Journal of Heredity 2011:102(1):55–66
doi:10.1093/jhered/esq094
Advance Access publication August 18, 2010

Strong Population Genetic Structure in a Broadcast-Spawning Antarctic Marine Invertebrate

Joseph I. Hoffman, Lloyd S. Peck, Katrin Linse, and Andrew Clarke

From the Department of Animal Behaviour, University of Bielefeld, Bielefeld, Germany (Hoffman); and the British Antarctic Survey, Natural Environment Research Council, Cambridge, UK (Peck, Linse, and Clarke).

Address correspondence to Joseph I. Hoffman, Department of Animal Behaviour, University of Bielefeld, Postfach 100131, 33501 Bielefeld, Germany, or e-mail: j_i_hoffman@hotmail.com.

Abstract

Although studies of population genetic structure are commonplace, a strong bias exists toward species from low latitudes and with relatively poor dispersal capabilities. Consequently, we used 280 amplified fragment length polymorphism bands to explore patterns of genetic differentiation among 8 populations of a high latitude broadcast-spawning marine mollusc, the Antarctic limpet *Nacella concinna*. Over 300 individuals were sampled along a latitudinal gradient spanning the Antarctic Peninsula from Adelaide Island to King George Island (67°–62°S), then to Signy Island (60°S) and South Georgia (54°S). Populations from the Antarctic Peninsula exhibited little genetic structure but were themselves strongly differentiated from both Signy and South Georgia. This finding was analytically highly robust and implies the presence of significant oceanographic barriers to gene flow in a species long regarded as a classic example of a widely dispersing broadcast spawner.

Key words: *amplified fragment length polymorphisms, Antarctic limpet, Antarctic Peninsula, climate change, larval dispersal, mollusc,* Nacella concinna, *phylogeography*

It has long been assumed that planktonic developing marine organisms are excellent dispersers, forming vast panmictic populations. However, with the majority of empirical studies to date having focused on species with relatively poor dispersal capabilities (Bradbury et al. 2008), this generalization has not yet been widely tested. In parallel, the scientific literature is also strongly biased toward organisms from low latitudes (Bradbury et al. 2008), probably at least in part due to the difficulty and expense of obtaining samples from polar regions. Fortunately, several recent genetic studies of Antarctic organisms have begun to redress this imbalance, for example by using mitochondrial DNA (mtDNA) to identify cryptic species (e.g., Linse et al. 2007; Wilson et al. 2007; Mahon et al. 2008) and to evaluate genetic connectivity across major oceanic features such as the Drake Passage (e.g., Shaw et al. 2004; Thornhill et al. 2008; Wilson et al. 2009). However, few studies to date have explored the impact of more subtle oceanographic features. An urgent need has also been identified for the development and application of faster evolving nuclear markers for fine-scale intraspecific studies in general but specifically for the Antarctic biota (Held and Leese 2009).

Antarctica provides an unparalleled opportunity to undertake studies of the origins and maintenance of biological diversity, at both species and population levels (Clarke 2000). Millions of years of isolation from warmer waters to the North by the Antarctic Circumpolar Current have led to the evolution of a diverse and abundant benthic fauna that is both highly endemic and cold adapted (Clarke and Johnston 2003; Barnes and Griffiths 2008). One of the most striking series of events in Antarctica were the Cenozoic glaciations (Zachos et al. 2001) when extensions of the ice sheets and coincidental reduction of available shelf habitats probably forced species into wide bathymetric ranges which still can be found in Antarctic shelf benthos (Brey et al. 1996). Instead of making the shelf fauna extinct, however, the advance and retreat of the ice sheet appears instead to have promoted speciation by separating populations for long periods of time and then recombining them (Clarke and Crame 1989), providing a mechanism for speciation analogous to the "climate diversity pump" of Valentine (1967).

Over the past decade, attention has increasingly focused on the Antarctic Peninsula, the most proximate projection of the Antarctic land mass to another continent and a region that is currently experiencing one of the fastest rates of regional climate change on earth. Here, mean annual air temperatures have increased by as much as 3 °C over the

past half century and winter minimum temperatures have risen by over 5 °C during the same period (King et al. 2003). This has driven the widespread retreat of glaciers, ice shelf collapse, and exposure of new habitats in both terrestrial and marine sites, some of which have resulted in the production of large amounts of novel biological productivity (Peck, Barnes, et al. 2009). Predicting the future impact of environmental change in this region is nontrivial but because benthic Antarctic organisms tend to have very limited capacities to cope with elevated temperature (Peck, Clark, et al. 2009), the ability of individuals to migrate to new sites that allow survival could become increasingly important to the persistence of populations or even species (Barnes et al. 2010). Consequently, there is a need to collect baseline genetic data from a variety of taxa inhabiting this region with a view toward better understanding the factors that influence population connectivity.

The Antarctic Peninsula is not only important in the above context but also constitutes, together with the adjacent islands of the Scotia Chain, an exceptional system for exploring the impact of a variety of factors including geographical separation, depth, and current systems on levels of gene flow. The Peninsula itself (Figure 1) is over 1500 km long and is bounded by the Bellingshausen Sea to the west and Weddell Sea to the east. There is a complex of currents close inshore along the west coast of the peninsula that moves both west and south over large areas. In addition, the main circumpolar current flows from the Antarctic Peninsula and South Shetland Islands to South Georgia. Most of the current bypasses the South Orkney Islands, but there is still significant water flow to this island group at the edge of the current and via eddy systems. Sea surface current models suggest that the probability of particles released at the tip of the Antarctic Peninsula reaching South Georgia may be as low as 0–10%, with the journey probably taking around 150–250 days (Thorpe et al. 2007), although a recent study by Matschiner et al. (2009) found that 13 of 52 passive drifters (25.0%) reached South Georgia from the Antarctic Peninsula within 4 months. The extended development times of several broadcast-spawning Antarctic marine species including brachiopods, echinoderms, molluscs, and fish potentially allow for periods of this duration (Peck and Robinson 1994; Peck et al. 1997; Stanwell-Smith and Peck 1998). However, the few empirical studies to have tested this prediction have yielded somewhat contradictory findings. For example, a recent mtDNA study of a circumpolar crinoid (*Promachocrinus kerguelensis*) with buoyant eggs capable of dispersing long distances revealed

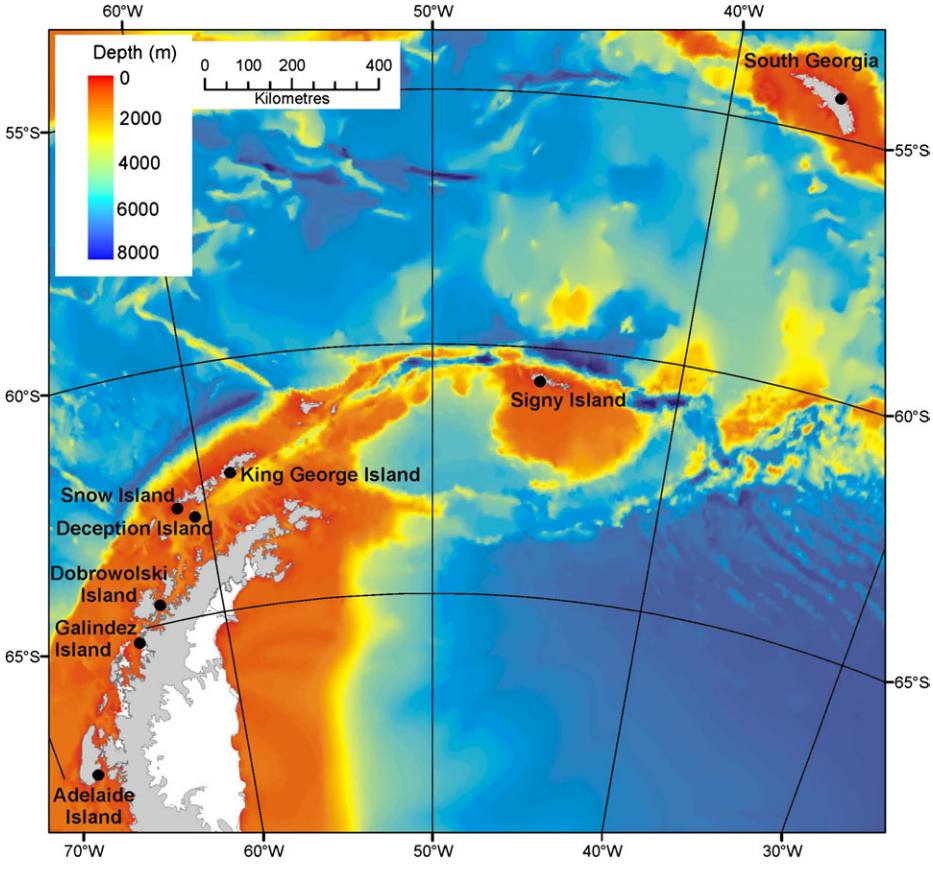

Figure 1. Map showing the sampling locations of 8 *Nacella concinna* populations with bathymetry depicted in color.

several distinct lineages, the largest and most widespread of which showed significant differences between the Antarctic Peninsula, South Sandwich Islands and South Georgia, suggesting restricted gene flow (Wilson et al. 2007). In contrast, no significant genetic differences were detected using either mtDNA or microsatellites among populations of a free spawning notothenioid fish (*Gobionotothen gibberifrons*) sampled from the tip of the Antarctic Peninsula, the South Sandwich Islands, and South Georgia (Matschiner et al. 2009), implying high levels of population connectivity.

The Antarctic limpet *Nacella concinna* provides an ideal model system with which to elucidate the genetic structure of a high latitude broadcast-spawning invertebrate. This species is one of the most dominant shallow-water macro-invertebrates found in the maritime Antarctic (Powell 1951; Walker 1972) and possesses a free-swimming planktotrophic veliger larval stage that can survive in the water column for at least 1–2 months (Bowden et al. 2006). Although it has already been subject to a number of genetic studies (Beaumont and Wei 1991; de Aranzamendi et al. 2008; Hoffman et al. 2010; González-Wevar et al., forthcoming), relatively little is currently known about the population structure of this species. This is primarily because previous work has focused on testing the hypothesis that 2 distinct morphotypes of *N. concinna*, an intertidal "polaris" form and a subtidal "concinna" form (Strebel 1908; Powell 1951) constitute genetically distinct races. A mixed picture has emerged, with Beaumont and Wei (1991) finding no genetic differences between the morphotypes using 5 allozymes, de Aranzamendi et al. (2008) reporting weak but statistically significant differences using 35 binary intersimple sequence repeat (ISSR) markers, and the latest and largest study by Hoffman et al. (2010) being unable to detect any genetic differences using 168 amplified fragment length polymorphisms (AFLPs).

AFLPs are useful markers for studying population genetic structure because they combine several desirable properties including high reproducibility, the potential to screen large numbers of loci, and the ability to resolve even extremely small genetic differences (Maughan et al. 1996; Mueller and Wolfenbarger 1999; Bensch and Akesson 2005; Meudt and Clarke 2007). AFLPs are also predominantly nuclear in origin and readily amplifiable across a variety of taxa without the need for any prior genomic information (Vos et al. 1995), making them particularly well suited to the study of organisms whose genomes are poorly characterized, such as the majority of Antarctic marine species. Here, to follow on from a previous study (Hoffman et al. 2010), we used AFLPs to genotype over 300 *N. concinna* individuals sampled from 8 subtidal populations spanning a latitudinal gradient of 13° and over 2000 km distance, from the base of the Antarctic Peninsula through Signy Island to South Georgia. Our null hypothesis was no genetic structure due to this species being a broadcast spawner. Our sampling scheme, however, was designed to explore any possible effects of both the deep waters of the Scotia Sea and local gyres and other hydrographic structure along the Antarctic Peninsula (Hoffman et al. 1996).

Table 1 Details of sampling locations and numbers of *Nacella concinna* samples collected at each site

Population	Latitude (S)	Longitude (W)	Number of samples
Adelaide Island	67°36′25	68°11′28	48
Galindez Island	65°14′00	64°14′00	48
Dobrolowski Island	64°36′52	64°55′06	48
Deception Island	62°56′79	60°39′40	36
Snow Island	62°46′25	61°14′03	48
King George Island	62°13′93	58°41′00	18
Signy Island	60°40′88	45°36′10	48
South Georgia	54°17′00	36°29′79	38
Total			332

Materials and Methods

Tissue Sample Collection

Antarctic limpet specimens were collected by SCUBA divers during the austral summer of 1999 from the shallow sublittoral zone off the islands of Adelaide, Galindez, Dobrolowski, Deception, Snow, King George, Signy and South Georgia (Table 1 and Figure 1). All the individuals sampled were of the subtidal morphotype described by Strebel (1908) and Powell (1951). Tissue samples were stored in 95% ethanol, initially for 4 months at −20 °C and thereafter at room temperature.

DNA Extraction and AFLP Genotyping

For each specimen, total genomic DNA was extracted from a small piece of foot tissue using the Qiagen DNeasy tissue extraction kit following the manufacturer's recommended protocols. The AFLP protocol was adapted from Vos et al. (1995) following Dasmahapatra et al. (2009). Briefly, 100–400 ng of genomic DNA was first digested using *Taq*I (5 U in a 10 μl volume at 65 °C for 2 h) and then with *Eco*RI (5 U in a 20 μl volume at 37 °C for 2 h). *Taq*I and *Eco*RI adapters (Ajmone-Marsan et al. 1997) were then ligated onto the digested DNA using T4 DNA ligase (1 U in a 50 μl volume at 37 °C for 3 h), and the resulting products diluted 10-fold in 10 mM Tris–HCL and ethylenediaminetetraacetic acid (EDTA) (0.1 mM, Ph 8.0). For the preamplification, 5 μl of ligation mix was added to a 50 μl polymerase chain reactions (PCRs) containing Tris–HCl (10 mM, pH 8.3), $MgCl_2$ (1.5 mM), KCl (50 mM), deoxynucleoside triphosphates (0.2 mM), *Taq* polymerase (1 U), and 50 ng each of the *Taq*I-C and *Eco*RI-A preamplification primers (the primer sequences were 5′-GATGAGTCCTGACCGAC-3′ and 5′-GACTGCGTACCAATTCA-3′ respectively). Following 30 preamplification 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 10 with 10 mM Tris–HCL and EDTA (0.1 mM, pH 8.0). For the selective amplification, 2.5 μl of the diluted preamplification 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), deoxyadenosine triphosphates, deoxythymidine triphosphate and deoxyguanosine triphosphate (0.2 mM each),

deoxycytidine triphosphate (dCTP) (0.04 mM), α^3 P-dCTP, *Taq* polymerase (0.2 U), *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. Seven different selective primer combinations were employed (Supplementary Table 1).

Quantification of the AFLP Genotyping Error Rate

As with other classes of genetic marker, AFLPs can be prone to genotyping error, with potential sources including DNA contamination, restriction artifacts, PCR inhibition and mistakes in allele calling (Polisky et al. 1975; Dyer and Leonard 2000; Bonin et al. 2004). Consequently, following Hoffman and Amos (2005), we estimated the genotyping error rate for our data set by independently reextracting, regenotyping, and blind scoring 32 individuals (approximately 10% of the samples). To ensure broad coverage of the data set, 4 individuals were selected at random from each of the populations. The error rate per reaction was then quantified as the number of mismatching genotypes divided by the number of polymorphic bands compared (Bonin et al. 2004).

Data Analyses

The *N. concinna* AFLP data set analyzed consisted of 80 012 binary characters representing the presence and absence genotypes of 332 individuals at 241 polymorphic AFLP bands. To explore patterns of genetic differentiation, we first calculated pairwise F_{st} values among the populations and generated F_{st} matrices for each of 1000 bootstrapped data sets using the program AFLP-SURV (Vekemans 2002). To relate genetic differences among the populations to their geographic proximity, we next conducted an isolation-by-distance analysis. Geographic distances among the populations were calculated using a geographic information system (ESRI ArcGis v 9.2). By applying distance allocation tools to a bathymetric digital elevation model (GEBCO: General Bathymetric Chart of the Ocean 2003) and excluding land from the analysis, the shortest sea route between each site was calculated. A Lambert Conformal Conic projection was used to ensure the least possible spatial distortion. The significance of correlations between genetic and geographic distance matrices was assessed using Mantel tests with 999 iterations implemented in GENALEX V6 (Peakall and Smouse 2005).

We next tested whether genetic structure could be detected without the need for a priori geographic data by conducting a Bayesian cluster analysis of the data set using the program STRUCTURE version 2.3.3 (Pritchard et al. 2000). This program can handle data from various different types of genetic marker, including unidominant AFLP bands, and uses a maximum likelihood approach to determine both the most likely number of distinct genetic groups in the sample (K) and which individuals are most likely to belong to each group (the membership of each individual is estimated as q, which varies between 0 and 1 with the latter indicating full membership). We ran 5 independent runs for $K = 1$–8 setting the option RECESSIVEALLELES to 1 (Falush et al. 2003) and using 10^6 Markov chain Monte Carlo 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 both the maximal average value of Ln $P(D)$, a model-choice criterion that estimates the posterior probability of the data and ΔK, an ad hoc statistic based on the second order rate of change of the likelihood function with respect to K (Evanno et al. 2005).

One potential criticism of STRUCTURE is that the output of this program can be difficult to interpret when levels of population structure are low. Consequently, we also analyzed our data using the LOCPRIOR model within STRUCTURE, which uses the sampling locations of individuals to assist the clustering process (for further details, see Hubisz et al. 2009). This model essentially favors clustering solutions that correlate with sampling locations, while ignoring the sampling information whenever this is uninformative about the ancestry of individuals. It tends to outperform the standard model when population structure is weak, generating more accurate estimates of K and substantially improving the accuracy of group membership coefficients. We ran this analysis as described above but setting the option LOCPRIOR to 1.

Finally, a variety of methods have been developed to distinguish neutral loci from those influenced by selection based on the principle that the latter often show different, "outlier" patterns of variation (Luikart et al. 2003; Bensch and Akesson 2005; Bonin et al. 2007). To test for signatures of natural selection in our AFLP data set, we used a recently developed Bayesian approach implemented within the program BAYESCAN (Foll and Gaggioti 2008). This approach generalizes the method of Beaumont and Balding (2004) to allow direct estimation of the posterior probability that a given locus is under selection. To summarize briefly, it is based on a logistic regression model of F_{st} that incorporates both locus and population as predictor variables, the latter accounting for variation both in the demographic histories of individual populations and in the magnitude of genetic drift among them. For each locus, the probability of being under selection is then inferred using the Bayes factor, which is calculated as the ratio of the posterior probabilities of 2 models, one that includes the locus and another that excludes it. These posterior probabilities are estimated using Reversible Jump Markov Chain Monte Carlo. Based on Jeffrey's (1961) scale of evidence, a Bayes factor between 32 and 100 (corresponding to a $\log_{10}$ Bayes factor of 1.5–2) is interpreted as "strong evidence" for one model being supported over the other, whereas a Bayes factor of 100 or greater ($\log_{10}$ Bayes

Table 2 Pairwise F_{st} values among *Nacella concinna* populations (above diagonal)

	Adelaide Island	Galindez Island	Dobrolowski Island	Deception Island	Snow Island	King George Island	Signy Island	South Georgia
Adelaide Island	*	0.007	0.004	0.001	0.003	0.003	0.041	0.122
Galindez Island	**0.003**	*	0.000	0.000	0.000	0.000	0.025	0.113
Dobrolowski Island	**0.021**	0.609	*	0.000	0.000	0.000	0.027	0.115
Deception Island	0.201	0.946	0.696	*	0.000	0.000	0.028	0.117
Snow Island	**0.042**	0.637	0.919	0.950	*	0.000	0.027	0.120
King George Island	0.104	0.427	0.712	0.747	0.348	*	0.021	0.107
Signy Island	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	*	0.102
South Georgia	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	*

P values, calculated using 1000 permutations of the data set, are given below the diagonal, with significant values highlighted in bold.

factor > 2) is interpreted as "decisive evidence." Foll and Gaggioti's (2008) approach has several advantages over previous methods, including a popular approach based on summary statistics implemented in DFDIST (Beaumont and Nichols 1996). Most obviously, by fitting population as a term in the logistic regression model, BAYESCAN is better able to cope with complex demographic scenarios that deviate from the standard symmetrical island model of Wright (1951). However, the program also circumvents the problem of multiple testing of a large number of genomic locations because the number of loci is taken into account through the prior distribution. For our analysis, the estimation of model parameters was automatically tuned on the basis of 10 pilot runs of 5000 iterations each. After an additional burn-in of 50 000 iterations, we then used a sample size of 10 000 iterations and a thinning interval to 50 as suggested by Foll and Gaggioti (2008), resulting in a total chain length of 550 000 iterations. Following Nielsen et al. (2009), a Bayes factor of infinity, corresponding to a posterior probability of 1, was assigned a $\log_{10}$ value of 5.

Results

We genotyped 332 Antarctic limpets from 8 populations at 7 selective primer combinations, yielding 280 AFLP bands that could be scored unambiguously across all the samples, of which 241 (86.1%) were polymorphic (Supplementary Table 1). To assess the genotyping error rate for our data set, 4 individuals were randomly selected from each population (total $n = 32$ samples, approximately 10% of the data set), reextracted, regenotyped and blind scored. The resulting error rate was low at 0.087% (66 differences observed out of 7614 comparisons). Of the discrepancies observed between the 2 sets of genotypes, 25 (37.9%) were attributed to scoring or data entry errors, and the remaining 41 (62.1%) were due to the stochastic appearance or disappearance of bands as similarly documented by Bonin et al. (2004).

Patterns of Genetic Structure

A permutation test for genetic differentiation among the 8 island populations based on 10 000 randomizations of the data set indicated a strong deviation from the null hypothesis of no genetic structure (overall $F_{st} = 0.0384$, $P < 0.0001$). F_{st} values for each of the pairwise population comparisons ranged from 0 to 0.122 (Table 2), and a highly significant relationship was obtained between the shortest geographic distance by sea among the populations and genetic distance (Figure 2, Mantel's $r = 0.92$, $n = 8$, $P = 0.003$). However, this appears largely driven by differences between the populations of the Antarctic Peninsula and those of Signy and South Georgia. Thus, excluding South Georgia from the regression, the r^2 value falls from 0.85 to 0.77 but the significance alters little (Mantel's $r = 0.88$, $n = 7$, $P = 0.002$), whereas r^2 drops to 0.17 and the overall relationship becomes no longer significant after excluding both Signy and South Georgia (Mantel's $r = 0.41$, $n = 6$, $P = 0.161$).

Bayesian STRUCTURE Analysis

To identify genetic groups without a priori knowledge of sample origin, we next used a Bayesian approach implemented in the program STRUCTURE (Pritchard et al. 2000;

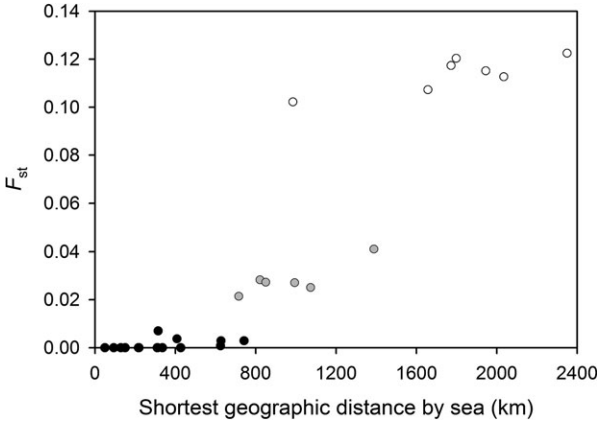

Figure 2. The relationship between shortest geographic distance by sea and genetic distance among 8 *Nacella concinna* populations. Black circles represent comparisons involving only the Antarctic Peninsula populations. Gray-filled and empty circles indicate comparisons involving Signy and South Georgia, respectively (with the far left empty circle representing the comparison between Signy and South Georgia).

Falush et al. 2003). This program works by partitioning the data set in such a way any departures from Hardy–Weinberg and linkage equilibrium within the resulting groups are minimized. Five runs were conducted for each possible number of groups (K), ranging from $K = 1$, implying no population differentiation, through to $K = 8$, which would imply that each of the populations were genetically distinct. The true number of groups present is most often identified using the maximal value of Ln $P(D)$, a model-choice criterion that estimates the posterior probability of the data. However, a simulation study by Evanno et al. (2005) found that once the true value of K is reached, Ln $P(D)$ often plateaus or continues to increase slightly at larger values of K. Our data set yielded just such a pattern, with Ln $P(D)$ starting low at $K = 1$, rising steeply to $K = 2$ and thereafter increasing slightly toward a peak at $K = 4$ before tailing off (Figure 3a). Consequently, we calculated ΔK an ad hoc statistic based on the second order rate of change of the likelihood function with respect to K that has been shown by Evanno et al. (2005) to be effective at detecting the uppermost hierarchical level of structure under most scenarios. This statistic peaked sharply at $K = 2$ (Figure 3a), indicating strong support for

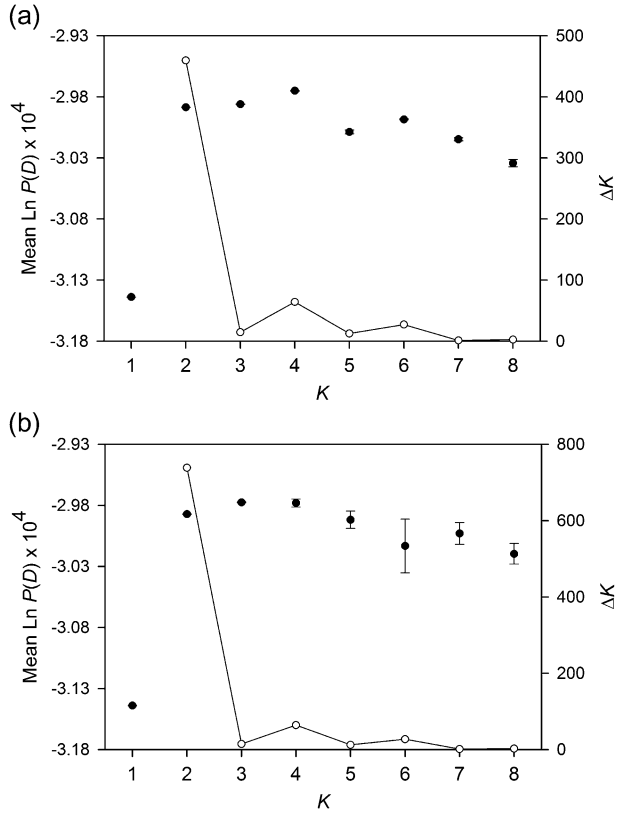

Figure 3. Results of the STRUCTURE (Pritchard et al. 2000) analysis showing mean ± standard error Ln $P(D)$ and ΔK values (filled and open circles, respectively) based on 5 replicates for each value of K, the hypothesized number of groups represented in the data, using (**a**) the standard model and (**b**) the LOCPRIOR model (for details, see Materials and Methods).

2 groups. Membership coefficients for these 2 inferred groups are summarized in Figure 4a, where each vertical bar represents a different individual and the relative proportions of the 2 shades of gray indicate the probabilities of belonging to each group. Classifying individuals according to their populations of origin, 2 groups are well defined, the first comprising individuals sampled from the Antarctic Peninsula and Signy Island (predominantly light gray) and the second comprising individuals from South Georgia (predominantly dark gray). The average membership coefficients for the 2 groups were high, at 0.993 and 0.988 respectively, indicating near-perfect assignment of the majority of individuals. Increasing the number of clusters to 3 ($K = 3$), the South Georgia group remained largely unaffected but a third distinct grouping was revealed (represented by medium gray in Figure 4b) that was present in varying proportions in individuals sampled from Signy Island as well as in several individuals originating from the Antarctic Peninsula. Increasing K further did not substantially alter this pattern, with any additional groups making negligible contributions other than to increase overall amounts of admixture (data not shown).

One potential drawback of STRUCTURE is that this program sometimes fails to provide a clear indication of population structure when the underlying signal is weak. Consequently, we reanalyzed our data set using a recently developed model within STRUCTURE that uses the sampling locations of individuals to inform cluster assignments (Hubisz et al. 2009). This algorithm has been shown to substantially improve the estimation of K as well as increase the accuracy of individual group membership coefficients when the data are relatively uninformative. Implementing this model, ΔK again peaked at $K = 2$, although this time Ln $P(D)$ was maximal at $K = 3$ (Figure 3b). Plotting individual cluster membership coefficients by population, the overall clustering result for $K = 3$ appears improved relative to when the standard STRUCTURE model was used, with the Antarctic Peninsula populations being substantially less admixed (average membership coefficient = 0.994) and individuals from Signy Island showing more consistent cluster membership values.

Analysis of Outlier AFLP Bands

Finally, loci that are under selection have the potential to bias phylogenetic inference (Luikart et al. 2003). Consequently, we used the Bayesian approach of Foll and Gaggioti (2008) to test for signatures of natural selection in our AFLP data set (for details, see Materials and Methods). A total of 26 out of 241 bands (10.8%) were identified as being influenced by selection (Supplementary Table 2), with Jeffrey's (1961) scale of evidence being "decisive" for 11 of these (4.6%) and "very strong" for a further 3 (1.2%). All of these loci had positive alpha coefficients, suggesting that they are likely to be subject to divergent rather than stabilizing selection. Excluding all 26 of these outliers from the AFLP data set and repeating the permutation test for overall genetic differentiation, the null hypothesis of no

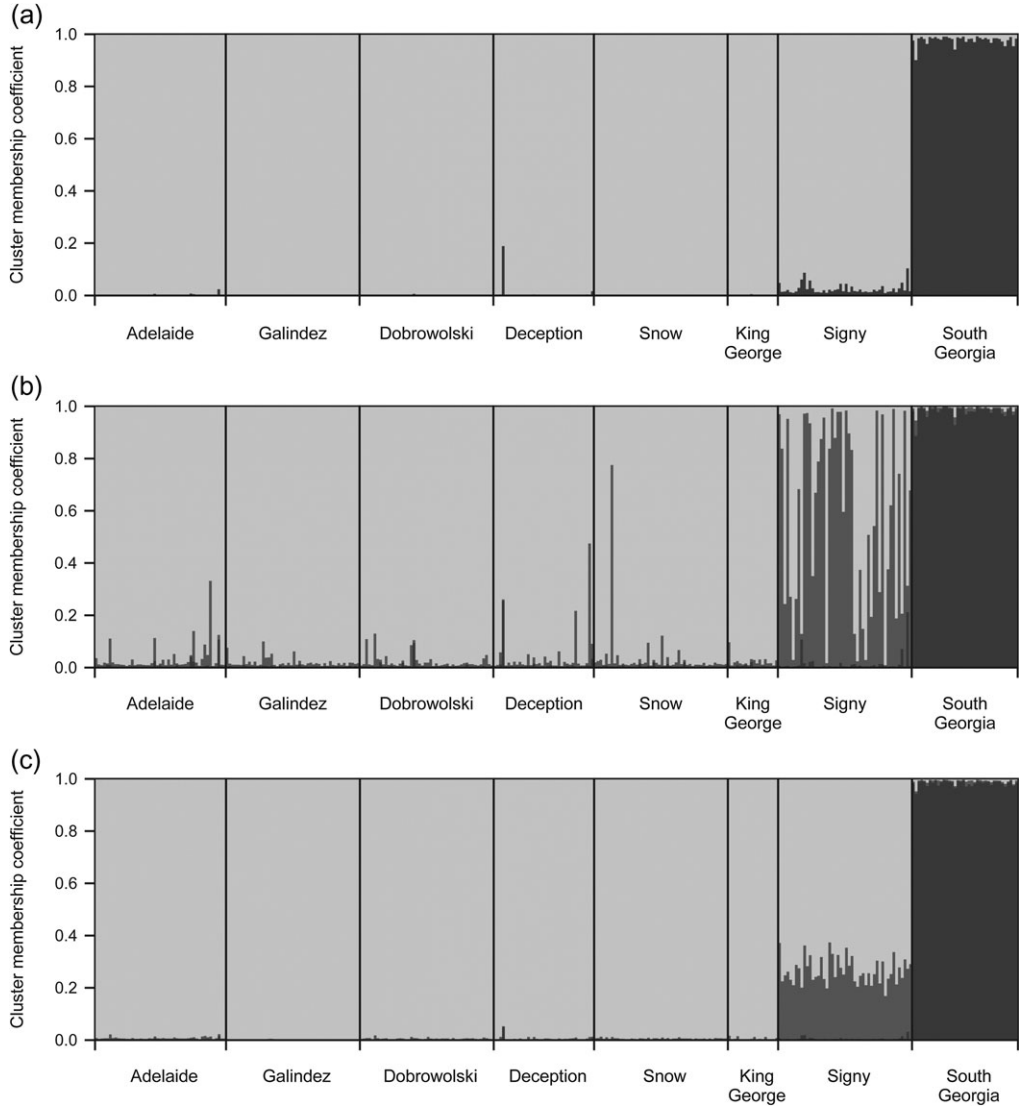

Figure 4. Group membership coefficients derived using the program STRUCTURE (Pritchard et al. 2000) for 332 *Nacella concinna* individuals for (**a**) $K = 2$ and (**b**) $K = 3$ using the standard model and (**c**) $K = 3$ using the LOCPRIOR model. Each individual is represented by a vertical line partitioned into light gray, medium gray, and dark gray segments, the lengths of which indicate the posterior probability of membership in each group.

genetic structure was again rejected ($F_{st} = 0.0143$, $P < 0.0001$ using 10 000 permutations of the data set) and the isolation-by-distance pattern remained highly significant (Mantel's $r = 0.86$, $n = 8$, $P = 0.003$). By implication, population structure in *N. concinna* appears robust to the influence of a small subset of outlier loci.

Discussion

Relatively few studies have explored the population genetic structure of broadcast-spawning species in any marine environment but particularly at high latitudes. Consequently, we used AFLPs to explore the population structure of the Antarctic limpet, an abundant Antarctic shallow-water macroinvertebrate with long-lived, free-swimming planktotrophic larvae. A highly significant correlation was found between genetic distance (F_{st}) and the shortest geographic distance by sea among populations. However, this pattern was driven by differences between the largely unstructured Antarctic Peninsula populations and the outlying islands of Signy and South Georgia, which are separated by deep water and strong currents. By implication, deep open-ocean channels appear to constitute a significant barrier to gene flow above and beyond any possible effects arising from isolation-by-distance.

Population Genetic Structure along the Antarctic Peninsula

Our first important finding was that all but 1 of the 6 limpet populations sampled from the Antarctic Peninsula were

undifferentiated at a panel of over 200 AFLP bands, and thus appear to constitute a single panmictic population. This observation is consistent with the broadcast-spawning life history of *N. concinna* and suggests that larval dispersal is sufficiently strong over much of the Antarctic Peninsula to counteract genetic drift. The exception was Adelaide Island, sampled from the base of the peninsula, which yielded weakly significant F_{st} values in comparisons with Galindez, Dobrolowski, and Snow Islands, although these did not remain significant following table-wide sequential Bonferroni correction for multiple statistical tests (data not shown). This is suggestive of restricted gene flow between Adelaide Island and more northerly sections of the Antarctic Peninsula. Although the Antarctic Circumpolar Current has a strong northerly flow along the continental slope and in deeper water offshore (Deacon 1984; Savidge and Amft 2009), there is also a strong southerly flow in the Antarctic Coastal Current close to the shore (Moffat et al. 2008). On the shelf itself, there are also indications of a series of semi-isolated gyres (Hoffman et al. 1996) that may be sufficient to impart a mild genetic structure by moderating larval transport.

Although consistent with what we know about *N. concinna*, the absence of discernable population structure among all but the most southerly of the Antarctic Peninsula populations could potentially be viewed as being at odds with a recent study by de Aranzamendi et al. (2008), which reported weak but statistically significant genetic differences using ISSR markers between 3 populations sampled within 2 km of one another at Potters Cove, King George Island. Unfortunately, we were unable to obtain samples from the same locations as de Aranzamendi et al. (2008), precluding a direct comparison. However, the single sample that we obtained from King George Island was undifferentiated from the adjacent Antarctic Peninsula populations. This suggests either that de Aranzamendi et al.'s (2008) observation was due to type I error, a possibility given the relatively small sample sizes both of individuals and loci or that fine-scale structure exists but at a resolution below the spatial scale of our sampling. Given that we are currently unable to discount the latter possibility, we believe that it could be of interest in the future to explore patterns of genetic variation in this species at a finer spatial scale.

Genetic Differentiation between the Antarctic Peninsula and Islands of the Scotia Arc

In contrast to our findings from the Antarctic Peninsula, pronounced genetic differences were observed between the peninsula populations and the islands of Signy and South Georgia. These were readily distinguishable using both traditional F_{st}-based approaches and Bayesian cluster analysis implemented within the program STRUCTURE (Pritchard et al. 2000). In the latter analysis, Evanno et al.'s (2005) ΔK statistic peaked at $K = 2$, suggesting that the uppermost hierarchical structure for the data set consists of 2 groups, whereas the maximal value of Ln $P(D)$ was obtained at either $K = 3$ or $K = 4$, depending on the specific clustering model used. Remarkably, the 2 groups identified using ΔK were found to perfectly reflect the deep split between South Georgia and the remaining populations, while increasing K to 3 revealed an additional group representative of Signy. Increasing K to 4 did not substantially alter this pattern (data not shown), suggesting that STRUCTURE was unable to meaningfully partition any of the remaining genetic variation.

Regressing F_{st} against the shortest geographic distance by sea among populations yielded a highly significant relationship. If we assume that the metapopulation is at equilibrium, this would imply restricted but ongoing gene flow between the Antarctic Peninsula, Signy Island, and South Georgia. This is broadly consistent with 2 observations; first, the Antarctic Circumpolar Current has been present longer than the taxon itself (González-Wevar et al., forthcoming), and second, unidirectional gene flow in the direction of the current has been observed in another species with a pelagic larval stage sampled over the same geographic range (Matschiner et al. 2009). In practice, however, very few natural populations are likely to achieve or remain at equilibrium for long, and similar patterns can also arise under nonequilibrium conditions (Slatkin 1993). Consequently, an alternative possibility that we are currently unable to exclude is that Signy and South Georgia could have become separated more recently, with any genetic differences between them being perpetuated by a lack of contemporary gene flow. Alternatively, it is possible that these 2 islands could have been founded or have received gene flow from divergent, unsampled populations. However, it is unclear where such populations could lie, with stepwise colonization of the Scotia Ridge from South America seeming unlikely owing to the fact that *N. concinna* diverged in the late Miocene, long after the formation of the Antarctic Polar Front (González-Wevar et al., forthcoming).

Although perhaps unexpected for a broadcast spawner, the discovery of significant genetic differences between the Antarctic Peninsula, Signy, and South Georgia is consistent with a previous study by Beaumont and Wei (1991) that reported differences at 5 allozyme loci between animals from Signy and South Georgia. Unfortunately, however, Beaumont and Wei (1991) were only able to obtain samples from these 2 islands due to logistic constraints. Moreover, because allozymes are not always selectively neutral, the potential exists for these markers to reveal contrasting patterns to, for example, microsatellites (Thelen and Allendorf 2001). Consequently, it is difficult to distinguish on the basis of the previous study between 2 opposing explanations, the first invoking genome-wide differentiation and the second selection on the markers themselves. Our study lends weight to the former explanation because the genetic differences previously reported by Beaumont and Wei (1991) are also manifest in a large panel of (putatively) neutral AFLP bands.

Placing our study into a broader context, the pattern of genetic structure observed in *N. concinna* is also consistent with several recent studies suggesting that physical oceanographic features, including major currents and open

stretches of deep water, can significantly impede gene flow in marine organisms. For example, the Drake Passage has been shown to constitute a major barrier to the exchange of individuals in a variety of species, including Patagonian toothfish *Dissostichus eleginoides*, ribbon worms *Parborlasia corrugatus*, brittle stars *Astrotoma agassizii*, and Antarctic sea slugs *Doris kerguelenensis*, irrespective of whether the species in question is a brooder or broadcast spawner (Shaw et al. 2004; Hunter and Halanych 2008; Thornhill et al. 2008; Wilson et al. 2009). However, the picture emerging from the Antarctic Peninsula and sub-Antarctic Islands, where oceanographic features are arguably more subtle, is less clear. For example, the circumpolar crinoid *P. kerguelensis* shows significant genetic differences between the Antarctic Peninsula, South Sandwich Islands, and South Georgia (Wilson et al. 2007), whereas the notothenioid fish *G. gibberifrons* exhibits a complete absence of structure over a very similar geographic range (Matschiner et al. 2009).

Perhaps the most obvious explanation for the contrasting patterns of genetic structure observed in 3 species with qualitatively similar life histories involves differences in larval longevity. Larvae of *N. concinna* and *P. kerguelensis* can survive for 1–2 months and up to 3 months respectively in the water column (Bowden et al. 2006; Wilson et al. 2007), whereas the larval stage of *G. gibberifrons* persists for around 4 months (Matschiner et al. 2009). These differences in larval duration would be expected to have little or no impact on population connectivity along the Antarctic Peninsula, where suitable shallow-water habitat is more-or-less continuous. However, larvae released at the tip of the Antarctic Peninsula face a lengthy journey across deep waters to South Georgia, data from passive drifters indicating a minimum time of around three and a half months (Matschiner et al. 2009), whereas estimates based on sea surface current models are slightly higher at 150–250 days (Thorpe et al. 2007). Such durations would theoretically permit the passive transport of living *G. gibberifrons* larvae to South Georgia but are considerably longer than the larval life spans of either *N. concinna* or *P. kerguelensis*.

It is important to note, however, that several other factors may also contribute toward variation in the pattern and strength of population structure, both within- and among-species. For example, Wilson et al. (2007) speculated that variation in the amount of genetic structure within different mitochondrial lineages of the Antarctic sea slug could potentially be attributable to their having utilized different refugia during the previous glacial maxima. According to this hypothesis, recolonization from the deep sea would likely produce different patterns of structure than recolonization from multiple shelf refugia. Population size could also be important because the rate of genetic drift is directly proportional to effective population size, being faster in small populations (Hartl 2000). Thus, higher levels of gene flow will be required to counteract drift in small populations, whereas highly abundant species with wide geographic ranges such as *N. concinna* may show a relative predisposition toward being unstructured. Finally, other factors could also be involved, a possibility supported by a recent study of 3 closely related Hawaiian limpet species that found biogeographical range and microhabitat specificity to be better predictors of dispersal than minimum larval duration (Bird et al. 2007).

Implications for Physiological Variation between Populations

Antarctic benthic marine species are characterized by markedly slowed physiological rates (Peck 2002), generating life-history characters of dramatically extended developmental periods and significantly longer times required to reach maturity than temperate or tropical species. They also tend to produce fewer embryos per reproductive event than lower-latitude congeners (Clarke 1987). Consequently, it has been argued that Antarctic marine species may be less able to evolve new characters in changing environments than equivalent species from lower latitudes (Barnes and Peck 2008) and hence that genetic connectivity could become increasingly important to the persistence of local populations or even entire species (Peck 2005; Barnes and Peck 2008). *N. concinna* is interesting in this context because it is highly stenothermal, being incapable of acclimatizing over a 2-month period to 3 °C (Peck et al., forthcoming). Recently, Morley et al. (2009) also found that limpets from South Georgia, where temperature variability and maxima are highest, have lower physiological critical temperature limits than individuals from Signy or Adelaide Islands. This is consistent with our finding that South Georgia is deeply genetically divergent from the other populations and also supports the hypothesis that populations at the edge of a species range may be more vulnerable to environmental change than those nearer the center.

Analysis of Outlier Bands

In respect of the physiological differences observed by Morley et al. (2009), it is interesting to consider if the patterns of genetic structure observed in *N. concinna* reflect genome-wide divergence or whether they could instead be driven by selection operating on a subset of outlier loci. A classic example of the latter comes from a study by Wilding et al. (2001) of the periwinkle *Littorina saxatilis*. This species possesses 2 distinct forms, a thick shelled "M" morph that occurs on the lower shore and is adapted to crab predation and the thin shelled "H" morph that occupies the upper shore and is resistant to wave action. Wilding et al. (2001) genotyped 4 geographically distant pairs of parapatric populations at 306 AFLP bands and constructed phylogenies both including and excluding 15 bands that were inferred to be under selection. Remarkably, the neighbor joining tree based on the full data set grouped the H and M morphotypes together, whereas removal of the outlier bands led to the populations clustering instead by sampling site. To similarly test for signatures of selection in our AFLP data set and to broadly ascertain the relative contributions of neutral and selected loci to the overall pattern of genetic structure, we used a recently developed Bayesian approach that is able to account for relatively complex population structure (Foll

and Gaggioti 2008). Twenty-six AFLP bands (10.8%) were identified as being influenced by selection, of which over half achieved a rating of very strong or above using Jeffrey's (1961) scale of evidence. This number is broadly consistent with the results of similar genome scans in other species, with for example Nielsen et al. (2009) identifying 8/98 loci (8.2%) as being under selection in the Atlantic cod and Manel et al. (2009) identifying 9/83 loci (10.8%) as outliers in pine weevils using the same approach. However, although many of the outliers identified in *N. concinna* may be good candidates for further study in relation to physiological adaptation, they should be treated with caution on an individual basis because of the possibility of there being some false positives (Foll and Gaggioti 2008). These can arise, for example, wherever populations have passed through a population bottleneck, leading to the fixation of alleles that are otherwise rare (Manel et al. 2009). Nevertheless, the possible inclusion of false positives only strengthens the conclusion that significant genetic structuring remains after excluding all of the outlier bands from our analyses. Consequently, the pattern of genetic differentiation we observe in *N. concinna* appears robust and not to be affected by the potential for artifact due to the inclusion of outlier bands that could conceivably be associated with phenotypic differences among populations.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

Funding

Natural Environment Research Council British Antarctic Survey Strategic Alliance Fellowship awarded to J.I.H.

Acknowledgments

Nacella concinna were collected by divers during the British Antarctic Survey Peninsula Gene flow cruise in 1999. We thank the dive team, officers, and crew of RRS Bransfield for their support in the collection of these samples and the late Martin White for considerable help and support with sample design and logistical aspects. We also wish to thank Peter Fretwell for calculating shortest geographic distances by sea among populations using a geographic information system and for preparing Figure 1.

References

Ajmone-Marsan J, Valentini A, Cassandro M, Vecchiotti-Antaldi G, Bertoni G, Kuiper M. 1997. AFLPTM markers for DNA fingerprinting in cattle. Anim Genet. 28:418–426.

Barnes DKA, Griffiths HJ. 2008. Biodiversity and biogeography of southern temperate and polar bryozoans. Global Ecol Biogeogr. 17: 84–89.

Barnes DKA, Peck LS. 2008. Examining vulnerability of Antarctic shelf biodiversity to predicted climate warming. Clim Res. 37:149–163.

Barnes DKA, Peck LS, Morley S. Forthcoming. Acute temperature sensitivity of Antarctic invertebrates determines colonisation potential, biogeography and resilience to environmental change. Glob Chang Biol. doi: 10.1111/j.1365–2486.2010.02176.x.

Beaumont MA, Balding DJ. 2004. Identifying adaptive genetic divergence among populations from genome scans. Mol Ecol. 13:969–980.

Beaumont MA, Nichols RA. 1996. Evaluating loci for use in the genetic analysis of population structure. P Roy Soc Lond B Bio. 263:1619–1626.

Beaumont AR, Wei JHC. 1991. Morphological and genetic variation in the Antarctic limpet *Nacella concinna* (Strebel, 1908). J Molluscan Stud. 57:443–450.

Bensch S, Akesson M. 2005. Ten years of AFLP in ecology and evolution: why so few animals? Mol Ecol. 14:2899–2914.

Bird CE, Holland BS, Bowen BW, Toonen RJ. 2007. Contrasting phylogeography in three endemic Hawaiian limpets (*Cellana* spp.) with similar life histories. Mol Ecol. 16:3173–3186.

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.

Bonin A, Ehrich D, Manel S. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Mol Ecol. 16:3737–3758.

Bowden DA, Clarke A, Peck LS, Barnes DKA. 2006. Antarctic sessile marine benthos: colonisation and growth on artificial substrata over three years. Mar Ecol Prog Ser. 316:1–16.

Bradbury IR, Laurel B, Snelgrove PVR, Bentzen P, Campana SE. 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. P Roy Soc Lond B Bio. 275: 1803–1809.

Brey T, Dahm C, Gorny M, Klages M, Stiller M, Arntz WE. 1996. Do Antarctic benthic invertebrates show an extended level of eurybathy? Antarct Sci. 8:3–6.

Clarke A. 1987. Temperature, latitude and reproductive effort. Mar Ecol. Progr Ser. 38:89–99.

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

Clarke A, Crame JA. 1989. The origin of the Southern Ocean marine fauna. In: Crame JA, editor. Origins and evolution of the Antarctic biota. London: The Geological Society. p. 253–268.

Clarke A, Johnston NM. 2003. Antarctic marine benthic diversity. Ocean Mar Biol Annu Rev. 41:47–114.

Dasmahapatra KK, Hoffman JI, Amos W. 2009. Pinniped phylogenetic relationships inferred using AFLP markers. Heredity. 103:168–177.

Deacon G. 1984. The Antarctic circumpolar ocean. Cambridge: Cambridge University Press.

De Aranzamendi M, Sahade R, Tatian M, Chiappero MB. 2008. Genetic differentiation between morphotypes in the Antarctic limpet *Nacella concinna* as revealed by inter-simple sequence repeat markers. Mar Biol. 154:875–885.

Dyer AT, Leonard KJ. 2000. Contamination, error, and nonspecific molecular tools. Phytopathology. 90:565–567.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 14:2611–2620.

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.

Foll M, Gaggioti O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genetics. 180:977–993.

Gonzáles-Wevae CA, Nakano T, Canete JI, Poulin E. Forthcoming. Molecular phylogeny and historical biogeography of Nacella (Patellogastropoda: Nacellidae) in the Southern Ocean. Mol Phylogenet Evol.

Hartl DL. 2000. A primer of population genetics. 3rd ed. Sunderland (MA): Sinauer Associates Inc.

Held C, Leese F. 2009. The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. Polar Biol. 30:513–521.

Hoffman JI, Amos W. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Mol Ecol. 14:599–612.

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.

Hofmann EE, Klinck JM, Lascara CM, Smith DA. 1996. Water mass distribution and circulation west of the Antarctic Peninsula and including Bransfield Strait. In: Ross RM, Hofmann EE, Quetin LB, editors. Foundations for ecological research west of the Antarctic Peninsula. Washington (DC): American Geophysical Union. p. 61–80.

Hubisz MJ, Falush D, Stephens M, Pritchard JK. 2009. Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour. 9:1322–1332.

Hunter RL, Halynch KM. 2008. Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the Drake Passage in the Southern Ocean. J Hered. 99:137–148.

Jeffreys H. 1961. Theory of probability. 3rd ed. New York: Oxford University Press.

King JC, Turner J, Marshall GJ, Connolley WM, Lachlan-Cope TA. 2003. Antarctic Peninsula climate variability and its causes as revealed by instrumental records. Antarct Res Ser. 79:17–30.

Linse K, Cope T, Lorz A-N, Sands C. 2007. Is the Scotia Sea a centre of Antarctic marine diversification? Some evidence of cryptic speciation in the circum-Antarctic bivalve *Lissarca notorcadensis* (Arcoidea: Philobryidae). Polar Biol. 30:1059–1068.

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P. 2003. The power and promise of population genomics: from genotyping to genome typing. Nat Rev Genet. 4:981–994.

Mahon AR, Arango CP, Halynch KM. 2008. Genetic diversity of *Nymphon* (Arthropoda: Pycnogonida: Nymphonidae) along the Antarctic Peninsula with a focus on *Nymphon australe* Hodgson 1902. Mar Biol. 155:315–323.

Manel S, Conord C, Depres L. 2009. Genome scan to assess the respective role of host-plant and environmental constraints on the adaptation of a widespread insect. BMC Evol Biol. 9:288.

Matschiner M, Hanel R, Salzburger W. 2009. Gene flow by larval dispersal in the Antarctic notothenioid fish Gobionotothen gibberifrons. Mol Ecol. 18:2574–2587.

Maughan P, Saghai Maroof MA, Buss GR, Huestis GM. 1996. Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. Theor Appl Genet. 93:392–401.

Meudt HM, Clarke AC. 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends Plant Sci. 12:106–108.

Moffat C, Beardsley RC, Owens B, van Lipzig NPM. 2008. A first description of the Antarctic Peninsula Coastal Current. Deep Sea Res II. 55:277–293.

Morley SA, Hirse T, Portner HO, Peck LS. 2009. Geographical variation in thermal tolerance within Southern Ocean marine ectotherms. Comp Biochem Physiol. 153:154–161.

Mueller UG, Wolfenbarger LL. 1999. AFLP genotyping and fingerprinting. TREE. 14:389–394.

Nielsen EE, Hemmer-Hansen J, Poulsen NA, Loeschcke V, Moen T, Johansen T, Mittelholzer C, Taranger G-L, Ogden R, Carvalho GR. 2009. Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (Gadus morhua). BMC Evol Biol. 9:276.

Peakall R, Smouse PE. 2005. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 6:288–295.

Peck LS. 2002. Ecophysiology of Antarctic ectotherms: limits to life. Polar Biol. 25:31–40.

Peck LS. 2005. Prospects for survival in the Southern Ocean; vulnerability of benthic species to temperature change. Antarct Sci. 17:495–505.

Peck LS, Barnes DKA, Cook AJ, Fleming AH, Clarke A. 2009. Negative feedback in the cold: ice retreat produces new carbon sinks in Antarctica. Glob Chang Biol. 16:2614–2623.

Peck LS, Clark MS, Morley SA, Massey A, Rossetti H. 2009. Animal temperature limits and ecological relevance: effects of size, activity and rates of change. Funct Ecol. 23:248–253.

Peck LS, Morely SA, Clark MS. Forthcoming. Poor acclimation capacities in Antarctic marine ectotherms. Mar Biol. Doi: 10.1007/s00227-010-1473-x.

Peck LS, Powell DK, Tyler PA. 1997. Very slow development in two Antarctic bivalve molluscs, the infaunal clam, *Laternula elliptica* and the scallop *Adamussium colbecki*. Mar Biol. 150:1191–1197.

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

Polisky B, Greene P, Garfin DE, McCarthy BJ, Goodman HM, Boyer HW. 1975. Specificity of substrate recognition by the EcoRI restriction endonuclease. Proc Natl Acad Sci U S A. 72:3310–3314.

Powell AWB. 1951. Antarctic and subantarctic mollusca: Pelecypoda and Gastropoda. Discovery Rep (USA). 26:49–196.

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

Savidge DK, Amft JA. 2009. Circulation on the West Antarctic peninsula derived from 6 years of shipboard ADCP transects. Deep Sea Res I. 56:1633–1655.

Shaw PW, Arkhipin AI, Al-khairulla H. 2004. Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep-water troughs as barriers to genetic exchange. Mol Ecol. 13:3293–3303.

Slatkin M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution. 47:264–279.

Stanwell-Smith DP, Peck LS. 1998. Temperature and embryonic development in relation to spawning and field occurrence of larvae of 3 Antarctic echinoderms. Biol Bull. 194:44–52.

Strebel. 1908. (Patellidae) at Signy Island, in relation to environmental variables. J Molluscan Stud. 64:123–127.

Thelen GC, Allendorf FW. 2001. Heterozygosity-fitness correlations in rainbow trout: effects of allozyme loci or associative overdominance? Evolution. 55:1180–1187.

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.

Thorpe SE, Murphy EJ, Watkins JL. 2007. Circumpolar connections between Antarctic krill (*Euphausia superba* Dana) populations: investigating the roles of ocean and sea ice transport. Deep Sea Res I. 54:792–810.

Valentine JW. 1967. Influence of climatic fluctuations on species diversity within the Tethyan provincial system. Aspects of Tethyan biogeography. Syst Assn Pub. 7:153–166.

Vekemans X. 2002. AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

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

Walker AJM. 1972. Introduction to the ecology of the Antarctic limpet *Patinigera polaris* (Hombron and Jaquinot) at Signy Island, South Orkney Islands. Brit Antarct Surv B. 28:49–71.

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

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

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

Wright S. 1951. The genetical structure of populations. Ann Eugen. 15:323–354.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science. 292:686–693.

Received April 19, 2010; Revised July 13, 2010;
Accepted July 19, 2010

Corresponding Editor: Stephen Karl