J. Mar. Biol. Ass. U.K. (1999), **79**, 949–952
Printed in the United Kingdom

Are *Pecten maximus* and *Pecten jacobaeus* different species?

Craig S. Wilding*, Andy R. Beaumont and John W. Latchford

School of Ocean Sciences, University of Wales Bangor, Menai Bridge, Gwynedd, LL59 5EY.
*Present address: School of Biology, University of Leeds, Leeds, LS2 9JT

Two members of the scallop genus *Pecten* (Bivalvia: Pectinidae) occur in European waters. *Pecten maximus* is largely an Atlantic species whilst *Pecten jacobaeus* is almost completely confined to Mediterranean waters despite slight overlap of distributions in the western Mediterranean. Genetic distances between these species were estimated using both allozyme electrophoresis and mitochondrial DNA PCR–RFLP and shown to be of a similar magnitude to intraspecific values, considerably lower than expected for congeneric species. These data are discussed in the light of recognised morphological differences, hybrid rearing trials and previous studies examining genetic distance by allozyme methodologies.

Within north European waters two species of *Pecten* support important fisheries; *Pecten maximus* and *P. jacobaeus*. *Pecten maximus* (L.) is largely an Atlantic species whilst its congener *P. jacobaeus* is limited to the Mediterranean, although only occurring in sufficient densities for exploitation in the northern Adriatic (Mattei & Pellizzato, 1996). The fossil record of these species is reasonable. Depéret & Roman (1902) document *P. maximus* fossils in quaternary series and *P. grandis* fossils (the hypothetical ancestor of *P. maximus*) in the Pliocene Red Crag of Britain. *P. jacobaeus* fossils are also described by Depéret & Roman (1902) as first appearing at the beginning of the Pliocene. The potential for differentiation of these taxa has existed for approximately five million years, since at this time the Mediterranean once again became habitable following the Messinian salinity crisis (Hsü, 1972, 1987; Pérès, 1985).

Although these species are morphologically similar there are distinguishing features (Wagner, 1991), but there are no published data on comparative multivariate studies of morphology. Similarly, studies examining genetic differences between the species are sparse. Data on population genetic structure are available for *P. maximus* but not for *P. jacobaeus*. Crucially, there has been only one comparative study of allozymes in the two European *Pecten* species. Beaumont (1991) used data on allele frequencies for *P. maximus* and *P. jacobaeus* (Huelvan, 1985) to calculate Nei's genetic distance of 0.05, a value more typical of separate populations than separate species (Thorpe & Solé-Cava, 1994). There has been, as yet, no comparative study of these two species using DNA markers. Here we further examine the genetic relationships of these two species using morphometrics, allozyme electrophoresis and the mtDNA PCR–RFLP approach previously applied to populations of *P. maximus* (Wilding et al., 1997, 1998).

A single sample of *Pecten jacobaeus* was collected from 65–75 m depth, 13 miles off the Oropesa coast, western Castellón, Spain and samples of *P. maximus* were taken from various locations around the UK, Eire and northern France (see Wilding et al., 1998 for locations and sources).

A number of measurements were taken from the shells of these animals. Rib number was counted from the upper valve and variation measured by one-way ANOVA. Significant differences ($F=7.22$, $P<0.001$) were detected between certain populations of *P. maximus* and *P. jacobaeus* with a mean (±SE) rib number of 12.881 ±0.026 in *P. maximus* and 13.818 ±0.193 in *P. jacobaeus*. Of 15 comparisons *P. jacobaeus* differed significantly from a *P. maximus* population (Scheffé's test) in eight, always with more ribs. Five shell measurements (height, length, width, shell mass and hinge length) were also taken and used as input for a principle component analysis after subjection to a geometric mean transformation (using $\sqrt[3]{}$shell mass). Table 1 summarizes the PCA output. Morphological differences in these mensural parameters were greater on the whole in congeneric comparisons than they were for intraspecific comparisons (although two *P. maximus* populations separate strongly on PC1 from all other populations and *P. jacobaeus*; Wilding et al., 1998). For PC1 and PC2 significant differences are detectable using ANOVA ($F=37.33$ $P<0.001$; $F=18.0$ $P<0.01$) and *P. jacobaeus* is significantly different in 10 and 15 (of 15) comparisons for PC1 and PC2 respectively using Scheffé's test. This is unsurprising given the notable differences in shell morphology between these species (Wagner, 1991).

Genetic variation at 7 enzyme loci was assessed by horizontal starch gel electrophoresis as described in Wilding et al. (1998). Allele frequencies from *P. jacobaeus* and the average values for *P. maximus* are given in Table 2 and additional summary statistics are provided in Table 3. Allele frequencies were bootstrapped

Table 1. *Summary of PCA performed on complete* Pecten maximus *and* P. jacobaeus *morphometric dataset using the covariance matrix. 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.*

Character	Eigenvector PC1	PC2	PC3	PC4
length	−0.241	−0.446	−0.365	0.640
depth	−0.193	0.760	−0.421	−0.095
height	−0.308	−0.428	−0.008	−0.723
weight	−0.147	0.188	0.829	0.237
hinge-l	0.888	−0.074	−0.051	−0.059
Eigenvalue	0.000764	0.000403	0.000170	0.000057
% Proportion	54.8	28.9	12.2	4.1
% Cumulative	54.8	83.8	95.9	100

Table 2. *Allele frequencies at seven allozyme loci, and mitochondrial DNA PCR–RFLP haplotype frequencies in* Pecten maximus *(averaged over populations) and* P. jacobaeus.

Locus	R.M.	*P. maximus*	*P. jacobaeus*	Haplotype	*P. maximus*	*P. jacobaeus*
Dia	94	0.022	0.075	AAAAAA	0.388	0.263
	100	0.692	0.500	AAAAAB	0.003	0
	105	0.251	0.425	AAAABA	0.031	0
	110	0.033	0.000	AAAACA	0.047	0.053
	116	0.002	0.000	AAAACB	0.003	0
	N	227	20	AAAADA	0.003	0
				AAAAEA	0.008	0
Est-D	64	0.002	0.000	AAAAFA	0.003	0
	75	0.005	0.000	AAAAGA	0.003	0
	84	0.016	0.000	AAAABB	0.003	0
	100	0.559	0.675	AABAAB	0.19	0.158
	108	0.034	0.175	AABAAA	0.064	0.158
	119	0.354	0.125	AAABAA	0.022	0
	125	0.027	0.025	AAABCB	0.003	0
	140	0.005	0.000	AAACHA	0.006	0
	N	222	20	AAACCA	0.003	0
				AAAGEA	0.003	0
Gpi	38	0.013	0.000	AAAGCA	0.008	0
	50	0.008	0.000	AAAGAA	0.003	0
	64	0.059	0.000	AAAGBA	0.006	0
	74	0.095	0.119	AAAFCC	0.003	0
	78	0.013	0.000	AAACAA	0.003	0.053
	87	0.193	0.071	AAACBA	0.003	0
	93	0.017	0.095	AABDCA	0.003	0
	100	0.244	0.429	AABABB	0.014	0
	105	0.008	0.000	AABFAB	0.003	0
	112	0.163	0.048	AABACB	0.008	0.053
	114	0.000	0.024	AABAAD	0.003	0
	115	0.021	0.024	AADAAA	0.028	0
	119	0.036	0.166	AABBAB	0.025	0.105
	124	0.102	0.000	AACGCA	0.003	0
	130	0.009	0.000	AABABA	0.003	0
	136	0.019	0.024	AABIAA	0.003	0
	N	264	21	AABEAB	0.003	0
				AADBAB	0.003	0
Gr	62	0.002	0.000	AABABB	0.003	0
	75	0.002	0.000	AABGAB	0.003	0
	85	0.047	0.100	AAEAAA	0.003	0
	96	0.008	0.000	AAFAAA	0.003	0
	100	0.529	0.475	AAFACA	0.003	0
	110	0.019	0.000	ABAAAA	0.003	0
	119	0.375	0.375	ABBACA	0.003	0
	125	0.004	0.050	AFBAAA	0.003	0
	130	0.008	0.000	ACAAAA	0.008	0
	140	0.006	0.000	ACABEA	0.003	0
	N	257	20	ACBAAB	0.003	0
				ACAACA	0	0.053
Odh	78	0.002	0.000	ACAGCA	0.003	0
	87	0.011	0.024	ACACCA	0.003	0
	90	0.277	0.143	ADBAAB	0.008	0
	100	0.691	0.810	AEAAAA	0.003	0
	108	0.019	0.024	AEABAA	0	0.053
	N	264	21	BAAAAA	0.011	0
				BABAAA	0.003	0
Pgd	52	0.008	0.000	BABAAB	0.006	0
	75	0.099	0.071	BCAAAA	0.003	0
	100	0.882	0.929	BCAACA	0.003	0
	125	0.011	0.000	BDAACA	0.003	0
	N	263	21	CAAAAA	0.006	0
				CAAEAA	0.003	0
Pgm	71	0.002	0.000	CABAAA	0.003	0
	77	0.017	0.024	CABAAB	0.003	0
	89	0.040	0.071	DABAAB	0	0.053
	100	0.892	0.881	N	358	19
	114	0.047	0.024			
	124	0.002	0.000			
	N	264	21			

N, sample size.

https://doi.org/10.1017/S0025315499001149 Published online by Cambridge University Press

Table 3. *Comparison of morphological and genetic data for* Pecten maximus *and* P. jacobaeus.

Character	*Pecten maximus*	*Pecten jacobaeus*
Morphological		
Number of ribs (Mean ±SE)	12.881 ±0.026	13.818 ±0.193
Mean PC1 score (Mean ±SE)	0.0629 ±0.001	0.0932 ±0.004
Mean PC2 score (Mean ±SE)	−0.659 ±0.001	−0.617 ±0.005
Mean PC3 score (Mean ±SE)	−0.900 ±0.001	−0.912 ±0.002
Allozyme electrophoresis		
Heterozygosity	0.443	0.440
Average Nei's D between *P. maximus* and *P. jacobaeus*	0.047	
Average Nei's D between *P. maximus* populations	0.021	
Average Nei's D between *P. maximus* and Mulroy Bay	0.027	
MtDNA		
Haplotype diversity	0.803	0.901
Nucleotide diversity	0.026	0.022
Average d between *P. maximus* and *P. jacobaeus*	0.045%	
Average d between *P. maximus* populations	0.022%	
Average d between *P. maximus* and Mulroy Bay	0.104%	

and used to calculate values of Nei's genetic identity and these were subsequently used for clustering via neighbour-joining methodology and construction of a consensus tree (Figure 1B). This shows good bootstrap support for differences between the species with *P. jacobaeus* clustering separately from *P. maximus*.

Mitochondrial DNA analysis followed the methods described by Wilding et al. (1997) using primer pair Pma1 (Wilding et al., 1997). As for the allozyme data, no high frequency private haplotypes were seen, and haplotype frequency differences were in the order of those seen between populations of *P. maximus* (Table 2). Nucleotide divergence between populations was clustered by UPGMA (Wilding et al., 1997). Due to tied trees, a consensus dendrogram was constructed (Figure 1B). This shows that Mulroy Bay *P. maximus* appear less similar to other *P. maximus* populations than *P. jacobaeus* does to *P. maximus*. Thus nucleotide divergence does not suggest genetic differences between the two species, neither is there evidence for differences in haplotype diversity (Table 3).

These species are believed to have last shared a common ancestor some 5 mya prior to the Messinian salinity crisis (T. Waller, Smithsonian Institute, NMNH, Washington DC, personal communication) and now have separate but partially overlapping ranges. Despite this supposed time period since separation, the calculated genetic differences are slight and more appropriate for intra- than interspecific comparisons. The value of genetic distance calculated by Beaumont (1991) is in the order of the value from this study and neither is typical of interspecific comparisons (Thorpe & Solé-Cava, 1994). Similarly from the mtDNA comparisons it seems that the interspecific differentiation is of only a similar magnitude to the amongst-population levels seen for *P. maximus* and is much less than the differences encountered in comparisons of *P. maximus* populations with the *P. maximus* population from Mulroy Bay, Eire (Wilding et al., 1997). The question is therefore raised as to why such a low genetic differentiation is evident in taxa which are currently recognised as valid morphological species. We suggest that there are three possibilities:

(1) These do represent valid species but the genetic distance measures calculated here do not reflect this. Waller (Department of Palaeobiology, Smithsonian Institution, NMNH, personal communication) suggests that although closely related, the species are valid based on a number of taxonomically significant morphological differences including rib profile, ear–hinge angle and costae development during ontogeny. However their recognition as true species is at odds with the calculated genetic distances given the time available for divergence (~5 my). Under the tenuous figure of mtDNA divergence at 2% per my (Brown et al., 1979) we would expect a nucleotide divergence of 10% and for allozymes, Nei (1987) calculated a t (time of divergence) $=5\times10^6$ D thus leaving us with an expected Nei's D of 1.0. Sarich (1977) modified this equation to t $=30\times10^6$ D which would lead us to expect a Nei's D in this case of 0.167. Instead we get figures of 0.045% for nucleotide divergence and 0.047 for Nei's D.

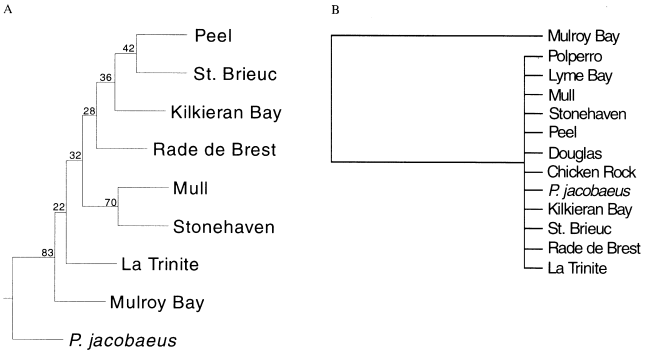

Figure 1. (A) Bootstrapped consensus neighbour-joining tree from allozyme frequencies of *Pecten maximus* and *P. jacobaeus*. Numbers at the nodes refer to the number of times the groups to the right of the branch point occurred together in 100 replicates. (B) Consensus dendrogram from all equally likely UPGMA dendrograms from clustered nucleotide divergence values calculated from PCR–RFLP data on the Pma1 amplified fragment (Wilding et al., 1997). See Wilding et al. (1998) for location of *P. maximus* sampling sites.

(2) *Pecten jacobaeus* is not a valid species—the higher allozyme differentiation (in pairwise comparisons involving *P. jacobaeus* compared to interspecific *P. maximus* comparisons) merely represents increased isolation by distance. The morphological differentiation would then have to be explained by environmental moulding. In order for this possibility to be given credence there would need to be not only overlap of ranges (see above) but

https://doi.org/10.1017/S0025315499001149 Published online by Cambridge University Press

evidence of capability of hybridization: Huelvan (1985) and J.C. Cochard (IFREMER, France, personal communication) demonstrated that *P. maximus* and *P. jacobaeus* could be induced to hybridise under laboratory conditions (male *P. jacobaeus*, female *P. maximus*). Under laboratory conditions the fertilisation rate (80–90%) and percentage of eggs developing to straight-hinge larvae (40–50%) were normal. After transplantation into the Bay of Brest there appeared to be no differences between the hybrids and *P. maximus* in growth rate and achievement of maturity. Hybrid shell structure was intermediate between the two species—deeper ridges than *P. maximus* but rounder than *P. jacobaeus*. Hybrid animals died due to *Polydora* infestation before further crosses could be carried out. However this does demonstrate that the species are capable of hybridization to produce viable adults.

(3) *Pecten jacobaeus* in this part of the Mediterranean are interbreeding with *P. maximus* whilst animals elsewhere are not and have therefore undergone more substantial differentiation. However, the collection point (The Oropesa coast, Castellón) is east of the Almeria–Oran frontal system (Prieur & Sournia, 1994) which argues against this. In previous studies of various species (see Pannacciulli et al., 1997 and references therein) genetic breaks have been detected at the position of this front. If *P. jacobaeus* follows a similar pattern we would expect to see greater differences in comparisons of *P. maximus* with *P. jacobaeus* from east of the front than with animals from west of the front. Thus, under this scenario, the genetic distance values calculated in this study would represent the upper limit of species differences. In *Mytilus galloprovincialis*, the genetic distance between populations either side of the front were slight (although significant) at approximately Nei's D=0.03 (Quesada et al., 1995). A similar value is evident between *P. jacobaeus* and *P. maximus* in a previous (Beaumont, 1991) and in the present study. Such differences are more likely in conspecific populations separated by a front, than between two species.

The present data are inconclusive. Determining which, if any, of these postulated possibilities holds true will require more rigorous large scale sampling of *P. jacobaeus* along the Mediterranean coasts of Spain and France.

We would like to thank Dr Juan Peña for provision of the sample of *Pecten jacobaeus*. C.S.W. was supported by a NERC studentship GT4/92/275A.

REFERENCES

Beaumont, A.R., 1991. Genetic distances between some scallop species. In *An international compendium of scallop biology and culture* (ed. S.E. Shumway and P.A. Sandifer), pp. 151–155. Baton Rouge, LA: World Aquaculture Society.

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.

Brown, W.M., George, M. Jr & Wilson, A.C., 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **76**, 1967–1971.

Depéret, C. & Roman. F., 1902. Monographie des Pectinidés Néogènes de l'Europe et des régions voisines. *Mémoires de la Sociétè Géologique de France. Paléontologie*, mémoire no. 26.

Huelvan, S., 1985. *Variabilite genetique de populations de* Pecten maximus *L. en Bretagne*. These du Doctorat de 3eme Cycle, Universite de Bretagne Occidentale, Brest, France.

Hsü, K.J., 1972. When the Mediterranean dried up. *Scientific American*, **December 1972**, 26–36.

Hsü, K.J., 1987. The desiccation of the Mediterranean Sea. *Endeavour*, **11**, 67–72.

Mattei, N. & Pellizzato, M., 1996. A population study on three stocks of a commercial Adriatic pectinid (*Pecten jacobaeus*). *Fisheries Research*, **26**, 49–65.

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

Pannacciulli, F.G., Bishop, J.D.D. & Hawkins, S.J., 1997. Genetic structure of populations of two species of *Chthamalus* (Crustacea: Cirripedia) in the north-east Atlantic and Mediterranean. *Marine Biology*, **128**, 73–82.

Pérès, J.M., 1985. History of the Mediterranean biota and the colonization of the depths. In *Key environments: western Mediterranean* (ed. R. Margalef), pp. 198–232. Oxford: Pergamon Press.

Prieur, L. & Sournia, A., 1994. Almofront-1 (April–May, 1991)—an interdisciplinary study of the Almeria–Oran geostrophic front, S.W. Mediterranean Sea. *Journal of Marine Systems*, **5**, 187–203.

Quesada, H., Zapata, C. & Alvarez, G., 1995. A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. *Marine Ecology Progress Series*, **116**, 99–115.

Sarich, V.M., 1977. Rates, sample sizes and the neutrality hypothesis for electrophoresis in evolutionary studies. *Nature, London*, **265**, 24–28.

Thorpe, J.P. & Solé-Cava, A.M., 1994. The use of allozyme electrophoresis in invertebrate systematics. *Zoologica Scripta*, **23**, 3–18.

Wagner, H.P., 1991. Review of the European Pectinidae. *Vita Marina*, **41**, 1–48.

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.

Wilding, C.S., Latchford, J.W. & Beaumont, A.R., 1998. An investigation of possible stock structure in *Pecten maximus* (L.) using multivariate morphometrics, allozyme electrophoresis and mitochondrial DNA PCR–RFLP. *Journal of Shellfish Research*, **17**, 131–139.

Submitted 15 July 1998. Accepted 12 January 1999.

Note in proof:

C. Rios, S. Sanz and J.B. Peña (personal communication) reported data at the Twelfth International Pectinid Workshop (Bergen, Norway) to show that the *AK* locus (EC 2.7.4.3) had diagnostic alleles for *Pecten maximus* and *P. jacobaeus*. Over 14 loci populations of the two species were separated by a mean Nei's D of 0.148.