Genetic Population Structure in the Antarctic Benthos: Insights from the Widespread Amphipod, *Orchomenella franklini*

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Abstract

Currently there is very limited understanding of genetic population structure in the Antarctic benthos. We conducted one of the first studies of microsatellite variation in an Antarctic benthic invertebrate, using the ubiquitous amphipod *Orchomenella franklini* (Walker, 1903). Seven microsatellite loci were used to assess genetic structure on three spatial scales: sites (100 s of metres), locations (1–10 km) and regions (1000 s of kilometres) sampled in East Antarctica at Casey and Davis stations. Considerable genetic diversity was revealed, which varied between the two regions and also between polluted and unpolluted sites. Genetic differentiation among all populations was highly significant ($F_{ST}$ = 0.086, $R_{ST}$ = 0.139, p < 0.001) consistent with the brooding mode of development in *O. franklini*. Hierarchical AMOVA revealed that the majority of the genetic subdivision occurred across the largest geographical scale, with $N_{m}$<1 suggesting insufficient gene flow to prevent independent evolution of the two regions, i.e., Casey and Davis are effectively isolated. Isolation by distance was detected at smaller scales and indicates that gene flow in *O. franklini* occurs primarily through stepping-stone dispersal. Three of the microsatellite loci showed signs of selection, providing evidence that localised adaptation may occur within the Antarctic benthos. These results provide insights into processes of speciation in Antarctic brooders, and will help inform the design of spatial management initiatives recently endorsed for the Antarctic benthos.

Introduction

Gene flow – or genetically effective migration – is one of the most important factors governing the evolution of species [1–3]. Gene flow can dampen localised adaptation yet spread advantageous alleles for the cohesive evolution of the species (e.g. in the face of climate change), whereas the absence of gene flow can lead to population divergence, and ultimately speciation [4–6]. Anthropogenic impacts have the potential to disrupt gene flow and alter genetic diversity of natural populations (e.g. [7]), thereby affecting the evolutionary potential of species. Understanding the genetic structure of populations will therefore shed light on how species may respond to these impacts. Moreover, genetic studies can help optimise the design of conservation efforts to preserve genetic diversity, in order to help ensure the long-term adaptability and persistence of species [8,9].

Marine fauna are currently threatened by a plethora of human activities including fisheries harvest, habitat destruction, localised pollution, introduced species and climate change [see 10,11]. Initiatives such as Marine Protected Areas (MPAs) are one of the most important tools for the conservation of marine populations, the success of which relies on ‘spillover’ of individuals from protected areas to replenish outside populations [12–14]. Estimates of genetic connectivity thus help determine the optimal size and placement of MPAs to achieve this desired broad-scale flux [15,16]. There is strong evidence of a correlation between the dispersal capacity inferred by a species’ pelagic larval phase and the genetic connectivity of populations (and subsequent potential for MPA connectivity), however exceptions are common, and patterns of marine genetic structure are far from predictable (reviewed in [17–19]). Furthermore, local adaptation has been increasingly emphasised for its role in structuring marine populations (e.g. [20–22]). Local adaptation may be particularly important in the face of localised marine pollution, which can alter allele frequencies or genetic diversity in exposed populations (e.g. [23–25]), in turn affecting speciation processes and ultimately, species fitness [26].

The Antarctic benthos represents one of the most isolated marine ecosystems on the planet, with particularly unique fauna (reviewed in [27,28]) that are considered vulnerable to future environmental change [29]. Intraspecific genetic structure in Antarctic benthic organisms is poorly understood, and the need for genetic research has been highlighted [30–33]. This is particularly pertinent given a recent endorsement by the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) to establish a network of MPAs in Antarctica [34]. Determining the location and size of these MPAs is still in progress and is hindered by a lack of existing baseline knowledge on Antarctic
marine fauna [35]. Improving our understanding of benthic gene flow and genetic diversity in Antarctica will not only help inform the design of these MPAs, but will also shed light on the high rates of speciation prevalent in Antarctic benthos [30,32,36]. Furthermore, studying these microevolutionary processes will help predict the potential for Antarctic organisms to adapt to existing threats, such as local pollution surrounding human settlements [37,30], and broad-scale climate change [39].

Studies of gene flow in Antarctic benthic fauna have focused primarily on large-scale connectivity over major hydrographic features such as the Polar Front, or abyssal depths between islands (e.g. [40–42]). Commonly, these studies have revealed highly distinct genetic lineages assumed to represent cryptic species (see [43] for recent summary). This partly explains why truly intraspecific genetic patterns remain much less explored. What has emerged from the limited population-level studies is that the unique hydrography of Antarctica may have an important influence on genetic structure. For instance, local circulation patterns are believed to play a role in isolating populations of species that display surprisingly fine-scale (<20 km) genetic subdivision, despite possessing pelagic larvae for dispersal (Notothenioidei: [44]; Bivalvia: [45]). Other unique mechanisms such as iceberg scouring and historical glaciation have been implicated when populations from different locations exhibit markedly different levels of genetic diversity (e.g. Amphipoda: [46]; Isopoda: [47]; Ascidiacea: [48]; Pycnogonida: [49]).

Brooding benthic organisms are particularly interesting candidates in which to address questions of gene flow and speciation in Antarctica, as their lack of a pelagic dispersal phase should lead to high genetic structuring of populations [50,51]. In Antarctica, brooding taxa are highly speciose and largely endemic, with several groups that have undergone intense radiation [52–55]. One such group is the amphipods, which are remarkably abundant crustaceans that occupy a wide range of ecological niches and play a significant role in Antarctic trophic exchanges [50,56–58]. We chose to study the ubiquitous amphipod Orchestophora franklini (Walker, 1903) to address the current paucity of knowledge on intraspecific genetic structure in Antarctic benthos. O. franklini often dominates Antarctic shallow water communities [59–62], and its presence in polluted bays adjacent to Antarctic research stations allowed us to investigate potential effects of contamination on genetic diversity.

We used microsatellite markers to investigate genetic variation in O. franklini. Microsatellites are tandem repeats of typically one to six nucleotides that mutate rapidly and are found at high frequency in most eukaryotic genomes [63,64]. The high variability of microsatellites enables resolution of genetic structure over fine (<100 km) spatial scales [32,63–67], which in Antarctica remain the least understood [32,68]. To our knowledge just a single study to date has used microsatellites to explore gene flow in an Antarctic benthic invertebrate, and this focused on large-scale migration between islands [48]. Thus we present the first known Antarctic study of microsatellite variation in a benthic invertebrate over small (100 s of metres), moderate (10 s of kilometres), and large (1000 s of kilometres) spatial scales.

Materials and Methods

Sampling

Samples of Orchestophora franklini were collected from two geographical regions, adjacent to the Australian research stations Casey (66°S, 110°E) and Davis (68°S, 78°E), in East Antarctica. Casey and Davis are separated by approximately 1400 km (Fig. 1), and were sampled during the summer months of 2009 and 2010 respectively. Within the Casey region samples were collected at eight locations (~1–30 km apart; Fig. 1). Within the Davis region samples were collected at four locations (~3–20 km apart; Fig. 1). Locations were classified as either polluted or unpolluted (Fig. 1, Table S1) based on proximity to known contaminated areas [69–71], and knowledge of the extent of dispersion of these
Antarctic Division.

AMLR 09-10-3051; voucher specimens are held at the Australian Museum. Collections from Davis were made under permit AMLR 08-3051 and collections from Casey were made under permit AMLR 08-81. Collections from Casey were made under permit AMLR 08-3051 obtained for the described field studies from the Commonwealth of Australia under the Antarctic Marine Living Resources Act 1981. All necessary permits were obtained for the described field studies from the Commonwealth of Australia under the Antarctic Marine Living Resources Act 1981.

Development of microsatellite loci

A genomic library of *O. franklini* was made by ecogenics GmbH (Zurich, Switzerland) based on a pooled sample of DNA from 15 individual amphipods. SNX forward and reverse linkers were ligated onto size-selected DNA following the procedure of [73] and these were enriched for (TAC)₇, (AAC)₁₀, (GT)₁₃, (CT)₁₃, and (ACAG)₇ oligonucleotide repeats by magnetic bead selection [74,75]. The enriched library was cloned and 1406 recombinant colonies screened for the presence of microsatellites by hybridisation. DNA inserts from 230 positive clones were subsequently sequenced and primers were designed for 23 microsatellite loci. DNA inserts from 230 positive clones were subsequently sequenced and primers were designed for 23 microsatellite loci and tested for polymorphism. Seven loci (Table 1) were considered suitable for population-level analysis.

Microsatellite genotyping

Whole specimens of *O. franklini* were used for DNA extractions due to small body size (2–8 mm). DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer’s instructions, with elution volume decreased to 120 μl to maximise DNA concentration. Microsatellite loci were amplified in three multiplex polymerase chain reactions (PCRs) (Table 1). For multiplex reactions A and B, 20 μl reactions using the QIAGEN Multiplex PCR kit were used, with a final concentration of 1× Qiagen Multiplex PCR Master Mix (provides 3 mM MgCl₂ and one unit HotStar Taq DNA Polymerase), 0.2 μM each primer (forward primers were fluorescently labeled), and approximately 100 ng template DNA. Capillary separation of amplified fragments occurred on an automated sequencer (CEQ 8000, Beckman Coulter), and alleles were scored (according to PCR fragment size) with CEQ 8000 Genetic Analysis System software version 0.0. Multiplex C was amplified at the Australian Genome Research Facility, with capillary separation occurring on an Applied Biosystems 3730 DNA Analyzer and alleles scored using Applied Biosystems GeneMapper software version 3.1. All fragment data were visually checked for allele scoring errors and stutter. Micro-checker 2.2.3 [76] was used to check data for the presence of null alleles. Loci were tested for linkage disequilibrium in Genepop 4.0.10 [77] with the critical level p<0.05 adjusted for multiple comparisons, using the sequential Bonferroni procedure [78].

Genetic diversity of populations

Measures of genetic diversity, including observed heterozygosity (*H*ₐ), unbiased expected heterozygosity (*H*ₑ) and allelic richness (standardised for sample size: *A*ₐₑ) at each locus were calculated in FSTAT 2.9.3 [79]. To determine whether diversity measures differed between Casey and Davis, and among polluted and unpolluted sites, permutation tests were performed on *H*ₐ, *H*ₑ and *A*ₐₑ in FSTAT. The number of private alleles (*P*ₐₑ) at each site was determined using Genalex 6.41 [80]. There is evidence that the occurrence of private alleles most closely follows a Poisson distribution [81], so we used a Poisson generalised linear model to assess the effect of region and pollution on *P*ₑ carried out in R 2.12.2 [82].

Table 1. Details for the seven microsatellite loci amplified in *Orchomenella franklini*.

| Multiplex | PCR thermal protocol | Locus | Primer sequence (5’-3’) | Fluorescent dye | Repeat motif | Total no. of alleles | Allele size range (bp) |
|-----------|----------------------|-------|------------------------|-----------------|--------------|---------------------|-----------------------|
| A         | 15 min at 95°C       | Orcfra4 | F: AGCATCCCTACAAAAGATAAG G | D2 (AAC)₇ | 9 | 97–116 |
|           | 30 min at 72°C       | Orcfra5 | F: GTGGGGCTAGCTAGAAAC | D3 (CAA)₉ | 6 | 138–159 |
|           | 30 min at 72°C       | Orcfra6 | R: TGGTTTGTATGCTCTGTAACATTAGG | D4 (CA)₉ | 8 | 228–249 |
|           | 30 min at 72°C       | Orcfra7 | R: TACATCCAGGACACAAAG | D3 (CTA)₉ | 7 | 113–128 |
|           | 30 min at 72°C       | Orcfra8 | R: CGATCTGCAACATAAACA | D4 (CA)₉ | 8 | 157–173 |
|           | 30 min at 72°C       | Orcfra9 | R: CGATCTGCAACATAAACA | D4 (CA)₉ | 8 | 157–173 |
|           | 30 min at 72°C       | Orcfra10 | R: TGGTTTGTATGCTCTGTAACATTAGG | D4 (CA)₉ | 8 | 157–173 |
|           | 30 min at 72°C       | Orcfra11 | R: TACATCCAGGACACAAAG | D3 (CAA)₉ | 6 | 138–159 |
|           | 30 min at 72°C       | Orcfra12 | R: TGGTTTGTATGCTCTGTAACATTAGG | D4 (CA)₉ | 8 | 157–173 |

PCR thermal protocols and corresponding primer details are given for each multiplex reaction. Fluorescent dyes were used to label the forward primers. The number of alleles provided is the total observed across all individuals.

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Exact tests (using default Markov chain parameters, performed in Genepop) were used to test for departures from Hardy-Weinberg Equilibrium, with Wright’s fixation index ($F_{ST}$) for each locus-site combination used to determine the nature of those departures (where $F_{ST} < 0$ indicates heterozygote excess and $F_{ST} > 0$ indicates heterozygote deficits). Significance levels were adjusted for multiple comparisons using the sequential Bonferroni procedure [78].

Cryptic species are common in Antarctic benthic invertebrate fauna [51], in part because many taxa are poorly studied. To check for the presence of cryptic species within *O. franklini*, Principal Coordinate Analysis (PCoA in Genalex) was used to examine the complete multilocus data set for evidence of distinct genetic groups [83,84].

**Population differentiation**

We calculated Weir and Cockerham’s $F_{ST}$ estimates [85] to examine genetic differentiation among all sites. Since $F_{ST}$ assumes an infinite allele model of mutation (IAM), we also calculated $R_{ST}$ which assumes a stepwise mutation model (SMM). Currently there is no consensus over which model is more appropriate for microsatellite data, so the conservative approach is to calculate both [86]. $F_{ST}$ and $R_{ST}$ were calculated in Arlequin 3.11 [87], using 50000 permutations to assess significance. The high variability of microsatellites can result in depressed estimates of $F_{ST}$, therefore we also calculated $F’_{ST}$ (a standardised measurement which accounts for high within-population variation), using RECODEDATA 0.1 [88]. Matrices of the pairwise differentiation (as both $F_{ST}$ and $R_{ST}$) between all sites were generated in Genepop.

The partitioning of genetic variation among regions, locations nested within regions, sites nested within locations and individuals nested within sites was determined using a four-level hierarchical analysis of molecular variance (AMOVA). AMOVA was performed using Hierfstat [89], which calculates variance components and $F$-statistics [85] for each hierarchical level according to [90]. Departures from values expected under panmixis (i.e. $F_{ST} = 0$) at each hierarchical level were determined with 10,000 permutations of the data. We estimated the migration occurring between Casey and Davis as $N_{m} = 1/4(1/F_{ST} – 1)$ [91], using the $F_{ST}$ among regions generated from hierarchical AMOVA. $F_{ST}$ varied considerably among loci (see results), so we tested for evidence of selection at each of the seven loci using Lustin [92]. Confidence intervals (99%) for neutral loci were determined using 20,000 simulations and the recommended ‘neutral mean $F_{ST}$’ option [93].

Coastal marine populations are unlikely to disperse according to the island model [94] so we tested for evidence of isolation by distance (which indicates a stepping-stone mode of dispersal; [95]) among *O. franklini* populations within the Casey and Davis regions. We examined the relationship between geographic distance (as the natural logarithm of the shortest water-based route) and genetic differentiation (as linearised $F_{ST}$; i.e. $F_{ST}/(1 – F_{ST})$) between all sites within each region, using Mantel tests implemented in Genepop. The shortest water-based route was an estimation of the direct linear distance (or combination of linear vectors) that an amphipod could feasibly travel between sites (there was insufficient data on ice cover or currents to allow for a more biologically-relevant estimate). Mantel tests were also performed assuming the SMM (i.e. using $R_{ST}/(1 – R_{ST})$ for genetic differentiation estimates). Data were permuted 10,000 times to determine significance.

**Results**

**Genetic diversity of populations**

A total of 718 *Orchomenella franklini* specimens were genotyped for the seven microsatellite loci (448 from Casey, 270 from Davis: Table S1). None of the loci showed evidence of linkage disequilibrium ($p > 0.05$ for all pairwise comparisons). The number of alleles observed at each locus ranged from 6 to 24 (Table 1). Average allelic richness, observed heterozygosity and expected heterozygosity were all significantly higher at Davis ($A_r = 4.46$, $H_o = 0.495$, $H_e = 0.574$), compared to Casey ($A_r = 3.62$, $H_o = 0.420$, $H_e = 0.447$; $p < 0.001$; Table 2). None of these diversity measures were found to differ significantly among polluted and unpolluted sites within either of the regions.

A total of 16 private alleles were observed across the entire dataset (Table S2). Standard diagnostics revealed that the Poisson generalised linear model was an appropriate model for the number of private alleles per population ($P_{h}$), with no evidence of overdispersion. The model revealed strong evidence that $P_{h}$ was significantly greater at Davis than at Casey ($p = 0.002$), and also significantly greater at unpolluted sites compared to polluted sites ($p = 0.005$; Table S2). There was no evidence of a significant interaction between the two factors ($p = 0.307$), and average sample size was almost identical for unpolluted and polluted populations, thus could be disregarded as a potential confounding factor.

Most populations were in Hardy-Weinberg Equilibrium; of the 175 tests across all loci by all sites, only eight were significant after bonferroni correction (Table S2). For just one of these significant departures from HWE did the $F_{IS}$ value represent an excess of heterozygotes (at locus *Orcfra13* for site NEa at Casey; Table S2). The remaining seven were heterozygote deficits at locus *Orcfra4* (Table S2) and all of these occurred at Davis sites. Heterozygote deficits can result from the presence of null alleles; indeed at *Orcfra4* we found evidence of null alleles for all nine sites at Davis, and for three of the 16 sites at Casey. We subsequently adjusted allele frequencies at *Orcfra4* to account for the presence of null alleles (using the Ooerhout correction algorithm in Micro-checker), but this made no difference to the significance of genetic differentiation or isolation by distance determined under either the IAM or SMM, therefore results for the raw data alone are presented for simplicity. Evidence of null alleles was also detected at *Orcfra5* (in five sites), and *Orcfra6* (in three sites). Adjusting allele frequencies at these loci was considered unnecessary because the null alleles were only detected in a small proportion of the total 25 sites.

There was no evidence of cryptic species within the samples of *O. franklini* from Casey and Davis. Although PCoA explained 67%
of the variation in the multilocus genetic data within the first three co-ordinates, it indicated just a single genetic group within the sample (Fig. S1).

### Population differentiation

Genetic differentiation among all sites was highly significant, regardless of the mutational model assumed ($F_{ST} = 0.086, \ p<0.001$; $R_{ST} = 0.139, \ p<0.001$; $F_{ST} = 0.162$; Table 3). Hierarchical AMOVA revealed that the majority of this genetic differentiation occurred between Casey and Davis (69%), with significant differentiation also occurring among locations within each region (2%), but not among sites within each location (Table 4). A considerable amount of the variation (29%) was also due to differences among individuals within each site (Table 4). This hierarchical structure was also reflected in pairwise differentiation estimates: $F_{ST}$ between Casey sites and Davis sites ranged from 0.120 to 0.199, whereas pairwise $F_{ST}$ estimates among all sites within Casey and Davis ranged from 0 to 0.031 (Table S3). Pairwise $R_{ST}$ estimates were consistently higher than $F_{ST}$ values, but showed the same pattern; ranging from 0.063 to 0.486 between Casey and Davis, from 0.000 to 0.119 within Casey, and from 0.000 to 0.129 within Davis (Table S3). The estimate of migration ($N_{m}$) occurring between Casey and Davis was 1.4 (both before and after the removal of loci under selection; see below).

Locus Orcfra4 produced the highest overall $F_{ST}$ values (Table 3), and we found evidence that this locus was under directional selection (as indicated by 99% confidence intervals). We also detected balancing selection at the two loci which produced the lowest overall $F_{ST}$ estimates: Orcfra5 and Orcfra12 (Table 3). We subsequently removed these three loci from the analyses, but the results remained unchanged (Tables 3 and 4). $F_{ST}$ estimates for the differentiation of locations within regions were not particularly high for Orcfra4, nor were they particularly low for Orcfra5 and Orcfra12. Indeed, when we tested loci for evidence of selection within the Casey and Davis datasets independently, all loci were found to be neutral. This suggests that selection associated with Orcfra4, Orcfra5 and Orcfra12 is occurring at the regional scale (i.e. between Casey and Davis) but not on a smaller scale (i.e. between populations within each of the regions).

We found evidence of isolation by distance indicative of stepping-stone dispersal among sites within both the Casey and Davis regions. Mantel tests indicated a significant correlation between genetic and geographic distance under both the IAM (Casey: $p = 0.002$; Davis: $p = 0.000$; Fig. 2A), and the SMM (Casey: $p = 0.003$; Davis: $p = 0.019$; Fig. 2B).

### Discussion

This study has revealed considerable genetic diversity and population differentiation in the ubiquitous Antarctic benthic invertebrate, *Orchomenella franklini*. Genetic differentiation was most pronounced across 1000 s of km between Casey and Davis, indicating that populations in these two regions are effectively isolated. At local scales, genetic differentiation was consistent with a stepping-stone model of dispersal and we conclude that individuals maintain gene flow over hundreds of metres, but that dispersal across larger distances occurs rarely. In addition, we found evidence of differential selection occurring between the Casey and Davis populations and suggest this represents localised adaptation in *O. franklini*. The lack of gene flow among populations and evidence of selection provide an insight into processes of speciation in Antarctic brooders, and should be considered in future management initiatives for the Antarctic benthos.

### Contrasting levels of genetic diversity in *O. franklini* populations

While microsatellite variation in all *O. franklini* populations was considerable, genetic diversity was significantly lower at Casey than at Davis. Contrasting levels of genetic diversity among populations of high latitude species are often suggested to reflect signatures of historical glaciation [31,46,96], however this is unlikely to explain our data, as microsatellite variation should reflect more recent demographic processes [97]. One potential explanation is differences in the spatial heterogeneity of Casey and Davis: it is generally accepted that greater environmental heterogeneity will maintain a

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**Table 3.** Estimates of genetic differentiation ($F_{ST}$, $R_{ST}$ and $F_{9ST}$) among all sites for *Orchomenella franklini*.

|                | $F_{ST}$ | $R_{ST}$ | $F_{9ST}$ |
|----------------|----------|----------|-----------|
| Orcfra3        | 0.091    | 0.177    | 0.254     |
| Orcfra4        | 0.206    | 0.234    | 0.205     |
| Orcfra5        | 0.012    | 0.001    | 0.013     |
| Orcfra6        | 0.056    | 0.027    | 0.146     |
| Orcfra12       | 0.049    | 0.147    | 0.128     |
| Orcfra13       | 0.089    | 0.133    | 0.159     |
| Orcfra26       | 0.154    | 0.161    | 0.190     |
| Overall        | 0.086*** | 0.139*** | 0.162     |

Estimates of genetic differentiation are given for each locus and over all loci. The overall estimate of $F_{ST}$ excluding loci potentially under selection (Orcfra4, Orcfra5 and Orcfra12) is provided in parentheses. Negative values have been converted to zero. Significance of differentiation is indicated as ***$p<0.001$.

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**Table 4.** The partitioning of genetic variation in *Orchomenella franklini* at each spatial level as indicated by hierarchical AMOVA.

|                | Over all loci | Excluding potentially selected loci |
|----------------|---------------|-----------------------------------|
|                | F-statistic   | var. component | % variance | F-statistic | var. component | % variance |
| Among regions  | 0.156***     | 0.640           | 68.7       | 0.152***    | 0.393           | 86.4       |
| Among locations within regions | 0.005**     | 0.019           | 2.0        | 0.006**     | 0.014           | 3.0        |
| Among sites within locations | 0          | 0               | 0          | 0          | 0               | 0          |
| Within sites   | 0.079        | 0.273           | 29.3       | 0.002       | 0.048           | 10.6       |

Results of the analysis excluding loci potentially under selection (Orcfra4, Orcfra5 and Orcfra12) are also provided. Negative values have been converted to zero. Significance is indicated as *$p<0.05$, **$p<0.01$, ***$p<0.001$.

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higher level of genetic variation within species (reviewed in [98]). While there is no published comparison of Casey and Davis environments, studies have generally emphasised the high heterogeneity of Davis benthic habitats, which include fjords and fjord mouths, open wave-exposed coast and significant quantities of wind blown sediment resulting from large ice-free areas [99,100]. Preliminary measurements of sediment properties also indicate greater heterogeneity at Davis, with a range in mean sediment grain size range among Davis locations of approximately 516 μm and a range among Casey locations of approximately 222 μm. Similarly, the total organic carbon content of sediments ranges up to 15% at Davis, and only up to 10% at Casey (the authors, unpublished data). Different levels of iceberg scouring [47] and interspecific competition [48] have also been proposed to explain contrasting genetic diversity among Antarctic benthic invertebrate populations, providing further plausible explanations for the observed differences between Casey and Davis.

There was some evidence of an effect of local anthropogenic pollution on genetic diversity. Although allelic richness and heterozygosity measures appeared unaffected, the number of private alleles per population was lower in polluted locations. Private alleles are an important measure of genetic diversity [101], and a reduction in private alleles may occur through many mechanisms including population bottlenecks, selection against sensitive genotypes or even depressed mutation rates due to contaminants [26]. While elucidating the contribution of these
processes to reduced genetic diversity in polluted *O. franklini* populations is beyond the scope of this study, the result indicates that further examination of the genetic effects of anthropogenic pollution on Antarctic benthos will be important. Interestingly, significantly lower genetic diversity has also been observed for contaminated populations of the amphipod *Orchomenella paugus* from the Arctic [102], suggesting that amphipods may be useful bioindicators of anthropogenic induced genetic change in polar regions.

**Restricted gene flow in *O. franklini***

Genetic differentiation among populations of *O. franklini* was greatest between the two major geographical regions Casey and Davis, which explained 69% of all microsatellite variation observed. Significant $F_{ST}$ of 0.16, pairwise $F_{ST}$ values of up to 0.2, and $R_{ST}$ values up to nearly 0.5 indicate that the two regions are effectively isolated. Importantly, this significant genetic differentiation was still evident after removal of loci under selection, confirming that drift due to restricted gene flow is important in driving this strong genetic subdivision between Casey and Davis. Estimated $N_m$ of 1.4 provides further evidence that there is insufficient exchange of individuals between these regions to prevent them from diverging on independent evolutionary trajectories [4,103].

Gene flow in *O. franklini* is also limited across relatively small spatial scales. Although there was no significant differentiation revealed between replicate sites within locations, indicating that animals are panmictic over 100 s of metres, we did find genetic differentiation among locations within regions (i.e. across distances of 1–30 km). There was a clear pattern of isolation by distance within both Casey and Davis, indicating that migration occurs primarily between adjacent populations [104]. This is one of the first reports of population differentiation over such a small distance for an Antarctic benthic invertebrate. Limited gene flow over these scales is consistent with the brooding development in *O. franklini*, which predicts highly restricted capacity for dispersal. Indeed, similar findings have been reported for brooding taxa from temperate and tropical regions (e.g. [105–108]). Whilst some similar findings have been reported for brooding taxa from temperate and tropical regions (e.g. [105–108]), the evolutionary potential of species may be compromised to broad-scale environmental change, as advantageous alleles will not have the opportunity to become widespread [4,5].

Rather, local adaptation is likely to facilitate speciation, as populations subject to differential selection pressures become more genetically isolated over time [122,123]. For *O. franklini*, the potential for speciation between Casey and Davis populations will ultimately be determined by the interplay of both directional and balancing selective forces, along with continued genetic drift in the face of restricted gene flow.

**Implications for conservation and future research**

The geographical isolation of *O. franklini* populations has important implications for the future design of Antarctic MPAs. To maintain connectivity in this species and replenish any diminished populations outside reserve boundaries, a very close spacing of protected areas would be required. Of course, final management designs must incorporate such information from a wide variety of taxa, nevertheless, the high prevalence of brooding in Antarctic benthic species [59,124] suggests that maintaining connectivity between reserves will emerge as a key design challenge. Of further importance to Antarctic benthic management is the different levels of genetic diversity observed within *O. franklini* (e.g. between Casey and Davis populations). Conserving genetic diversity within species is crucial as it provides the raw material for adaptation to changing conditions, hence facilitating long-term persistence [8,125]. Thus, if management efforts inadvertently protect populations with lower genetic diversity, as has already been shown to occur in one established marine reserve (see [9]), the evolutionary potential of species may be compromised. Our study also provided preliminary evidence of a loss of genetic diversity in polluted populations, which may further increase their susceptibility to any ongoing stressors [126,127]. Such indications of anthropogenic induced genetic change require further attention in the Antarctic, where pollutants are highly localised [38], yet their effects on marine fauna are largely unknown [128].

Clearly, intraspecific genetic structure is a field that warrants increased research in the Antarctic benthos. To date this has been hampered by the logistical difficulties of sampling such an extreme environment [129], as well as by the common discovery of cryptic species, which drastically lowers intraspecific sample size. Our results highlight that future research should address intraspecific gene flow over several spatial scales, as mechanisms acting over one scale may not be apparent over another. Despite Antarctica’s suite of remarkably stable environmental features and long-held views of a homogenous fauna, our study suggests that populations may be adapted to local selection pressures within the Antarctic benthic environment, and this may help explain the high rates of speciation in amphipods and other Antarctic brooders. The
continued use of microsatellites and other highly variable molecular markers should further illuminate such microevolutionary patterns in the Antarctic benthos [32], although increased research on the underlying ecology of species will help interpret the patterns revealed, in particular the processes driving local adaptation. Consequently, this will improve our understanding of Antarctic benthic species responses to environmental change, and how best to manage this unique environment.

Supporting Information

Figure S1 Results of Principal Coordinate Analysis on multilocus genotypes of *Orchomenella franklini*. Over 50% of genetic variation is explained within the first two coordinates; however, there is no single group sufficiently discrete to indicate cryptic species in the dataset. (TIF)

Table S1 Regions, locations, and sites sampled for *Orchomenella franklini*, with corresponding sample size (n). Locations (and respective sites) in bold have been classified as polluted. (DOC)

Table S2 Inbreeding coefficients by locus (*F*<sub>IS</sub>) and the number of private alleles (*P*<sub>4</sub>) for each population of *Orchomenella franklini*. Asterisks indicate significant departures from Hardy-Weinberg Equilibrium (*p*<0.05) after Bonferroni correction. Dashes indicate that a locus was monomorphic, hence *F*<sub>IS</sub> could not be estimated. Polluted sites are in bold. (DOC)

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Author Contributions

Conceived and designed the experiments: HPB KJM JSS. Performed the experiments: HPB. Analyzed the data: HPB KJM. Contributed reagents/materials/analysis tools: HPB KJM JSS. Wrote the paper: HPB. Data interpretation: HPB KJM. Provided editorial revisions on manuscript: KJM JSS. Approved final version of manuscript: KJM JSS.

References

1. Slatkin M (1994) Gene flow and population structure. In: Real LA, ed. Ecological Genetics. Princeton, USA: Princeton University Press. pp 3–17.
2. Morton CL, Riebege LK (2004) How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Molecular Ecology 13: 1341–1356.
3. Bohonak AJ (1999) Dispersal, gene flow and population structure. The Quarterly Review of Biology 74: 21–45.
4. Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236: 787–792.
5. Levins R (1964) The theory of fitness in a heterogeneous environment. IV. The adaptive significance of gene flow. Evolution 18: 635–638.
6. Gooch JL (1975) Mechanisms of evolution and population genetics. In: Kinne O, ed. Marine Ecology: A Comprehensive, Integrated Treatise on Life in Oceans and Coastal Waters: 2 Physiological Mechanisms. London, UK: Wiley-Interscience. pp 349–409.
7. Allendorf FW, Englund PR, Laikhter G, Ritchie PA, Ryman N (2008) Genetic effects of harvest on wild animal populations. Trends in Ecology & Evolution 23: 327–337.
8. Bowen BW (1999) Preserving genes, species, or ecosystems? Healing the fractured foundations of conservation policy. Molecular Ecology 8: S5–S10.
9. Bell JJ, Okamura B (2005) Low genetic diversity in a marine nature reserve: re-evaluating diversity criteria in reserve design. Proceedings of the Royal Society of London B: Biological Sciences 272: 1067–1074.
10. Gray JS (1997) Marine biodiversity: patterns, threats and conservation needs. Biodiversity and Conservation 6: 153–175.
11. Costello MJ, Coll M, Danovaro R, Halpin P, Ojaveer H, et al. (2010) A census of Antarctic marine species. Trends in Ecology & Evolution 25: S1–S7.
12. Agardy TM (1994) Advances in marine conservation: the role of marine protected areas. Trends in Ecology & Evolution 9: 267–270.
13. Balan L, Belhaji A, Magalhães S, Spindler DJ, Kuklinski S, et al. (2010) Genetic connectivity in the Antarctic Benthos. PLoS ONE 5: e12110. doi:10.1371/journal.pone.0012110
14. Frame SM (2009) Assessing and managing the impact of marine protection for biomonitoring and ecotoxicology. Mutation Research/Reviews in Mutation Research 685: 33–51.
15. Picken GB (1985) Benthic research in Antarctica: past, present and future. In: Gray JS, Christiansen ME, eds. Marine Biology of Polar Regions and Effects of Stress on Marine Organisms. Chichester, UK: John Wiley & Sons. pp 157–194.
16. De Wet H, Bhat R, Backeljau T (2004) The population genetic structure of *Lithotrya lithosa* (Motulaca: Gastropoda) along a pollution gradient in the Scheldt estuary (The Netherlands) using RAPD analysis. Science of The Total Environment 325: 39–69.
17. Held C, Leese F (2007) The utility of fast evolving molecular markers for evaluating diversity criteria in reserve design. Proceedings of the Royal Society of London B: Biological Sciences 272: 1067–1074.
18. Hilbish TJ (1996) Population genetics of marine species: the interaction of natural selection and historically differentiated populations. Journal of Experimental Marine Biology and Ecology 200: 67–83.
19. Donnelly CL, Cowles DL, Carter RL (2000) Effect of pollution on genetic diversity in the bay mussel *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*. Marine Environmental Research 50: 559–563.
20. Slatkin M (1994) Gene flow and population structure. In: Real LA, ed. Ecological Genetics. Princeton, USA: Princeton University Press. pp 3–17.
21. Refstie T, Gjermoe AS, Bentsen R, Olesen M (2000) Evaluation of the use of *Mytilus galloprovincialis* for biomonitoring and ecotoxicology. Mutation Research/Reviews in Mutation Research 463: 33–51.
22. Amundsen PR, Hovland JR, Johnsen JS (1998) On the origin of Antarctic marine ecosystems. Journal of Experimental Marine Biology and Ecology 228: 167–184.
23. Antz WE, Brey T, Gallardo VA (1994) Antarctic zoobenthos. Oceanography and Marine Biology: an Annual Review 32: 241–304.
24. Picken GB (1985) Benthic research in Antarctica: past, present and future. In: Gray JS, Christiansen ME, eds. Marine Biology of Polar Regions and Effects of Stress on Marine Organisms. Chichester, UK: John Wiley & Sons. pp 157–194.
25. De Wet H, Bhat R, Backeljau T (2004) The population genetic structure of *Lithotrya lithosa* (Motulaca: Gastropoda) along a pollution gradient in the Scheldt estuary (The Netherlands) using RAPD analysis. Science of The Total Environment 325: 39–69.
26. Hadfield JD, Addinall S, Boul C, Moreau P, Malone S (2000) Effect of pollution on genetic diversity in the bay mussel *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*. Marine Environmental Research 50: 559–563.
27. Bickham JW, Sandhu S, Hebert PB, Chidli I, Athwal R (2000) Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. Mutation Research/Reviews in Mutation Research 463: 33–51.
28. Amundsen PR, Hovland JR, Johnsen JS (1998) On the origin of Antarctic marine ecosystems. Journal of Experimental Marine Biology and Ecology 228: 167–184.
29. Hold C, Leese F (2007) The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. Polar Biology 30: 513–521.
30. Hofman JI, Picken GB, Lanne R, Clarke A (2010) Strong population genetic structure in a broadcast-spawning Antarctic marine invertebrate. Journal of Heredity.
31. Commission for the Conservation of Antarctic Marine Living Resources (2005) 16th meeting. Kuala Lumpur, Malaysia: CCMAR. p S1–S7.
32. Held C, Leese F (2007) The utility of fast evolving molecular markers for studying speciation in the Antarctic benthos. Polar Biology 30: 513–521.
95. Kimura M (1953) “Stepping stone” model of population. Annual Report of the National Institute of Genetics 3: 62–63.

96. Marko PB (2004) “What’s larvae got to do with it?” Diapause patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. Molecular Ecology 13: 597–611.

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

98. Hedrick PW (1986) Genetic polymorphism in heterogeneous environments: a decade later. Annual Review of Ecology and Systematics 17: 535–566.

99. Everett DA, Poore GCB, Pickard J (1980) Marine benthos from Davis Station, Eastern Antarctica. Australian Journal of Marine and Freshwater Research 31: 829–836.

100. O’Brien PE, Stark JS, Johnstone G, Smith J, Riddle MJ (2011) Seabed contamination: a field study on an arctic amphipod. PhD Thesis. Roskilde, Denmark: Olsen and Olsen. pp 265–272.

101. Bastidas C, Benzie JAH, Fabricius KE (2002) Genetic differentiation among populations of the brooding soft coral Cladaura batikiren on the Great Barrier Reef. Coral Reefs 21: 233–241.

102. De Broyer C, Jazewski K (1993) Contribution to the marine biodiversity inventory: a checklist of the Amphipoda (Crustacea) of the Southern Ocean. Documents de Travail de l’Institut royal des Sciences naturelles de Belgique 73: 1–154.

103. Nielsen EE, Hansen MM, Meldrup D (2006) Evidence of microsatellite hitchhiking selection in Atlantic cod (Gadus morhua L.): Implications for inferring population structure in nonmodel organisms. Molecular Ecology 15: 3219–3229.

104. Bach L (2009) Costs and consequences for populations to adapt to life in contaminated environments – a field study on an arctic amphipod. PhD Thesis. Roskilde, Denmark: Roskilde Universitet.

105. Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? Molecular Ecology 19: 3038–3051.

106. De Broyer C, Jazewski K (1993) Contribution to the marine biodiversity inventory: a checklist of the Amphipoda (Crustacea) of the Southern Ocean. Documents de Travail de l’Institut royal des Sciences naturelles de Belgique 73: 1–154.

107. Carvalho GR (1989) Microgeographic genetic differentiation and dispersal capacity in the intertidal isopod, Jaera albifrons. In: Ryland JS, Tyler PA, eds. Reproduction, Genetics and Distributions of Marine Organisms. London, UK: Academic Press. pp 421–461.

108. Hodgson JW (1970) Marine biogeography of the Antarctic regions. In: Holdgate MW, ed. Antarctic Ecology. London, UK: Academic Press. pp 97–104.

109. Wilson AB, Boates JS, Snyder M (1997) Genetic isolation of populations of the brooding holothuroid. Journal of Experimental Marine Biology and Ecology 122: 187–194.

110. Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49: 561–576.

111. Cohen AS, Johnston MR (1987) Speciation in brooding and poorly dispersing taxa. Evolution 41: 1135–1147.

112. Larsson LC, Larke L, Pahn S, André C, Carvalho GR, et al. (2007) Concordance of allozyme and microsatellite differentiation in a marine fish, but evidence of selection at a microsatellite locus. Molecular Ecology 16: 1135–1147.

113. Nevo E, Noy R, Lavie B, Beiles A, Muchtar S (1986) Genetic diversity and resistance to marine pollution. Biological Journal of the Linnean Society 29: 139–144.

114. Andre C, Larsson LC, Larke L, Bekkved D, Brigham J, et al. (2011) Detecting population structure in a high gene-flow species, Atlantic herring (Clupea harengus): direct, simultaneous evaluation of neutral vs putatively selected loci. Heredity 106: 270–280.

115. Vasemägi A, Primmer CR (2005) Challenges for identifying functionally important genetic variation: the promise of combining complementary research strategies. Molecular Ecology 14: 3623–3642.