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Genetic diversity and population structure of the commercially harvested sea urchin *Paracentrotus lividus* (Echinodermata, Echinoidea)

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Abstract

The population structure of the edible Atlanto-Mediterranean sea urchin *Paracentrotus lividus* is described by analysing sequence variation in a fragment of the mitochondrial gene cytochrome *c* oxidase subunit I in 127 individuals from 12 localities across south-west Europe. The study revealed high levels of genetic diversity but low levels of genetic structure, suggesting a large degree of gene flow between populations and panmixis within each, the Mediterranean and Atlantic basins. However, we found significant genetic differentiation between the two basins probably due to restricted gene flow across the geographical boundary imposed by the area of the Strait of Gibraltar. Populations of *P. lividus* appeared to have experienced a recent demographic expansion in the late Pleistocene. We provide new evidence on the population structure of this commercial species, predicting a healthy stock of this sea urchin on the Mediterranean and Atlantic coasts.

Keywords: Atlanto-Mediterranean distribution, cytochrome *c* oxidase subunit I, mtDNA, *Paracentrotus lividus*, population genetic structure, sea urchin

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Introduction

Most marine invertebrates spend part of their life cycle in open waters as free-moving gametes, larvae or adults. Even in species with low mobility in adult stages, opportunities for moderate to high gene flow may be the norm except where strong ecological or biogeographical barriers to dispersal exist. The expected pattern is that species with high dispersal potential (i.e. with planktotrophic larvae) have little genetic structure and high gene flow (Palumbi & Wilson 1990; Lacson 1992; Ovenden *et al.* 1992; Russo *et al.* 1994; Uthicke & Benzie 2003). Conversely, species with low dispersal potential (i.e. with lecitotrophic larvae) are expected to have clear patterns of genetic structure (Janson & Ward 1984; Day & Bayne 1988; McMillan *et al.* 1992; Duffy 1993; Hunt 1993; Duran *et al.* 2004b). Nevertheless, there are examples showing that such expectations may be unfounded (Solé-Cava *et al.* 1994; Grant & da Silva-Tatley 1997; Uthicke & Benzie 2000; Lazoski *et al.* 2001), because a variety of additional factors (biological, physical, ecological, etc.) might contribute to the shaping of the population structure of marine invertebrates through space and time.

One of the most interesting biogeographical boundaries in the world's oceans occurs between the Mediterranean Sea and the Atlantic Ocean at the Strait of Gibraltar, separating the Mediterranean region (to the East), the Lusitanian region (to the north-west) and the Mauritanian region (to the south-west) (Briggs 1974). The closure of the Rifean and Baetic gateways between the Atlantic Ocean and the Mediterranean Sea some 6 million years ago (Mya) led to the so-called Messinian salinity crisis (Maldonado 1985; Duggen *et al.* 2003), whereby the Mediterranean Sea was reduced to a series of hypersaline lakes with thick evaporite deposition. Most present-day fauna has colonized the Mediterranean since the opening of the Strait of Gibraltar

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some 5 Mya, at the end of the Miocene. Afterwards, the interplay between glacial and interglacial periods during the Quaternary, with associated marine regressions and transgressions has provided opportunities for diversification and speciation.

Atlanto-Mediterranean species of marine invertebrates might present restricted gene flow through the Strait of Gibraltar, which has been considered an important area of endemism for several groups such as, for example, molluscs (Gofas 1998) and ascidians (Naranjo *et al.* 1998). Studies analysing the genetic structure of Atlanto-Mediterranean marine species report moderate to strong genetic discontinuity between each side of the Strait of Gibraltar (Borsa *et al.* 1997; Chikhi *et al.* 1997; Pannacciulli *et al.* 1997; McFadden 1998; Quesada *et al.* 1998; Hawkins *et al.* 2000; Duran *et al.* 2004a) although in the case of the Norwegian lobster (*Nephrops norvegicus*) there were no signs of an Atlantic–Mediterranean division (Stamatis *et al.* 2004). The Strait of Gibraltar is not the main barrier between the Mediterranean and Atlantic basins because of the existence of a surface current of North Atlantic water reaching as far as the Alboran Sea, where a density front has been described (Tintore *et al.* 1998). This front (Almeria–Oran line) may mark the main barrier between Atlantic and Mediterranean populations, as seen in diverse invertebrate groups (Maldonado & Uriz 1995; Quesada *et al.* 1995; Pannacciulli *et al.* 1997).

In temperate rocky bottoms, sea urchins are known to exert a major role in shaping benthic communities through their grazing activity (Palacín *et al.* 1998; Sala *et al.* 1998). *Paracentrotus lividus* is found throughout the Mediterranean, as well as in the Northeast Atlantic from Ireland to the coasts of Morocco, the Canary Islands and the Azores (Boudouresque & Verlaque 2001). In the Mediterranean sublittoral, *P. lividus* is the most important invertebrate grazer and it is subject to important commercial fisheries both in the Mediterranean Sea and more recently in the Atlantic Ocean (Barnes & Crook 2001). Spawning in *P. lividus* features one or two annual peaks (Lozano *et al.* 1995). In the north-western Mediterranean most larvae settle in spring-early summer (López *et al.* 1998; Hereu *et al.* 2004), although smaller settlement episodes do occur in the fall (Pedrotti 1993; Tomàs *et al.* 2004). The planktonic life span of the larvae is of 20–40 days (Pedrotti 1993) allowing this species to disperse over great distances. However, biological (larval behaviour, predation, food availability, etc.), oceanographic (current boundaries) and biogeographical (physical barriers) factors could result in population differentiation despite having a long larval stage. Hereu *et al.* (2004) showed striking spatial heterogeneity in settlement of *P. lividus* larvae, implying that there could be barriers to larval movement.

Sequence data from a 644 bp fragment of the cytochrome *c* oxidase subunit I (COI) region of the mitochondrial DNA (mtDNA) was used to determine the genetic structure of *P. lividus* in south-west Europe, where the species is subjected to intensive commercial exploitation. The COI marker has proved to be highly polymorphic in different echinoid species (Lessios *et al.* 1999; Debenham *et al.* 2000; McCartney *et al.* 2000; Lessios *et al.* 2001b; Lessios *et al.* 2003) and allows the detection of both historical and contemporary gene flow (Avise *et al.* 1987). The data are used to investigate the differentiation of Atlantic and Mediterranean populations and provide information to assist management and conservation on this ecologically important marine invertebrate.

Materials and methods

Sampling

We analysed a total of 127 individuals from six populations from each of the Eastern Atlantic and Western Mediterranean coasts (Table 1 and Fig. 1) covering a significant range of the species' distribution. Specimens were collected by scuba or snorkelling. A gonad from each individual was extracted, and preserved in absolute ethanol at –20 °C until processed.

DNA extraction, polymerase chain reaction (PCR) amplification and DNA sequencing

Total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen) following the instructions of the supplier. A fragment of the mtDNA COI gene was amplified using the primers described in Arndt *et al.* (1996) (COIe-F: 5′-ATA ATG ATA GGA GGR TTT GG-3′; COIe-R: 5′-GCT CGT GTR TCT ACR TCC AT-3′). These primers amplified 644 nucleotides of the echinoderm COI gene, corresponding to positions (5′–3′): 6001–6674 of the mitochondrial genome

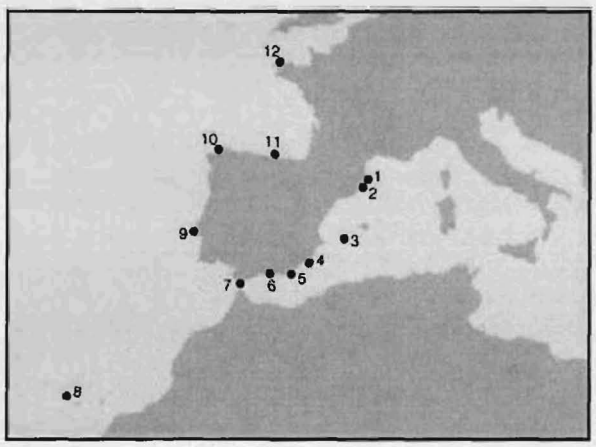

Fig. 1 Map showing the 12 sampling sites of *Paracentrotus lividus*. See Table 1 for details.

Table 1 Diversity measures for the populations of *Paracentrotus lividus* studied

Population	Code	*n*	Nh	Np	*h*	π
Illes Medes	1	10	9	14	0.977 (0.054)	0.0058 (0.0036)
Blanes	2	12	8	13	0.924 (0.057)	0.0052 (0.0032)
Eivissa	3	11	10	18	0.981 (0.046)	0.0071 (0.0042)
Cabo de Palos	4	10	10	19	1.000 (0.044)	0.0098 (0.0057)
Cabo de Gata	5	10	6	7	0.844 (0.102)	0.0035 (0.0024)
La Herradura	6	9	8	19	0.972 (0.064)	0.0078 (0.0047)
Tarifa	7	11	8	10	0.945 (0.053)	0.0055 (0.0034)
Tenerife	8	11	9	18	0.945 (0.065)	0.0079 (0.0046)
Cascais	9	11	7	12	0.909 (0.065)	0.0067 (0.0040)
Ferrol	10	11	9	17	0.963 (0.051)	0.0071 (0.0042)
Santander	11	11	10	19	0.981 (0.046)	0.0085 (0.0050)
Roscoff	12	10	9	17	0.977 (0.054)	0.0089 (0.0053)
Mediterranean		62	36	43	0.942 (0.017)	0.0064 (0.0036)
Atlantic		65	33	38	0.963 (0.009)	0.0074 (0.0040)
Total		127	65	63	0.961 (0.009)	0.0071 (0.0004)

Note: Population code (as in Fig. 1), sample size (*n*) number of haplotypes (Nh), number of polymorphic sites (Np), haplotype diversity (*h*) and nucleotide diversity (π). Standard deviation are given in parenthesis.

of the echinoid *Strongylocentrotus purpuratus* (Jacobs *et al.* 1988). Amplifications were carried out in a 50-μL volume reaction, with 1.25 units of AmpliTaq DNA Polymerase (PerkinElmer), 200 μM of dNTPs and 1 μM of each primer. The PCR program consisted of an initial denaturing step at 95 °C for 2 min, 30 amplification cycles (95 °C for 30 s, 45 °C for 30 s and 72 °C for 1 min) and a final step at 72 °C for 8 min in a GeneAmp PCR System 9700 (Applied Biosystems). All PCR products were checked for the presence of products on 1.5% agarose gels.

The PCR amplified samples were purified with the QIAquick PCR Purification Kit (Qiagen). Cycle-sequencing with AmpliTaq DNA Polymerase, FS (PerkinElmer) using dye-labelled terminators (ABI PRISM BigDye version 3.0 Terminator Cycle Sequencing Ready Reaction Kit) was performed in a GeneAmp PCR System 9700 (Applied Biosystems). The sequencing reaction was carried out in a 10 μL volume reaction: 2 μL of Terminator Ready Reaction Mix, 2 μL of 5× sequencing buffer (supplied with BigDye), 10–30 ng/mL of PCR product, 5 pmol of primer and distilled water (10 μL). The cycle-sequencing program consisted of an initial step at 94 °C for 3 min, 25 sequencing cycles (94 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min) and a rapid thermal ramp to 4 °C and hold. The BigDye-labelled PCR products were cleaned with AGTC Gel Filtration Cartridges (Edge BioSystems) and directly sequenced using an automated ABI PRISM 3100 Genetic Analyser (Applied Biosystems). Chromatograms obtained from the automated sequencer were read and contigs assembled using the sequence editing software SEQUENCHER version 4.0 (Gene Codes Corporation). Sequences were edited and aligned with BIOEDIT Sequence Alignment Editor (Hall 1999). No indels were observed.

Population genetics analyses

Haplotype and nucleotide diversity values were calculated using ARLEQUIN version 2.001 (Schneider *et al.* 2000). The same program was used to calculate the pairwise genetic distances (F_{ST}) and their significance by performing 10 000 permutations among the individuals between populations. We also performed an analysis of molecular variance (AMOVA) to examine hierarchical population structure pooling the populations in Mediterranean and Atlantic groups, as well as without grouping populations. We executed 16 000 permutations to guarantee having less than 1% difference with the exact probability in 99% of cases (Guo & Thompson 1992) and used our a priori expectation of a genetic division between the Mediterranean and Atlantic populations. An exact test of population differentiation based on haplotype frequencies (Raymond & Rousset 1995) was performed to test the null hypothesis that observed haplotype distribution is random with respect to sampling location. The significance of individual tests was estimated by comparison with simulated distributions constructed from 10 000 random permutations of the original data matrix.

Relationships among haplotypes were analysed in a parsimony network estimated with TCS version 1.12 (Clement *et al.* 2000) using the statistical parsimony procedure (Templeton *et al.* 1992; Crandall *et al.* 1994). This method estimates the unrooted tree and provides a 95% plausible set for all sequence type linkages within the unrooted tree.

Correlation of genetic distance (as $F_{ST}/(1 - F_{ST})$) over geographical distances for all pairs of populations was tested with the Mantel permutation procedure available in GENETIX version 4.04 (Belkhir *et al.* 2001).

Demographic analysis

To determine the historical demography of the populations we analysed the mismatch distributions (i.e. the frequency distribution of pairwise differences among all haplotypes in a sample) with the models of Rogers & Harpending (1992) and Rogers (1995). We assessed the fit of mismatch distributions to the theoretical distribution in an expansion scenario by Montecarlo simulations of 10 000 random samples using ARLEQUIN. The sum of squared deviations between observed and expected mismatch distributions was used as a test statistic and its *P*-value represents the probability of obtaining a simulated sum of squared deviations larger or equal to the observed one. Given the fact that mismatch distributions have been found to be very conservative (Ramos-Onsins & Rozas 2002) and in order to have a wider view of the evolutionary scenario we also assessed the history of effective population size by means of other statistics such as Tajima's *D*-test (Tajima 1989), Fu's (1997) F_S test, and the recently developed Ramos-Onsins and Rozas's R_2 test (Ramos-Onsins & Rozas 2002), using DNA_{SP} version 400.4 (Rozas & Rozas 1999).

Finally, the relationship $\tau = 2ut$ (Rogers & Harpending 1992) was used to estimate the approximate time of expansion in generations (t) for *P. lividus* populations, where τ is the date of the growth or decline measured in units of mutational time and u is the mutation rate per sequence and per generation. The value of u was calculated from $u = 2\mu k$, where μ is the mutation rate per nucleotide and k is the number of nucleotides of the analysed fragment. Mutation rates for echinoid COI have been reported to range from 1.6 to 3.5% per million years (Lessios *et al.* 1999; McCartney *et al.* 2000; Lessios *et al.* 2001b).

All the analyses were performed for all populations as well as for the populations pooled into two regions: Atlantic and Mediterranean. Although geographically located at the limit between the Atlantic Ocean and the Mediterranean Sea, we considered Tarifa an Atlantic population because it lies west of the Almeria–Oran line.

Results

Haplotype diversity

We obtained 644 bp sequences of COI from 127 individuals of *P. lividus* from 12 populations along the western Mediterranean and eastern Atlantic coasts (Fig. 1 and Table 1). All the sequences have been deposited in the GenBank Data Base (Accession numbers AY630792–AY630918). From the 127 individuals, a total of 65 haplotypes were found, indicating a high degree of polymorphism. We found a total of 63 polymorphic sites and 67 mutations out of 644 bp (9.5% variable sites). The number of nucleotide differences between any two sequences ranged from 1 to 13 substitutions and all the mutations resulted in synonymous substitutions. The number of haplotypes per site ranged from 6 to 10 (Table 1) and there was no trend in the number of haplotypes related to geographical location. The most common haplotype (Seq1) was found in every locality we sampled. The Atlantic and Mediterranean regions shared four haplotypes (Seq1, Seq4, Seq14 and Seq19) (see Appendix 1 for complete information on haplotype frequencies for each sampling site and geographical location). It was found that 80% of haplotypes were population specific and occurred at very low frequencies.

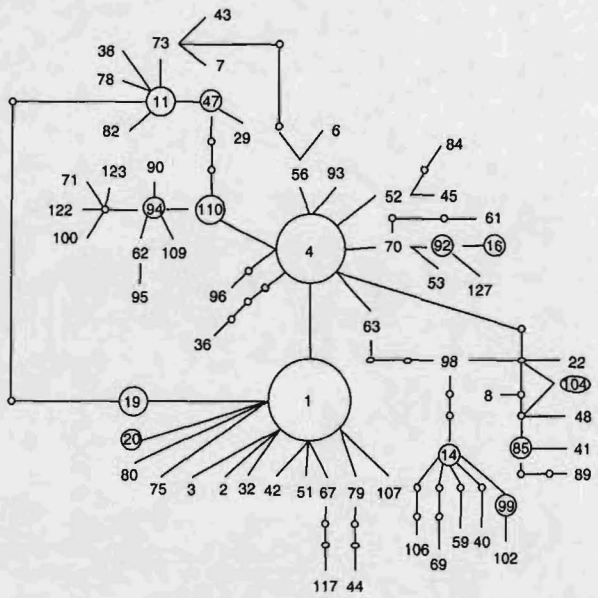

Fig. 2 Statistical parsimony network based on the cytochrome *c* oxidase subunit I (COI) sequences of *Paracentrotus lividus*. Each haplotype is defined by its corresponding number (see Appendix). The area of each circle is proportional to the number of individuals bearing more than one haplotype. Small empty circles indicate intermediate haplotypes that are not present in the samples but are necessary to link the observed haplotypes in the network. Each line in the network represents one mutational step.

The statistical parsimony procedure yielded one network with several ambiguous connections (Fig. 2). The most frequent haplotypes presented a central position in the network and the remaining ones were, in general, closely connected to the common haplotypes.

Diversity and population structure

All populations showed high values of haplotype diversity (mean 0.961 ± 0.009) and low values of nucleotide diversity (mean 0.007 ± 0.00043) (Table 1).

All pairwise F_{ST} values were smaller than 0.08 and Tenerife vs. Cabo de Gata was the only significant pairwise

Table 2 Pairwise F_{ST} values between populations of *Paracentrotus lividus*

	1	2	3	4	5	6	7	8	9	10	11	12
1	0											
2	−0.0005	0										
3	0.0020	−0.0225	0									
4	−0.0090	−0.0210	−0.0282	0								
5	0.0307	−0.0211	0.0145	0.0083	0							
6	−0.0086	−0.0234	−0.0182	−0.0202	0.0038	0						
7	0.0207	0.0284	0.0201	0.0095	0.0342	0.0115	0					
8	0.0297	0.0508	0.0283	0.0186	0.0706*	0.0317	0.0222	0				
9	0.0394	0.0471	0.0386	0.0099	0.0540	0.0307	0.0243	0.0410	0			
10	0.0021	−0.0046	0.0025	−0.0187	0.0243	−0.0193	0.0211	0.0375	−0.0300	0		
11	−0.0072	−0.0061	−0.0067	−0.0186	−0.0152	−0.0182	−0.0051	0.0201	0.0137	−0.0059	0	
12	0.0123	0.0334	0.0111	0.0011	0.0409	0.0140	0.0116	0.0207	0.0305	0.0204	−0.0363	0

*Significant at $P < 0.05$ after a 10 000 permutation of haplotypes between localities. Populations coded as in Table 1.

Table 3 Analysis of molecular variance for the cytochrome *c* oxidase subunit I (COI) sequences of *Paracentrotus lividus*. Analyses are presented pooling populations in Mediterranean and Atlantic basins and for the whole area without grouping

		AMOVA with Mediterranean and Atlantic groups			
Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among groups	1	0.939	0.00708 Va	1.46*	F_{CT}: 0.0146
Among populations within groups	10	4.896	0.00131 Vb	0.27	F_{ST}: 0.0173
Within populations	115	54.709	0.47573 Vc	98.27	F_{SC}: 0.0027
Total	126	60.543	0.48412		
AMOVA without grouping					
Among populations without grouping	11	5.835	0.00517 Va	1.08	F_{ST}: 0.0107
Within populations	115	54.709	0.47573 Vb	98.92	
Total	126	60.543	0.48090		

Note: Groups correspond to the Mediterranean Sea and the Atlantic Ocean.
*Significant at $P < 0.05$ after 16 000 permutations.
Va, Vb and Vc are the associate covariance components. F_{CT}, F_{ST} and F_{SC} are the *F*-statistics.

comparison after the permutation process (Table 2). Similarly, exact tests on population differentiation showed no significant difference ($P > 0.05$) for any pair of populations (data not shown).

The AMOVA analysis pooling sampling sites according to the Atlantic and Mediterranean geographical regions showed that 98% of the genetic variance observed was within populations (Table 3) but the variance component was not significant ($P = 0.09$). Therefore the overall F_{ST} value (0.01733) was not larger than those obtained from random permutations of haplotypes between populations, indicating no genetic structure and panmixis within the two geographical regions. The AMOVA without grouping samples in two basins also showed that there is no genetic structure in the whole area studied. However, genetic variance between Mediterranean and Atlantic regions differed significantly ($P = 0.001$) indicating some degree of genetic differentiation between the two basins.

As expected from the lack of geographical structure and the presence of large local differentiation, we found no correlation between geographical and genetic distances. A Mantel test on the correlation between $F_{ST}/(1 - F_{ST})$ and geographical distance matrices was not significant ($P = 0.18$).

Demographic history

Given the differentiation found between the two seas and the lack of internal structure we performed one set of analyses considering each geographical region as a single panmictic metapopulation for the demographic analysis, pooling the 62 sequences from the six populations in the Mediterranean Sea separately from the 65 sequences from the 6 populations in the Atlantic Ocean. Another set of analyses were performed pooling all the sequences together as the results obtained pooling populations in two groups were very similar.

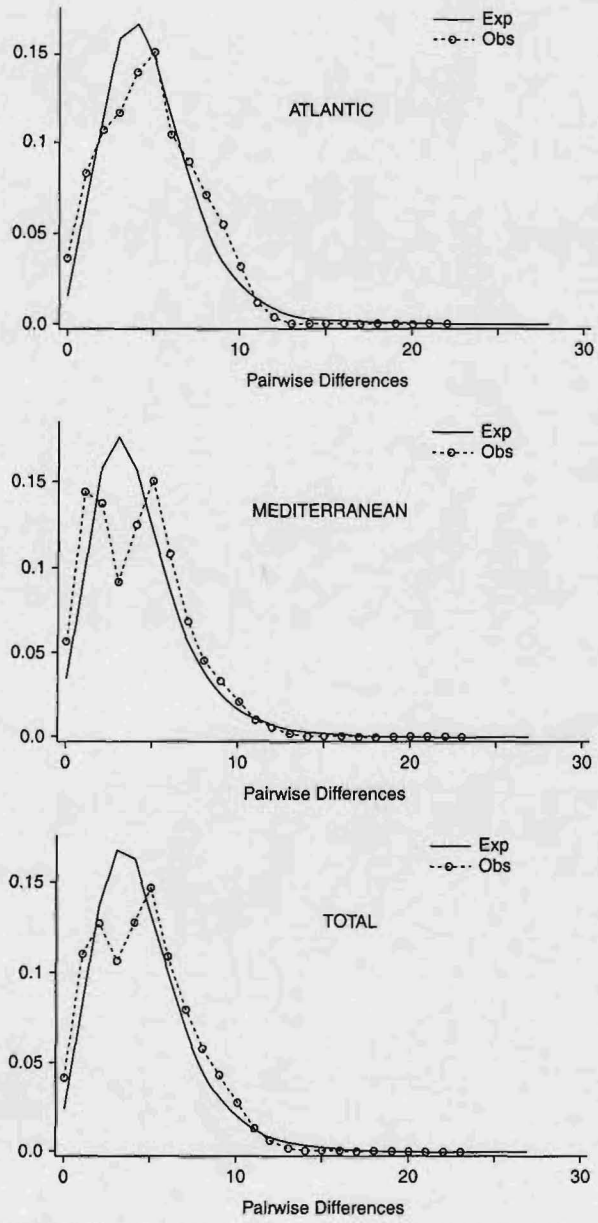

Fig. 3 Mismatch distributions of haplotypes of *Paracentrotus lividus* for the Atlantic Ocean and the Mediterranean Sea as well as for the total area studied. The solid line depicts the mismatch distribution expected from a sudden expansion model with parameters shown in Table 4. The dashed line describes the observed distribution.

The mismatch distributions were not significantly different from the sudden expansion model of Rogers & Harpending (1992) for the two geographical regions and for the whole area (Fig. 3 and Table 4). For the statistics of the other neutrality tests, Tajima's *D*, Fu's F_S and Ramos-Onsins and Rozas's R_2 were all significant for the Mediterranean Sea, but only Fu's F_S was significant for the Atlantic Ocean.

Table 4 Neutrality tests for *Paracentrotus lividus* pooled into Mediterranean and Atlantic groups as well as in the whole area studied

Parameters	Mediterranean	Atlantic	Total
τ	4.183	3.873	3.974
θ_0	1.158	1.671	1.515
θ_1	10.348	23.274	17.264
Goodness of fit test			
SSD	0.0062	0.0012	0.00219
P	0.6169	0.8436	0.7199
Tajima's *D*-test	−1.88874	−1.32386	−1.98169
P	< 0.05	> 0.05	< 0.05
Fu's F_S test	−30.024	−20.229	−71.130
P	< 0.001	< 0.001	< 0.001
Ramos-Onsins & Rozas's R_2 test	0.0426	0.0577	0.0333
P	< 0.02	> 0.05	< 0.05

The parameters of the model of sudden expansion (Rogers & Harpending 1992) are presented, as well as the goodness of fit test of the model. SSD, sum of squared deviations. *P*-values for rejection of the sudden expansion model are based on a comparison of the sum of squares of expected and observed distributions, using parametric bootstrapping with 10 000 replicates (Schneider & Excoffier 1999). Tajima's *D*, Fu's F_S and Ramos-Onsis and Rozas's R_2 values and their statistical significance are shown.

We estimated an approximate time of expansion (t) for the two geographical regions. The expansion was estimated to have taken place approximately 46 000–101 000 generations ago in the Mediterranean Sea and 43 000–94 000 generations ago in the Atlantic Ocean, depending on the estimate of mutation rates (between 1.6 and 3.5% per million years). The expansion was estimated to be 44 000–96 000 generations ago if we consider the whole studied area without groupings. Data from the gonadosomatic index suggests that *P. lividus* attains full maturity in a period of *c.* 3 years (Lozano *et al.* 1995; Turon *et al.* 1995). Using this figure as an estimate of the generation time, then the expansion events occurred between 138 000 and 303 000 years ago in the Mediterranean Sea and 129 000 and 282 000 years ago in the Atlantic Ocean. If we consider that only one expansion occurred in the whole area it might happened between 132 000 and 289 000 years ago.

Discussion

Population structure

Analysis of mtDNA sequences of the Atlanto-Mediterranean sea urchin *P. lividus* suggests that gene flow is occurring over large distances with no evidence for isolation by distance acting at the scales studied. Patterns described for echinoderms in other areas are similar (Palumbi & Wilson

1990; Lessios *et al.* 1999; Debenham *et al.* 2000; McCartney *et al.* 2000; Lessios *et al.* 2001a,b, 2003; Uthicke & Benzie 2003).

Populations within the Atlantic Ocean and Mediterranean Sea appear to be panmictic as there are no statistically significant differences in haplotype frequencies among their respective populations. Nevertheless, AMOVA detected a slight but significant pattern of genetic differentiation between the two basins, probably owing to restrictions in larval exchange across the Strait of Gibraltar. The Strait and associated areas have been shown to constitute a physical boundary to gene flow resulting in population subdivision in several invertebrates (Quesada *et al.* 1998; Zane *et al.* 2000; Launey *et al.* 2002; Pérez-Losada *et al.* 2002; Duran *et al.* 2004a), with species sometimes presenting completely different haplotypes in the two regions.

The lack of population structure may not be solely the result of exchange of individuals sufficient to prevent population divergence, as the slight genetic differences found among populations of *P. lividus* are also consistent with a recent divergence.

Although we found high levels of variability (65 haplotypes and *c.* 10% variable positions), most of the populations shared the two dominant haplotypes (Seq1 and Seq4) and these are found in 25% of the individuals studied. The rest of haplotypes are present at lower frequencies. This pattern has been reported in several marine crustaceans (Bucklin & Wiebe 1998; Benzie 2000; Zane *et al.* 2000; Barber *et al.* 2002; Stamatis *et al.* 2004) and also in some continental species (Ball *et al.* 1988). It has been suggested that the occurrence of a large number of low-frequency haplotypes can result from the enormous population sizes of marine organisms, causing retention of numerous haplotypes during population growth or expansion (Watterson 1984). It has also been shown that demographic expansions can lead to star-shaped genealogies (Slatkin & Hudson 1991) translating into an excess of rare mutations and into unimodal mismatch distributions (Rogers & Harpending 1992).

Demographic structure

We found high haplotype diversity and low nucleotide diversity in the populations of *P. lividus* (mean $h = 0.961$ and $\pi = 0.0071$). These values are comparable to those of other echinoderms (Debenham *et al.* 2000; McCartney *et al.* 2000; Uthicke & Benzie 2003) and crustaceans (Barber *et al.* 2002). In many marine organisms this combination of high levels of haplotype diversity and low levels of nucleotide diversity has frequently been attributed to expansion after a period of small effective population size, retaining new mutations (Avise *et al.* 1984; Watterson 1984), and often related to episodes of marine level oscillations (e.g. Barber *et al.* 2002). It is believed that many of these marine species originated in the Pliocene or early Pleistocene, but their mtDNA genealogies coalesce on a more recent scale of around a few hundred-thousand years (Grant & Bowen 1998). The expansion in *P. lividus* is also supported by the distribution of the number of nucleotide differences observed in the comparisons of the haplotypes of the two geographical regions studied, which can be attributed to mutation-drift disequilibrium caused by explosive population growth (Rogers & Harpending 1992).

Our data suggest that such an expansion may have happened in the two geographical regions investigated, and the estimates of time of expansion are largely similar dates. Accordingly, we suggest a population expansion in both sea basins by the late Pleistocene, prior to the most recent glaciation (18 000 years ago). By that time the Mediterranean was already configured as we now know it and the Mediterranean climate was well established in this region (Blondel & Aronson 1999).

In addition to the mismatch distribution, which is based in the pairwise sequence differences, there are other statistics based on the mutation (segregating site) frequencies (i.e. Ramos-Onsins and Rozas's R_2, Tajima's D) and haplotype distribution (Fu's F_S), which might be less popular, but have been suggested to be more appropriate and powerful for detecting population growth events (Ramos-Onsins & Rozas 2002). The Mediterranean expansion and an expansion for the whole area studied is well supported by these three statistics, while only Fu's F_S is significant for the expansion in the Atlantic. Different timing and intensity on the expansion or other evolutionary processes, such as genetic hitchhiking, could have caused those different results.

Implications for management

Our results suggest that *P. lividus* behaves as two randomly mating populations within the western Mediterranean and eastern Atlantic, and that panmixis exists within the two geographical areas investigated. The presence of a long dispersal larval phase, large genetic pool of mitochondrial sequences, and the broad distribution of the major haplotypes predict a healthy stock of this sea urchin on the Mediterranean and Atlantic coasts. If so, local depletion of the populations linked to intense harvesting (Le Gall 1987; Régis 1987; Byrne 1990) or the devastating effects of diseases such as the bald-urchin disease (Boudouresque 1980; Azzolina 1987) may not have compromised the future of this widespread species of sea urchin.

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Appendix 1

Absolute haplotype frequencies for each population and region studied

	Populations												Regions	
Haplotypes	1	2	3	4	5	6	7	8	9	10	11	12	Med	Atl
Seq1	1	2	1	1	4	1	1	1	1	1	2	1	10	7
Seq2		1											1	
Seq3	1	1											2	
Seq4	1	3	1	1	2	2	1		1	2	1		10	5
Seq6		1											1	
Seq7		1											1	
Seq8		1											1	
Seq11		2	2	1	1								6	
Seq14				1					2	1			1	3
Seq16									3	2				5
Seq19					1		2		2		1	1	1	6
Seq20								3	1					4
Seq22									1					1
Seq29					1								1	
Seq32					1								1	
Seq36				1									1	
Seq38				1									1	
Seq40				1									1	
Seq41				1									1	
Seq42				1									1	
Seq43				1									1	
Seq44			1										1	
Seq45			1										1	
Seq47			1			1							2	
Seq48			1										1	
Seq51			1										1	
Seq52			1										1	
Seq53			1										1	
Seq56										1				1
Seq59										1				1
Seq61										1				1
Seq62										1				1
Seq63										1				1
Seq67						1							1	
Seq69						1							1	
Seq70						1							1	
Seq71						1							1	
Seq73						1							1	
Seq75	1												1	
Seq78	1												1	
Seq79	1												1	
Seq80	2												2	
Seq82	1												1	
Seq84	1												1	
Seq85							2	1						3
Seq89								1						1
Seq90								1						1
Seq92								1				1		2
Seq93								1						1
Seq94							1	1						2
Seq95								1						1
Seq96											1			1
Seq98											1			1
Seq99											1	2		3

Appendix 1 *Continued*

Haplotypes	Populations												Regions	
	1	2	3	4	5	6	*7*	*8*	*9*	*10*	*11*	*12*	Med	*Atl*
Seq100											*1*			*1*
Seq102											*1*			*1*
Seq104											*1*	*1*		*2*
Seq106											*1*			*1*
Seq107							*1*							*1*
Seq109							*1*							*1*
Seq110							*2*							*2*
Seq118												*1*		*1*
Seq122												*1*		*1*
Seq123												*1*		*1*
Seq127												*1*		*1*
Total	10	12	11	10	10	9	*11*	*11*	*11*	*11*	*11*	*10*	62	*65*

Populations coded as in Table 1 Atlantic populations shown in italic type.