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Genetic structure of the deep-sea coral *Lophelia pertusa* in the northeast Atlantic revealed by microsatellites and internal transcribed spacer sequences

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Abstract

The azooxanthellate scleractinian coral *Lophelia pertusa* has a near-cosmopolitan distribution, with a main depth distribution between 200 and 1000 m. In the northeast Atlantic it is the main framework-building species, forming deep-sea reefs in the bathyal zone on the continental margin, offshore banks and in Scandinavian fjords. Recent studies have shown that deep-sea reefs are associated with a highly diverse fauna. Such deep-sea communities are subject to increasing impact from deep-water fisheries, against a background of poor knowledge concerning these ecosystems, including the biology and population structure of *L. pertusa*. To resolve the population structure and to assess the dispersal potential of this deep-sea coral, specific microsatellites markers and ribosomal internal transcribed spacer (ITS) sequences ITS1 and ITS2 were used to investigate 10 different sampling sites, distributed along the European margin and in Scandinavian fjords. Both microsatellite and gene sequence data showed that *L. pertusa* should not be considered as one panmictic population in the northeast Atlantic but instead forms distinct, offshore and fjord populations. Results also suggest that, if some gene flow is occurring along the continental slope, the recruitment of sexually produced larvae is likely to be strongly local. The microsatellites showed significant levels of inbreeding and revealed that the level of genetic diversity and the contribution of asexual reproduction to the maintenance of the subpopulations were highly variable from site to site. These results are of major importance in the generation of a sustainable management strategy for these diversity-rich deep-sea ecosystems.

Keywords: cold-water coral, ITS sequencing, *Lophelia*, microsatellite, northeast Atlantic, population structure

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Introduction

Lophelia pertusa is an azooxanthellate scleractinian coral thought to be distributed throughout the world oceans, except in polar seas (Zibrowius 1980; Cairns 1994) and forms deep-water reefs on continental slopes, mid-oceanic ridges and fjords (Rogers 1999). It is the main reef-building species in the northeast Atlantic (Freiwald 1998). The shallowest record for *L. pertusa* is in a Norwegian fjord (39 m) (Rapp & Sneli 1999). On the Mid-Norwegian Shelf, in a water depth of 250–350 m, on the Sula Ridge, *L. pertusa* forms one of the largest deep-water reefs in the northeast Atlantic (Freiwald *et al*. 1999; Fosså *et al*. 2002; Freiwald *et al*. 2002). In the Northern Rockall Trough, numerous small seabed mounds (the Darwin Mounds) support clumps of *L. pertusa* colonies, at 1000-m depth (Bett 2001; Masson *et al*. 2003). North and west of Shetland, at 250–300-m depth, *L. pertusa* forms small, scattered colonies (Wilson 1979a; Bett 1997; Roberts *et al*. 2003). On the Rockall Bank, at depths ranging from 130 to 400 m (Wilson 1979b), in the Southern Rockall Trough, between 500 and 1200 m deep (Kenyon *et al*. 2002), and in the Porcupine Seabight, at depths between 400 and 1000 m (Hovland *et al*. 1994; Kenyon *et al*. 1998; de Mol *et al*. 2002), much larger reefs have been described. In the North Sea, *L. pertusa* was

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observed and collected around an oil platform, at about 100-m depth (Beryl Alpha) (Roberts 2002). *Lophelia pertusa* reefs are also present off Brittany (Le Danois 1948) and along the northern and northwestern shelves off the Iberian Peninsula (Le Danois 1948).

These deep-sea reefs play a major ecological role, notably by increasing the habitat complexity on the continental shelf, slope and seamounts, and are associated with a highly diverse fauna, consisting of epi- and endobionts (Fosså & Mortensen 1998; Rogers 1999; Fosså *et al*. 2002). *Lophelia pertusa* has low growth rates and larger reefs may take thousands of years to grow (Freiwald *et al*. 2002). However, these habitats are under threat from human activities, particularly the development of fisheries targeting deep-sea fish aggregations in the vicinity of the reefs, using 'rockhopper' and other specialized gears (Hall-Spencer *et al*. 2002). Damage to *L. pertusa* from trawling includes the direct mechanical impacts of trawls and trawl-doors (e.g. Fosså *et al*. 2002) and the resuspension of sediments and resettlement over the coral polyps and reef-associated fauna (Roberts, personal communication).

Recent investigations of the reproductive biology of *L. pertusa* reveal that it is a gonochoristic species, with a seasonal gamete production, and a lecithotrophic larval stage (Waller & Tyler 2004). However, it is not known how far the larvae of this species can disperse and whether larva-mediated gene flow is sufficient to maintain the genetic cohesiveness of European populations. These data are important because if gene exchange exists between populations then the loss of areas of reef will not be damaging to the overall genetic diversity of the species. Furthermore, sites damaged by trawling or other activities may be recolonized over time by sexually produced larvae. The analysis of the genetic structure of northeast Atlantic *L. pertusa* populations presented here was a first attempt to understand whether there is genetic cohesion among the European populations, to generate a sustainable management strategy for these diversity-rich deep-sea ecosystems.

Microsatellite markers, because of their high mutation rates (Bruford & Wayne 1993; Jarne & Lagoda 1996) have high levels of polymorphism and are powerful tools for studying population structure (Estoup & Angers 1998) and for identifying individuals or clones (Palsbøll *et al*. 1997; Sunnucks *et al*. 1997); thus they are useful for the investigation of the clonal structure and genetic diversity of asexually reproducing organisms (e.g. Gomez & Carvalho 2000; Reusch *et al*. 2000). A preliminary study, describing the development of 10 microsatellite markers for *L. pertusa* (Le Goff & Rogers 2002) showed the potential of this high-resolution molecular marker and suggested that an important part was played by asexual reproduction, and that a marked genetic structure existed at the level of the six sampling sites investigated. The ribosomal internal transcribed spacers (ITS) are noncoding regions that, because of their high rate of evolution, are useful for elucidating relationships at the level of genus (Chen *et al*. 1996, 2002) and population (Vogler & Desalle 1994; Caporale *et al*. 1997); they have also been used to investigate species complexes in corals (Odorico & Miller 1997; Medina *et al*. 1999).

Here, we present the results from screening 334 individuals taken from 10 different sites distributed along the European margin and in Scandinavian fjords, with the microsatellite markers developed for *L. pertusa*. In addition, 77 individuals from these 10 sites were sequenced for the ITS1 and ITS2 regions. The results are interpreted in terms of the genetic structure of the *L. pertusa* population at both wide and local scales and their significance for management of this species in the northeast Atlantic is discussed.

Materials and methods

Samples

Specimens of *Lophelia pertusa* were collected at 10 sites, distributed along the European margin, and in fjords, using different methods (Table 1). The locations of the sampling

Table 1 Sampling areas and their abbreviations, collecting methods and sample sizes (*n*) for the microsatellites (M) and ITS1 and ITS2 sequencing

Sampling areas	Abbreviation	Collecting methods	*n* M	*n* ITS1 and ITS2
Kosterfjord	KF	ROV*	2	2
Osterfjord	OF	camera-assisted grab	15	5
Trondheimfjord	TF	dredge	6	2
Sula Ridge	SR	dredge and submersible	6	3
North Sea (Beryl Alpha platform)	NS	ROV	10	10
North Rockall Trough (Darwin Mounds)	NRT	Agassiz trawl	53	21
South Rockall Trough	SRT	trawl and ROV	20	6
Porcupine Seabight	PS	trawl and ROV	49	19
La Chapelle	LC	dredge	8	2
La Galicia	LG	trawl	165	7

*ROV, remotely operated vehicle.

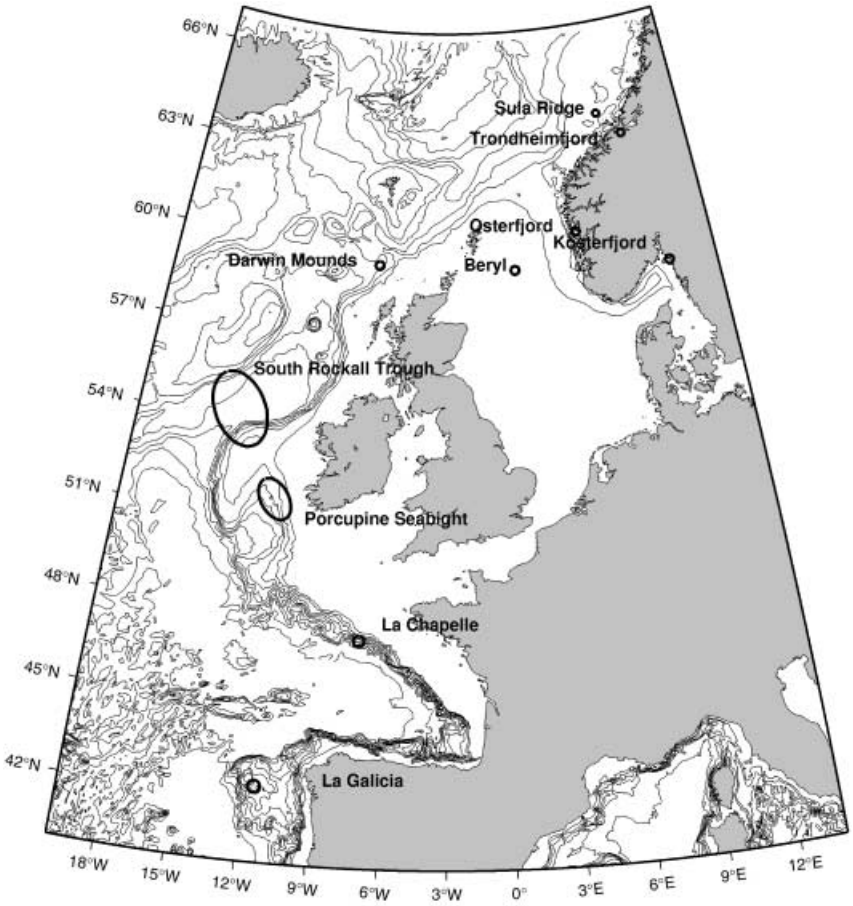

Fig. 1 Sampling sites for *Lophelia pertusa* in the northeast Atlantic marked as circles. This map was built using bathymetric data extracted from the Gebco database (International Hydrographic Bureau 1991) and then processed with GMT (Wessel & Smith 2002). It uses a Cassini projection and a 1–16 000 000 scale.

sites are shown in Fig. 1. Two to three polyps were taken per colony and either placed into 95% ethanol or frozen.

DNA extraction

Two methods were used to extract DNA from the frozen or ethanol-preserved tissue: a high-salt extraction protocol, as described in Le Goff-Vitry *et al*. (2004) and the Qiagen QIAquick DNA extraction kits, following the manufacturer's instructions.

Microsatellites

The 10 microsatellite markers developed for *L. pertusa* (Le Goff & Rogers 2002) were amplified and genotyped following the procedure described in the article referenced above.

Data analyses

Studies of the morphology of *Lophelia* colonies in the northeast Atlantic have revealed that both sexual and asexual reproduction are important in the development of *Lophelia* populations. This coral produces dispersing planula larvae, which settle and metamorphose on a stable substrate (Wilson 1979b). The colony grows mostly through unequal intratentacular budding (Cairns 1995) and new colonies can be formed by fragmentation, when portions of the coral break off and fall onto the sediment (Wilson 1979b). Hence, genetically identical individuals produced asexually need to be distinguished from genetically identical individuals produced sexually by random recombination of the population alleles. A multilocus genotype (MLG) was assigned to each individual. For each MLG occurring more than once, the likelihood of observing it at least as many times in a sexually reproducing population (P_{sex}) was calculated with the binomial expression described by Parks & Werth (1993; eqn 2), using the program MLGSIM (Stenberg *et al*. 2003). This program also calculates the sampling distribution of the P_{sex}-values through simulation to obtain the corresponding critical values (10 000 000 simulations were used in this study). This approach identified the specific MLGs observed more than once that are unlikely to be the result of sexual reproduction. When the P_{sex}-value calculated for a MLG occurring more than once was lower than the critical value obtained for a significance level of $P = 0.05$, the observed MLG was assumed to be a result of asexual reproduction. The individuals estimated to be clones from the same colony were excluded from subsequent analyses because it is only amongst sexually produced individuals that genetic diversity is related to drift and recombination (Reusch *et al*. 2000).

Due to a too-small sample size (two individuals), the site of Kosterfjord was not used for further analyses.

The MICROSATELLITE TOOLKIT (Park 2001) was used to generate input files for ARLEQUIN version. 2.000 (Schneider *et al*. 1997) and FSTAT version 2.9.3.2 (Goudet 2001) software. The sequential Bonferroni test (Rice 1989) was used each time multiple tests were simultaneously carried out. The computer program kindly provided by W. R. Rice (Rice 1989) was used to calculate the minimum table-wide significance of component test statistics.

An exact test of Hardy–Weinberg equilibrium, for each subpopulation, at each locus, was calculated using ARLEQUIN (Schneider *et al*. 1997). The allelic richness was calculated for each locus and for each subppulation using FSTAT version 2.9.3.2 (Goudet 2001).

The program BOTTLENECK version 1.2.02 (Cornuet & Luikart 1996) was used to detect recent reduction of the effective population size for each subpopulation, using the two-phase model of mutation model, with 10% of non-stepwise mutation model mutations, and Wilcoxon test.

Weir & Cockerham's (1984) estimators of inbreeding (f and F, respectively, equivalent to Wright's (1951) F_{IS} and F_{IT}) and their statistical significance were calculated with GENETIX version 4.02 (Belkhir *et al*. 2001) for each subpopulation. The gene diversity (Nei 1987) was calculated for each subpopulation using ARLEQUIN (Schneider *et al*. 1997).

The overall genetic differentiation among all subpopulations was assessed through an analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992), using ARLEQUIN (Schneider *et al*. 1997) and by performing a *G*-test of population differentiation by permuting genotypes (Goudet *et al*. 1996) using FSTAT version 2.9.3.2 (Goudet 2001). These analyses were performed using all subpopulations and a second round of tests was carried out without the fjord subpopulations.

To estimate the genetic differentiation between each pair of subpopulations, θ (Weir & Cockerham 1984) and *Rho* from averaging variance components (Goodman 1997) were calculated using, GENETIX version 4.02 (Belkhir *et al*. 2001) and RST CALC version 2.2 (Goodman 1997), respectively. The statistical significance was tested with permutation tests performed using these software packages.

Stepwise weighted genetic distances (D_{SW}) (Shriver *et al*. 1995) were calculated between subpopulations using the program MICROSAT version 1.5b (Minch *et al*. 1996); this measure was chosen because of its relatively low variance at moderate sample sizes (Ruzzante 1997). They subsequently underwent a multidimensional scaling analysis, with the commercial package PRIMER version 5.2.0.

The hypothesis of 'isolation by distance' (Wright 1943) among the subpopulations distributed along the European margin was tested through a Mantel test (1967), using the program GENETIX Ver. 4.02 (Belkhir *et al*. 2001). The correlation between the matrix of pairwise θ (Weir & Cockerham 1984) and the matrix of geographical distances was assessed through the comparison of Mantel's coefficient (Z), with figures obtained by random permutations. Pairwise geographical distances were calculated using the Gebco database (International Hydrographic Bureau 1991), following the direction of surface currents estimated from New *et al*. (2001), among the following subpopulations: SR, NRT, SRT, PS, LC and LG. These areas were estimated to be separated by a maximum distance of 4396.5 km. Adjacent localities were between 568 and 1140 km apart.

Sequencing of ITS1 and ITS2 regions

Polymerase chain reaction (PCR) amplification of the ITS1 and part of the 5.8S region was carried out using the primers from Chen *et al*. (1996): 1S (5′-GGTACCCTTT-GTACACACCGCCCGTCGCT-3′), A4 (5′-ACACTCAGACA-GACATG-3′). Amplification of the ITS2 region was carried out using the primers ITS2FA (5′-CGAATCTTTGAACGC-AAATG-3′) and ITS2RA (5′-ACAGACGGGGTTGTCACC-3′). These were designed from ITS2 sequences for *L. pertusa*, obtained with primers A7 and 2SS from Chen *et al*. (1996), using Primer 3 (Rozen & Skaletsky 1998). These primers produced more consistent amplifications and cleaner sequences than those from Chen *et al*. (1996) for ITS2 in *L. pertusa*.

PCR reactions were carried out using 20-μL reaction volumes with 2 μL 10× PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 50 pmol of each primer, 2 ng of template DNA and 1 U? *Taq* polymerase (all reagents Qiagen).

Amplification for ITS-1 was carried out using the protocol of Chen *et al*. (1996): one cycle at 95 °C (3 min), 50 °C (1 min), 72 °C (2 min); four cycles at 94 °C (30 s), 50 °C (1 min), 72 °C (2 min); 25 cycles at 94 °C (30 s), 57 °C (1 min), 72 °C (2 min); one cycle at 75 °C (10 min). This was modified for ITS-2 to: one cycle at 95 °C (3 min), 53 °C (1 min), 72 °C (2 min); four cycles at 94 °C (30 s), 53 °C (1 min), 72 °C (2 min); 25 cycles at 94 °C (30 s), 57 °C (1 min), 72 °C (2 min); and one cycle at 75 °C (10 min).

The PCR products were then purified using Qiagen QIAquick spin colums. A cycle-sequencing reaction was carried out using DYEnamic ET terminator reagent premix (Amersham Pharmacia) and 4 μL DNA template. The products were purified using an ethanol precipitation method according to the manufacturer's instructions (Amersham Pharmacia). A MegaBACE 200 (Amersham Pharmacia) automated sequencer was used for the sequencing run. The samples were sequenced in both directions for sequence checking.

Sequence analysis

The DNA fragments obtained after amplification of the ITS1 and ITS2 regions were joined together and analysed as a single sequence for each individual. The sequences were aligned using the multiple alignment program PARALLEL PRRN version 3.0a for UNIX (Gotoh 1996). In a second alignment,

Scapophyllia cylindrica (Odorico & Miller 1997) was chosen as an outgroup because it was the closest sequence for this range of DNA regions available on GenBank. Phylogenetic analyses were carried out with this outgroup, and, in a second set of analyses without it, using the midpoint rooting option. Phylogenies were constructed using PAUP* version 4.0b10 for UNIX (Swofford 2001). The data were analysed using maximum likelihood criteria. The two-parameter Hasegawa–Kishino–Yano nucleotide substitution model (Hasegawa *et al.* 1985) was specified, with a gamma distribution of rate heterogeneity among sites, using parameters provided by the program MODELTEST (Posada & Crandall 1998). Analyses were performed including insertions and deletions (indels), and, in a second round, excluding them. Because of the weakness of the phylogenetic signal of these data, the maximum likelihood tree was used without performing any bootstrap analysis, as a visual representation of the genetic variability present within the population. Trees were displayed using the software TREEVIEW version 1.6.0 (Page 1996).

Results

Microsatellites

The number of MLGs occurring more than once in a subpopulation was variable from site to site; also, the number of such MLGs assumed to result from asexual reproduction was variable (Table 2). A very high proportion

Table 2 In each subpopulation, for each microsatellite MLG occurring more than once, number of individuals showing the same microsatellite MLG, with the corresponding P_{sex}-value and its statistical significance, number of individuals assumed to be asexually produced, and proportion of clonemates

	Microsatellite MLGs occurring more than once				
Sampling area	MLGs	No. individuals with same MLG	P_{sex}-values	No. clonemates detected	Proportion of clonemates
KF	0			0	0
OF	1	2	0.005	0	0
	2	3	< 0.001		
	3	2	< 0.001		
	4	2	< 0.001		
TF	0			0	0
SR	0			0	0
NS	0			0	0
NRT	1	26	< 0.001*	26	0.491
	2	2	< 0.001		
	3	2	< 0.001		
	4	2	< 0.001*		
	5	2	< 0.001		
SRT	0			0	0
PS	1	4	< 0.001*	10	0.204
	2	5	< 0.001*		
	3	2	< 0.001*		
	4	3	< 0.001*		
LC	1	2	< 0.001	0	0
LG	1	2	< 0.001*	25	0.152
	2	10	< 0.001*		
	3	2	< 0.001*		
	4	2	< 0.001*		
	5	2	< 0.001*		
	6	3	< 0.001*		
	7	2	< 0.001*		
	8	2	< 0.001*		
	9	2	< 0.001*		
	10	2	< 0.001*		
	11	2	< 0.001*		
	12	2	< 0.001*		
	13	2	< 0.001*		
	14	2	< 0.001*		
	15	3	< 0.001*		

$P <$ critical value for $P = 0.05$.

Table 3 For each subpopulation, expected and observed heterozygosity, and allelic richness at each locus, inbreeding coefficients (*f*-value) (Weir & Cockerham 1984), and gene diversity (mean ± standard deviation) (Nei 1987), obtained with microsatellite data

Locus		OF	TF	SR	NS	NRT	SRT	PS	LC	LG
1	H_E	0.522	0.924	0.929	0.805	0.744	0.928	0.929	0.788	0.917
	H_O	0.600	0.833	0.750	0.300**	1.000**	0.917**	0.462**	0.833	0.678**
	Allelic richness	1.434	1.909	1.893	1.747	1.744	1.928	1.918	1.773	1.916
2	H_E	/	0.378	/	0.425	0.481	0.484	0.508	0.643	0.442
	H_O		0.200		0.375	0.667**	0.222	0.436	0.250	0.241**
	Allelic richness	1.000	1.200	1.000	1.325	1.454	1.392	1.507	1.607	1.432
3	H_E	/	/	0.833	0.542	0.187	0.314	0.450	/	0.432
	H_O			1.000	0.000*	0.200	0.222	0.436*		0.376**
	Allelic richness	1.000	1.000	1.833	1.433	1.187	1.216	1.450	1.000	1.432
4	H_E	/	0.455	/	0.621	0.748	0.795	0.774	0.733	0.821
	H_O		0.000		0.500	0.720**	0.800	0.615**	0.500	0.554**
	Allelic richness	1.000	1.303	1.000	1.621	1.731	1.795	1.757	1.633	1.819
5	H_E	0.657	0.727	0.800	0.868	0.810	0.892	0.906	0.883	0.909
	H_O	0.067**	0.500	0.000	0.900*	0.826**	0.333**	0.763**	1.000**	0.604**
	Allelic richness	1.563	1.621	1.533	1.868	1.805	1.869	1.902	1.883	1.906
6	H_E	0.632	0.758	0.924	0.879	0.629	0.892	0.837	0.835	0.894
	H_O	1.000**	0.833	0.833	0.900*	0.440**	0.846**	0.795**	0.714	0.722**
	Allelic richness	1.632	1.742	1.909	1.879	1.628	1.874	1.836	1.802	1.891
7	H_E	0.517	0.682	0.848	0.774	0.744	0.801	0.882	0.867	0.854
	H_O	1.000**	0.500	0.167**	0.800*	1.000**	0.917	0.897**	0.500*	0.550**
	Allelic richness	1.517	1.530	1.742	1.726	1.744	1.801	1.880	1.800	1.852
8	H_E	0.549	0.803	0.894	0.905	0.792	0.927	0.880	0.825	0.944
	H_O	1.000**	0.000*	0.667	0.600**	0.960**	0.789**	0.641**	0.625*	0.765**
	Allelic richness	1.549	1.667	1.848	1.879	1.792	1.916	1.869	1.758	1.943
9	H_E	0.517	0.788	0.742	0.700	0.528	0.736	0.737	0.658	0.841
	H_O	1.000**	1.000	0.500	0.800	0.960**	0.450**	0.897*	0.625	0.633**
	Allelic richness	1.517	1.788	1.652	1.658	1.528	1.712	1.737	1.508	1.839
10	H_E	/	0.682	0.894	0.753	0.767	0.409	0.844	0.683	0.710
	H_O		1.000	0.833	0.300**	0.783**	0.385	0.692**	0.625	0.679**
	Allelic richness	1.000	1.682	1.864	1.721	1.760	1.348	1.839	1.533	1.708
f		−0.476	0.115	0.264*	0.211**	−0.190	0.146**	0.133**	0.110	0.251**
Gene diversity		0.9149 ± 0.0252	0.9848 ± 0.0403	1.0000 ± 0.0340	0.9895 ± 0.0193	0.9829 ± 0.0086	0.9962 ± 0.0066	0.9980 ± 0.0024	0.9833 ± 0.0278	0.9991 ± 0.0004

/monomorphic locus; *H_O is significantly different from H_E at $P = 0.05$ level; ** at $P = 0.01$ level, after sequential Bonferroni correction.

of clones was detected for the site of NRT, suggesting the prevalence of asexual reproduction there. In contrast to other sites, where similar MLGs were shared by a few individuals, one clone dominates the Darwin Mounds subpopulation. For the Osterfjord subpopulation, we could not reject the hypothesis that the MLGs occurring more than once were the result of sexual reproduction, and the identical MLGs might be the result of recombination.

The exact test of Hardy–Weinberg equilibrium (Guo & Thompson 1992) revealed strong deviations for all subpopulations and at most of the loci (Table 3). Both excesses and deficits of heterozygotes were reported. Some subpopulations showed a global deficit of heterozygotes (NS, SRT, PS, LG), others a global excess of heterozygotes (OF, NRT).

There was a significant excess of observed gene diversity compared to the expected equilibrium gene diversity for two subpopulations: Osterfjord (at $P = 0.01$ level, after the Bonferroni correction), and Darwin Mounds (at $P = 0.05$ level, but not after the Bonferroni correction). This could suggest that these subpopulations have experienced a genetic bottleneck (Luikart *et al*. 1998). For the Trondheimfjord, Sula Ridge and La Chapelle subpopulations, this test could not be performed, as a minimum of 10 individuals per subpopulation is required (Cornuet & Luikart 1996).

The *F*-value (Weir & Cockerham 1984) was significant (at $P = 0.05$ level) and positive (0.264), revealing an excess of homozygotes in the compound population. When performed over all subpopulations, the *G*-test of population differentiation (Goudet *et al*. 1996) revealed a significant differentiation among subpopulations ($P = 0.001$). When the fjords populations were excluded from the analysis, a significant differentiation among subpopulations was still reported ($P = 0.001$).

Table 4 Pairwise estimates of genetic differentiation: θ (Weir & Cockerham 1984) (above diagonal) and *Rho* from averaging variance components (Goodman 1997) (below diagonal) obtained with microsatellite data

	OF	TF	SR	NS	NRT	SRT	PS	LC	LG
OF		0.4811**	0.4631**	0.3965**	0.3669**	0.3519**	0.2837**	0.4385**	0.2510**
TF	0.4899*		0.2652**	0.1905**	0.1973**	0.1663**	0.1500**	0.2519**	0.1224**
SR	0.4850*	0.1857*		0.1633*	0.2394**	0.1156*	0.0635*	0.1647*	0.1018*
NS	0.4939*	0.2619*	0.0961*		0.1558**	0.1084**	0.0792**	0.1604**	0.0653**
NRT	0.5100*	0.1810*	0.0545*	0.1651*		0.1516**	0.1289**	0.2044**	0.0831**
SRT	0.4692*	0.2442*	−0.0110*	0.1651*	0.0928*		0.0580**	0.0551*	0.0454**
PS	0.6096*	0.1475*	0.0233*	0.1389*	0.1179*	0.1173*		0.0820**	0.0442**
LC	0.4969*	0.1609*	0.0254*	0.0699*	0.0183*	0.0416*	0.0457*		0.0656**
LG	0.5069*	0.1868*	0.0412*	0.0902*	0.0810*	0.0806*	0.0534*	0.0293*	

θ estimates: *$P < 0.05$; **$P < 0.01$, after sequential Bonferroni correction.
Rho estimates: *$P < 0.05$.

When all subpopulations were included, the AMOVA (Excoffier *et al*. 1992) showed that 12.93% of the variation was distributed among subpopulations, and the fixation index was significant (Weir & Cockerham 1984) ($F_{ST} = 0.1293$; with $P = 0.0000$). Without the fjords subpopulations, 9.98% of the variation was distributed among subpopulations and the fixation index was still significant ($F_{ST} = 0.0998$; with $P = 0.0000$).

The calculation of pairwise fixation indices (Table 4) revealed a moderate genetic differentiation between the subpopulations distributed along the continental margin, and a great to very great genetic differentiation between the two fjords and between the fjords and the other sites.

The multidimensional scaling of genetic distances has proved to be a useful approach in population structure studies (Lessa 1990). Here, this treatment performed on the stepwise weighted genetic distances (D_{SW}) (Shriver *et al*. 1995) revealed the high genetic differentiation of the fjords subpopulations from the others (data not shown). The points corresponding to each of the fjords were far apart from one another, and from those points representing the continental margin sites. The latter were all tightly grouped together. The low stress value (0) reveals the high significance of this representation.

Mantel's correlation coefficient was not found to be statistically significant, as a result of the permutation test ($P = 0.5844$). This result suggests that the observed genetic differentiation among the subpopulations distributed along the European margin cannot be explained solely by geographical isolation.

ITS1 and ITS2 sequences

The length of the sequences obtained varied between 834 and 1004 base pairs (bp), corresponding to a partial sequence of the gene coding for the 18S ribosomal RNA, the ITS1 region, the gene encoding for the 5.8S ribosomal RNA, the ITS2 region, and a partial sequence of the gene coding for the 28S ribosomal RNA. The aligned sequences were 1235 bp long.

The alignment is available on GenBank (accession numbers: AY257253 to AY257337, AY354196 and AY354197).

The topologies obtained with the outgroup and without it were identical, and the topology obtained when the indels were considered and when they were excluded were very similar. The topology presented in Fig. 2 results from the analysis performed with the outgroup, and with indels removed. The tree did not show any clear geographical pattern. Some common haplotypes were found across multiple locations (LG, LC and OF), and more divergent and rare haplotypes were present at different locations, especially NS, NRT and PS. The individuals from the fjords were found in clades where individuals from the same fjord origin were present, and these clades were distinct one from another. However, the haplotypes found in the fjords were not distinct from those found in the offshore sites.

Discussion

Proportion of clonemates and genetic diversity

The proportion of clonemates varied dramatically from site to site, and was greater at the Darwin Mounds site than at any other. *Lophelia* does not form large reefs at the Darwin Mounds but is found as small colonies, aggregated on the top of small sand mounds created by fluid escape (Masson *et al*. 2003). Corals are rare, and form small and widely scattered colonies on gravel substrates in areas adjacent to the mounds. *Lophelia* benefits from an elevated position on the mounds, associated with an enhanced bottom current velocity, important for suspension-feeding organisms (Masson *et al*. 2003). The low number of MLGs encountered in this area may result from low recruitment

Fig. 2 Phylogenetic relationships obtained with the ITS1 and ITS2 sequences, resulting from the maximum likelihood searches. On the scale bar, the units are substitution per site.

rates of sexually produced larvae, caused by the patchiness of suitable habitat, and the high number of clonemates could be explained by the subsequent colonization of the available space through asexual reproduction. Investigations on the reproductive biology of *Lophelia pertusa* have revealed a complete absence of reproductive corals at this site (Waller & Tyler 2004). This area is intensively affected by bottom trawling (Bett 2001) which, through the mechanical destruction of the reefs, may impede sexual reproduction by fragmentation to below the minimum size for reproduction (Waller & Tyler 2004).

The gene diversity indices (Nei 1987), calculated on the basis of sexually produced individuals only, revealed a spatially heterogeneous distribution of genetic variability. The lowest value was reported for the Osterfjord. At this site, several similar MLGs were encountered, although the sample size was very small; these were not statistically unlikely to be the result of sexual reproduction, and might be the result of genetic recombination. Such a situation could reflect a bottleneck arising from the founding of the population. Among the continental margin subpopulations, the Darwin Mounds area had the lowest gene diversity, consistent with the hypothesis formulated above.

Deviations from Hardy–Weinberg equilibrium

Strong deviations from Hardy–Weinberg equilibrium were detected for all subpopulations, for a majority of loci. The presence of null alleles may result in heterozygote deficits (Pemberton *et al*. 1995), but it is unlikely that all of the studied loci could be affected by this phenomenon. Both excesses and deficits of heterozygotes are encountered, with strong differences among sites. Potential bottlenecks were detected for the Osterfjord and Darwin Mounds subpopulations, although the mechanisms for these are not obvious, except in the case of the Osterfjord subpopulation, thought to result from a small founding population. The significant heterozygosity excess in NRT could also be caused by the importance of asexual reproduction to the maintenance of this subpopulation. High heterozygosities have been shown in asexual lineages of aphids (Sunnucks *et al*. 1996; Simon *et al*. 1999; Wilson *et al*. 1999). Delmotte *et al*. (2002) have shown that asexual populations of the aphid *Rhopalosiphum padi* were characterized by a lower allelic polymorphism, and a higher heterozygosity at most microsatellite loci, than sexual populations. This could be explained by the rapid increase of neutral sequence divergence between two alleles of a locus within individual genomes in asexual lineages, which is hampered by recombination and large population size in sexually reproducing populations (Birky 1996). Inbreeding indices have proved statistically significant for many sites (Sula Ridge, Beryl, South Rockall Trough, Porcupine Seabight and La Galicia) and may be explained by a spatially restricted effective gene flow and a predominance of self-recruitment of sexually produced larvae within subpopulations. Heterozygote deficits have also been described in many shallow-water coral populations (e.g.

Ayre & Hughes 2000; Ridgway *et al*. 2001) and have often been hypothesized as a consequence of localized recruitment via restricted dispersal of gametes or larvae and inbreeding.

Geographic differentiation

The microsatellite data clearly indicate genetic structuring among the sampled locations, that is greater among fjord populations than those in the open sea.

Indirect estimates of gene flow were not calculated using the statistics of population differentiation because natural populations very rarely meet the underlying assumptions (Bossart & Prowell 1998; Whitlock & McCauley 1999). The *Lophelia* subpopulations studied clearly violate these assumptions; some of them have undergone recent bottlenecks, they are not in Hardy–Weinberg equilibrium and they show differences in the level of population differentiation between subpopulations. *F*-statistics were simply used as unbiased estimators to quantify the distribution of genetic variation among subpopulations and to qualify the level of gene flow occurring among them.

The moderate genetic differentiation reported among the subpopulations distributed along the European margin suggests that a certain genetic cohesion is maintained over the open slope over long periods of time. Because of asexual proliferation and the longevity of the clones, even a low-level and sporadic recruitment of sexually produced larvae from outside a given subpopulation might be sufficient to maintain such cohesion.

Wide-scale allozyme studies of the population structure of shallow-water corals have shown a range of genetic structures (e.g. Adjeroud & Tsuchiya 1999; Ayre & Hughes 2000). Ayre & Hughes (2000) showed that, whatever the breeding mode (brooder or spawner), some species did not display any genetic differentiation along the 1200 km of the Great Barrier Reef, whereas others did. Because genetic subdivision was found among sites on each reef, they hypothesized that large-scale genetic structures are determined by historical patterns of gene flow and chance long-distance dispersal events (Ayre & Hughes 2000).

Lophelia pertusa is gonochoristic, with a seasonal broadcast of gametes, and a high fecundity (3327 oocytes cm^{-2}), the planulae might be lecithotrophic, as the oocyte size could reach an average of 350 μm (Waller & Tyler 2004). These larvae could be transported all along the European margin by the Shelf Edge Current, a surface current which travels northwards from the Iberian margin to the Norwegian Sea (New *et al*. 2001). In the northeast Atlantic the cold temperatures, increased disturbance and low levels of phytodetritus available in the water column for planktotrophic larvae to feed on around January/February, when spawning is supposed to occur, imply that the larvae need their own food support, depending on the amount of time the embryo takes to develop to the feeding stage (Waller & Tyler 2004). Long-distance dispersal is likely to play a major role over long time scales, and the colonization of man-made structures by *L. pertusa*, such as submarine cables, ship wrecks, as well as the Brent Spar oil-storage buoy and moorings from the Beryl Alpha oil production platform (Roberts 2002), are consistent with sporadic long-distance dispersal.

No correlation between genetic differentiation and geographical distance was found among the subpopulations distributed along the European margin. This result could be explained by continuing long-distance genetic exchange, disequilibria between gene flow and drift, or barriers to gene flow (see Hellberg 1995).

The fjord subpopulations were highly genetically differentiated from the continental margin subpopulations, suggesting a very low gene flow between these groups. Genetic differentiation between fjord organisms and offshore ones has been described for many species (e.g. Turan *et al*. 1998; Drengstig *et al*. 2000; Suneetha & Nævdal 2001; Suneetha & Salvanes 2001). Local geological and hydrological characteristics (Farmer & Freeland 1983) can explain the limited gene flow between the fjord populations and those in offshore waters, and the genetic differentiation that has developed in the 10 000 years since the creation of the fjords. The partial isolation of fjord subpopulations may represent incipient allopatric speciation (Suneetha & Nævdal 2001).

The results obtained with the ITS1 and ITS2 sequences support some of the conclusions reached with the microsatellite data. The fjord populations appeared genetically distinct from one another but a higher genetic exchange among the subpopulations distributed along the European margin was shown by the distribution of individuals from all these sites throughout the topology. The fact that individuals from the fjords grouped with individuals from the continental margin could be a result of the colonization of the fjords by migrants originating from offshore populations. The presence of only one haplotype in the Osterfjord is consistent with the bottleneck and low gene diversity described with the microsatellites.

However, some results obtained with the ITS1 and -2 sequences are not consistent with those obtained with the microsatellites. At La Galicia, only one haplotype was encountered, and it corresponds to a haplotype found across most of the sites, which suggests a very low genetic diversity at this site. In contrast, the site of Darwin Mounds showed a high diversity of haplotypes, and some highly divergent ones, which is contradictory with the microsatellite results. Some ambiguous base-calling on the sequences could be interpreted as cases of heterozygosity, or intragenomic variability. Although concerted evolution is expected to homogenize all copies of the rDNA families within individuals (Arnheim *et al*. 1980), intraindividual variability

has been detected through the cloning and subsequent sequencing of ITS PCR products (e.g. Vogler & DeSalle 1994; Odorico & Miller 1997; Harris & Crandall 2000; Chen *et al*. 2002). The degree of intraindividual variation in the ITS varies from species to species, depending on the balance between the rate of homogenization and the rate of new mutation (Ohta & Dover 1983). Because the use of ITS sequences as a molecular marker relies on the assumption that the rDNA array is evolving as a single molecule, the analysis of nonhomogeneous sequences for population or phylogenetic investigation is to be interpreted cautiously (see Harris & Crandall 2000). The results obtained in this study through the direct sequencing of ITS 1 and 2 regions could be biased by the presence of intraindividual variability.

Conservation implications

Deep-sea coral communities are being increasingly affected by human activities, mainly through the direct and indirect effects of expanding deep-sea fisheries, both worldwide (Probert *et al*. 1997; Koslow & Gowlett-Holmes 1998; Koslow *et al*. 2001) and in many areas of the northeast Atlantic (Bett 2001; Gage 2001; Fosså *et al*. 2002; Roberts *et al*. 2003). In the latter region, *L. pertusa* appears to be of major importance, as the main reef-building species, and species diversity on *Lophelia* reefs is three times higher than in the surrounding soft-bottoms (UK Biodiversity Group 2000). Video observations have revealed the dense aggregations of gravid redfish females on the reefs and fishermen have reported the influence of the presence of reefs on fish abundance (Fosså *et al*. 2002). Understanding the population structure, dispersal capacities, and development pattern of *L. pertusa* along the European margin is fundamental in the generation of a sustainable management strategy for these diversity-rich deep-sea ecosystems

This study has revealed that *L. pertusa*, distributed from off the Iberian margin to the Scandinavian fjords, cannot be considered as one panmictic population, but is formed by genetically distinct, offshore and fjord, subpopulations. Along the continental slope, the subpopulations are moderately differentiated. Although larvae might be dispersed along the European margin through surface currents, the gene flow occurring among these subpopulations is likely to be moderate, and must be considered in the light of the age of these coral communities, the prevalence of asexual reproduction in the development of the reefs and the longevity of individual clones. Inbreeding was shown at several sites, suggesting that there is a high degree of self-recruitment in these subpopulations.

Effective conservation measures, such as the designation of a protected area where bottom trawling is banned, would be necessary to protect vulnerable populations. The Darwin Mounds have recently been protected by an emergency measure (European Commission Regulation no. 1475/2003), which prohibits the use of bottom-trawl or similar bottom-towed nets in this area, and might be designated as a Special Area of Conservation, under the European Habitats Directive (92/43/EEC).

Despite the low number of samples available from fjords, these subpopulations have been shown to be highly differentiated. Careful monitoring of the fjords subpopulations should be undertaken, as they are particularly sensitive to genetic drift, because of their small sizes, and are vulnerable to coastal pollution.

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References

Adjeroud M, Tsuchiya M (1999) Genetic variation and clonal structure in the scleractinian coral *Pocillopora damicornis* in the Ryukyu Archipelago, southern Japan. *Marine Biology*, **134**, 753–759.

Arnheim N, Krystal M, Schmickel R *et al*. (1980) Molecular evidence for genetic exchanges among ribosomal genes on non-homologous chromosomes in man and apes. *Proceedings of the National Academy of Sciences of the USA*, **77**, 7323–7327.

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution*, **54**, 1590–1605.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) Genetix: logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions: CNRS UMR. 5000, Université de Montpellier II, Montpellier.

Bett BJ (1997) *Cruise report 7. RRS Charles Darwin cruise 101C leg 2. 14 July–20 August 1996 Atlantic Margin Environmental Survey: Seabed Survey of the Shelf Edge and Slope West of Scotland*. Challenger Division for Seafloor Processes, Southampton Oceanography Centre, Southampton.

Bett BJ (2001) UK Atlantic margin environmental survey: introduction and overview of bathyal benthic ecology. *Continental Shelf Research*, **21**, 917–956.

Birky CW (1996) Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics*, **144**, 427–437.

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution*, **13**, 202–206.

Bruford M, Wayne R (1993) Microsatellites and their applications to population genetic studies. *Current Opinion in Genetics and Development*, **3**, 939–943.

Cairns SD (1994) *Scleractinia of the Temperate North Pacific*. Smithsonian Institution Press, Washington DC.

Cairns SD (1995) The marine fauna of New Zealand: Scleractinia (Cnidaria: Anthozoa). *New Zealand Oceanographic Institute Memoirs*, **103**, 1–210.

Caporale DA, Beal BF, Roxby R, Beneden RJ (1997) Population structure of *Mya arenaria* along the New-England coastline. *Molecular Marine Biology and Biotechnology*, **6**, 33–39.

Chen CA, Willis BL, Miller DJ (1996) Systematic relationships between tropical corallimorpharians (Cnidaria: Anthozoa: Corallimorpharia): utility of the 5.8s and internal transcribed spacer (ITS) regions of the rRNA transcription unit. *Bulletin of Marine Science*, **59**, 196–208.

Chen CA, Chen CP, Fan TY, Yu JK, Hsieh HL (2002) Nucleotide sequences of ribosomal internal transcribed spacers and their utility in distinguishing closely related *Perinereis* polychaetes (Annelida; Polychaeta; Nereididae). *Marine Biotechnology*, **4**, 17–29.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Delmotte F, Leterme N, Gauthier J-P, Rispe C, Simon J-C (2002) Genetic architecture of sexual and asexual populations of the aphid *Rhopalosiphum padi* based on allozymes and microsatellite markers. *Molecular Ecology*, **11**, 711–723.

Drengstig A, Fevolden S-E, Galand PE, Aschan MM (2000) Population structure of the deep-sea shrimp (*Pandalus borealis*) in the north-east Atlantic based on allozyme variation. *Aquatic Living Ressources*, **13**, 121–128.

Estoup A, Angers B (1998) Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. In: *Advances in Molecular Ecology* (ed. Carvalho G), pp. 55–86. IOS Press, Amsterdam.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplogroups: applications to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Farmer DM, Freeland HJ (1983) The physical oceanography of the fjords. *Progress in Oceanography*, **12**, 147–220.

Fosså JH, Mortensen PB (1998) Artsmangfoldet pa *Lophelia*-korallrev og metoder for kartlegging og overakning (Diversity of species associated with *Lophelia* coral reefs and methods for mapping and monitoring). *Fisken Og Havet*, **2**, 1–94.

Fosså JH, Mortensen PB, Furevik DM (2002) The deep-water coral *Lophelia pertusa*. Norwegian waters: distribution and fishery impacts. *Hydrobiologia*, **471**, 1–12.

Freiwald A (1998) *Geobiology of Lophelia Pertusa (Scleractinia) Reefs in the North Atlantic*. Universitat Bremen, Bremen.

Freiwald A, Wilson JB, Henrich R (1999) Grounding Pleistocene iceberg shape recent deep-water coral reefs. *Sedimentary Geology*, **125**, 1–8.

Freiwald A, Huhnerbach V, Lindberg B, Wilson JB, Campbell J (2002) The Sula Reef Complex, Norwegian Shelf. *Facies*, **47**, 179–200.

Gage JD (2001) Deep-sea benthic community and environment impact assessment at the Atlantic frontier. *Continental Shelf Research*, **21**, 957–986.

Gomez A, Carvalho GR (2000) Sex, parthenogenesis and genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. *Molecular Ecology*, **9**, 203–214.

Goodman SJ (1997) Rst Calc: a collection of computer programs for calculating unbiaised estimates of genetic differentiation and determining their significance for microsatellite data. *Molecular Ecology*, **6**, 881–885.

Gotoh O (1996) Significant improvement in accuracy of multiple protein sequence alignments by iterative refinement as assessed by reference to structural alignmemts. *Journal of Molecular Biology*, **264**, 823–838.

Goudet J (2001) *Fstat: a Program to Estimate and Test Gene Diversities and Fixation Indices*. Institute of Ecology, Biology Building, UNIL, Lausanne, Switzerland.

Goudet J, Raymond M, de Meeiis T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Guo S, Thompson E (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.

Hall-Spencer J, Allain V, Fosså JH (2002) Trawling damage to Northeast Atlantic ancient coral reefs. *Proceedings of the Royal Society (B)*, **269**, 507–511.

Harris DJ, Crandall KA (2000) Intragenomic variation within ITS1 and ITS2 of crayfish (Decapoda: Cambaridae): implications for phylogenetic and microsatellite studies. *Molecular Biology and Evolution*, **17**, 284–291.

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitchondrial DNA. *Journal of Molecular Evolution*, **22**, 160–174.

Hellberg ME (1995) Stepping-stone gene flow in the solitary coral *Balanophyllia elegans*: equilibrium and non-equilibrium at different spatial scales. *Marine Biology*, **123**, 573–581.

Hovland M, Croker PF, Martin M (1994) Fault-associated seabed mounds (carbonate knolls?) off western Ireland and north-west Australia. *Marine and Petroleum Geology*, **11**, 232–246.

International Hydrographic Bureau (1991) *Gebco Database*. International Hydrographic Bureau, Monaco.

Jarne P, Lagoda PJL (1996) Microsatellites from molecules to populations and back. *Trends in Ecology and Evolution*, **11**, 424–429.

Kenyon NH, Ivanov MK, Akmetzhanov AM (1998) *Cold Water Carbonate Mounds and Sediment Transport on the Northeast Atlantic Margin*. UNESCO, Paris.

Kenyon NH, Akmetzhanov AM, Wheeler AJ *et al.* (2002) Giant carbonate mud mounds in the southern Rockall Trough. *Marine Geology*, **3264**, 1–26.

Koslow JA, Gowlett-Holmes K (1998) *The Seamount Fauna Off Southern Tasmania: Benthic Communities, Their Conservation and Impacts of Trawling*. Final report to Environment Australia and the Fisheries Reseach and Development Corporation. CSIRO, Hobart, Tasmania.

Koslow JA, Gowlett-Holmes K, Lowry J (2001) Seamount benthic macrofauna off southern Tasmania: community structure and impacts of trawling. *Marine Ecology Progress Series*, **213**, 111–125.

Le Danois E (1948) *Les Profondeurs de la Mer*. Ed. Payot, Paris.

Le Goff MC, Rogers AD (2002) Characterization of 10 microsatellite loci for the deep-sea coral *Lophelia pertusa* (Linnaeus 1758). *Molecular Ecology Notes*, **2**, 164–166.

Le Goff-Vitry M, Rogers AD, Baglow D (2004) A deep-sea slant on the molecular phylogeny of the Scleractinia. *Molecular Phylogenetics and Evolution*, **30**, 167–177.

Lessa EP (1990) Multidimensional analysis of geographic genetic structure. *Systematic Zoology*, **39**, 242–252.

Luikart GL, Allendrof FW, Cornuet J-M, Sherwin W (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238–247.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Masson DG, Bett BJ, Billet DSM *et al.* (2003) The origin of deep-water, coral-topped mounds in the northern Rockall Trough, Northeast Atlantic. *Marine Geology*, **194**, 159–180.

Medina M, Weil E, Szmant AM (1999) Examination of the *Monastrea annularis* species complex (Cnidaria: Scleractinia) using ITS and COI sequences. *Marine Biotechnology*, **1**, 89–97.

Minch E, Ruiz-Linares A, Goldstein D, Feldman M, Cavalli-Sforza LL (1996) *Microsat Version 1.5: a Computer Program for Calculating Various Statistics on Microsatellite Allele Data*. Stanford University, Stanford, CA.

de Mol B, van Rensbergen P, Pillen S *et al.* (2002) Large deep-water coral banks in the Porcupine Basin, southwest of Ireland. *Marine Geology*, **188**, 193–231.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

New A, Barnard S, Herrmann P, Molines J-M (2001) On the origin and pathway of the saline inflow to the Nordic Seas: insights from models. *Progress in Oceanography*, **48**, 255–287.

Odorico DM, Miller DJ (1997) Variation in the ribosomal internal transcribed spacers and 5.8s rDNA among five species of *Acropora* (Cnidaria; Scleractinia): patterns of variation consistent with reticulate evolution. *Molecular Biology and Evolution*, **14**, 465–473.

Ohta T, Dover GA (1983) Population genetics of multigene families that are dispersed into two or more chromosomes. *Proceedings of the National Academy of Sciences of the USA*, **80**, 4079–4083.

Page RDM (1996) TEEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, **12**, 357–358.

Palsbøll P, Allen J, Berube M (1997) Genetic tagging of humpback whales. *Nature*, **388**, 767–769.

Park SDE (2001) *Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection*. PhD Thesis, University of Dublin, Ireland.

Parks JC, Werth CR (1993) A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany*, **80**, 537–544.

Pemberton JM, Slate J, Bancroft DR, Barrett JA (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology*, **4**, 249–252.

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Probert PK, McKnight DG, Grove SL (1997) Benthic invertebrate by-catch from a deep-water trawl fishery, Chatham Rise, New Zealand. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **7**, 27–40.

Rapp HT, Sneli JA (1999) *Lophelia pertusa* — myths and reality abstract only. In: *2nd Nordic Marine Sciences Meeting*, Hirtshals, Denmark 2nd–4th March 1999.

Reusch TBH, Stam WT, Olsen JL (2000) A microsatellite-based estimation of clonal diversity and population subdivision in *Zostera marina*, a marine flowering plant. *Molecular Ecology*, **9**, 127–140.

Rice WR (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.

Ridgway T, Hoegh-Guldberg O, Ayre DJ (2001) Panmixia in *Pocillopora verrucosa* from South Africa. *Marine Biology*, **139**, 175–181.

Roberts JM (2002) The occurrence of the coral *Lophelia pertusa* and other conspicuous epifauna around an oil platform in the North Sea. *Journal of the Society for Underwater Technology*, **25**, 83–91.

Roberts JM, Long D, Wilson JB, Mortensen PB, Gage JD (2003) The cold-water coral *Lophelia pertusa* (Scleractinia) and enigmatic seabed mounds along the north-east Atlantic margin: are they related? *Marine Pollution Bulletin*, **46**, 7–20.

Rogers AD (1999) The biology of *Lophelia pertusa* (Linnaeus 1758) and other deep-water reef-forming corals and impacts from human activities. *International Revue of Hydrobiology*, **84**, 315–406.

Rozen S, Skaletsky HJ (1998) Primer 3. Code available at: http://www-genome.wi.mit.edu/genome_software/other/primer3.thml.

Ruzzante DE (1997) A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1–14.

Schneider S, Kueffer JM, Roessli D, Excoffier L (1997) *Arlequin: A Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Shriver MD, Jin L, Boerwinkle E, *et al.* (1995) A novel measure of genetic distance for highly polymorphic tandem repeat loci. *Molecular Biology and Evolution*, **12**, 914–920.

Simon JC, Baumann S, Sunnucks P *et al.* (1999) Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. *Molecular Ecology*, **8**, 531–545.

Stenberg P, Lundmark M, Saura A (2003) MLGsim: a program for detecting clones using a simulation approach. *Molecular Ecology Notes*, **3**, 329–331.

Suneetha KB, Nævdal G (2001) Genetic and morphological stock structure of the pearlside, *Maurolicus muelleri* (Pisces, Sternoptychidae), among Norwegian fjords and offshore area. *Sarsia*, **86**, 191–201.

Suneetha KB, Salvanes AGV (2001) Population genetic structure of the glacier lanternfish, *Benthosema glaciale* (Myctophidae) in Norwegian waters. *Sarsia*, **86**, 203–212.

Sunnucks P, de Barro PJ, Lushai G, MacLean N, Hales D (1997) Genetic structure of an aphid using microsatellites: cyclic parthenogenesis, differentiated lineages, and host specialization. *Molecular Ecology*, **7**, 339–353.

Sunnucks P, England PR, Taylor AC, Hales DF (1996) Microsatellite and chromosome evolution of parthenogenic *Sitobion* aphids in Australia. *Genetics*, **144**, 747–756.

Swofford DL (2001) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, MA.

Turan C, Carvalho GR, Mork J (1998) Molecular genetic analysis of Atlanto-Scandian herring (*Clupea harengus*). *Journal of Marine Biology Association of UK*, **78**, 269–283.

UK Biodiversity Group (2000) *Tranche 2 Action Plans, V –. Maritime Species and Habitats*. UK Biodiversity Group, Peterborough.

Vogler AP, DeSalle R (1994) Evolution and phylogenetic information content of the ITS-1 region in the tiger bettle *Cincindela dorsalis*. *Molecular Biology and Evolution*, **11**, 393–405.

Waller RG, Tyler PA (2004) The reproductive ecology of two deep-sea reef-building scleractinlans from the NE Atlantic Ocean. *Coral Reefs*, in press.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1360.

Wessel P, Smith WHF (2002) GMT: The Generic Mapping Tools ANSI-, POSIX-, and Y2k-compliant free software and data. Code available at: http://www.soest.hawaii.edu/gmt.

Whitlock M, McCauley D (1999) Indirect measures of gene flow and migration: F_{ST} not equal 1/(4*Nm* + 1). *Heredity*, **82**, 117–125.

Wilson JB (1979a) The distribution of the coral *Lophelia pertusa* (L.) [*L. prolifera* (Pallas)] in the north east Atlantic. *Journal of the Marine Biology Association of the United Kingdom*, **59**, 149–162.

Wilson JB (1979b) 'Patch' development of the deep-water coral *Lophelia pertusa* (L.) on Rockall Bank. *Journal of the Marine Biology Association of the United Kingdom*, **59**, 165–177.

Wilson AAC, Sunnucks P, Hales D (1999) Microevolution, low clonal diversity and genetic affinities of parthenogenetic *Sitobion* aphids in New Zealand. *Molecular Ecology*, **8**, 1655–1666.

Wright S (1943) Isolation by distance. *Genetics*, **28**, 139–156.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 114–138.

Zibrowius H (1980) Les Scléractiniaires de la Méditerranée et de l'Atlantique nord-oriental. *Mémoires de l'Institut Oceanographique, Monaco*, **11**, 1–227.

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