.6690	1.0855	—	0.0289	−0.0375
M	5.3125*	4.7270*	0.7099	5.1928*	1.9678	—	0.0586*
P	0.8826	1.0394	3.5138*	1.1122	0.1864	2.7540	—

B, Bradda Inshore; C, Chickens; D, East Douglas; R, Ramsey; T, Targets; M, Mulroy Bay; P, Plymouth. *, $P<0.05$, **, $P<0.01$ before Bonferroni correction.

combined fragments DPM and PMA are referred to as DPM+PMA. The main composite haplotypes were AAAAAAAAAA, which occurred in 34.16% of all individuals, and AABBABBAAB in 12.42%, while unique haplotypes (occurring in a single individual) had a combined frequency of 26.70% (Table 5). The sample from Plymouth had the highest number of haplotypes of DPM+PMA (16), while Mulroy had the fewest haplotypes (11) (Table 6). Amongst the Manx samples, East Douglas had the highest number of haplotypes (16), Targets the lowest (12). Diagnostic haplotypes were not identified in any sample. The overall number of haplotypes was 63.

Haplotype diversity for DPM+PMA was lower in Mulroy (0.71) than in any other sample, while the value for Plymouth was highest (0.93) (Table 6). Amongst the Manx samples, Targets showed the lowest value (0.85), and Ramsey the highest (0.92). The lowest nucleotide diversity was again exhibited by Mulroy (0.80). East Douglas also showed low nucleotide diversity (0.85). Chickens had the highest value with 1.41. The χ^2 analysis for frequencies of haplotypes resulted in a non-significant value.

Pairwise analysis of nucleotide divergence, including negative values, gave lowest values for Ramsey and Bradda, Ramsey and Chickens, and Targets and Plymouth (Table 7). In the consensus tree based on nucleotide divergence with the negative values adjusted to zero, Bradda, Chickens, Ramsey and Plymouth formed a cluster, Targets clustered separately, and East Douglas plus Mulroy formed a loose group (Figure 2).

The variance component in AMOVA was negative (non-significant) for the analysis among regions, low, but significant among the Manx populations, and significant among individuals within locations (Table 8). A Bartlett's test between all populations revealed significant heteroscedasticity ($P=0.0130$). However, the pairwise Bartlett's tests showed significant values only before Bonferroni correction (Table 9). Many of the pairwise phi $_{ST}$ values were negative, and none remained significant after Bonferroni correction (Table 9).

Mantel tests indicated that no significant correlation existed between DPM+PMA nucleotide divergence between populations and pairwise distances based on RAPD fragment frequencies (Heipel et al., 1998), either

https://doi.org/10.1017/S0025315498000848 Published online by Cambridge University Press

for all seven localities (P=0.210) or for the five Manx localities alone (P=0.154).

DISCUSSION

The application of PCR-based technology proved to be very successful in providing high yields of amplified mtDNA and enabling all digests to be carried out on the same PCR product, thus overcoming problems with the small amount of mtDNA obtained when extracting and digesting the whole mtDNA molecule. Restriction analysis of the DPM fragment with the enzymes TaqI, MspI, HaeIII, DraI/PvuII and RsaI resulted in a slightly higher number of individuals displaying the main haplotype and fewer haplotypes overall than the analysis of the PMA fragment with the same enzymes. This is particularly notable because DPM is the longer fragment. Furthermore, the values for mean nucleotide sequence diversity were generally much higher for PMA than for DPM. Both trends are probably due to the DPM fragment including rRNA-genes, which are known to exhibit lower mutation rates than other mtDNA genes (Meyer, 1994). Hence, any potential population differentiation is more likely to show up in the PMA fragment; this was apparently the case for the χ^2 analysis of PMA fragment haplotype frequencies, which gave values closer to significance than for the DPM fragment. For both fragments, the two most common haplotypes were found in all populations (with the exception of PMA haplotype BBAAB in East Douglas), suggesting that these haplotypes are relatively ancient in origin (Neigel & Avise, 1993), and that there has been, and maybe still is, extensive gene flow between all locations. Within the analyses of the two fragments separately, the frequency of the most common composite haplotype (AAAAA) showed considerable variation between locations (DPM 22.7–70.8%; PMA 33.3–58.3%), in keeping with expected random sampling errors (standard deviation of frequency estimate for each sample equivalent to approximately 10%). Wilding (1996) reported similarly variable values between locations: 25–53% of specimens in the different samples possessed the most frequent haplotype of PmaI (=PMA). Values for mean nucleotide sequence diversity for PMA were relatively high in the present study, the highest being 2.62% (Chickens), while the lowest Manx sample was East Douglas with 1.41%. Wilding et al. (1997) found values of comparable magnitude for the same fragment: mean nucleotide sequence diversity for Chickens of 2.43%, and for Douglas of 1.84%. The values for the DPM fragment in the present study were lower, ranging from 0.54 to 0.89%. Nucleotide sequence diversities reported in a study of the total mitochondrial genome of two other pectinids were similar to or lower than the values for DPM reported here. Blake & Graves (1995), in a survey of haplotypes among five genetically differentiated populations of *Argopecten irradians*, calculated values of mean nucleotide sequence diversity ranging from 0.22 to 0.53%, and also reported values of 0.54 and 0.69% for two populations of *Argopecten gibbus*.

No population-specific haplotypes were found in *Pecten maximus*, but unique haplotypes were present at all locations. If populations were truly isolated, then mutations within haplotype lineages could result in 'private' haplotypes characteristic of a particular population (Slatkin, 1985). Since the unique haplotypes were, by definition, only encountered once in all samples, no statement can be made about their 'private' character without additional sampling.

In the present study, AMOVA revealed that almost all genetic variation (>95% of total variance) was distributed within populations. Accordingly, most pairwise phi$_{ST}$ values were close to zero or even negative. Furthermore, χ^2 values for heterogeneity of *P. maximus* haplotype frequencies proved non-significant, and high nucleotide sequence diversity, characteristic of extensive gene flow, suggested little population differentiation. However, there are some notable trends in the data, opposing the conclusion that *P. maximus* represents an entirely panmictic population; although small, the variance component between Manx locations was significant. The highest nucleotide diversity was exhibited by Chickens, which is of interest since Chickens is the most open-sea location of the Isle of Man sample sites (Figure 1), and thus perhaps subject to larval input from the greatest variety of sources. Wilding et al. (1997) similarly reported higher nucleotide diversity for PmaI (=PMA) from this ground than from Douglas. For both fragments, samples from Mulroy and East Douglas showed lower values of haplotype diversity, and even more clearly, of mean nucleotide sequence diversity than other samples, indicating greater homogeneity within each of these two populations. Furthermore, Mulroy and East Douglas formed a cluster distinct from the other Manx populations and from Plymouth in the UPGMA diagram. This clustering reflected the fact that, compared with the other localities, these two samples shared higher frequencies of the commonest haplotypes (AAAAA) for both fragments, and lower proportions of the second commonest haplotypes. However, East Douglas and Mulroy Bay differed markedly from each other in the unique and rare haplotypes present, suggesting that these populations are not in fact closely related.

Mulroy Bay is an enclosed body of water, with an entrance sill and a further sill separating the upper and lower lochs (Milne, 1972). The structure of such lochs results in reduced water transfer in and out, potentially causing confinement of larvae and self-recruitment. Scallop spat settlement decreased dramatically in Mulroy Bay linked to the use of tributyltin (TBT) on salmon nets in the mid 1980s, but recovered subsequently following the ban on use of this anti-foulant (Minchin et al., 1987). This event might have affected genetic variability. The Mulroy Bay population has indeed been found to be genetically different from samples around the UK in previous studies (Wilding et al., 1997; Heipel et al., 1998). *Pecten maximus* from Mulroy Bay have also been reported to differ in colour from other Irish populations (Minchin, 1991).

The results of the present mtDNA analyses may be compared with those of an earlier investigation of predominantly nuclear markers (RAPD: Randomly Amplified Polymorphic DNA) in the same samples (Heipel et al., 1998). In both cases, the great majority of genetic variation, estimated by AMOVA, was found between individuals within samples. Nevertheless, in both analyses there was slight but significant differentiation between the Manx samples. For both types of marker, East Douglas was relatively distinct from all other Manx locations. However, inter-relationships between the remaining

Manx locations were not similar in the two analyses, showing considerable concordance with geographical proximity in the RAPD data, but not with mtDNA. Significant divergence between the three geographical regions (Isle of Man, Mulroy Bay and Plymouth) was also shown by RAPD markers. In contrast, AMOVA on mtDNA showed no differentiation attributable to these three regions, since Plymouth closely resembled some Manx samples while East Douglas was most similar to Mulroy Bay. These differences in the patterns of differentiation of the two classes of molecular marker are reflected in the non-significant Mantel statistics obtained. Mulroy Bay showed the lowest level of genetic diversity of any location for mtDNA and marginally the lowest for RAPD markers, while East Douglas did not show low diversity for RAPD (unpublished analyses), unlike the present findings for mtDNA.

The reason for the East Douglas population showing less mitochondrial DNA diversity than the other Manx populations is not obvious, but could be related to long water residence times locally (Heaps & Jones, 1977) or circular current systems and gyres leading to some retention of larvae in Douglas Bay (Aldridge & Davies, 1993), thus making the East Douglas population at least partially self-sustaining. Heavy fishing of *P. maximus* has substantially reduced population size in East Douglas (Brand & Prudden, 1997), and if the grounds are partly self-sustaining, this could possibly have resulted in a transient bottleneck (which would not affect nuclear diversity as much as mitochondrial diversity). Obviously, if populations are only partly self-sustaining, and the restraining event has occurred only recently, differences between them might not be very pronounced. Mulroy Bay and East Douglas are distinct from the other populations studied, and for each amplified fragment both samples have higher proportions of the commonest haplotype and lower proportions of the second commonest, compared to the other locations. (These localities show similar trends, in comparison with other Manx sites and Polperro in south-west England, in the data of Wilding et al. (1997) for Pma1.) However, Mulroy Bay and East Douglas are not otherwise similar, having different unique or rare haplotypes in the two populations (particularly for DPM), suggesting different sources of recruitment.

In addition to the present results and those of the earlier RAPD investigation (Heipel et al., 1998), the Mulroy Bay population has been found to be genetically different from samples around the UK in a previous mtDNA study (Wilding et al., 1997). The prospect is raised that *P. maximus* stocks in enclosed habitats may generally have less genetic variability than open-water stocks, and potentially be genetically divergent from them. This possibility may be particularly significant given the importance of fjords, sea-loughs and rias for pectinid aquaculture and as centres of commercial spat collection and export (various papers in Shumway, 1991). Mulroy Bay itself is a major source of *P. maximus* spat exported for commercial ongrowing at various sites (Slater, 1995).

The financial support of the Isle of Man Department of Agriculture, Fisheries and Forestry is gratefully acknowledged. The authors would like to thank the crew of the RV 'Roagan' for collecting the Isle of Man samples and Roger Swinfen and the crew of RV 'Squilla' for the Plymouth specimens. Dr D. Roberts kindly arranged the supply of scallops from Mulroy Bay. We are grateful to Drs Craig Wilding and Geir Dahle for advice and discussions. D.A.H. received a studentship from the University of Liverpool and additional funding from the Marine Biological Association, Plymouth.

REFERENCES

Aldridge, J.N. & Davies, A.M., 1993. A high-resolution three-dimensional hydrodynamic tidal model of the eastern Irish Sea. *Journal of Physical Oceanography*, **23**, 207–224.

Allison, E.H., 1993. *The dynamics of exploited populations of scallops (*Pecten maximus *L.) and queens (*Chlamys opercularis *L.) in the North Irish Sea*. PhD thesis, University of Liverpool.

Beaumont, A.R., 1982. Geographic variation in allele frequencies at three loci in *Chlamys opercularis* from Norway to the Brittany coast. *Journal of the Marine Biological Association of the United Kingdom*, **62**, 243–261.

Beaumont, A.R., Morvan, C., Huelvan, S., Lucas, A. & Ansell, A.D., 1993. Genetics of indigenous and transplanted populations of *Pecten maximus*—no evidence for the existence of separate stocks. *Journal of Experimental Marine Biology and Ecology*, **169**, 77–88.

Blake, S.G. & Graves, J.E., 1995. Mitochondrial DNA variation in the bay scallop, *Argopecten irradians* (Lamarck, 1819), and the Atlantic calico scallop, *Argopecten gibbus*. *Journal of Shellfish Research*, **14**, 79–85.

Brand, A.R., Allison, E.H. & Murphy, E.J., 1991. North Irish Sea scallop fisheries: a review of changes. In *An international compendium of scallop biology and culture* (ed. S.E. Shumway and P.A. Sandifer), pp. 204–218. Baton Rouge: World Aquaculture Society.

Brand, A.R. & Prudden, K.L., 1997. *The Isle of Man scallop and queen fisheries: past, present and future*. Isle of Man: Port Erin Marine Laboratory, University of Liverpool.

Excoffier, L., Smouse, P.E. & Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Fevolden, S.E., 1992. Allozymic variability in the Iceland scallop *Chlamys islandica* geographic variation and lack of growth heterozygosity correlations. *Marine Ecology Progress Series*, **85**, 259–268.

Gjetvaj, B., Cook, D.I. & Zouros, E., 1992. Repeated sequences and large-scale size variation of mitochondrial DNA: a common feature among scallops (Bivalvia: Pectinidae). *Molecular Biology and Evolution*, **9**, 106–124.

Heaps, N.S. & Jones, J.E., 1977. Density currents in the Irish Sea. *Geophysical Journal of the Royal Astronomical Society*, **51**, 393–429.

Heipel, D.A., Bishop, J.D.D., Brand, A.R. & Thorpe, J.P., 1998. Population genetic structure of the great scallop *Pecten maximus* (L.) in the northern Irish Sea investigated by randomly amplified polymorphic DNA. *Marine Ecology Progress Series*, **162**, 163–171.

Lewis, R.I. & Thorpe, J.P., 1994. Temporal stability of gene frequencies within genetically heterogeneous populations of the queen scallop *Aequipecten (Chlamys) opercularis* (L.). *Marine Biology*, **121**, 117–126.

McElroy, D., Moran, P., Bermingham, E. & Kornfield, I., 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. *Journal of Heredity*, **83**, 157–158.

Meyer, A., 1994. Molecular phylogenetic studies of fish. In *Genetics and evolution of aquatic organisms* (ed. A.R. Beaumont), pp. 219–249, London: Chapman & Hall.

Milne, P.H., 1972. *Hydrography of Scottish west coast sea lochs*. Edinburgh: HMSO. [Department of Agriculture and Fisheries for Scotland, Marine Research, no. 3.]

Minchin, D., 1991. Observations on shell colour in the scallop, *Pecten maximus* (L.). *Journal of Conchology*, **34**, 41–46.

Minchin, D., Duggan, C.B. & King, W., 1987. Possible effects of organotins on scallop recruitment. *Marine Pollution Bulletin*, **18**, 604–608.

Nei, M., 1987. *Molecular evolutionary genetics*. New York: Columbia University Press.

Nei, M. & Miller, J.C., 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. *Genetics*, **125**, 873–879.

Nei, M. & Tajima, F., 1981. DNA polymorphisms detectable by restriction endonucleases. *Genetics*, **97**, 145–163.

Nei, M. & Tajima, F., 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction site data. *Genetics*, **105**, 207–217.

Neigel, J.E. & Avise, J.C., 1993. Application of a random walk model to geographic distributions of animal mitochondrial DNA variation. *Genetics*, **135**, 1209–1220.

Palumbi, S.R., 1992. Marine speciation on a small planet. *Trends in Ecology and Evolution*, **7**, 114–118.

Palumbi, S.R., 1995. Using genetics as an indirect estimator of larval dispersal. In *Marine invertebrate larvae* (ed. L. McEdward), pp. 369–387, New York: CRC Press.

Rigaa, A., Le Gal, Y.L. & Sellos, D., 1993. Mapping and repeated sequence organization of mitochondrial DNA in scallop, *Pecten maximus*. *Molecular Marine Biology and Biotechnology*, **2**, 218–224.

Rigaa, A., Monnerot, M. & Sellos, D., 1995. Molecular cloning and complete nucleotide sequence of the repeat unit and flanking gene of the scallop *Pecten maximus* mitochondrial DNA: putative replication origin features. *Journal of Molecular Evolution*, **41**, 189–195.

Roff, D.A. & Bentzen, P., 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Molecular Biology and Evolution*, **6**, 539–545.

Rohlf, F.J., 1993. *NTSYS-pc: numerical taxonomy and multivariante analysis system, version 1.8*. Setauket, New York: Exeter Software.

Shumway, S.E., ed., 1991. *Scallops: biology, ecology and aquaculture*. Amsterdam: Elsevier.

Slater, J., 1995. Scallop spat collection in Mulroy Bay, Ireland, 1979–1994. In *Book of Abstracts, 10th International Pectinid Workshop, Cork, Ireland, 26 April–2 May* (ed. G. Burnell), pp. 68–69. Cork, Ireland: University College Cork.

Slatkin, M., 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.

Stewart, C.N. Jr & Excoffier, L., 1996. Assessing population genetic structure and variability with RAPD data: application to *Vaccinum macrocarpon* (American cranberry). *Journal of Evolutionary Biology*, **9**, 153–171.

Wilding, C.S., 1996. *Population genetics of the scallop* Pecten maximus*: morphological, allozyme electrophoresis and mitochondrial DNA approaches*. PhD thesis, University of Wales, Bangor.

Wilding, C.S., Beaumont, A.R. & Latchford, J.W., 1997. Mitochondrial DNA variation in the scallop *Pecten maximus* (L.) assessed by a PCR-RFLP method. *Heredity*, **79**, 178–189.

Zhang, D.X. & Hewitt, G.M., 1996. Highly conserved nuclear copies of the mitochondrial control region in the desert locust *Schistocerca gregaria*: some implications for population studies. *Molecular Ecology*, **5**, 295–300.

Submitted 12 February 1998. Accepted 28 October 1998.