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ORIGINAL PAPER

Fine-scale spatial genetic structure in the brooding sea urchin *Abatus cordatus* suggests vulnerability of the Southern Ocean marine invertebrates facing global change

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Abstract The Southern Ocean benthic communities are characterized by their levels of endemism and their diversity of invertebrate brooding species. Overall, biological processes acting within these species remain poorly understood despite their importance to understand impacts of ongoing global change. We take part in filling this gap by studying the genetic structure over different spatial scales (from centimeters to tens of kilometers) in *Abatus cordatus*, an endemic and brooding sea urchin from the Kerguelen Islands. We developed three microsatellites and two exon-primed intron crossing markers and conducted a two-scale sampling scheme (from individuals to patches) within two dense localities of *Abatus cordatus*. Between patches, all pairwise comparisons, covering distances from few meters (between patches within locality) to 25 km (between localities), revealed significant genetic differentiation, a higher proportion of the molecular variance being explained by the comparisons between localities than within localities, in agreement with an isolation by distance model. Within patches, we found no significant correlation between individual pairwise spatial and genetic distances, except for the most polymorphic locus in the patch where the largest range of geographical distances had been analyzed. This study provides an estimation of the dispersal capacities of *Abatus cordatus* and highlights its low recolonization ability. Similar low recolonization capacities are thus expected in other Antarctic and Subantarctic brooding invertebrate species and suggest a high vulnerability of these species facing global change.

Keywords *Abatus cordatus* · Microsatellites · Introns · Brooding · Genetic structure · Heterozygote deficiency

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Introduction

The Southern Ocean ecosystem is characterized by extreme and fluctuating environmental conditions and by a moderate level of biodiversity compared to its area (Gutt et al. 2004; Costello et al. 2010; Krug et al. 2010). Nonetheless, around 50% of the 8,200 inventoried species are considered as endemic (Griffiths 2010) likely because of the physical isolation of the Southern Ocean by the Antarctic circumpolar current that also acts as a complex barrier (geographic, oceanographic, bathymetric, and even physiological) to most marine organisms (e.g., Gérard et al. 2008 in *Mytilus*

spp., González-Wevar et al. 2010 in *Nacella spp.*, Diaz et al. 2011 in *Sterechinus spp.*; see also Thatje 2005). Recent studies argue that this distinctive component of Earth biodiversity is threatened by global change (Agustí et al. 2010) questioning its future and calling for the characterization of the processes involved in its evolution to ensure its conservation (Smetacek and Nicol 2005; Wilson et al. 2007; Griffiths 2010).

To date, questions regarding identity, diversity, and distribution of Southern Ocean biodiversity were mainly tackled above the species level through the use of community ecology (e.g., Teixido et al. 2002, 2007; see Thatje et al. 2005 for review) or phylogenetic approaches (e.g., Held and Wägele 2005; Stevens and Hogg 2006; see Rogers 2007 for review). For example, focusing on invertebrate species, the benthic communities display an uncommonly high proportion of brooding compared to broadcasting species (Thomson 1878; Murray 1885; Dell 1972). Poulin and Féral (1996), Poulin et al. (2002) and Féral and Poulin (2011) suggested that this dominance of brooding development could be explained by species-level selection over geological times due to fluctuations in levels of permanent sea ice (see Thatje et al. 2005 and Aronson et al. 2008 for discussion). Comparatively, the processes acting within species remain poorly understood (Held and Leese 2007), in spite of their importance for biodiversity evolution and conservation (Hendry et al. 2010).

Among the processes acting at the population level, dispersal is central (Epperson 1995; Broquet and Petit 2009) as it impacts reproductive strategies, demographic interactions between individuals and population structure (Ronce 2007). It was also recently emphasized as a key evolutionary factor with direct effects on community structure due to its role in local adaptation, evolvability, or species range (Lavergne et al. 2010). However, our knowledge regarding dispersal and population structure in Antarctic and Subantarctic marine invertebrates is limited (Hoffman et al. 2011) despite recent improvements in genetic tools to tackle these questions (Féral 2002; Chenuil 2006; Selkoe and Toonen 2006). The development of polymorphic markers such as microsatellites combined with adapted sampling allows refining conclusions regarding population structure (Jarne and Lagoda 1996; e.g., Estoup et al. 1998) and accurately estimating processes such as dispersal (Broquet and Petit 2009; e.g., Ledoux et al. 2010b). Moreover, to date, the existing studies were mainly focused on pelagic species such as krill (*Euphausia superba* Zane et al. 1998; *Euphausia crystallorophias* Jarman et al. 2002) and only marginally on benthic organisms (but see Thornhill et al. 2008 in *Parborlasia corrugatus* or Wilson et al. 2009 in *Doris kerguelenensis*). There is therefore a need to pursue efforts to develop species-specific molecular markers to characterize the patterns and processes at stake within these species. Filling this gap will enhance our knowledge regarding the evolutionary history of Southern Ocean invertebrates diversity (Held and Leese 2007) and accordingly will help to predict the consequences of global change.

The Echinoidea *Abatus cordatus* (Verrill 1876) belongs to the order Spatangoida, family Schizasteridae. It is a deposit feeder endemic to the Kerguelen Islands that burrows few centimeters below the surface of sandy sediments. It is mainly found in the shallow waters (0–3 m) of sheltered bays where tidal and wave actions are weak (Poulin and Féral 1995). In these habitats, *Abatus cordatus* individuals are clustered in discontinuous demes of high density (>200 ind m^{-2}; named "localities" in the following text) that should be temporally stable due to regular recruitment (Féral and Poulin 1994). Scattered individuals were also observed until 560 m depth (De Ridder et al. 1993). This brooding sea urchin is characterized by a direct development (i.e., no larval phase). After spending around 9 months protected in the four dorsal marsupia of the females (Schatt and Feral 1991), juveniles are released on the seafloor near their mothers. Considering dispersal strategies in benthic marine invertebrates as a continuum from low to extensive dispersal (Poulin et al. 2001), *Abatus cordatus* should be on the "low dispersal" edge. Previous genetic studies conducted in this species using two allozyme loci (Hexokinase and Phosphoglucoisomerase; Poulin and Féral 1994; Poulin 1996) confirmed this hypothesis. They demonstrated that the populations of *Abatus cordatus* were significantly differentiated at the kilometric scale and that they were structured through isolation by distance (IBD). They also suggested that each locality of high density corresponded to a genetic population due to the lack of differentiation between samples taken within the same locality (see Poulin and Féral 1994 for details).

The major goal of the present study was to go further in the characterization of the genetic structure of *Abatus cordatus* to gain data on the population processes in this Subantarctic benthic invertebrate with protected development (Poulin et al. 2001). Using a combination of two types of genetic markers, microsatellites and exon-primed intron crossing (EPIC) markers, developed specially for *Abatus cordatus* and a two-scale sampling scheme (from individuals to patches), we addressed three main objectives: (1) to test for Hardy–Weinberg equilibrium within patches, (2) to look for spatial genetic structure (SGS) between individuals within patches, and (3) to define the level of genetic structure between patches. Our results demonstrated that null alleles should be the main factor in the observed heterozygote deficiencies within patches. We also showed that populations of *Abatus cordatus* are structured even at the scale of ten meters highlighting the

reduced dispersal and recolonization abilities of the species. Because *Abatus cordatus* is a species with an extreme dispersal strategy, our results could be used as a landmark for the study of dispersal and genetic structure in Antarctic and Subantarctic marine invertebrates and thus, shed new light on the evolution and conservation of the Southern Ocean biodiversity.

Materials and methods

Sampling and DNA extraction

Sampling was conducted at two scales (individuals and patches) in two different localities characterized by a high density of individuals and situated in the "*Golfe du Morbihan*" in the southeastern part of the Kerguelen Islands: *Halage des Swains* (HS, 49°32′57″ S; 69°48′05″ E) and *Ile Haute* (IH, 49°23′13″ S; 69°56′20″ E) (Fig. 1). These two localities are separated by 25 km following the shortest waterway between them. In each locality, individuals were exhaustively collected within two patches of 70 × 70 cm (0.49 m^2) in 2002 (HS-02: $N = 92$; IH-02 $N = 41$) and 2003 (HS-03 $N = 136$; IH-03 $N = 105$). Because of the size of the localities, we consider that the two patches within each locality are separated by around 10 m. During the sampling conducted in 2003, spatial positions of most of the individuals (121 for HS-03 and 105 for IH-03; Fig. 2) were precisely mapped to obtain pairwise distance matrices. Due to meteorological conditions, the patch IH-03 was only partially sampled (see Fig. 2b).

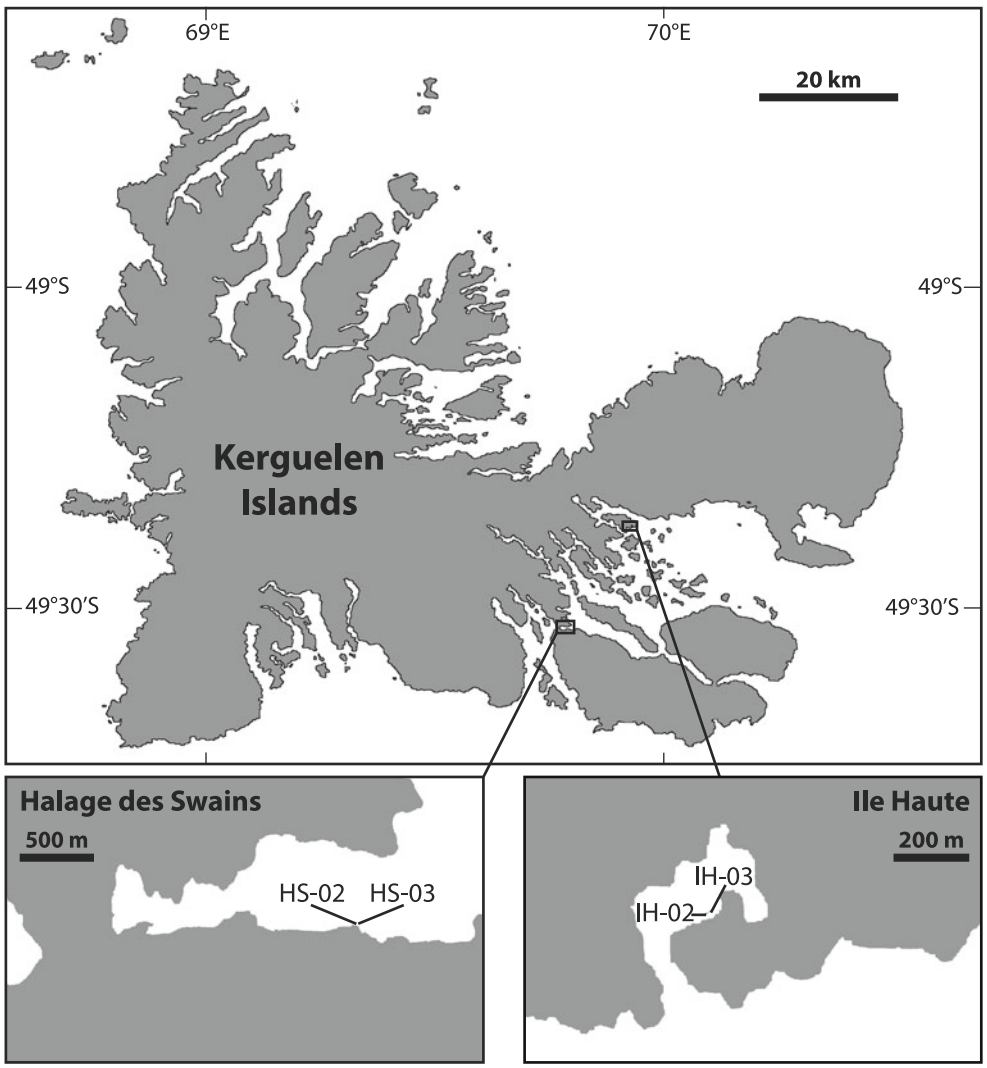

Fig. 1 The Kerguelen Islands with the positions of the two localities with high density of *Abatus cordatus*, *Halage des Swains* (HS), and *Ile Haute* (IH). These two localities are separated by 25 km, whereas the two patches in each locality (HS-02, HS-03, and IH-02, IH-03) are separated by around 10 m

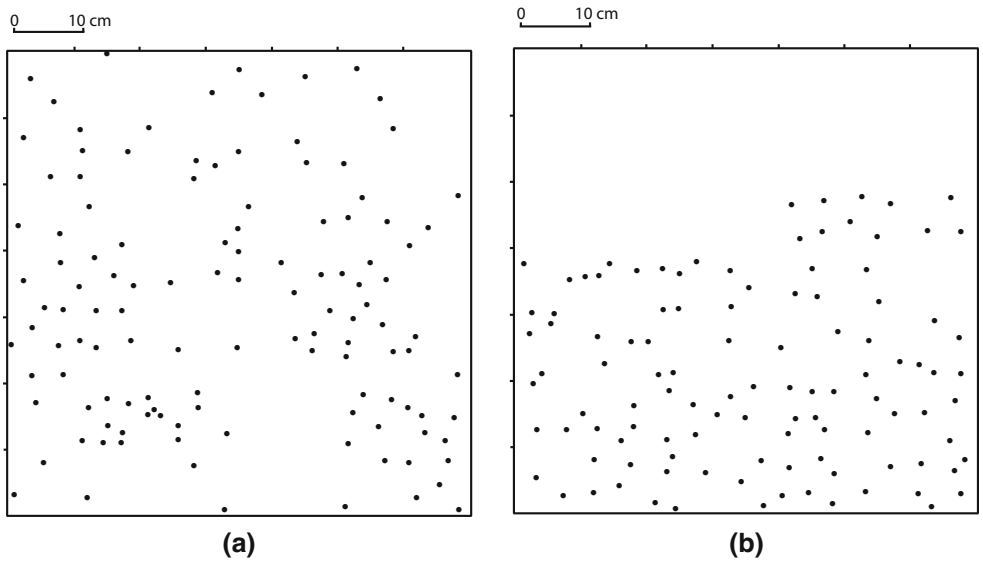

Fig. 2 Positions of individuals of *Abatus cordatus* in patches HS-03 (**a**) and IH-03 (**b**)

All the 374 individuals were preserved in 95% ethanol and stocked at −20°C until DNA extraction. Perioral podia and muscle spines were used to extract DNA using the Nucleon PhytoPure™ kits following manufacturer's recommendations. For the sample HS-03, the diameter of 67 individuals was measured. Four different size classes were defined using the Bhattacharya's method (1967): C1 ($N = 9$, mean diameter ± SD = 11.1 ± 1.2 mm), C2 ($N = 22$, mean diameter ± SD = 19.2 ± 1.2 mm), C3 ($N = 15$, mean diameter ± SD = 24 ± 1.1 mm), and C4 ($N = 21$, mean diameter ± SD = 29.7 ± 1.7 mm). These four size classes were considered as rough estimates of different cohorts thus allowing for testing temporal genetic differentiation within patch (see below).

Microsatellite and EPIC markers amplification

Following the enrichment library protocol described in Chenuil et al. (2003), we isolated five microsatellite loci in *Abatus cordatus* (Abc1, Abc3, Abc5, Abc6, and Abc7; Table 1). We retained 3 loci for the remaining analyses: Abc1, Abc5, and Abc7. These loci were chosen because their migration patterns were clear and because they showed enough polymorphism as well as moderate frequencies of null alleles (see below). To increase the number of codominant markers available for our study, we also tested eight putatively universal EPIC loci (i29, i30, i34, i36, i43, i48, i50, and i51) using the corresponding primer pairs developed by Chenuil et al. (2010) for metazoan taxa. Amplification products of these loci were size-selected on agarose gel and purified using the Qiagen Gel Extraction Kit. Purified fragments were then re-amplified and cloned using the pGEM-T easy vector system (Promega) and DH5 alpha *Escherichia coli* cells (Invitrogen), following the manufacturer's guidelines. Recombinant clones were amplified and sequenced with universal T7/SP6 primers. Internal primers were then defined for five loci (i30, i34, i43, i48, and i50). The size polymorphism of these loci was investigated by the amplification of eight individuals. Based on the levels of polymorphism and migration profiles, we retained two of these markers (i34 and i48; Table 1). These two loci also amplified and showed promising size polymorphisms in five related species belonging to the Schizasterids (*Abatus elongatus*, *Amphipneustes marsupialis, Amphipneustes lorioli*, *Amphipneustes rostratus,* and *Amphipneustes similis*) (Chenuil et al. unpublished data).

For the two types of markers, PCR reactions were performed in a final volume of 10 µl containing 1 µl of DNA, 2 µl of 5× Colorless GoTaq Flexi Buffer (Promega), 0.2 mM of dNTPs, 0.25 U of GoTaq DNA Polymerase (Promega), and 1 µM of each primer with the forward primer fluorescently labeled. The concentration of $MgCl_2$ was 3 mM for the microsatellites and i34 and 1.5 mM for i48. The PCR for microsatellites were done as follow: 95°C for 2 min followed by 30 cycles of 94°C for 30 s, annealing temperature (see Table 1) for 30 s without elongation and a final step of 72°C during 20 min. The PCR program for EPIC loci was 94°C for 2 min followed by 14 cycles of 94°C for 1 min, 62–48°C (−1°C per cycle) for 1 min and 72°C for 1 min followed by 25 cycles of 94°C for 40 s, 48°C for 45 s and 72°C for 1 min, and a final step of 72°C for 3 min. In both cases, electrophoreses were conducted on genetic analyzer ABI3130 (Applied Biosystem) using internal size standard for sizing (Genescan 600 LIZ, Applied Biosystem) and three reference

Table 1 Primer sequences and PCR conditions of the different loci tested in *Abatus cordatus*

Locus type	Locus Name	Primer sequence 5'-3'	Repeat motif	Number of cycles	Tm (°C)	Size range	Number of alleles	Ho	He	*f*	r	Genbank accession number
Microsatellites	Abc1	F: 6FAM-CAGGTAGATAAATTCATTTATGAT R: AAAGGCCACCATTGTGCTCTAT	(TG)x	30	52	180-283	31	0.65	0.92	0.31**	0.28	JN828969
	Abc3	F: CAATTTATAATTATCTTCATCATGA R: ATATGTGCTGTACAGAGCGT	(CA)x	30	49							JN828970
	Abc5	F: VIC-GCCGGAATAAAGACCATCATT R: ATCAAACGCCGCGTCTCCT	(CA)x	30	49	343-398	17	0.69	0.77	0.12**	0.3	JN828971
	Abc6	F: CTTTCATGATTTGCCGCCGTACAT R: TGAGTGCATAGAGCTAGCTAGTA	(CA)x	30	49							JN828968
	Abc7	F: 6FAM-TTGACTTATTACACATCTATGGAT R: ATAATAGCCCAGTAGTCTATGGAT	(G)x	28	52	82-91	7	0.8	0.77	-0.05	0.12	JN828972
EPIC	i34	F: PET- GACTGAGGATAAAGTGCCTTGCCC R:CCTTAATCATCATTAGTTTACACAGTC		#	#	214-222	5	0.12	0.18	0.08		
	i48	F : 6FAM-GATTAGACCCTTGACATTTTCATGTC R: GATAAATTGTTATTCCCATGTAAGCTG		#	#	117-122	4	0.07	0.1	0.01		

The genetic characteristics are shown only for the five retained loci. H_o: observed heterozygosity; H_e: gene diversity (Nei 1973); *f*: Weir and Cockerham (1984) estimator of F_{IS}; *r*: null allele frequency (** significant deviation from panmixia at 0.01 after FDR). Showed values correspond to the mean value over all samples

individuals for each migration. Electropherograms were analyzed with GENEMAPPER v.3 (Applied Biosystem).

Data analyses

Loci characteristics

For the microsatellites, we used MICROCHECKER v.2.2.3 (Van Oosterhout et al. 2004) to test for large allele dropout and scoring errors due to stutters. We estimated frequencies of null alleles (*r*) for each microsatellite in each sample by the expectation maximization algorithm (Dempster et al. 1977) implemented in FREENA (Chapuis and Estoup 2007). Null alleles could induce artificial heterozygote deficiencies (Chakraborty et al. 1992). They could also lead to an overestimation of genetic distances (Chapuis and Estoup 2007) and an underestimation of statistics summarizing genetic diversity (Chapuis et al. 2008).

The total number of alleles (N_a), size range, observed (H_o), and unbiased expected (H_e, Nei 1973) heterozygosities were computed for each of the two types of loci using FSTAT v.2.9.3 (Goudet 2001).

Testing for panmixia and addressing alternative explanations for heterozygote deficiencies

We tested the null hypothesis of linkage equilibrium for each pair of loci in each patch with a permutation procedure ($N = 1{,}000$) as implemented in GENETIX v.4.05 (Belkhir et al. 2004). The *f* estimator of F_{IS} (Weir and Cockerham 1984) was computed for each patch and each locus using GENEPOP v.4.0 (Rousset 2008). We tested the significance of the departures from panmixia using score test for heterozygote deficiencies and Markov chain algorithm (Guo and Thompson 1992; Raymond and Rousset 1995) with default parameters as implemented in GENEPOP.

To further investigate the cause of observed heterozygote deficiencies (see Results), we looked for putative cryptic population structure within patches. We used the Bayesian clustering method implemented in STRUCTURE v.2.2 (Pritchard et al. 2000; Falush et al. 2003; 2007). The number of genetic clusters *K* within each patch was inferred from the individual genotype dataset while optimizing Hardy–Weinberg (HW) and linkage equilibrium within each cluster. We performed ten replicates of each *K* with *K* ranging from 1 to 5 using admixture and the correlated allele frequencies model considering non-amplified genotypes as missing data. Each replicate was run for 200,000 iterations following a burn-in of 50,000. An estimate of the logarithm of the likelihood ("*LnP(D)*") of observing the data was computed for each *K* value. These values were then plotted as a function of the putative number of clusters (*K*). We selected one *K* value using the standard method (Pritchard et al. 2000) by looking for the *K* value that captured the major structure in the data (Pritchard et al. 2007). We also computed the $rxy_{Identity}$ identity relatedness coefficient for each pair of individuals within each patch using IDENTIX v.1.1 (Belkhir et al. 2002). Using permutation procedure of genotypes ($N = 1{,}000$), we tested whether the mean and variance values for each patch significantly differed from their null expectations under panmixia. Significantly higher means would suggest that individuals are more inbred than expected under random mating (i.e., biparental inbreeding), whereas significantly higher

variance would suggest that multiple groups of related individuals (i.e., families) had been sampled (Belkhir et al. 2002). We complemented these tests using the Robust Multilocus Estimation of Selfing (RMES) software (David et al. 2007) that allows distinguishing the effect of null alleles (specific of each locus) from the effect of inbreeding (which should, in general, equally affect all markers) in the observed heterozygote deficiencies. In gonochoric species, the selfing rate (s) computed with RMES is interpreted as biparental inbreeding rate (e.g., Tarnowska et al. 2010). This rate is deduced from an estimator of the 2-locus heterozygosity disequilibrium over all pairs of loci ($\hat{g_2}$) under the assumption of inbreeding and linkage equilibrium. The null hypothesis $s = \hat{g_2} = 0$ (i.e., no biparental inbreeding) was tested by resampling single-locus genotypes independently 1,000 times.

Spatial and temporal genetic structure between individuals within patches

Following Vekemans and Hardy (2004), we estimated genetic distances between individuals within HS-03 and IH-03 using the Nason's estimator of kinship coefficient (Loiselle et al. 1995). Kinship values (F_{ij}) were then regressed on the natural logarithm of the distance between individuals ($\ln(d_{ij})$), and the significance of the slope of the regression (b_{Ld}) was tested using 1,000 permutations of the spatial position of individuals under the hypothesis of no correlation between genetic and geographic distances. These computations were done in SPAGEDI v.1.3 (Hardy and Vekemans 2002).

The temporal genetic structure within patch was investigated using the θ estimator of F_{ST} (Weir and Cockerham 1984) as a measure of pairwise genetic differentiation between the four size classes defined for HS-03. Genotypic differentiation was tested using an exact test with default parameters in GENEPOP.

Genetic structure between patches

We quantified global and pairwise genetic differentiation between patches using θ and tested for genotypic differentiation with an exact test in GENEPOP. Analysis of molecular variance (AMOVA; Excoffier et al. 1992) conducted in ARLEQUIN (Excoffier et al. 2005) was used to quantify genetic variation among localities (F_{CT}), among patches within locality (F_{SC}), and within patches (F_{ST}). Significance of F-statistics was tested using a permutation procedure ($N = 1{,}000$).

When multiple tests were conducted, the levels of significance were adjusted by the false discovery rate (FDR) corrections (Benjamini and Hochberg 1995).

Results

Loci characteristics

No evidence of scoring errors due to stuttering or large allele dropout was found in the microsatellite dataset. Frequencies of null alleles computed with FREENA were 0.12 for Abc7, 0.28 for Abc1, and 0.30 for Abc5 with a mean frequency over loci and populations equal to 0.23 (Table 1).

All loci were polymorphic in all populations at the 0.05 level except i48 that did not amplify in IH-02. Total number of alleles was 4 and 5 for intron loci i48 and i34, and was 7, 17, and 31 for microsatellite loci Abc7, Abc5, and Abc1 with a mean of 12.8 alleles per locus considering the two types of markers. The observed and unbiased expected heterozygosity values varied from 0.07 (i48) to 0.80 (Abc7) and from 0.10 (i48) to 0.92 (Abc1), respectively (Table 1). The mean observed heterozygosity H_o over loci was 0.50, whereas the mean H_e was 0.57. Taking into account only the three microsatellites loci, the mean H_o and mean H_e were 0.71 and 0.83, respectively, whereas they were 0.17 and 0.18 for the EPIC markers. Considering all samples, the null hypothesis of linkage equilibrium among loci was not rejected.

Deviation from panmixia

Linkage disequilibrium was observed for one pair of loci (i34, Abc5) in HS-03 and for two pairs of loci (i34, Abc1 and Abc1, Abc5) in IH-03 but was not significant after FDR corrections. Multilocus F_{IS} estimator ranged between 0.10 for HS-03 to 0.17 for IH-02 (Table 2). The heterozygote deficiencies overall loci were significant in all samples ($P < 0.01$). Considering each locus separately, f values were variable ranging from -0.23 for i34 in IH-02 to 0.44 for Abc1 in IH-03. The results of score test for heterozygote deficiencies varied between loci and samples except for Abc1 and Abc5 that showed significant heterozygote deficiencies in all samples. For the STRUCTURE analysis and for each patch, the solution with the highest likelihood was $K = 1$ suggesting the absence of cryptic structure within patch (not shown). None of the four patches displayed mean or variance pairwise relatedness coefficient significantly different from expected values under the hypothesis of random mating within each patch (Table 2). The biparental inbreeding rates (s) computed with RMES were not significantly different from 0 (all $P > 0.05$; not shown).

Spatial and temporal genetic structure between individuals within patches

The slope of the regression between kinship values (F_{ij}) computed over all loci and spatial distances ($\ln(d_{ij})$) for

Table 2 Sampling size (*N*) and measures of genetic diversity for four patches of *Abatus cordatus* based on five nuclear markers

Sample name	*N*	*f*						$rxy_{Identity}$	
		i34	i48	Abc1	Abc5	Abc7	Overall	Mean	Variance
HS-02	92	**0.32**	−0.01	**0.29**	**0.13**	−0.12	**0.12**	0.31	0.05
IH-02	41	−0.23		**0.17**	**0.21**	0.02	**0.12**	0.23	0.04
HS-03	136	**0.1**	−0.1	**0.28**	**0.13**	−0.1	**0.1**	0.32	0.06
IH-03	105	−0.08	**0.27**	**0.44**	**0.03**	**0.05**	**0.17**	0.45	0.14

f: Weir and Cockerham (1984) estimator of F_{IS} computed for each loci and overall loci in each patch. Values in *bold* are significant at the 0.05 level after FDR correction. $rxy_{Identity}$: mean and variance of the identity relatedness coefficient between each pair of individuals within each sample computed using IDENTIX v.1.1

individuals sampled in HS-03 was −0.002 and not significant ($P = 0.23$). When considering each locus separately, the same trend (i.e., not significant slope of regression) was globally observed except when F_{ij} was computed based on Abc1 genotypes. In this case, the slope of regression was −0.01 and remained significant after FDR correction. Regarding the individuals sampled in IH-03, the regression slope was 0.004 and not significant ($P = 0.86$) for the computation implying all loci. None of the regression slopes computed locus by locus was significant.

None of the tests for pairwise differentiations between the four size classes, which were carried out for HS-03 and considering all loci, was significant (all $P > 0.05$; not shown).

Genetic structure between patches

The global value of θ over all loci was 0.03, and the test of genotypic differentiation for all samples was significant ($P < 0.01$). Considering each locus separately, θ varied between 0.01 for i34 and Abc7 and 0.07 for Abc5 and the tests for monolocus genotypic differentiation for all samples were always significant ($P < 0.01$).

Pairwise multilocus θ between samples varied between 0.01 for HS-02 versus HS-03 and 0.05 for IH-02 versus HS-03 (Table 3). Significant genotypic differentiations were observed for all the comparisons even for those implying two samples from the same locality ($P < 0.01$). Monolocus pairwise θ ranged between −0.01 when comparing IH-03 versus HS-03 for i34 to 0.14 for IH-02 versus HS-03 for i34. The number of significant *p* values for the tests of pairwise monolocus genotypic differentiation varied between pairwise comparisons. Fifty percent of the tests were significant for comparisons involving IH-02 versus IH-03 (all $P < 0.05$ except $P = 0.08$ and 0.44 for Abc7 and i34) and HS-03 versus IH-02 (all $P < 0.05$ except $P = 0.53$ and 0.27 for Abc1 and i34). All tests except one (80%) were significant ($P < 0.05$) for HS-02 versus HS-03 ($P = 0.38$ for Abc7), HS-02 versus IH-02 ($P = 0.02$ for i34), HS-02 versus IH-03 ($P = 0.59$ for i43), and HS-03 versus IH-03 ($P = 0.96$ for i34). Accordingly, the number of significant tests was different between loci: from 33% for i34 to 100% for Abc5 with a higher mean percentage of significant tests for microsatellites (83%) compared to EPIC markers (33%) (Table S1).

The AMOVA showed that the percentage of total variation explained by grouping the patches according to the locality they belonged was higher than the variation explained by the difference among patches within locality (2.6 and 1.5%, respectively). The main part of the total variation was observed within patches (95.9%). The corresponding *F*-statistics were highly significant for all hierarchical levels ($P < 0.001$).

Discussion

Molecular markers and genetic diversity

In this study, we used for the first time microsatellite and EPIC markers in the Subantarctic Echinoidea, *Abatus cordatus*. Overall loci, the levels of genetic diversity parameters were moderate ($H_e = 0.56$) although higher than those observed previously in the same species with allozymes ($H_e = 0.23$; computed from data in Poulin 1996) (see below). When considering each type of locus separately, the observed values for microsatellites fall within the range of values reported in Echinoidea species (e.g., Carlon and Lippé 2007 in *Tripneustes gratilla* or Calderón et al. 2009 in *Paracentrotus lividus*) or more generally in other echinoderms (e.g., Zulliger et al. 2008 in *Astropecten aranciacus*). Moreover, the mean frequency of null alleles over microsatellite loci and populations ($r = 0.23$) corresponds to an intermediate level and should not significantly affect the diversity and genetic differentiation analyses according to Chapuis et al. (2008) (see below for discussion regarding their impact on heterozygote deficiencies). Comparison of our EPIC markers with published data is less straightforward as these markers are still infrequently used for population studies despite their

Table 3 Pairwise genetic differentiations

Overall loci	HS-02	IH-02	HS-03	IH-03
HS-02		**	**	**
IH-02	0.04		**	**
HS-03	0.01	0.05		**
IH-03	0.04	0.02	0.04	

Pairwise F_{ST} values overall loci are shown below the diagonal

Results of the exact tests (** significance at 0.01 after FDR) are above the diagonal

potential utility (Berrebi et al. 2005). Our values nevertheless are concordant with those reported (e.g., Bierne et al. 2000 in *Paeneus spp.*; Berrebi et al. 2005 in *Zacco platypus*; Boissin et al. 2008 in *Amphipholis squamata*). In spite of the occurrence of null alleles, these loci are thus efficient tools for characterizing intraspecific diversity in *Abatus cordatus*. They may also be used in related species (e.g., *Abatus nimrodi* or *Abatus cavernosus*) to investigate their evolutionary history.

Heterozygote deficiencies and reproductive biology

We reported significant deviation from panmixia resulting in heterozygote deficiency in each patch. Deviations from panmixia are commonly observed in marine invertebrates (e.g., David et al. 1997) and may involve biological or technical factors (Castric et al. 2002). Considering that our markers are supposed to be neutral, a Wahlund effect (i.e., the presence of distinct spatial [e.g., Dupont et al. 2009], temporal [e.g., Dupont et al. 2007], or familial [e.g., Pudovkin et al. 1996] units differing in allele frequency in a sample) and biparental inbreeding (i.e., mating between related individuals) are the two most common biological sources of heterozygote deficiencies (Hoarau et al. 2005). The absence of cryptic structure within patches and the lack of temporal variation of allele frequencies in HS-03 ruled out the spatial and the temporal Wahlund effects. The variances of pairwise relatedness coefficients between individuals being not significantly different from expectations under random mating, the family Wahlund effect could also be rejected. Concerning the inbreeding hypothesis, the individuals in the sample did not appear more related than expected under random mating as demonstrated by the computations of the mean values of the relatedness coefficients and the rates of biparental inbreeding (Belkhir et al. 2002; David et al. 2007). These results are consistent with the absence of significant linkage disequilibrium between loci in each sample. However, they should be taken with caution because of the low number of loci analyzed. For example, simulation studies demonstrated that assignment methods such as implemented in STRUCTURE are more efficient when at least ten loci are used (Cornuet et al. 1999). Moreover, some of these effects depend on the spatial scale investigated and may become significant when considering a larger spatial scale (e.g., inbreeding see Fenster et al. 2003). Regarding putative technical factors, the occurrence of null alleles is the main source of artifact leading to homozygote excess (Chapuis and Estoup 2007) and was reported several times in echinoderms (e.g., McCartney et al. 2004). In the present study, we observed null homozygotes (i.e., individuals that do not amplify) for all loci in all populations and accordingly, the estimated frequencies of null alleles as estimated for microsatellite loci by FREENA were not negligible in most of the locus–sample combinations. Null alleles should thus be the main factor implied in the observed significant deviation from panmixia.

Considering that null alleles are a technical bias and that we did not obtain evidence for the occurrence of biological factors leading to heterozygote deficiencies, panmixia is thus not unlikely at the studied scale (i.e., half a square meter). This hypothesis could be counterintuitive regarding the brooding strategy of *Abatus cordatus*. Nonetheless, Addison and Hart (2005) have shown that the developmental modes (larval vs. no larval dispersal) and F_{IS} values were not significantly associated. Different studies such as parentage analysis, monitoring of individual dispersal behavior combined with improvement of PCR amplifications to reduce the frequencies of null alleles (see Lemer et al. 2011) would be appropriate to test this hypothesis.

Lack of spatial genetic structure between individuals within patches?

One of the main objectives of this study was to test the occurrence of a SGS between individuals sampled within half a square meter. This was motivated by the occurrence of protected development (Schatt and Feral 1991) combined with the temporal stability of dense populations (Féral and Poulin 1994) suggesting that individuals should span their life in their native area. Overall loci, none of the

two studied patches (IH-03 and HS-03) showed significant SGS between individuals. However, a significant SGS was observed for Abc1 (the most polymorphic, so the most discriminating locus) in HS-03 (the patch with the highest number of individuals and the largest range of geographic distances surveyed; see Rousset 1997, 2000 for discussion).

Significant SGS between individuals were mainly reported in sessile organisms from terrestrial plants (e.g., Fenster et al. 2003 in *Chamaecrista fasciculata*), trees (e.g., Born et al. 2008 in *Aucoumea klaineana*) to marine invertebrates (e.g., Ledoux et al. 2010b in *Corallium rubrum*). Comparatively, reports of SGS in mobile invertebrate species are rare. To our knowledge, they only concerned terrestrial species (e.g., Arnaud et al. 2001 in *Helix aspersa*; Watts et al. 2007 in *Coenagrion mercuriale*) and implied higher geographic distances (i.e., from 10 s to 100 s of meters). Therefore, based on the significant result observed for Abc1 in HS-03 combined with the significant differentiation observed between the two patches belonging to the same locality (see below), we argue that complementary studies of SGS in *Abatus cordatus* based on sampling involving more individuals and/or more loci should be conducted (see Leblois et al. 2003 for discussion).

Population genetic structure: estimates of dispersal range and inferences on population functioning

We demonstrated that patches belonging to the same locality and separated by around ten meters were significantly differentiated. To our knowledge, such fine-scale genetic structure has never been investigated in echinoderms species except in the brooding brittle star *Amphipholis squamata* (Féral et al. 2003; Boissin et al. 2008). Indeed, most of the studies focused on echinoderms with a protected developmental mode are based on genetic markers with low resolving power (see below) such as mitochondrial DNA (e.g., Arndt and Smith 1998 in *Cucumaria pseudocurata*) or allozymes (e.g., Hunt 1993 in *Patiriella exigua*) precluding the study of fine-scale spatial genetic structure. On the other hand, markers with high resolving power such as microsatellites have been mainly developed in broadcasting echinoderms (i.e., with putative extensive dispersal capacities) in which studies of genetic structure at the scale of tens of meters is likely irrelevant (e.g., Calderón et al. 2009 in *Paracentrotus lividus*; Yasuda et al. 2009 in *Acanthaster planci*). Overall, such fine-scale genetic structures were mainly reported in sessile organisms (e.g., Ledoux et al. 2010a in *Corallium rubrum*; see Weersing and Toonen 2009 for review) strengthening the novelty of our result.

The significant genetic structure between patches belonging to the same locality also contrasts with previous studies conducted in *Abatus cordatus* (Poulin and Féral 1994; Poulin 1996). Based on two allozyme loci with low numbers of alleles (five for Hexokinase and four for Phosphoglucoisomerase), Poulin and Féral (1994) reported that sample differentiation within locality was not significant. In analyses using allele frequencies estimates, the number of alleles is a good indicator of the precision of genetic distance estimates (Kalinowski 2002). The resolving power of allozymes is thus theoretically limited compared to highly variable markers such as microsatellites (see Jarne and Lagoda 1996; Ryman et al. 2006; Selkoe and Toonen 2006). Refinements in the scale of genetic structure because of the use of different molecular markers have been reported in various species (e.g., Reusch et al. 2000 in *Zostera marina*; Aurelle et al. 2002 in *Salmo trutta*). Our study also illustrated the difference in the resolving power of different types of genetic markers due to their levels of polymorphism. The EPIC markers show a smaller mean number of alleles than the microsatellites (4.5 vs. 18.3 alleles per locus). As theory predicts, the number of significant tests of genetic differentiation between patches is lower for the EPIC compared to microsatellites (33 vs. 83% of significant tests). The substantial genetic variation and the lower distance of significant genetic differentiation observed overall loci in our study (i.e., around 10 m) compared to Poulin and Féral (1994) is thus mainly induced by the microsatellite loci in accordance with their intrinsic properties.

This significant genetic differentiation between patches within localities challenged the statement of Poulin and Féral (1994) who suggested that each dense locality corresponded to a "genetic population" (i.e., a group of individuals that interbreed [see Waples and Gaggiotti 2006; Waples et al. 2008]). The significant pairwise F_{ST} between the two patches belonging to the same locality (HS-02 vs. HS-03 and IH-02 vs. IH-03) demonstrated that the genetic interactions between the involved individuals are not sufficient to prevent genetic differentiation within a locality. The dispersal should thus be very low at this scale. Accordingly, we suggest that each dense locality of *Abatus cordatus* may correspond to an assortment of small groups of interbreeding individuals. Different biological factors such as sperm limitation (*sensu* Yund 2000) or pseudocopulation behaviors (e.g., Slattery and Bosch 1993) may explain the occurrence of these differentiated breeding units. However, our knowledge regarding the mating behaviors of *Abatus* species and more generally of Southern Ocean brooding invertebrate species is generally scarce (Gil et al. 2009). Paternity analyses conducted in *Abatus nimrodi* (Chenuil et al. 2004) suggest that more than one sire fertilize a female. Nonetheless, technical limitations linked to the dominant features of the molecular markers used (RAPD) prevent the authors to go further in their

analysis. Thus, paternity analyses based on codominant markers such as microsatellites are needed to test the hypothesis of different breeding units within dense localities of *Abatus cordatus.*

Taking into account larger geographical scales, Poulin and Féral (1994) demonstrated that the populations of *Abatus cordatus* are structured following isolation by distance. Due to the restricted number of populations analyzed in our study, we were not able to formally test this pattern. However, the results of the AMOVA are consistent with an increase in the genetic distance with the geographic distance between patches. Indeed, the percentage of the total genetic variance explained by differences among localities separated by 25 km was about twice the percentage of the total genetic variance explained by the differences between patches within localities. The occurrence of isolation by distance between populations of marine species was recently emphasized as a frequent pattern of structure and a thorough way to estimate ecologically relevant dispersal distances (Palumbi 2003 but see Bradbury and Bentzen 2007). A similar study but based on a higher number of localities should be the next step in the investigation of the ecology of *Abatus cordatus*.

Conclusion

Our study based on a two-scale sampling scheme and two different types of molecular markers allows refining our knowledge regarding the processes acting within populations of *Abatus cordatus*. Besides the new hypothesis regarding its reproductive biology, we propose that most of the dispersal of this species should occur at a scale below ten meters highlighting the importance of local processes in its population biology. These results have significant implications regarding the evolution and conservation of this Subantarctic invertebrate species with a protected developmental mode. On a long-term temporal scale, the low gene flow between populations reinforces the likelihood of speciation event through vicariance (Poulin et al. 2002). Over short-term temporal periods, such a restricted dispersal induces that the recolonization capacities of *Abatus cordatus* are very low.

The Southern ecosystems are particularly impacted by global change with warming trend in some regions several times higher than the global average (Vaughan et al. 2003; Griffiths 2010). Moreover, the Southern Ocean should be the first place where acidification will be observed by the year 2100 (Orr et al. 2005). In this context, our study shed new light on the vulnerability of Antarctic and Subantarctic benthic communities characterized by a high number of brooding species (Poulin et al. 2002). Generalizing our results, these brooding invertebrates may display restricted recolonization capacities. These species should thus be particularly sensitive to disturbances questioning their abilities to deal with the current level of environmental changes. The development of research at the population level should therefore be pursued in Southern Ocean brooding invertebrate species to better grasp the impact of ongoing global change on this distinct component of Earth biodiversity.

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References

Addison J, Hart M (2005) Spawning, copulation and inbreeding coefficients in marine invertebrates. Biol Bull 1:450–453

Agustí S, Sejr MK, Duarte CM (2010) Impacts of climate warming on polar marine and freshwater ecosystems. Polar Biol 33:1595–1598

Arnaud JF, Madec L, Guiller A, Bellido A (2001) Spatial analysis of allozyme and microsatellite DNA polymorphisms in the land snail *Helix aspersa* (Gastropoda: Helicidae). Mol Ecol 10:1563–1576

Arndt A, Smith MJ (1998) Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Mol Ecol 7:1053–1064

Aronson RB, Thatje S, Clarke A, Peck LS, Blake DB, Wilga CD, Seibel BA (2008) Climate change and invasibility of the Antarctic benthos. Annu Rev Ecol Evol Syst 38:129–154

Aurelle D, Cattaneo-Berrebi G, Berrebi P (2002) Natural and artificial secondary contact in brown trout (*Salmo trutta*, L.) in the French Western Pyrenees assessed by allozymes and microsatellites. Heredity 89:171–183

Belkhir K, Castric V, Bonhomme F (2002) IDENTIX, a software to test for relatedness in a population using permutation methods. Mol Ecol Notes 2:611–614

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, populations, interactions, CNRS UMR 5000. Université de Montpellier II, Montpellier, France

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B-Stat Methodol 57:289–300

Berrebi P, Boissin E, Fang F, Cattaneo-Berrebi G (2005) Intron polymorphism (EPIC-PCR) reveals phylogeographic structure of *Zacco platypus* in China: a possible target for aquaculture development. Heredity 94:589–598

Bhattacharya CG (1967) A simple method of resolution of a distribution into Gaussian components. Biometrics 23:115–135

Bierne N, Lehnert SA, Bédier E, Bonhomme F, Moore SS (2000) Screening for intron-length polymorphisms in penaeid shrimps using exon-primed intron-crossing (EPIC)-PCR. Mol Ecol 9:233–235

Boissin E, Hoareau TB, Feral JP, Chenuil A (2008) Extreme selfing rates in the cosmopolitan brittle star species complex

Amphipholis squamata: data from progeny-array and heterozygote deficiency. Mar Ecol Prog Ser 361:151–159

Born C, Hardy OJ, Chevallier MH, Ossari S, Attéké C, Wickings EJ, Hossaert-McKey M (2008) Small-scale spatial genetic structure in the Central African rainforest tree species *Aucoumea klaineana*: a stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. Mol Ecol 17:2041–2050

Bradbury IR, Bentzen P (2007) Non-linear isolation by distance: implications for dispersal estimation in anadromous and marine fish populations. Mar Ecol Prog Ser 340:245–257

Broquet T, Petit EJ (2009) Molecular estimation of dispersal for ecology and population genetics. Annu Rev Ecol Evol Syst 40:193–216

Calderón I, Palacin C, Turon X (2009) Microsatellite markers reveal shallow genetic differentiation between cohorts of the common sea urchin *Paracentrotus lividus* (Lamarck) in northwest Mediterranean. Mol Ecol 18:3036–3049

Carlon DB, Lippé C (2007) Eleven new microsatellite markers for the tropical sea urchin *Tripneustes gratilla* and cross-amplification in *Tripneustes ventricosa*. Mol Ecol Notes 7:1002–1004

Castric V, Belkhir K, Bernatchez L, Bonhomme F (2002) Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. Heredity 89:27–35

Chakraborty R, Andrade MD, Daiger SP, Budowle B (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. Ann Hum Genet 56:45–57

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631

Chapuis MP, Lecoq M, Michalakis Y, Loiseau A, Sword GA, Piry S, Estoup A (2008) Do outbreaks affect genetic population structure? A worldwide survey in *Locusta migratoria*, a pest plagued by microsatellite null alleles. Mol Ecol 17:3640–3653

Chenuil A (2006) Choosing the right molecular genetic markers for studying biodiversity: from molecular evolution to practical aspects. Genetica 127:101–120

Chenuil A, Le Gac M, Thierry M (2003) Fast isolation of microsatellite loci of very diverse repeat motifs by library enrichment in echinoderm species, *Amphipholis squamata* and *Echinocardium cordatum*. Mol Ecol Notes 3:324–327

Chenuil A, Gault A, Feral JP (2004) Paternity analysis in the Antarctic brooding sea urchin *Abatus nimrodi*: a pilot study. Polar Biol 27:177–182

Chenuil A, Hoareau TB, Egea E, Penant G, Rocher C, Aurelle D, Mokhtar-Jamai K, Bishop JDD, Boissin E, Diaz A, Krakau M, Luttikhuizen PC, Patti FP, Blavet N, Mousset S (2010) An efficient method to find potentially universal population genetic markers, applied to metazoans. BMC Evol Biol 10:276

Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000

Costello MJ, Coll M, Danovaro R, Halpin P, Ojaveer H, Miloslavich P (2010) A census of marine biodiversity knowledge, resources, and future challenges. PLoS One 5:e12110

David P, Perdieu M, Pernot A, Jarne P (1997) Fine-grained spatial and temporal population genetic structure in the marine bivalve *Spisula ovalis*. Evolution 51:1318–1322

David P, Pujol B, Viard F, Castella V, Goudet J (2007) Reliable selfing rate estimates from imperfect population genetic data. Mol Ecol 16:2474–2487

De Ridder C, David B, Larrain A (1993) Antarctic and subantarctic echinoids from 'Marion Dufresne' expeditions MD03, MD04, MD08 and from the 'Polarstern' expedition Epos III. Bull Mus Nat Hist Nat Paris 14:405–441

Dell RK (1972) Antarctic benthos. Adv Mar Biol 10:1–216

Dempster A, Laird N, Rubin D (1977) Maximum likelihood from incomplete data via the EM algorithm. J R Stat Soc Ser B Stat Methodol 39:1–38

Diaz A, Féral JP, David B, Saucède T, Poulin E (2011) Evolutionary pathways among shallow and deep sea echinoids of the genus *Sterechinus* in the Southern Ocean. Deep Sea Res Part II 58 SI:205–211

Dupont L, Bernas D, Viard F (2007) Sex and genetic structure across age groups in populations of the European marine invasive mollusc, *Crepidula fornicata* L. (Gastropoda). Biol J Linnean Soc 90:365–374

Dupont L, Viard F, Dowell MJ, Wood C, Bishop JDD (2009) Fine- and regional-scale genetic structure of the exotic ascidian *Styela clava* (Tunicata) in southwest England, 50 years after its introduction. Mol Ecol 18:442–453

Epperson B (1995) Fine-scale spatial structure: correlation for individual genotypes differ from those for local gene-frequencies. Evolution 49:1022–1026

Estoup A, Rousset F, Michalakis Y, Cornuet JM, Adriamanga M, Guyomard R (1998) Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). Mol Ecol 7:339–353

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

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567–1587

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: Dominant markers and null alleles. Mol Ecol Notes 7:574–578

Fenster CB, Vekemans X, Hardy OJ (2003) Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaecrista fasciculata* (Leguminosae). Evolution 57:995–1007

Féral JP (2002) How useful are the genetic markers in attempts to understand and manage biodiversity? J Exp Mar Biol Ecol 268:121–145

Féral JP, Poulin E (1994) Growth, recruitment and age structure of *Abatus cordatus*, a brood protecting schizasterid. SCAR sixth biology symposium. Antarctic communities: species, structure and survival, Venice, 30 May–3 June 1994. Univ. Padova, Padova, p 91

Féral JP, Poulin E (2011) Kerguelen Islands: a living laboratory to understand the benthic biodiversity of the Antarctic. In: Duhamel G, Welsford D (eds) The Kerguelen plateau, marine ecosystem and fisheries. Société Française d'Ichtyologie, Paris, pp 151–156

Féral JP, Villard AM, Barré N, Chenuil A (2003) What is the smallest distance of genetic differentiation in the brood protecting ophiuroid *Amphipholis squamata* from Western Mediterranean? In: Féral JP, David B (eds) Echinoderm research. Proceedings of 6th European conference on Echinoderm, Banyuls-sur-mer, France. Swets and Zeitlinger Publishers, Lisse, pp 23–27

Gérard K, Bierne N, Borsa P, Chenuil A, Féral JP (2008) Pleistocene separation of mitochondrial lineages of *Mytilus spp.* mussels from Northern and Southern Hemispheres and strong genetic differentiation among southern populations. Mol Phylogenet Evol 49:84–91

Gil DG, Zaixso HE, Tolosano JA (2009) Brooding of the sub-Antarctic heart urchin, *Abatus cavernosus* (Spatangoida: Schizasteridae), in Southern Patagonia. Mar Biol 156:1647–1657

González-Wevar CA, Nakano T, Cañete JI, Poulin E (2010) Molecular phylogeny and historical biogeography of *Nacella* (Patellogastropoda: Nacellidae) in the Southern Ocean. Mol Phylogenet Evol 56:115–124

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995)

Griffiths HJ (2010) Antarctic marine biodiversity—what do we know about the distribution of life in the Southern Ocean? PLoS One 5:e11683

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

Gutt J, Sirenko BI, Smirnov IS, Arntz WE (2004) How many macrozoobenthic species might inhabit the Antarctic shelf? Antarct Sci 16:11–16

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Held C, Leese F (2007) The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. Polar Biol 30:513–521

Held C, Wägele JW (2005) Cryptic speciation in the giant Antarctic isopod *Glyptonotus antarcticus* (Isopoda: Valvifera: Chaetiliidae). Sci Mar 69:175–181

Hendry AP, Lohmann LG, Conti E, Cracraft J, Crandall KA, Faith DP, Hauser C, Joly CA, Kogure K, Larigauderie A, Magallon S, Moritz C, Tillier S, Zardoya R, Prieur-Richard AH, Walther BA, Yahara T, Donoghue MJ (2010) Evolutionary biology in biodiversity science, conservation and policy: a call to action. Evolution 64:1517–1528

Hoarau G, Boon E, Jongma DN, Ferber S, Palsson J, Van der Veer HW, Rijnsdorp AD, Stam WT, Olsen JL (2005) Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). Proc R Soc B Biol Sci 272:497–503

Hoffman JI, Clarke A, Linse K, Peck LS (2011) Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs. Mar Biol 158:287–296

Hunt A (1993) Effects of contrasting patterns of larval dispersal on the genetic connectedness of local populations of two intertidal starfish, *Patiriella calcar* and *P. exigua*. Mar Ecol Prog Ser 92:179–186

Jarman SN, Elliott NG, Nicol S, McMinn A (2002) Genetic differentiation in the Antarctic coastal krill *Euphausia crystallorophias*. Heredity 88:280–287

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. Trends Ecol Evol 11:424–429

Kalinowski ST (2002) How many alleles per locus should be used to estimate genetic distances? Heredity 88:62–65

Krug AZ, Jablonski D, Roy K (2010) Differential extinction and the contrasting structure of polar marine faunas. PLoS One 5:e15362

Lavergne S, Mouquet N, Thuiller W, Ronce O (2010) Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. Annu Rev Ecol Evol Syst 41:321–350

Leblois R, Estoup A, Rousset F (2003) Influence of mutational and sampling factors on the estimation of demographic parameters in a "continuous" population under isolation by distance. Mol Biol Evol 20:491–502

Ledoux JB, Mokthar-Jamaï K, Roby C, Féral JP, Garrabou J, Aurelle D (2010a) Genetic survey of shallow populations of the Mediterranean red coral [*Corallium rubrum* (Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. Mol Ecol 19:675–690

Ledoux JB, Garrabou J, Bianchimani O, Drap P, Féral JP, Aurelle D (2010b) Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, *Corallium rubrum*. Mol Ecol 19:4204–4216

Lemer S, Rochel E, Planes S (2011) Correction method for null alleles in species with variable microsatellite flanking regions, a case study of the black-lipped pearl oyster *Pinctada margaritifera*. J Hered 102:243–246

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, Sychotria officinalis (Rubiaceae). Am J Bot 82:1420–1425

McCartney MA, Brayer K, Levitan DR (2004) Polymorphic microsatellite loci from the red urchin, *Strongylocentrotus franciscanus,* with comments on heterozygote deficit. Mol Ecol Notes 4:226–228

Murray J (1885) Notes on the reproduction of certain echinoderms from the Southern Ocean. Rep Sci Res 'Challenger' Expedition (Her Majesty's Stationery Office) 1:379–396

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner GK, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig MF, Yamanaka Y, Yool A (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–686

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13:S146–S148

Poulin E (1996) Signification adaptative et conséquences évolutives de l'incubation chez un invertébré marin benthique subantarctique, *Abatus cordatus* (Verrill, 1876). PhD thesis, Université Montpellier II

Poulin E, Féral JP (1994) The fiction and the facts of antarctic incubation—population genetics and phylogeny of schizasterid echinoids. In: David B, Guille A, Féral JP, Roux M (eds) Echinoderms through time. Proceedings of the 8th international echinoderm conference, Dijon, France. Balkema, Rotterdam, pp 837–843

Poulin E, Féral JP (1995) Pattern of spatial distribution of a brood-protecting schizasterid echinoid, *Abatus cordatus*, endemic to the Kerguelen Islands. Mar Ecol Prog Ser 118:179–186

Poulin E, Féral JP (1996) Why are there so many species of brooding Antarctic echinoids? Evolution 50:820–830

Poulin E, von Boletzky S, Feral JP (2001) Combined ecological factors permit classification of developmental patterns in benthic marine invertebrates: a discussion note. J Exp Mar Biol Ecol 257:109–115

Poulin E, Palma AT, Féral JP (2002) Evolutionary versus ecological success in Antarctic benthic invertebrates. Trends Ecol Evol 17:218–222

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pritchard JK, Wen X, Falush D (2007) Documentation for the STRUCTURE software, Version 2. Chicago. Available at http://pritch.bds.uchicago.edu. Accessed March 2011

Pudovkin AI, Zaykin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygote-excess in progeny. Genetics 144:383–387

Raymond M, Rousset F (1995) GENEPOP (ver. 1.2): a population genetics software for exact test and ecumenicism. J Hered 86:248–249

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

Rogers AD (2007) Evolution and biodiversity of Antarctic organisms: a molecular perspective. Philos Trans R Soc B Biol Sci 362:2191–2214

Ronce O (2007) How does it feel to be like a rolling stone? Ten questions about dispersal evolution. Annu Rev Ecol Evol Syst 38:231–253

Rousset F (1997) Genetic differentiation and estimation of gene flow from F statistics under isolation by distance. Genetics 145:1219–1228

Rousset F (2000) Genetic differentiation between individuals. J Evol Biol 13:58–62

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8:103–106

Ryman N, Palm S, André C, Carvalho GR, Dahlgren TG, Jorde PE, Laikre L, Larsson LC, Palmé A, Ruzzante DE (2006) Power for detecting genetic divergence: differences between statistical methods and marker loci. Mol Ecol 15:2031–2045

Schatt P, Feral JP (1991) The brooding cycle of *Abatus cordatus* (Echinodermata, Spatangoida) at Kerguelen Islands. Polar Biol 11:283–292

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629

Slattery M, Bosch I (1993) Mating-behavior of a brooding antarctic Asteroid, *Neosmilaster georgianus*. Invertebr Reprod Dev 24:97–102

Smetacek V, Nicol S (2005) Polar ocean ecosystems in a changing world. Nature 437:362–368

Stevens MI, Hogg ID (2006) Contrasting levels of mitochondrial DNA variability between mites (Penthalodidae) and springtails (Hypogastruridae) from the Trans-Antarctic Mountains suggest long-term effects of glaciation and life history on substitution rates, and speciation processes. Soil Biol Biochem 38:3171–3180

Tarnowska K, Chenuil A, Nikula R, Féral JP, Wolowicz M (2010) Complex genetic population structure of the bivalve *Cerastoderma glaucum* in a highly fragmented lagoon habitat. Mar Ecol Prog Ser 406:173–184

Teixido N, Garrabou J, Arntz WE (2002) Spatial pattern quantification of Antarctic benthic communities using landscape indices. Mar Ecol Prog Ser 242:1–14

Teixido N, Garrabou J, Gutt J, Arntz WE (2007) Iceberg disturbance and successional spatial patterns: the case of the shelf Antarctic benthic communities. Ecosystems 10:142–157

Thatje S (2005) The future fate of the Antarctic marine biota? Trends Ecol Evol 20:418–419

Thatje S, Hillenbrand CD, Larter R (2005) On the origin of Antarctic marine benthic community structure. Trends Ecol Evol 20:534–540

Thomson CW (1878) Notice of some peculiarities on the mode of propagation of certain echinoderms of the southern seas. Zool J Linn Soc 13:55–79

Thornhill DJ, Mahon AR, Norenburg JL, Halanych KM (2008) Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae). Mol Ecol 17:5104–5117

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 6:255–256

Vaughan DG, Marshall GJ, Connolley WM, Parkinson C, Mulvaney R, Hodgson DA, King JC, Pudsey CJ, Turner J (2003) Recent rapid regional climate warming on the Antarctic Peninsula. Clim Change 60:243–274

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. Mol Ecol 13:921–935

Verrill AE (1876) Contribution to the natural history of Kerguelen Island. Annelids and Echinoderms. Bull US Natl Mus 3:64–75

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15:1419–1439

Waples RS, Punt AE, Cope JM (2008) Integrating genetic data into management of marine resources: how can we do it better? Fish Fish 9:423–429

Watts PC, Rousset F, Saccheri IJ, Leblois R, Kemp SJ, Thompson DJ (2007) Compatible genetic and ecological estimates of dispersal rates in insect (*Coenagrion mercuriale*: Odonata: Zygoptera) populations: analysis of 'neighbourhood size' using a more precise estimator. Mol Ecol 16:737–751

Weersing K, Toonen RJ (2009) Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393:1–12

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Wilson NG, Hunter RL, Lockhart SJ, Halanych KM (2007) Multiple lineages and absence of panmixia in the "circumpolar" crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. Mar Biol 152:895–904

Wilson NG, Schrodl M, Halanych KM (2009) Ocean barriers and glaciation: Evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). Mol Ecol 18:965–984

Yasuda N, Nagai S, Hamaguchi M, Okaji K, Gérard K, Nadaoka K (2009) Gene flow of *Acanthaster planci* (L.) in relation to ocean currents revealed by microsatellite analysis. Mol Ecol 18:1574–1590

Yund PO (2000) How severe is sperm limitation in natural populations of marine free-spawners? Trends Ecol Evol 15:10–13

Zane L, Ostellari L, Maccatrozzo L, Bargelloni L, Battaglia B, Patarnello T (1998) Molecular evidence for genetic subdivision of Antarctic krill populations. Proc R Soc B-Biol Sci 265:2387–2391

Zulliger D, Ruch M, Tanner S, Ribi G (2008) Characterization of nine microsatellite loci in the sea star *Astropecten aranciacus* and cross-species amplification for related taxa. Mol Ecol Resour 8:634–636