Polar Biol (2012) 35:919–929
DOI 10.1007/s00300-011-1139-2

ORIGINAL PAPER

Widespread amplification of amplified fragment length polymorphisms (AFLPs) in marine Antarctic animals

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Received: 12 October 2011 / Revised: 18 November 2011 / Accepted: 23 November 2011 / Published online: 20 December 2011

Abstract Although recent years have witnessed a rapid growth in the number of genetic studies of Antarctic organisms, relatively few studies have so far used nuclear markers, possibly due to the perceived cost and difficulty of isolating markers such as microsatellites. However, an often overlooked alternative is to use amplified fragment length polymorphisms (AFLPs), a versatile and low-cost method capable of generating large numbers of predominantly nuclear loci in virtually any organism. We conducted a literature review of population genetic studies of Antarctic organisms, finding that fewer than 10% used AFLPs. Moreover, a strong taxonomic bias was found, with studies employing mitochondrial DNA or microsatellites focussing predominantly on animals, while those using AFLPs were mostly of plants or lower organisms. Consequently, we explored the extent to which AFLPs amplify across a range of Antarctic marine animal taxa by genotyping eight individuals each of twelve different species, ranging from echinoderms through soft corals to pelagic fish, at four selective primer combinations. AFLPs readily amplified across all of the taxa, generating between 32 and 84 loci per species, with on average 56.5% of these being polymorphic. In general, levels of polymorphism bore little relationship with expectations based on larger populations of broadcast-spawning species being more variable, though we did find a tentative positive correlation between the number of AFLP bands amplified and a measure of effective population size. Our study lends further support for the utility and ease of use of AFLPs and their suitability for studies of Antarctic species across a wide range of taxa.

Keywords Genetic marker · Antarctica · Polar · Marine biota · Population structure · Genetic diversity · Life history

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Introduction

Antarctica provides an important setting for population genetic studies, not only because of its complex biogeography but also because it comprises an unrivalled natural laboratory for studying the effects of climate change (Clarke 2000; Meredith and King 2005). Consequently, with increasing ease of access to Antarctica itself and the Sub-Antarctic islands, molecular studies have proliferated (Rogers 2007). So far, the majority of these studies have used mitochondrial DNA (mtDNA), probably reflecting the convenience of this marker together with its utility for reconstructing deep phylogenetic histories (Ballard and Whitlock 2004). Moreover, this trend appears set to continue thanks to substantial investment into initiatives such as the Polar Barcode of Life (PolarBOL) project (http://www.polarbarcoding.org). However, although barcoding will undoubtedly bring important insights into the diversity and phylogeography of Antarctic organisms, it is based on effectively a single marker, making data from other genomic regions desirable. Thus, if the marker used for barcoding comes under selection, has an unusual mutation profile or is driven by some other factor that is non-representative

of the rest of the genome, a misleading picture could result (e.g. Dasmahapatra et al. 2010).

Recently, Held and Leese (2007) identified an urgent need for the development and application of nuclear markers for studying Antarctic marine organisms. However, they placed a firm emphasis on microsatellites and largely dismissed alternative classes of genetic marker that could also offer benefits. Microsatellites are powerful markers, being both codominant and often highly polymorphic (Ellegren 2004). However, they are relatively costly and time-consuming to develop (Zane et al. 2002), can only conveniently be screened in modest numbers (Slate et al. 2009) and are prone to genotyping errors caused by null alleles, allelic dropout and human error (Bonin et al. 2004; Hoffman and Amos 2005). A further issue is that microsatellites are often selected on the basis of high polymorphism in the focal species, thereby creating the potential for ascertainment bias (Brandstrom and Ellegren 2008) in that levels of variability are likely to be lower when the same marker is used on non-focal species. The net result of these effects can be a distorted picture of how genetic diversity is distributed and which species are most variable, though these problems may be reduced as increasing numbers of studies use next-generation sequencing to obtain a relatively unbiased picture.

Amplified fragment length polymorphisms (AFLPs) offer an alternative but often overlooked class of predominantly nuclear marker. AFLP loci are generated through the polymerase chain reaction (PCR) amplification of a random but reproducible set of genomic fragments (Vos et al. 1995). The protocols are relatively simple and are capable of generating hundreds of loci at modest cost and with a short start-up time (Mueller and Wolfenbarger 1999; Bensch and Akesson 2005). AFLPs have been used widely for a variety of applications from the determination of population genetic structure through linkage mapping and parentage analysis to phylogenetic analysis. The development, modes of application, advantages and disadvantages of these markers have been thoroughly reviewed elsewhere (Savelkoul et al. 1999; Mueller and Wolfenbarger 1999; Gerber et al. 2000; Bensch and Akesson 2005; Bonin et al. 2007; Meudt and Clarke 2007).

As with all genetic markers, AFLPs have their downsides, the greatest of these probably being their unidominant inheritance which makes them less attractive for applications such as parentage analysis (Bensch and Akesson 2005; Bonin et al. 2007). Another potential downside is the possibility of inadvertently amplifying non-specific DNA, such as that from gut endosymbionts (Dyer and Leonard 2000; Vorwerk and Forneck 2007). However, this issue can generally be controlled by careful tissue selection (e.g. the foot muscle of molluscs) or by scoring only loci known to originate from the host. The third main issue concerns homoplasy. With large numbers of essentially arbitrarily sized bands, one can seldom be sure that any given band originates from a unique locus. The size of this problem is unclear, but several studies suggest that it can be minimized by avoiding scoring small fragments that tend to show greater homoplasy (Vekemans et al. 2002), by not scoring too many fragments per primer combination (Caballero et al. 2008), or by applying a relatively simple protocol for detecting homoplasious fragments (O'Hanlon and Peakall 2000). Moreover, for many analyses the primary consequence seems to involve a minor loss of statistical power (see Caballero et al. 2008 for details).

Putting aside these drawbacks, AFLPs offer a number of clear advantages over and above their reduced development time and the number of informative bands they generate. For example, other classes of marker can be biased in the genomic regions they target. Most obviously, mitochondrial markers only track female lineages, but even microsatellites may be non-random. For example, microsatellite development tends to favour loci with higher levels of polymorphism, and these may derive preferentially from regions of the genome experiencing balancing selection (Huang and Yu 2003; Santucci et al. 2007), while dinucleotide repeats rarely occur in translated coding regions due to the frameshifts they would cause (Li et al. 2004) so may preferentially derive from gene-poor regions. In contrast, AFLPs are likely to embrace sites distributed throughout the genome (Vekemans et al. 2002). In combination with the possibility of screening many hundreds rather than tens of loci, this makes AFLPs well suited to genome-wide analyses. One attractive possibility is the detecting of genomic regions under selection, for example, as a consequence of environmental gradients (e.g. Bonin et al. 2006; Nunes et al. 2011) or even potentially climate change (e.g. Jump et al. 2006). A second is unbiased assessment of genetic diversity, not only at the species level (Milot et al. 2007) but also for populations (Mariette et al. 2002; Hoffman et al. 2009) and even individuals (Dasmahapatra et al. 2007). Despite this, AFLP variability has not previously been explored in the context of interspecific life-history variation or within an Antarctic context. One hypothesis amenable to testing is that broadcast-spawning species, which tend to have greater dispersal capacities, larger population sizes and greater local abundance, should harbour more genetic diversity than otherwise equivalent brooding species.

Here, we explore the usefulness of AFLPs for population genetic studies of Antarctic marine organisms by applying a standard laboratory protocol to amplify AFLPs in twelve different species, chosen to represent a wide variety of Antarctic marine animal taxa from echinoderms through soft corals to pelagic fish. We also use AFLPs to quantify levels of intraspecific diversity and then present a preliminary test

of the hypothesis that levels of diversity correlate with life-history predictors of effective population size.

Materials and methods

Survey of Antarctic genetic studies

Bensch and Akesson (2005) previously identified a general trend in which, although AFLPs are applied widely to plants, bacteria and fungi, they are less often applied to animals. To determine whether the same bias could also apply to studies of Antarctic organisms, we conducted ISI Web of Science searches in June 2011 using 'Mitochondria* AND Antarctic*', '(plastid* OR chloroplast*) AND Antarctic*', 'Microsatellite* AND Antarctic*', 'Tandem repeat* AND Antarctic*', 'VNTR* AND Antarctic*' and 'AFLP* AND Antarctic*' as search terms in the 'Topic' field. Although these terms are unlikely to recover every genetic study of an Antarctic organism, the recovered data set is large and, we believe, unbiased.

Tissue sample collection and DNA extraction

Eight specimens each of twelve different species were collected from South Cove and Hangar Cove at Rothera Point on Adelaide Island, Antarctica (Table 1, Fig. 1). Samples were collected by SCUBA divers during the austral summer of 2009 from the shallow sublittoral zone and stored in 95% ethanol at room temperature. Total genomic DNA was extracted from a small piece of each specimen using the Qiagen DNeasy tissue extraction kit following the manufacturer's recommended protocols.

AFLP genotyping

The AFLP protocol was adapted from Vos et al. (1995) and is described in detail by Dasmahapatra et al. (2009). Briefly, 100–400 ng of genomic DNA was first digested using *Taq*I (5U in a 10 µl volume at 65°C for 2 h) and then with *Eco*RI (5U in a 20 µl volume at 37°C for 2 h). *Taq*I and *Eco*RI and adapters (Table 3) were then ligated onto the digested DNA using T4 DNA ligase (1U in a 50 µl volume at 37°C for 3 h) and the resulting products diluted tenfold in 10 mM Tris HCL and EDTA (0.1 mM, Ph 8.0). For the pre-amplification, 5 µl of ligation mix was added to a 50 µl PCR containing Tris–HCl (10 mM, pH 8.3), $MgCl_2$ (1.5 mM), KCl (50 mM), dNTPs (0.2 mM), *Taq* polymerase (1U) and 50 ng each of the *Taq*I-C and *Eco*RI-A pre-amplification primers (Table 3). Following 30 pre-amplification cycles (30 s at 94°C, 60 s at 50°C and 60 s at 72°C), the products were diluted by a factor of ten with 10 mM Tris HCL and EDTA (0.1 mM, Ph 8.0). For the selective amplification, 2.5 µl of the diluted pre-amplification product was added to a 12.5 µl reaction containing Tris–HCl (10 mM, pH 8.3), $MgCl_2$ (1.5 mM), KCl (50 mM), dATPs, dTTP amd dGTP (0.2 mM each), dCTP (0.04 mM), α^{33}P-dCTP, *Taq* polymerase (0.2U), *Taq*I selective primer (30 ng) and *Eco*RI selective primer (5 ng). Samples were then subjected to 13 selective amplification cycles (30 s at 94°C, 60 s at 65°C, reducing by 0.7°C each cycle, and 60 s at 72°C), followed by a further 23 cycles (30 s at 94°C, 60 s at 56°C and 60 s at 72°C). PCR products were resolved by electrophoresis on standard 6% polyacrylamide sequencing gels and detected by autoradiography. Exposed X-ray films were assessed and if required, a second exposure was made for an adjusted time period. Gels were scored by eye, and genotypes were entered manually into a Microsoft excel spreadsheet. The following four different selective primer combinations were employed: *Taq*I-CTG & *Eco*RI-AGC, *Taq*I-CAG & *Eco*RI-AGC, *Taq*I-CGA & *Eco*RI-AGC and *Taq*I-CAC & *Eco*RI-AGC (see Table 2 for primer sequences). Scoring was conducted by a highly experienced observer, and although the genotyping error rate was not directly quantified, three previous studies using exactly the same protocol in the same laboratory all obtained low error rates (0.008–0.021 per reaction, Hoffman et al. 2010, 2011a, b). Finally, we quantified for each species the total number of loci that PCR amplified and the proportion of these loci that were polymorphic.

Data analysis

Genetic diversity among AFLP markers may be reflected in several different ways, of which we considered three. First, there is the number of bands amplified, which might be expected to reflect genome size, all other things being equal. Deviations from this expectation may be caused by differences in base composition or the presence of repetitive DNA, though these should tend to be revealed much more in some enzyme/selective primer combinations than others. Second, there is the proportion of polymorphic bands, which probably relates to the underlying SNP density in the sequences that are amplified. Third, one might consider the heterozygosity of the bands themselves. Consequently, all three response variables were calculated within the program AFLPSURV (Vekemans et al. 2002) and analysed using general linear models (GLMs) within R (Ihaka and Gentleman 1996). The following three categorical predictor variables were fitted together in each of the GLMs: (i) life-history strategy (with brooders coded as 1, mixed strategists as 2 and broadcasters as 3); (ii) mobility (coded as 1 = sessile and 2 = mobile); and (iii) abundance (coded on a scale of 1–4 with 1 = present and 4 = highly abundant). Using standard deletion-testing procedures (Crawley 2002), each term was then progressively dropped from models

Table 1 Details of the twelve Antarctic marine species genotyped at four selective AFLP combinations

Species	Group	Classification	Mode of development	Type of larvae	Duration of larval phase	Local abundance	Motility	Substratum	Type of feeder	References
Sterechinus neumayeri	Sea urchin	Echinodermata, Echinoidea	Broadcast	Planktotrophic	>100 days	Very abundant	Mobile	Hard	Omnivore/ scavenger	Bosch et al. (1987), Stanwell-Smith and Peck (1998), Pearse and Giese (1966)
Odontaster meridionalis	Sea star	Echinodermata, Asteroidea	Broadcast	Planktotrophic	>100 days	Present	Mobile	Hard	Predator/ scavenger	Bosch and Pearse (1990), Dayton et al. (1974)
Ophionotus victoriae	Brittle star	Echinodermata, Ophiuridae	Broadcast	Planktotrophic	~100 days	Abundant	Mobile	Soft and hard	Scavenger	Grange et al. (2004), Bowden et al. (2009), Dearborn (1977)
Nacella concinna	Limpet	Mollusca, Gastropoda	Broadcast	Planktotrophic	>50 days	Abundant	Mobile	Hard	Grazer	Shabica (1971, 1976)
Margarella antarctica	Top snail	Mollusca, Gastropoda	Brooding	NA	NA	Common	Mobile	Hard	Scavenger[a]	Poutiers (1998)
Yoldia eightsi	Bivalve	Mollusca, Bivalvia	Broadcast	Pelagic lecithotropic[b]	3 days	Common	Mobile	Soft	Suspension feeder	Colman (unpublished report), Nakaoka (1994), pers. comm.); Davenport (1988)
Laternula elliptica	Bivalve	Mollusca, Bivalvia	Brooding followed by pelagic phase	Lecithiotrophic	Unknown	Abundant	Sessile	Soft	Suspension feeder	Ahn (1994), Bosch and Pearse (1988), Urban and Mercuri (1998)
Liothyrella uva	Brachiopod	Brachiopoda, Rhynchonellata	Brooding followed by pelagic phase	Planktotrophic	Variable[c]	Present	Sessile	Hard	Suspension feeder	Peck (2001), Peck and Robinson (1994), Peck et al. (2005)
Alcyonium antarcticum	Soft coral	Cnidaria, Anthozoa	Brooding followed by very short pelagic phase	NA	NA	Common	Sessile	Hard	Predator[d]	Slattery and McClintock (1997), Dayton and Oliver (1977)
Parborlasia corrugatus	Ribbon worm	Nemertea, Anopla	Broadcast	Planktotrophic	>100 days	Abundant	Mobile	Hard	Predator	Peck (1993), Obermüller et al. (2010)
Barrukia cristata	Scale worm	Annelida, Polychaeta	Brooding followed by pelagic phase	Planktotrophic	Unknown[e]	Abundant	Mobile	Hard	Predator/ scavenger[f]	Gambi et al. (2001), Fauchald and Jumars (1979)
Harpagifer antarcticus	Fish	Chordata, Hapagiferidae	Brooding followed by pelagic phase	Pelagic planktotrophic	140–150 days	Common	Mobile	Hard	Predator	Casaux (1998), White and Burren (1992)

[a] data only at the level of Trochidae, [b] data from *Yoldia notabilis*, [c] Variable from immediate to 65 days, [d] data from related Octocoral plus Peck (pers obs), [e] unkown for this species because all other members of the *Harmothoe* are brooders, [f] data from related polychaetes

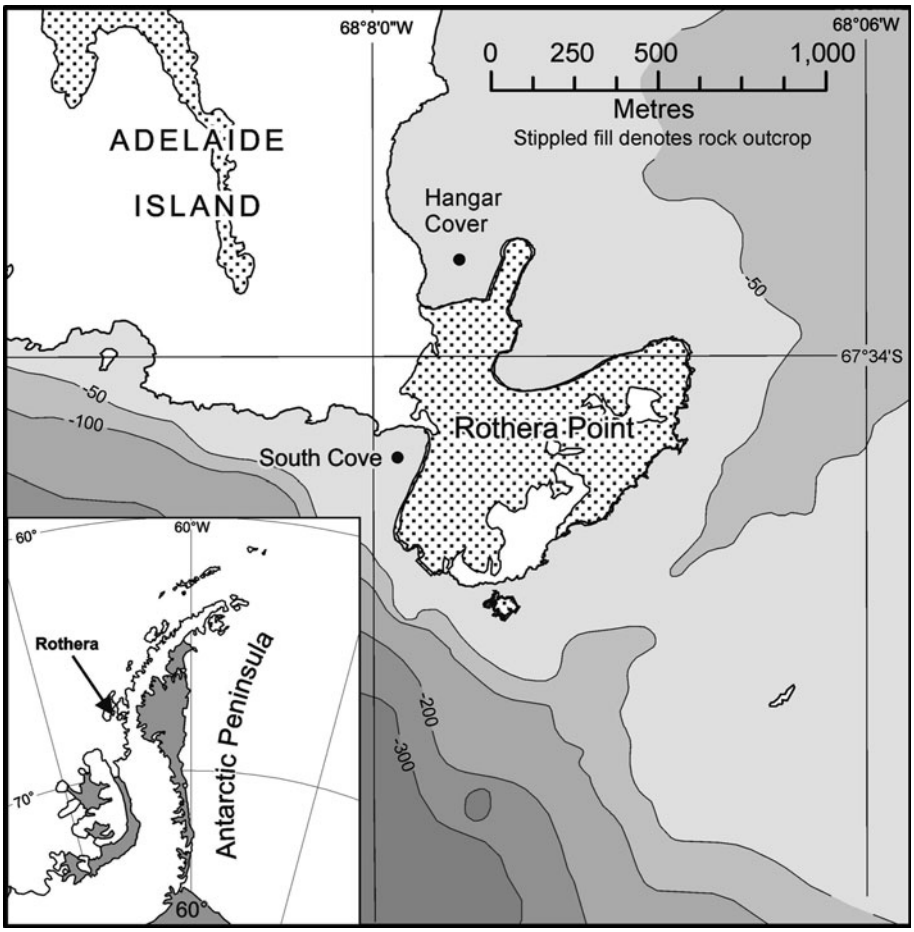

Fig. 1 Map showing sampling locations at Rothera Point, Adelaide Island

Table 2 Adapter and primer sequences used for AFLP analysis

Adapter/primer	Sequence (5′–3′)
TaqI adapter	GACGATGAGTCCTGAC
	CGGTCAGGACTCAT
EcoRI adapter	AATTGGTACGCAGTCTAC
	CTCGTAGACTGCGTACC
TaqI pre-selective primer	GATGAGTCCTGACCGAC
EcoRI pre-selective primer	GACTGCGTACCAATTCA
TaqI selective primers	GATGAGTCCTGACCGACTG
	GATGAGTCCTGACCGACAG
	GATGAGTCCTGACCGACGA
	GATGAGTCCTGACCGACAC
EcoRI selective primer	GACTGCGTACCAATTCAGC

unless doing so significantly reduced the amount of deviance explained (deviance is analogous to sums of squares in standard regression analysis). The change in deviance between full and reduced models was distributed as χ^2 with degrees of freedom equal to the difference in degrees of freedom between the models with and without the term in question. Finally, we attempted to summarize all of the predictors in a single composite measure that embraces life-history strategy, abundance and mobility by simply summing the scores above for each species. We then constructed three final GLMs of diversity fitting this measure as a categorical predictor variable.

Results

Given that many Antarctic marine organisms derive from taxa that are poorly characterized genetically, we would expect them to be prime candidates for using AFLPs. We therefore conducted a bibliographic survey of Antarctic population genetic and phylogenetic studies, recovering a total of 334 records (see "Materials and methods" for details). The majority of these studies employed mitochondrial/plastid DNA sequencing ($n = 239$, 71.6%) or microsatellites ($n = 72$, 21.6%), whereas only 6.9% ($n = 23$) used AFLPs (Fig. 2). By examining the titles, abstracts and where necessary the paper itself, each record was then broadly classified into taxonomic groupings. A strong bias was detected, with the majority of studies using mtDNA or microsatellites being of animals ($n = 192$, 97.0% and $n = 65$, 90.3%, respectively) whereas the reverse was true

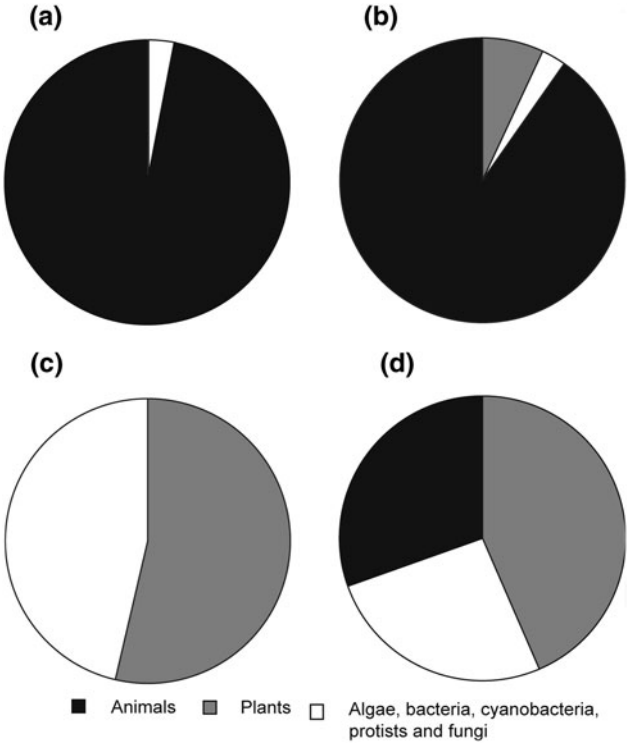

Fig. 2 Results of Web of Knowledge search (see "Materials and methods" for details) for **a** mitochondrial DNA ($n = 198$), **b** microsatellites ($n = 72$), **c** plastid DNA ($n = 41$) and **d** AFLPs ($n = 23$)

for plastids and AFLPs (only seven AFLP-based studies were of animal taxa, 30.4%).

To demonstrate the potential broad utility of AFLP markers for studying Antarctic marine animals, we genotyped twelve species representing 7 phyla and 10 classes (Table 1) at four different selective AFLP primer combinations (Table 2). Summary statistics including the total number of bands that could be reliably scored and the proportion of these that were polymorphic within each species are shown in Table 3. AFLPs successfully amplified in all twelve species, although the total number of loci generated varied considerably, from 32 in *Liothyrella uva* to 84 in *Parborlasia corrugatus*. The proportion of loci that were polymorphic was also highly variable, ranging from 19.3% in *Harpagifer antarcticus* to 80.7% in *Alcyonium antarcticum*, but with a moderately high average of 56.5%. Neither of these genetic traits seemed to relate obviously to life-history characteristics (Fig. 3).

In general, one might expect measures of variability to increase with species abundance, with broader rather than narrower dispersal and with greater rather than less mobility. To test these assumptions, we constructed GLMs of the number of loci amplified, the proportion of polymorphic loci and expected heterozygosity (Hj), fitting as predictor variables in each of the models life-history strategy, abundance and mobility. The only statistically significant trends detected were a weak negative relationship between the number of loci amplified and life-history strategy ($\chi^2 = 4.56$, df = 2, $P = 0.010$) and a positive relationship between the number of loci amplified and mobility ($\chi^2 = 9.15$, d$f = 1$, $P = 0.002$). The former is contrary to our a priori expectations and would suggest that brooders and mixed strategists harbour greater diversity than pure broadcast spawners. No relationships were found between life-history characteristics and the proportion of polymorphic loci. Since our life-history scoring was crude and different species could reach the same effective population size in different ways (e.g. a large sessile population versus a small mobile one), we next tried combining the trait scores into a single measure that would be expected to correlate with effective population size. For this, we simply summed the individual trait scores for each species. The resulting composite score was found to positively correlate with the number of bands scored (Fig. 4, $\chi^2 = 3.03$, d$f = 5$, $P = 0.009$) but again not with the proportion of polymorphic

Table 3 Summary statistics for AFLP amplification of eight individuals each of twelve different Antarctic marine taxa

Species	Total number of loci amplified	Number of polymorphic loci	Number of monomorphic loci	Percentage of polymorphic loci
Sterechinus neumayeri	64	41	23	64.1
Odontaster meridionalis	66	47	19	71.2
Ophionotus victoriae	81	44	37	54.3
Nacella concinna[a]	64	38	26	59.4
Margarella antarctica[b]	81	56	25	69.1
Yoldia eightsi	58	23	35	39.7
Laternula elliptica	51	15	36	29.4
Liothyrella uva	32	15	17	46.9
Alcyonium antarcticum	57	46	11	80.7
Parborlasia corrugatus	84	54	30	64.3
Barrukia cristata	64	51	13	79.7
Harpagifer antarcticus	57	11	46	19.3

[a] Data from Hoffman et al. (2010), [b] Hoffman et al. (2011a)

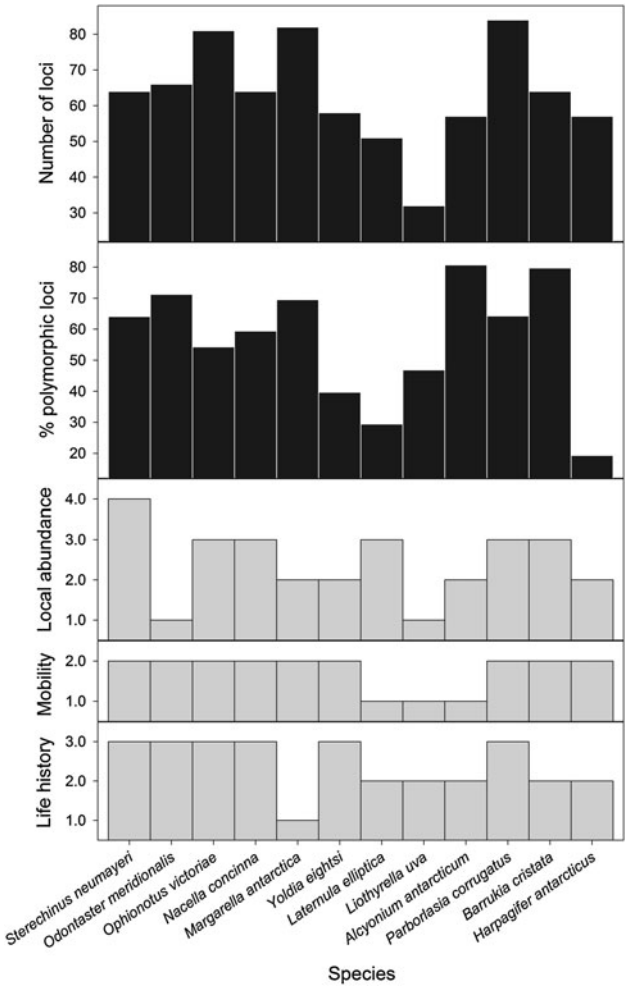

Fig. 3 Summary of genetic and life-history traits for twelve Antarctic marine animal taxa

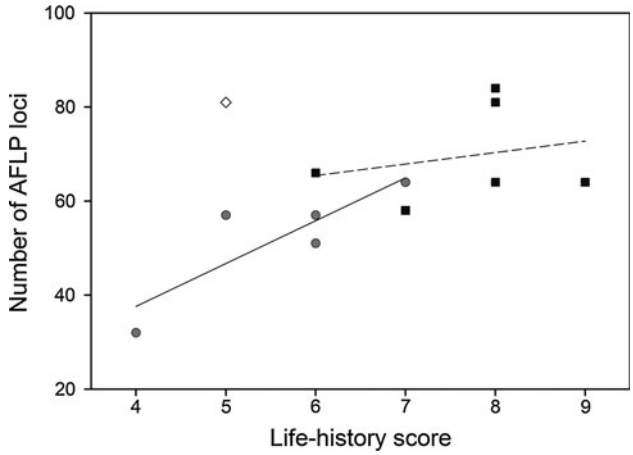

Fig. 4 Relationship between life-history score (a composite of life-history strategy, abundance and mobility, see "Materials and methods" for details) and the number of AFLP loci amplified, summarized for twelve Antarctic marine animal taxa. *Black squares* indicate broadcast spawners, *grey circles* mixed brooding and spawning strategists, while the *white diamond* indicates a single brooding species

loci. However, this relationship is not particularly strong, and it is entirely possible that it could be a type I error due to the number of tests we conducted.

Discussion

The results presented here suggest that AFLPs have not been widely applied to Antarctic organisms, in which a clear need for nuclear markers has been identified (Held 2006) and where genomic information is usually lacking. However, all twelve of the different Antarctic marine animal species tested here for AFLP amplification yielded polymorphic banding profiles, suggesting that AFLPs should work well for most taxa. We also present preliminary and counter-intuitive evidence that genetic diversity, as reflected by the number of loci amplifying, may be higher in brooding or mixed strategy species than pure broadcast spawners.

The need for nuclear markers

Antarctic organisms have evolved in extreme isolation and therefore tend to be genetically highly divergent from comparable temperate or tropical species. Moreover, there are no model Antarctic species and whole genome sequencing has not yet reached the stage where it is economically viable to apply it routinely to new non-model eukaryotic species. Consequently, those wishing to study Antarctic species are for the moment restricted to the use of single markers (mtDNA or plastid DNA), small panels of markers (i.e. microsatellites) or, for much wider genomic coverage, anonymous markers such as AFLPs. The latter are easy to use and are perhaps the only system offering accessible genome-wide coverage at low cost. For example, the current work was conducted in a standard molecular laboratory and at a cost of around £500. We used direct incorporation of radio-labelled nucleotides, which can be highly cost-effective for small-scale studies, but fluorescent AFLP genotyping is not much more expensive and offers greater efficiency for medium and large-scale projects.

AFLP amplification and levels of polymorphism

In this particular study, our protocol successfully amplified polymorphic AFLP profiles in all twelve species, demonstrating the utility of the method for a broad range of taxa and with no special optimization. Given the logistic difficulties associated with sampling Antarctic organisms, we analysed only modest sample sizes of each species obtained from a single location. For AFLP studies, small sample

sizes are not necessarily problematic because the number of bands scored per individual is large, but increasing both the number of samples and the geographic range of sampling would allow refinement of our current estimates. However, we can none the less ask questions about whether genetic diversity correlates with what we know about population size and population structure. A priori expectations would be that variability should increase from brooders, where dispersal is limited, through mixed strategists to broadcasters, which have the potential for very large population sizes. In practice we find that no correlation exists and that, if anything, brooders seem to have the most variability. Perhaps the most convincing trend we identified was a general positive relationship between the number of bands amplified and a composite measure based on life history, population size and mobility. All things being equal, the number of AFLP loci amplified should tend to correlate with the amount of DNA in the genome. Several complicating factors would undermine this relationship, most obviously factors such as base composition and the presence of repetitive sequences that influence the number of times a restriction enzyme cuts. On the other hand, if a given species shows higher band numbers across several different restriction enzymes and primer combinations, the larger genome explanation would seem more likely.

Brooding species appearing to exhibit higher levels of polymorphism than broadcasters contradicts our initial expectations, but is consistent with a previous meta-analysis of over a hundred studies in which F_{is} was found to be higher in free-spawning planktotrophic species than in brooding species (Addison and Hart 2005). A number of possible explanations can be put forward. First, all of the species examined have potentially huge population sizes in which genetic drift will operate extremely slowly. This is likely to be especially true for Antarctic species that have long generation times, slow growth and deferred maturity (Peck et al. 2006). Current measures may therefore reflect evolutionary events that happened literally millions of generation ago, predating changes in the ocean currents and temperature and even, perhaps, some speciation events. Second, many believe that mutation rate is selected to generate the right balance of novelty versus disruption (Metzgar and Wills 2000; Giraud et al. 2001; Møller and Cuervo 2003; Amos 2009). If so, species and taxa with long-term extremely large populations might evolve greatly reduced mutation rates to compensate, and the relative stability of the marine environment would only help this. It would be interesting to measure mutation rates directly to see whether this is so. Finally, the specimens were collected from sites that are shallower than 20 m depth, many of which will only be a few 100 years old, having recently emerged following glacial retreat (Golledge et al. 2010). This raises the possibility that founder effects could potentially have influenced our results.

The importance of quality control

As outlined in the introduction, AFLPs offer both advantages and disadvantages. A full account of these lies beyond the scope of this paper, but details can be obtained from a number of thorough reviews (e.g. Mueller and Wolfenbarger 1999; Savelkoul et al. 1999; Gerber et al. 2000; Bensch and Akesson 2005; Bonin et al. 2007; Meudt and Clarke 2007). However, an important caveat to the use of AFLPs is that, although these markers are in principle easy to amplify, to generate high-quality data sets requires careful quality control throughout the genotyping workflow. This is partly because AFLPs can be sensitive to DNA quality, although DNA concentration seems less critical (Bensch and Akesson 2005). Another problem can arise if certain bands do not amplify consistently across batches, although this can be compensated for by randomizing the order of the samples with respect to the groups or populations under comparison (Bensch and Akesson 2005). Finally, although parentage analyses indicate reasonably consistent Mendelian segregation (e.g. Huang et al. 2007), the unidominant nature of AFLPs makes it difficult to evaluate deviations from Hardy–Weinberg equilibrium, a key step in the quality control process for codominant markers such as microsatellites. As with other markers including microsatellites (Hoffman and Amos 2005), it is therefore strongly recommended that data quality be evaluated by replicating a randomly selected subset of samples from scratch (Bonin et al. 2004).

Emerging genomic approaches

The recent emergence of next-generation sequencing approaches capable of generating many millions of bases of sequence data (e.g. Margulies et al. 2005) has made it increasingly feasible to identify large numbers of putative genetic markers. For example, protocols now exist to develop assays for tens to hundreds of single nucleotide polymorphisms (SNPs) based on the in silico mining of transcriptome libraries (Vera et al. 2008). However, although these and related approaches are capable of generating similar marker densities to AFLPs, they also have drawbacks. For example, to develop a transcriptome typically requires animals to be sacrificed (although see Hoffman 2011), which may not be appropriate for every species. Moreover, sequence assembly and SNP genotyping usually need to be outsourced due to the prohibitive cost of the hardware required. Finally, SNPs themselves have various analytical drawbacks, for instance being prone to ascertainment bias when derived from small panels of individuals

(Brumfield et al. 2003; Morin et al. 2004; Helyar et al. 2011). We therefore feel it is unclear when AFLPs will be replaced by other techniques, not least because these alternatives have a long way yet to come down in price before they are affordable by most graduate students and in many developing countries.

Conclusions

AFLPs offer an under exploited resource that seems particularly applicable to the study of Antarctic species. Thus, although it appears inevitable that approaches based on next-generation sequencing will become increasingly prominent as prices continue to fall, AFLPs will likely remain an inexpensive and technologically simple technique for the genetic analysis of polar organisms for some time to come.

Acknowledgments We thank all members of the Rothera dive team for providing samples, Peter Fretwell for preparing Fig. 1 and Elaine Fitzcharles for performing the DNA extractions. Overall dive support was provided by the Natural Environment Research Council (NERC) National Facility for Scientific Diving at Oban. JIH was funded by a NERC British Antarctic Survey (BAS) Strategic Alliance Fellowship. This work was conducted by MSC and LSP within the Adaptations and Physiology Work Package as part of the BAS Polar Sciences for Planet Earth programme.

References

Addison JA, Hart MW (2005) Spawning, copulation and inbreeding coefficients in marine invertebrates. Biol Lett 1:450–453. doi:10.1098/rsbl.2005.0353

Ahn I-Y (1994) Ecology of the Antarctic bivalve *Laternula elliptica* (King and Broderip) in Collins Harbour, King George Island: benthic environment and an adaptive strategy. Mem Natl Inst Polar Res 50:1–10

Amos W (2009) Sexual selection does not influence minisatellite mutation rate. BMC Evol Biol 9:5. doi:10.1186/1471-2148-9-5

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. Mol Ecol 13:729–744. doi:10.1046/j.1365-294X.2003.02063.x

Bensch S, Akesson M (2005) Ten years of AFLP in ecology and evolution: why so few animals? Mol Ecol 14:2899–2914. doi:10.1111/j.1365-294X.2005.02655.x

Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P (2004) How to track and assess genotyping errors in population genetic studies. Mol Ecol 13:3261–3273. doi:10.1111/j.1365-294X.2004.02346.x

Bonin A, Taberlet P, Miaud C, Pompanon F (2006) Explorative genome scan to detect candidate loci for adaptation along a gradient of altitude in the common frog (*Rana temporaria*). Mol Biol Evol 23:773–783. doi:10.1093/molbev/msj087

Bonin A, Ehrich D, Mane S (2007) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Mol Ecol 16:3737–3758. doi:10.111 1/j.1365-294X.2007.03435.x

Bosch I, Pearse JS (1988) Seasonal pelagic development and Juvenile recruitment of the bivalve *Laternula elliptica* in McMurdo sound, Antarctica. Am Zool 28:A89–A89

Bosch I, Pearse JS (1990) Developmental types of shallow-water asteroids of McMurdo Sound, Antarctica. Mar Biol 104:41–46

Bosch I, Beauchamp KA, Steele ME, Pearse JS (1987) Development, metamorphosis, and seasonal abundance of embryos and larvae of the Antarctic Sea-Urchin *Sterechinus neumayeri*. Biol Bull 173:126–135

Bowden DA, Clarke A, Peck LS (2009) Seasonal variation in the diversity and abundance of pelagic larvae of Antarctic marine invertebrates. Mar Biol 156:2033–2047. doi:10.1007/s00227-009-1235-9

Brandstrom M, Ellegren H (2008) Genome-wide analysis of microsatellite polymorphism in chicken circumventing the ascertainment bias. Genome Res 18:881–887. doi:10.1101/gr.075242.107

Brumfield RT, Beerli P, Nickerson DA, Edwards SV (2003) The utility of single nucleotide polymorphisms in inferences of population history. Tree 18:249–256. doi:10.1016/S-169-5347(03)00018-1

Caballero A, Quesada H, Rolán-Alvarez E (2008) Impact of AFLP fragment size homoplasy on the estimation of population genetic diversity and the detection of selective loci. Genetics 179:539–554. doi:10.1534/genetics.107.083246

Casaux R (1998) The contrasting diet of *Harpagifer antarcticus* (Notothenioidei, Harpagiferidae) at two localities of the South Shetland Islands, Antarctica. Polar Biol 19:283–285. doi:10.1007/s003000050246

Clarke A (2000) Evolution in the cold. Antarct Sci 112:257

Colman JG. Life history biology of the Antarctic Bivalve *Yoldia eightsi*. BAS unpublished report H/1990/N3, Cambridge, UK

Crawley MJ (2002) Statistical computing, an introduction to data analysis using S-plus. Wiley, Chichester

Dasmahapatra KK, Lacy RC, Amos W (2007) Estimating levels of inbreeding using AFLP markers. Heredity 100:286–295. doi:10.1038/sj.hdy.6801075

Dasmahapatra KK, Hoffman JI, Amos W (2009) Pinniped phylogenetic relationships inferred using AFLP markers. Heredity 103:168–177. doi:10.1038/hdy.2009.25

Dasmahapatra KK, Elias M, Hill RI, Hoffman JI, Mallet J (2010) Mitochondrial DNA barcoding detects some species that are real, and some that are not. Mol Ecol Resour 10:264–273. doi:10.1111/j.1755-0998.2009.02763.x

Davenport J (1988) The Feeding Mechanism of *Yoldia* (=*Aequiyoldia*) *eightsi* (Courthouy). Proc Roy Soc Lond B Biol 232:431–442

Dayton PK, Oliver JS (1977) Antarctic soft-bottom benthos in oligotrophic and eutrophic environments. Science 197:55–58

Dayton PK, Robillia Ga, Paine RT, Dayton LB (1974) Biological accommodation in the benthic community at McMudo Sound, Antarctica. Ecol Monogr 44:105–128

Dearborn JH (1977) Foods and feeding characteristics of Antarctic asteroids and ophiuroids. In: Llano GA (ed) Adaptations within Antarctic ecosystems. Proceedings of the third SCAR symposium on Antarctic Biology, Smithsonian Institution. Gulf Publishing, Washington, DC, pp 293–326

Dyer AT, Leonard KJ (2000) Contamination, error, and nonspecific molecular tools. Phytopathol 90:565–567. doi:10.1094/PHYTO.2000.90.6.565

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. Nat Rev Genet 5:435–445. doi:10.1038/nrg1348

Fauchald K, Jumars P (1979) The diet of worms: a study of polychaete feeding guilds. Oceanogr Mar Biol Ann Rev 17:193–284

Gambi MC, Patti FP, Micaletto G, Giangrande A (2001) Diversity of reproductive features in some Antarctic polynoid and sabellid polychaetes, with a description of *Demonax polarsterni* sp (Polychaeta, Sabellidae). Polar Biol 24:883–891. doi:10.1007/s003000100287

Gerber S, Mariette S, Streiff R, Bodenes C, Kremer A (2000) Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. Mol Ecol 9:1037–1048. doi:10.1046/j.1365-294x.2000.00961.x

Giraud M, Matic I, Tenaillon O, Radman M, Fons M, Taddei F (2001) Costs and benefits of high mutation rates: adaptive evolution of bacteria in the mouse gut. Science 291:2606–2608. doi:10.1126/science.1056421

Golledge NR, Everest JD, Bradwell T, Johnson JS (2010) Lichenometry on Adelaide Island, Antarctic Peninsula: size frequency studies, growth rates and snow patches. Geografiska Annular A 91:111–124. doi:10.1111/j.1468-0459.2010x

Grange LJ, Tyler PA, Peck LS, Cornelius N (2004) Long-term interannual cycles of the gametogenic ecology of the Antarctic brittle star *Ophionotus victoriae*. Mar Ecol Prog Ser 278:141–155. doi:10.3354/meps278141

Held C, Leese F (2007) The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. Polar Biol 30:513–521. doi:10.1007/s00300-006-0210-x

Helyar SJ, Hemmer-hansen J, Bekkevold D, Taylor MI, Ogden R, Limborg MT, Cariani A, Maes GE, Diopere E, Carvalho GR, Nielsen EE (2011) Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. Mol Ecol Res 11:123–136. doi:10.1111/j.1755-0998.2010.02943.x

Hoffman JI (2011) Gene discovery in the Antarctic fur seal (*Arctocephalus gazella*) skin transcriptome. Mol Ecol Resour 11:703–710. doi:10.1111/j.1755-0998.2011.02999.x

Hoffman JI, Amos W (2005) Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Mol Ecol 14:599–612. doi:10.1111/j.1365-294X. 2004.02419.x

Hoffman JI, Dasmahapatra KK, Amos W, Phillips CD, Gelatt TS, Bickham JW (2009) Contrasting patterns of genetic diversity at three different genetic markers in a marine mammal metapopulation. Mol Ecol 18:2961–2978. doi:10.1111/j.1365-294X.2009.04246.x

Hoffman JI, Peck LS, Hillyard G, Zieritz A, Clark MS (2010) No evidence for genetic differentiation between Antarctic limpet *Nacella concinna* morphotypes. Mar Biol 157:765–778. doi:10.1007/s00227-009-1360-5

Hoffman JI, Clarke A, Linse K, Peck L (2011a) Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs. Mar Biol 158:287–296. doi:10.1007/s00227-010-1558-6

Hoffman JI, Peck L, Linse K, Clarke AC (2011b) Strong population genetic structure in a broadcast-spawning Antarctic marine invertebrate. J Hered 102:55–66. doi:10.1093/jhered/esq094

Huang SW, Yu HT (2003) Genetic variation of microsatellite loci in the major histocompatibility complex (MHC) region in the southeast Asian house mouse (*Mus musculus castaneus*). Genetica 119:201–218

Huang CW, Cheng YS, Rouvier R, Yang KT, Wu CP, Huang MC (2007) AFLP fingerprinting for paternity testing in ducks. Br Poult Sci 48:323–330. doi:10.1080/00071660701370459

Ihaka R, Gentleman GR (1996) A language for data analysis and graphics. J Comput Graph Stat 5:299–314

Jump AS, Hunt JM, Martinez-Izquierdo JA, Penuelas J (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. Mol Ecol 15:3469–3480. doi:10.1111/j.1365-294X.2006.03027.x

Li Y-C, Korol AB, Fahima T, Nevo E (2004) Microsatellites within genes: structure, function, and evolution. Mol Biol Evol 21:991–1007. doi:10.1093/molbev/msh073

Margulies M et al (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380. doi:10.1038/nature03959

Mariette S, LeCorre V, Austerlitz F, Kremer A (2002) Sampling within the genome for measuring within-population diversity: trade-offs between markers. Mol Ecol 11:1145–1156. doi:10.1046/j.1365-294X.2002.01519.x

Meredith MP, King JC (2005) Rapid climate change in the ocean west of the Antarctic Peninsula during the second half of the 20th century. Geophys Res Lett 32:L19604. doi:10.1029/2005GL024042

Metzgar D, Wills C (2000) Evidence for the adaptive evolution of mutation rates. Cell 101:581–584

Meudt HM, Clarke AC (2007) Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends Plant Sci 12:106–108. doi:10.1016/j.tplants.2007.02.001

Milot E, Weimerskirch H, Duchesne P, Bernatchez L (2007) Surviving with low genetic diversity: the case of albatrosses. Proc Roy Soc Lond B Biol 274. doi:10.1046/j.1365-2540.2001.00940.x

Møller AP, Cuervo JJ (2003) Sexual selection, germline mutation rate and sperm competition. BMC Evol Biol 3:6. doi:10.1186/1471-2148-3-6

Morin PA et al (2004) SNPs in ecology, evolution and conservation. Tree 19:208–216. doi:10.1016/j.tree.2004.01.009

Mueller UG, Wolfenbarger LL (1999) AFLP genotyping and fingerprinting. Trends Ecol Evol 14:389–394

Nakaoka M (1994) Size-dependent reproductive traits of *Yoldia notabilis* (Bivalvia, Protobranchia). Mar Ecol Prog Ser 114:129–137

Nunes VL, Beaumont MA, Butlin RK, Paulo OS (2011) Multiple approaches to detect outliers in a genome scan for selection in ocellated lizards (*Lacerta lepida*) along an environmental gradient. Mol Ecol 20:193–205. doi:10.1111/j.1365-294X.2010.04936.x

O'Hanlon PC, Peakall R (2000) A simple method for the detection of size homoplasy among amplified fragment length polymorphism fragments. Mol Ecol 9:815–816. doi:10.1046/j.1365-294x.2000.00924.x

Obermüller BE, Morley SA, Barnes DKA, Peck LS (2010) Seasonal physiology and ecology of Antarctic marine benthic predators and scavengers. Mar Ecol Prog Ser 415:109–126. doi:10.3354/meps08735

Pearse JS, Giese AC (1966) Food, reproduction, and organic constitution of the common Antarctic echinoid *Sterechinus neumayeri* (Meissner). Biol Bull 130:387–401

Peck LS (1993) Larval development in the Antarctic Nemertean *Parborlasia corrugatus* (Heteronemertea, Lineidae). Mar Biol 116:301–310

Peck LS (2001) Ecology of articulates. In: Carlson S, Sandy M (eds) Short course on brachiopods. Geological Society of the USA and University of Kansas, pp 171–184

Peck LS, Robinson K (1994) Pelagic larval development in the brooding Antarctic brachiopod *Liothyrella uva*. Mar Biol 120:279–286

Peck LS, Barnes DKA, Willmott J (2005) Responses to extreme seasonality in food supply: diet plasticity in Antarctic brachiopods. Mar Biol 147:453–463. doi:10.1007/s00227-005-1591-z

Peck LS, Clarke A, Chapman AL (2006) Metabolism and development of pelagic larvae of Antarctic gastropods with mixed reproductive strategies. Mar Ecol Prog Ser 318:213–220. doi:10.3354/meps318213

Poutiers JM (1998) Gastropods in: FAO species identification guide for fisheries purposes: the living marine resources of the Western Central Pacific Volume 1. Seaweeds, corals, bivalves and gastropods. Pub. Rome FAO, p 399

Rogers A (2007) Evolution and biodiversity of Antarctic organisms: a molecular perspective. Philos Trans R Soc Lond [Biol] 362:2191–2214. doi:10.1098/rstb.2006.1948

Santucci F, Ibrahim KM, Bruzzone A, Hewit GM (2007) Selection on MHC-linked microsatellite loci in sheep populations. Heredity 99:340–348. doi:10.1038/sj.hdy.6801006

Savelkoul PHM, Aarts HJM, De Haas J, Dijkshoorn L, Dium B, Otsen M, Rademaker JLW, Schouls L, Lenstra JA (1999) Amplified-fragment length polymorphism analysis: the state of an art. J Clin Microbiol 37:3083–3091

Shabica SV (1971) The general ecology of the Antarctic limpet *Patinigera polaris*. Antarct J US 6:160–162

Shabica SV (1976) The natural history of the Antarctic limpet *Patinigera polaris* (Humbron & Jacquinot) PhD Diss. Oregon State University, Corvallis

Slate J, Gratten J, Beraldi D, Stapley J, Hale M, Pemberton JM (2009) Gene mapping in the wild with SNPs: guidelines and future directions. Genetica 136:97–107

Slattery M, McClintock JB (1997) An overview of the population biology and chemical ecology of three species of Antarctic soft corals. In: Battaglia B, Valencia J, Walton DWH (eds) Antarctic Communities. Species, structure and Survival. Pub. CUP, pp 309–315

Stanwell-Smith D, Peck LS (1998) Temperature and embryonic development in relation to spawning and field occurrence of larvae of three Antarctic echinoderms. Biol Bull 194:44–52

Urban HJ, Mercuri G (1998) Population dynamics of the bivalve *Laternula elliptica* from Potter cove, King George Island, South Shetland islands. Antarct Sci 10:153–160

Vekemans X, Beauwens T, Lemaire M, Roldan-Ruiz I (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Mol Ecol 11:139–151. doi:10.1046/j.0962-1083.2001.01415.x

Vera JC, Wheat CW, Fescemyer HW, Frilander MJ, Crawford DL, Hanski I, Marden JH (2008) Rapid transcriptome characterization for a nonmodel organism using 454 pyrosequencing. Mol Ecol 17:1636–1647. doi:10.1111/j.1365-294X.2008.03666.x

Vorwerk S, Forneck A (2007) Analysis of genetic variation within clonal lineages of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) using AFLP fingerprinting and DNA sequencing. Genome 50:660–667. doi:10.1139/G07-046

Vos P, Hogers R, Bleker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

White MG, Burren PJ (1992) Reproduction and larval growth of *Harpagifer antarcticus* Nybelin (Pisces, Notothenioidei). Antarct Sci 4:421–430

Wilding CS, Butlin RK, Grahame J (2001) Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. J Exp Biol 14:611–619

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16. doi:10.1046/j.0962-1083.2001.01418.x