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Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs

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Abstract Life-history characteristics exert a profound influence upon the population structure of many marine organisms. However, relatively few genetic studies have compared direct with indirect-developing species in the same ecosystem or geographical region, and none to our knowledge within an Antarctic setting. To address this issue, we have collected novel amplified fragment length polymorphism (AFLP) data from the direct-developing top shell *Margarella antarctica* to form a comparison with previously published data for the broadcast-spawning Antarctic limpet *Nacella concinna*. We scored 270 loci in 240 *M. antarctica* individuals sampled from five populations spanning the full length of the Antarctic Peninsula. Profound differences were identified in the strength and pattern of population structure between the two species, consistent with gene flow being highly restricted in *M. antarctica* relative to *N. concinna*.

Introduction

It is widely believed that direct developing species exhibit restricted dispersal relative to indirect developers and should therefore tend to show lower levels of population genetic connectivity (e.g. Hellberg et al. 2002; Kinlan and Gaines 2003; Palumbi 2003). However, surprisingly few studies have tested this hypothesis directly by generating comparable empirical datasets, across similar geographical ranges, for species with contrasting life histories that are otherwise ecologically equivalent. Moreover, although some of these studies have found greater levels of population structure in direct developers in support of theoretical expectations (e.g. Nishikawa et al. 2003; Watts and Thorpe 2006; Sherman et al. 2008; Underwood et al. 2009), others have shown that it is not unusual for species with direct development to disperse long distances (e.g. Ayre and Hughes 2000; Richards et al. 2007). To explain this, authors have invoked the passive transport of individuals via fronds of seaweed or fragments of sponge dislodged during violent storms, or upon driftwood (e.g. Thiel and Gutow 2005a, b; Richards et al. 2007; Waters et al. 2008).

Another feature of previous comparative studies is that the majority have focussed on tropical species, with the primary emphasis being on taxa inhabiting coral reefs (e.g. Ayre and Hughes 2000; Bay et al. 2006; Nishikawa et al. 2003; Underwood et al. 2009). A few studies have also examined temperate marine species (e.g. Galarza et al. 2009), but to our knowledge none have yet explicitly compared the population structure of direct with indirect developers within a polar setting. This is important because, with polar regions currently warming faster than anywhere else on the planet (Meredith and King 2005), there is now a pressing need to evaluate how factors such as life history, geographical isolation and oceanographic barriers to gene flow interact to shape the population genetic structure of polar marine organisms.

The Antarctic marine environment has been relatively isolated since the formation of the Antarctic Circumpolar

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Current around 24–41 Mya (Lawver and Gahagan 2003; Pfuhl and McCave 2005; Scher and Martin 2006) and this has generated large numbers of endemic species on the Southern Ocean continental shelf (Clarke and Johnston 2003). Many of these have unique adaptations, such as the loss of haemoglobin in Channichthyd fish (di Prisco et al. 1998) and the widespread loss of the heat shock response (Clark and Peck 2009; Clark et al. 2008). Due to low temperatures in the Southern Ocean, physiological rates also tend to be very slow, with feeding and meal processing taking ten times longer in Antarctic predatory snails than in similar tropical species (Harper and Peck 2003) and development in benthic species also being an order of magnitude slower than tropical species (Peck et al. 2006). Protected development is also a more common attribute in Southern Ocean species than elsewhere (Clarke and Johnston 2003; Pearse and Lockhart 2004; Pearse et al. 2009), suggesting that a relatively large proportion of Antarctic marine species may possess poor dispersal capacities.

In practice, genetic studies have yielded considerable support for the above prediction, with many having found population structure strong enough even to warrant the delimitation of previously undescribed cryptic species (e.g. Krabbe et al. 2010; Linse et al. 2007; Wilson et al. 2007; Mahon et al. 2008; Thornhill et al. 2008). However, other studies paint a contradictory picture with, for example, little or no population structure being detected in circum-Antarctic populations of silverfish (Zane et al. 2006) or shrimp (Raupach et al. 2010). Life-history variation probably explains many of these interspecific differences, but unfortunately direct comparisons among species are difficult to make owing to the use of different sampling schemes and/or genetic markers. Consequently, there is a need to evaluate the role of life-history variation within an Antarctic setting by designing comparative studies that control for as many incidental variables as possible.

The Antarctic Peninsula and Scotia arc provide an excellent opportunity for exploring the relative impact of life-history variation, geographical isolation and oceanography upon the population structure of polar benthic invertebrates. The west coast of the Antarctic Peninsula forms a continuous coastline over 1,500 km long, spanning over 10° of latitude (Fig. 1). It is bordered by a string of islands that extend northwards to the South Shetland and South Orkney groups, and then on through the Scotia Arc and South Georgia to South America. The main oceanic current runs northwards along the Antarctic Peninsula, through the South Shetlands and then on to the South Orkney Islands (Deacon 1984; Savidge and Amft 2009), while near-shore coastal currents also flow around the tip of the peninsula and travel southwards for several hundred kilometres (Moffat et al. 2008; Thompson et al. 2009). The resulting complex system of eddies and gyres (Hofmann et al. 1996), together with discontinuous continental shelves and variation in the depth of the sea bed, forms an ideal natural experimental system in which to evaluate differences in dispersal capability due to reproductive mode.

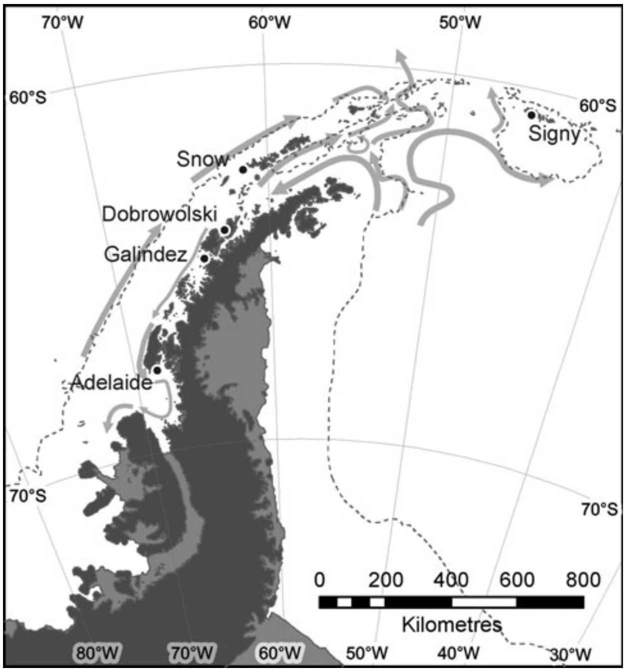

Fig. 1 Map of the Antarctic Peninsula showing the sampling locations of paired *M. antarctica* and *N. concinna* populations. A schematic of the main currents is also shown (based on data presented by Moffat et al. 2008, Savidge and Amft 2009, Thompson et al. 2009), with *arrow* thickness being proportional to the strength of flow

To explore the impact of life history on the population structure of organisms inhabiting the shallow coastal waters of the Antarctic Peninsula and the Scotia Arc, we compared two molluscan species with contrasting reproductive modes that are both abundant and widespread, *Margarella antarctica* and *Nacella concinna*. New data are provided here for *M. antarctica*, and these are compared against a previously published data set for *N. concinna* (Hoffman et al. 2010b), allowing elucidation of the effect of reproductive mode on gene flow. These species were chosen because they are from the same class (Gastropoda) and also have several ecological similarities including being members of the same trophic guild of surface film grazers (Linse et al. 2006). However, reproductively *M. antarctica* is a small trochid that exhibits non-pelagic reproduction, producing egg capsules which it attaches to rock or seaweed substrates from which crawling juveniles emerge (Picken 1979). In contrast, *N. concinna* is a broadcast-spawner with a free-swimming planktotrophic veliger larval stage that can survive in the water column for up to 2 months (Bowden et al. 2006).

To facilitate the comparison of these two species, we used amplified fragment length polymorphisms (AFLPs, Vos et al. 2008). This technique is based on the PCR amplification of a subset of restriction fragments that are generated using a pair of restriction enzymes. AFLPs tend to be highly polymorphic, rendering them capable of detecting even very minor genetic differences (Maughan et al. 1996). They are also well suited to interspecific comparisons such as the study we have undertaken because they readily amplify in virtually any species, thereby providing a means of generating parallel data sets without the need for multiple rounds of expensive and time-consuming marker development.

Here, we genotyped 240 *M. antarctica* individuals sampled from five populations spanning the full length of the Antarctic Peninsula at over 200 polymorphic AFLP loci. These data were analysed together with a previously published AFLP data set for *N. concinna* comprising an identical number of individuals sampled from the same five locations. Our aims were to test the hypothesis that *M. antarctica* exhibits stronger population structure than *N. concinna*, and to examine the effect of the deep waters of the Scotia Sea and the possible impact of local gyres and other hydrographical structures (Hofmann et al. 1996) on the genetic connectivity of shallow-water species along the Antarctic Peninsula.

Materials and methods

Tissue sample collection

We collected 240 *M. antarctica* samples during the 1999 ‘Peninsula Geneflow Cruise’ from the shallow sublittoral zone off the islands of Adelaide, Galindez, Dobrolowski, Snow and Signy (Table 1; Fig. 1). This cruise was designed both to sample sites along a latitudinal transect and to evaluate the effects of any potential barriers to gene flow, including current systems and deep water. A major advantage of this study was the collection of matched samples for the two species with contrasting life histories, the trochid top shell *M. antarctica* and the limpet *N. concinna*. Samples were collected by SCUBA divers in a series of four to ten dives at each site that were randomly distributed over an area of approximately 5 km^2. Tissue samples were stored in 95% ethanol, and total genomic DNA was subsequently extracted from a small piece of foot tissue using the Qiagen DNeasy tissue extraction kit following the manufacturer’s recommended protocols. Voucher tissue specimens of both species are available on request from the British Antarctic Survey.

Table 1 Details of sampling locations and numbers of *M. antarctica* and *N. concinna* individuals collected at each site

Sampling site	Latitude (S)	Longitude (W)	Number of *M. antarctica* samples	Number of *N. concinna* samples
Adelaide Island	67°36′25	68°11′28	48	48
Galindez Island	65°14′00	64°14′00	48	48
Dobrolowski Island	64°36′52	64°55′06	48	48
Snow Island	62°46′25	61°14′03	48	48
Signy Island	60°40′88	45°36′10	48	48
Total			240	240

AFLP genotyping

The AFLP protocol was adapted from Vos et al. (1995) as described in Hoffman et al. (2010a). Briefly, 100–400 ng of genomic DNA was sequentially digested using *Taq*I (5U in a 10 μl volume at 65°C for 2 h) and then *Eco*RI (5U in a 20 μl volume at 37°C for 2 h). *Taq*I and *Eco*RI and adapters (Ajmone-Marsan et al. 1997) 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 were diluted by a factor of ten 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 reactions 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 (the primer sequences were 5′GATGAGTCCTGACCGAC-3′ and 5′-GACTGCGTACCAATTCA-3′ respectively). 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 10 times 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 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). Seven different selective primer combinations were employed (Table 2), five of which were the same as those used for *N. concinna*.

PCR products were resolved by electrophoresis through 6% polyacrylamide gels and exposed to X-ray film for 5 days. These were then developed using a universal X-ray developer (Xograph Healthcare Ltd.) and, if required, a second exposure was made for an adjusted time period. All bands in the approximate size range of 75–300 bp were scored manually by an experienced operator (JIH). Only clear bands with minimal size variation that could be scored in all individuals were included, these being recorded as 1 = present and 0 = absent. Pairs of bands that were clearly

Table 2 Primer combinations used for the AFLP selective amplification and numbers of AFLP loci generated in 240 individuals each of *M. antarctica* and *N. concinna*

*Taq*I primer (5′–3′)	*Eco*RI primer (5′–3′)	*M. antarctica*			*N. concinna*		
		No. loci	No. polymorphic loci	% polymorphic loci	No. loci	No. polymorphic loci	% polymorphic loci
GATGAGTCCTGACCGA–CTG	GACTGCGTACCAATTC–AGC	46	41	89.1	40	33	82.5
GATGAGTCCTGACCGA–CGA	GACTGCGTACCAATTC–AGC	42	37	88.1	27	18	66.7
GATGAGTCCTGACCGA–CAG	GACTGCGTACCAATTC–AGC	29	21	72.4	21	18	87.5
GATGAGTCCTGACCGA–CAC	GACTGCGTACCAATTC–AGC	31	24	77.4	33	28	84.8
GATGAGTCCTGACCGA–CCA	GACTGCGTACCAATTC–AAC	28	19	67.9	28	21	75.0
GATGAGTCCTGACCGA–CGA	GACTGCGTACCAATTC–ATG	44	38	86.4	–	–	–
GATGAGTCCTGACCGA–CAG	GACTGCGTACCAATTC–ATG	50	42	84.0	–	–	–
GATGAGTCCTGACCGA–CCA	GACTGCGTACCAATTC–ACA	–	–	–	20	18	90.0
GATGAGTCCTGACCGA–CAC	GACTGCGTACCAATTC–ATG	–	–	–	44	38	86.4
Total		270	222	82.2	213	174	81.7

non-independent were scored as single traits. It was assumed that AFLP bands that were the same size across individuals represented homologous markers. Data were entered manually into a Microsoft excel spreadsheet. The genotyping error rate was estimated following Hoffman and Amos (2005) by independently re-extracting, re-genotyping and blind-scoring 24 randomly selected individuals (10% of the samples). The error rate per reaction, calculated following Bonin et al. (2004) as the number of mismatching genotypes divided by the number of polymorphic bands compared, was 2.1% (111 differences observed out of 5,217 comparisons).

A large AFLP data set was also available for *N. concinna*, comprising 332 individuals sampled from eight populations spanning the continental shelves of the western Antarctic Peninsula, Signy Island and South Georgia (Hoffman et al. 2010b). Both species were genotyped using exactly the same methodology within the same laboratory. To allow a direct comparison of the two species, we restricted the *N. concinna* data set to 240 individuals sampled from the five populations that were common to both studies (Table 1). However, because more than 60 unique AFLP loci were found in limpets from South Georgia, the exclusion of this population resulted in a significant reduction in the total number of polymorphic loci relative to the former study (Table 2). The genotyping error rate for the restricted *N. concinna* data set, assessed by re-genotyping 20 individuals, was 1.4% (48 differences observed out of 3,414 comparisons).

Data analysis

The program Aflp-Surv (Vekemans 2002) was used to calculate pairwise F_{st} values among the populations and to generate F_{st} matrices for each of 1,000 bootstrapped datasets. A neighbour-joining tree was then generated for each species using the Neighbour, Consense and Fitch modules in Phylip (Felsenstein 1993). Aflp-Surv was also used to conduct permutation tests for overall genetic differentiation using 10,000 randomisations of the data set. Geographical distances among populations were calculated using a Geographic Information System (ESRI ArcGis v 9.2) as described in detail by Hoffman et al. (2010b). The significance of correlations between genetic and geographical distance was assessed using Mantel tests with 999 iterations implemented in Genalex v6 (Peakall and Smouse 2005). Finally, cluster analysis of the AFLP data sets was conducted using the program Structure version 2.3.3 (Pritchard et al. 2000). Structure uses an iterative approach to cluster the genotypes into *K* populations without using the geographical locations of individuals. The approach essentially subdivides the genetic data in a way that maximises Hardy–Weinberg equilibrium and linkage equilibrium within the resulting clusters. The membership of each individual in a population is then estimated as q, which varies between 0 and 1 with the latter indicating full population membership. We ran five independent runs for $K = 1$–10 with RECESSIVEALLELES set to 1 (Falush et al. 2003) and using 10^6 MCMC iterations after a burn-in of 10^5, the correlated allele frequencies model and assuming admixture. The most likely number of groups was evaluated using the maximal average value of Ln $P(D)$, a model-choice criterion that estimates the posterior probability of the data.

Results

To explore the impact of life history on population structure, we used seven selective AFLP primer combinations to genotype *M. antarctica* individuals from five populations spanning the Antarctic Peninsula. The resulting data set consisted of 53, 280 binary characters representing the presence and absence genotypes of 240 individuals at 222 polymorphic loci (Table 2). For comparison, AFLP data were also available for 240 *N. concinna* individuals sampled from the same five locations and genotyped at 174 polymorphic loci (Table 2, Hoffman et al. 2010b).

Phylogeographical patterns

Although appreciable levels of genetic structure were detected in both species, global F_{st} was over seven times higher in *M. antarctica* (0.0887, $P < 0.0001$) than in *N. concinna* (0.0120, $P < 0.0001$). Similarly, all of the F_{st} values obtained in pairwise population comparisons were individually significant at $P < 0.0001$ in *M. antarctica*, whereas only a subset of comparisons involving Adelaide and Signy Islands were significant in *N. concinna* (Table 3). Plotting the shortest geographical distance by sea among populations against genetic distance yielded significant isolation-by-distance patterns in both species (Fig. 2, *M. antarctica*, Mantel's $r = 0.790$, $n = 5$, $P = 0.010$; *N. concinna*, Mantel's $r = 0.950$, $n = 5$, $P = 0.044$). However, significance was lost in *N. concinna* when Signy was excluded from the analysis (Mantel test, $r = 0.403$, $n = 4$, $P = 0.352$), whereas the relationship between genetic and geographical distance remained marginally significant in *M. antarctica* even using a conservative Mantel test on only four populations ($r = 0.863$, $n = 4$, $P = 0.046$).

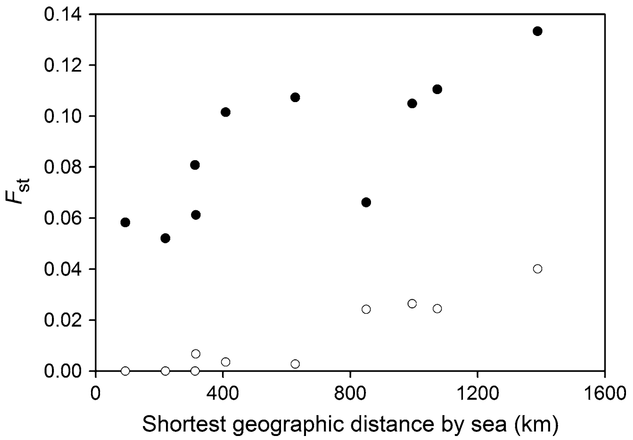

Fig. 2 The relationship between geographical and genetic distance (F_{st}) among five *M. antarctica* (*filled circles*) and *N. concinna* (*empty circles*) populations

To further explore patterns of population structure, we next constructed consensus neighbour-joining trees for each species using 1,000 bootstrapped F_{st} matrices. The resulting topography was well resolved in *M. antarctica*, with all of the nodes gaining >80% bootstrap support and perfect concordance being found between genetic divergence and the geographical distribution of populations (Fig. 3a). In contrast, only Signy and Adelaide Islands

Table 3 Pairwise F_{st} values among populations of *M. antarctica* and *N. concinna* (above diagonal). P values, calculated using 10,000 permutations of the data, are given below the diagonal, with significant values highlighted in bold

	Adelaide Island	Galindez Island	Dobrolowski Island	Snow Island	Signy Island
M. antarctica					
Adelaide Island	*	0.060	0.100	0.106	0.1325
Galindez Island	**<0.0001**	*	0.058	0.076	0.109
Dobrolowski Island	**<0.0001**	**<0.0001**	*	0.046	0.104
Snow Island	**<0.0001**	**<0.0001**	**<0.0001**	*	0.0676
Signy Island	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	*
N. concinna					
Adelaide Island	*	0.007	0.004	0.003	0.040
Galindez Island	**0.001**	*	0.000	0.000	0.025
Dobrolowski Island	**0.001**	0.630	*	0.000	0.026
Snow Island	**0.032**	0.802	0.953	*	0.024
Signy Island	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	*

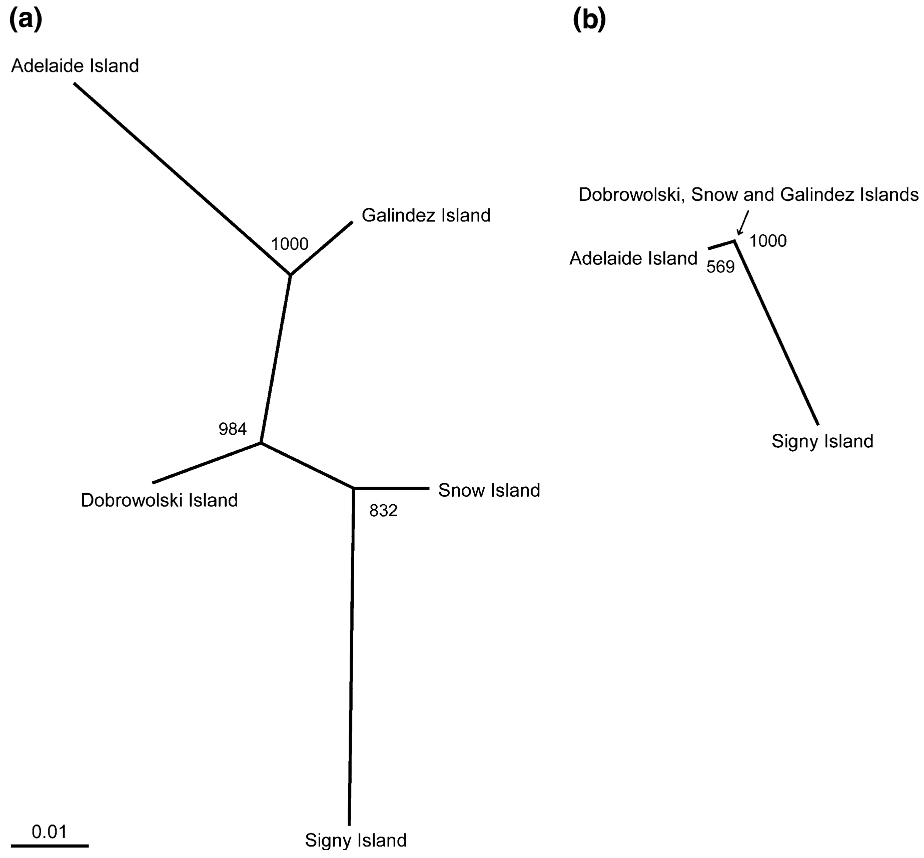

Fig. 3 Consensus F_{ST} neighbour-joining trees for five paired populations of **a** *M. antarctica* and **b** *N. concinna*. *Numbers* indicate nodes with greater than 50% support for 1,000 bootstrap replicates. Both trees are plotted on the same scale

could be resolved with >50% bootstrap support in the *N. concinna* tree, and branch lengths were also relatively short indicating lower levels of differentiation (Fig. 3b).

Bayesian cluster analysis

Arguably, the most powerful tests of genetic structure need not rely on a priori knowledge of the sampling locations of individuals. Consequently, we implemented Bayesian cluster analysis of the two AFLP data sets using the program Structure (Pritchard et al. 2000). Five replicate runs were conducted for each possible number of groups (K) ranging from one, implying no population differentiation, through to five, which would imply that each of the populations were genetically distinct. The highest average log likelihood value was associated with $K = 5$ in *M. antarctica* (Fig. 4a), providing support for the presence of five distinct groups. Membership coefficients for these inferred groups are summarised in Fig. 4c, in which each vertical bar represents a different individual and the relative proportions of the different shades from black to white indicate the probabilities of belonging to each group. By inspection, there was almost perfect concordance between the five groups identified by Structure and the five source populations from which the individuals were sampled. Moreover, when individuals were assigned to the groups in which their membership coefficients were greatest, all but six of the 240 individuals (97.5%) were successfully allocated to their populations of origin. A contrasting pattern was found for *N. concinna* in which mean Ln $P(D)$ peaked at $K = 1$ (Fig. 4b), suggesting that the most likely scenario was an absence of population structure. However, exploring a possible secondary peak at $K = 3$ revealed an additional cluster (shown in white) that was largely indicative of individuals sampled from Signy Island (Fig. 4d).

Discussion

Comparative population genetic studies of sympatric direct and indirect-developing taxa are relatively few, and although some have yielded results broadly consistent with theoretical expectations (e.g. Nishikawa et al. 2003; Sherman et al. 2008; Underwood et al. 2009), others have revealed unexpected patterns (e.g. Ayre and Hughes 2000; Richards et al. 2007). Consequently, we analysed the population structure of two benthic marine Antarctic molluscs, the direct-developing top shell *M. antarctica* and the indirect-developing limpet *N. concinna*. Population connectivity was found to be markedly lower in the top shell, consistent with the prediction that life history is a major determinant of the population genetic structure of benthic marine species.

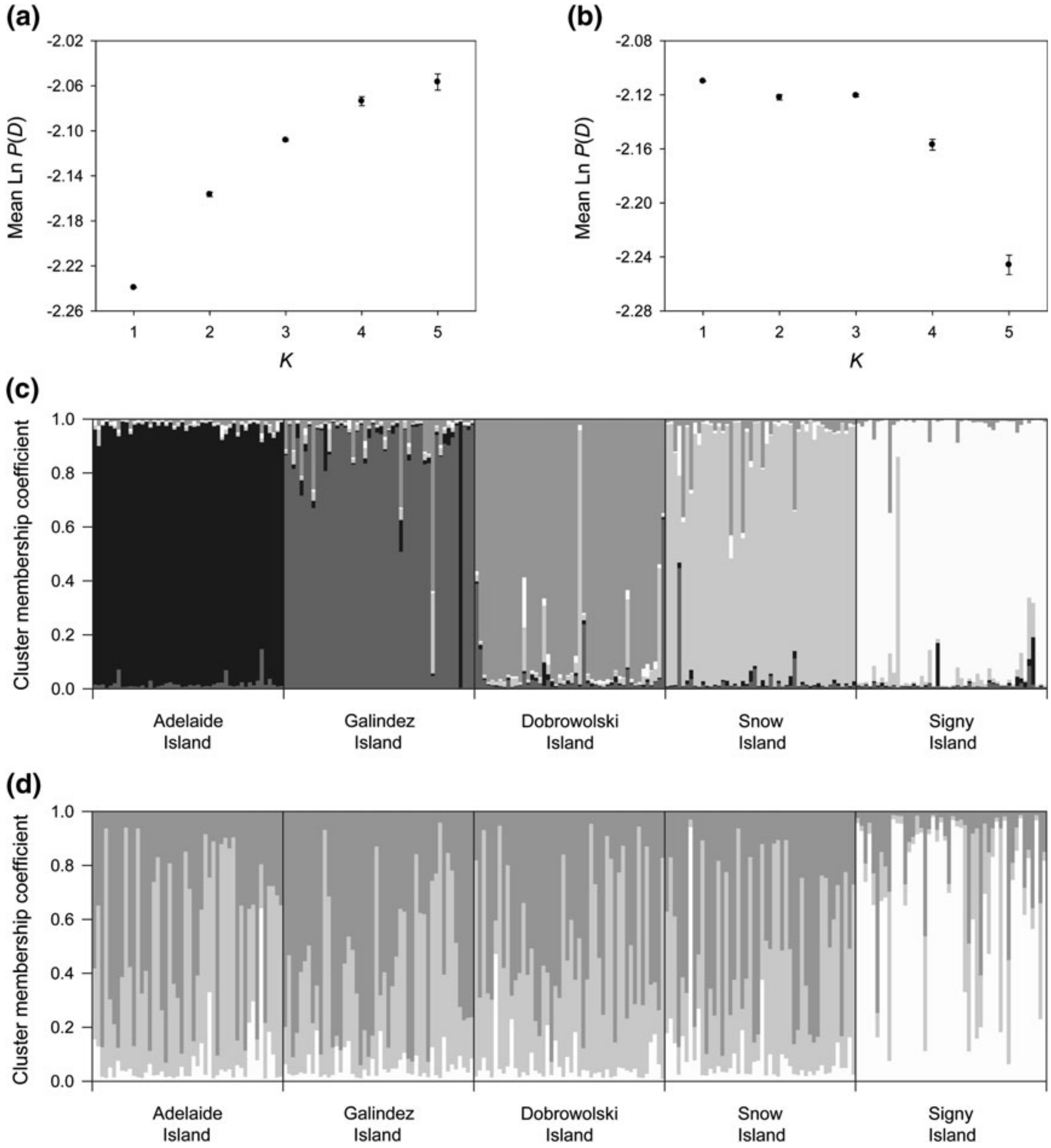

Fig. 4 Results of the STRUCTURE (Pritchard et al. 2000) analyses. The upper panels show mean ± SE Ln $P(D)$ values based on five replicates for each value of K, the hypothesised number of groups in the data, for **a** *M. antarctica* and **b** *N. concinna*. The *lower panels* show group membership coefficients derived for 240 individuals of **c** *M. antarctica* ($K = 5$) and **d** *N. concinna* ($K = 3$), where each individual is represented by a *vertical line* partitioned into segments of *different colour*, the lengths of which indicate the posterior probability of membership in each group

Strength and patterns of genetic structure

Although significant population partitioning was detected in both species, global F_{st} was over seven times higher in *M. antarctica*. This is important because, by sampling both species from the same geographical locations, our study design effectively controlled not only for the effect of geographical isolation but also for the possible presence of any physical barriers to gene flow. The most parsimonious interpretation of any differences between the two species is thus their contrasting life histories. Interestingly, the two species not only differed in the overall magnitude of F_{st} but also more subtly in the distribution of genetic variation among populations. For example, a highly significant regression was obtained between geographical and genetic distance in *M. antarctica*, whereas the equivalent relationship in *N. concinna* was only marginally significant and driven entirely by samples from Signy Island. Similarly, the consensus neighbour-joining tree for *M. antarctica* showed strong bootstrap support for all of the nodes and relatively long branches, while only two populations were discernable in the *N. concinna* tree, lending it the appearance of a 'hockey stick' (Fig. 3). Taken together, these observations suggest that *M. antarctica* populations are highly structured throughout the geographical range studied, whereas *N. concinna* is essentially panmictic, with the exception of the two populations sampled from the extremes of the range. This conclusion is supported by a larger *N. concinna* data set (Hoffman et al. 2010b) from which it is also possible to infer that the deeper waters of the Scotia Sea present a disproportionately strong barrier to gene flow in this species, helping to explain why Signy Island is more strongly differentiated.

Unfortunately, we were unable to obtain *M. antarctica* samples from all of the populations from which *N. concinna* was collected, not least because South Georgia lies outside the species' range boundary (Linse 2002), precluding a better understanding of how variation in depth impacts population connectivity in this species. Similarly, with only 10 pairwise population comparisons and no samples collected at spatial scales finer than 100 km, we are unable to more precisely delimit the shape of the isolation-by-distance profile. This would be of interest because Sherman et al. (2008) found that a distance of several kilometres was just as effective a barrier to dispersal to a direct-developing cushion star as far larger regions of unsuitable habitat. However, even with only five populations it is possible to deduce that, as with *N. concinna*, the two *M. antarctica* populations sampled from the extremes of the range are also the most strongly differentiated. This suggests that similar geographical factors may be to some extent jointly impacting both species.

Perhaps unsurprisingly given the relatively weak population structure found in the broadcast-spawning *N. concinna*, the program STRUCTURE was unable to discriminate among populations of this species in the absence of any a priori information about the geographical origins of individuals, although there was the suggestion of very weak population structure being present when we explored a possible secondary peak at $K = 3$. In contrast, STRUCTURE was not only able to resolve all five populations in the brooding *M. antarctica*, but when individuals were assigned to the groups in which their membership coefficients were greatest, all but six specimens (234/240, 97.5%) were correctly assigned to their populations of origin. This program therefore provides good independent analytical support for *M. antarctica* populations being strongly structured relative to *N. concinna*.

One unavoidable source of difference in our study is that although we made considerable efforts to collect matched sample sizes of individuals in the original collections, greater numbers of informative loci were obtained in *M. antarctica* ($n = 222$) than in *N. concinna* ($n = 174$) for the same experimental effort (i.e. the screening of seven different selective primer combinations). However, we believe that this difference in resolution is unlikely to account for the contrasting strength and patterns of genetic structure observed in the two species. This is not only because the smaller panel of 174 loci is still large, but also because AFLPs are sensitive to very small genetic differences (e.g. Maughan et al. 1996). However, to discount this possibility, we randomly selected a subset of 174 loci in *M. antarctica* and repeated all the analyses. The results were qualitatively very similar (data not shown), suggesting that variation in the strength and pattern of population genetic structure between the two species is not readily explicable by differences in genetic resolution. Similarly, we obtained very similar results regardless of whether we analysed all of the available AFLP data, or just those from the five AFLP selective combinations that were common to both species.

Our findings are in line with several previous studies that have compared the population structure of direct with indirect developers, although these were mostly investigations of coral reef-dwelling species. For example, Nishikawa et al. (2003) and Underwood et al. (2009) recorded higher levels of genetic structure in brooding relative to broadcast-spawning corals, and Watts and Thorpe (2006) and Sherman et al. (2008) found the same in bryozoa and cushion stars, respectively. Similarly, a direct developing species of coral reef fish exhibited markedly stronger population structure than seven otherwise comparable fish species possessing dispersive larval stages (Bay et al. 2006). However, similar comparisons elsewhere have yielded results that are less clear cut. For example, a genetic study of five brooding and four broadcast-spawning corals sampled from the Great Barrier Reef did not support the simple prediction that brooding species have more restricted dispersal than broadcast-spawners (Ayre and Hughes 2000). Instead, these authors found that three of the five brooders studied were panmictic across a geographical range of over 1,200 km, an outcome that is indicative of long-distance gene flow. In a similar comparison involving a broadcast-spawning brittle star and two brooding amphipods sampled along a 350-km stretch of coral reef in Florida, Richards (2007) was unable to detect any genetic structure in either of the amphipods. This was attributed to the intermittent long-distance transport of individuals within fragments of sponge dislodged and transported during strong storms or hurricanes. Effects of drifting or rafting have also been discussed for other species in a broad context (e.g. Waters 2008; Thiel and Gutow 2005a, b).

Prior to our study, it was thought possible that *M. antarctica* might be subject to occasional passive transport. This is because, although freshly deposited egg masses are most usually seen attached to bedrock or to the undersides of stones (Picken 1979), one of us (KL, pers. obs.) has observed fresh egg masses on the lower blades of red seaweed (Rhodophyta). Plausibly, this could permit passive gene flow via the rafting of eggs on seaweed fronds dislodged during violent storms or by iceberg impacts (e.g. Barnes et al. in press). However, our genetic data allow us to refute this hypothesis for *M. antarctica*, with strong population structure instead suggesting either that such dispersal events are extremely uncommon, or that they rarely if ever result in the effective transfer of genetic material between populations. Possible explanations for this could be that the egg masses of *M. antarctica* are too fragile to survive long-distance transport in strong currents, or that they are negatively buoyant to the extent that algal fragments with egg masses would sink.

Differences in the strength and pattern of population structure among species can also arise independently of life-history variation. For example, Bird et al. (2007) found sharply contrasting patterns of population structure in three different Hawaiian limpet species possessing qualitatively very similar life histories. In their study, population genetic differentiation was more strongly correlated with biogeographical range and microhabitat specificity than with larval duration. Similarly, Galarza et al. (2009) found no relationship between either egg type or larval duration and the strength of genetic structure across seven littoral fish species in the western Mediterranean, leading to these authors suggesting that genetic connectivity could be influenced either by unstudied biological characteristics such as larval or adult behaviour, or perhaps alternatively by fine-scale spatiotemporal variability in the physical environment. Consequently, it would be of interest in the future to extend our comparative approach to embrace a more phylogenetically diverse panel of species.

Conclusion

We tested the impact of reproductive mode on population connectivity by comparing two common Antarctic benthic surface grazing gastropods with contrasting developmental strategies, the direct developing top shell *M. antarctica* and the broadcast-spawning Antarctic limpet *N. concinna*. Our findings support the widely held view that life- history variation strongly impacts population connectivity in marine species. Future studies should aim to sample additional taxa with contrasting reproductive modes from the Antarctic Peninsula to determine the generality of our findings.

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