

1 **RAD sequencing and a hybrid Antarctic fur seal genome assembly**
2 **reveal rapidly decaying linkage disequilibrium, global population**
3 **structure and evidence for inbreeding**

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ABSTRACT

25 Recent advances in high throughput sequencing have transformed the study of wild organisms
26 by facilitating the generation of high quality genome assemblies and dense genetic marker
27 datasets. These resources have the potential to significantly advance our understanding of
28 diverse phenomena at the level of species, populations and individuals, ranging from patterns
29 of synteny through rates of linkage disequilibrium (LD) decay and population structure to
30 individual inbreeding. Consequently, we used PacBio sequencing to refine an existing
31 Antarctic fur seal (*Arctocephalus gazella*) genome assembly and genotyped 83 individuals
32 from six populations using restriction site associated DNA (RAD) sequencing. The resulting
33 hybrid genome comprised 6,169 scaffolds with an N50 of 6.21 Mb and provided clear evidence
34 for the conservation of large chromosomal segments between the fur seal and dog (*Canis*
35 *lupus familiaris*). Focusing on the most extensively sampled population of South Georgia, we
36 found that LD decayed rapidly, reaching the background level of $r^2 = 0.09$ by around 26 kb,
37 consistent with other vertebrates but at odds with the notion that fur seals experienced a strong
38 historical bottleneck. We also found evidence for population structuring, with four main
39 Antarctic island groups being resolved. Finally, appreciable variance in individual inbreeding
40 could be detected, reflecting the strong polygyny and site fidelity of the species. Overall, our
41 study contributes important resources for future genomic studies of fur seals and other
42 pinnipeds while also providing a clear example of how high throughput sequencing can
43 generate diverse biological insights at multiple levels of organisation.

44

45

INTRODUCTION

46 Advances in short read sequencing technologies, in particular Illumina sequencing, have
47 made it possible to generate genome assemblies as well as dense genetic marker datasets
48 for practically any organism (Ekblom and Galindo 2011; Ellegren 2014). However, assemblies
49 based solely on short read data tend to be highly fragmented, even with assembly strategies
50 that incorporate medium length insert libraries (Gnerre *et al.* 2011). Consequently, although
51 such assemblies can be generated rapidly and cheaply, there has been growing interest in
52 technologies that incorporate longer range information to improve scaffold length and
53 contiguity. For example, Pacific Biosciences (PacBio) single molecule real-time (SMRT)
54 sequencing generates read lengths in the order of several kilobases (kb) that have proven
55 effective in gap filling, resolving complex repeats and increasing contig lengths across diverse
56 taxa (English *et al.* 2012; Conte and Kocher 2015; Pootakham *et al.* 2017).

57 In parallel to these and related developments in genome sequencing technologies, reduced
58 representation sequencing approaches such as restriction site associated DNA (RAD)
59 sequencing (Baird *et al.* 2008; Peterson *et al.* 2012) are providing unprecedented levels of
60 genetic resolution for population genetic and genomic studies (Morin *et al.* 2004; Stapley *et*
61 *al.* 2010; Seeb *et al.* 2011). By sequencing and assembling short stretches of DNA adjacent
62 to restriction cut sites and interrogating the resulting tags for sequence polymorphisms, RAD
63 sequencing can facilitate the acquisition of large genome-wide distributed single nucleotide
64 polymorphism (SNP) datasets incorporating multiple individuals.

65 The above approaches show great promise for studying wild populations where genomic
66 resources are typically absent. For example, information from model organisms with well-
67 characterised genomes can facilitate studies of their wild relatives as long as patterns of
68 synteny between the two can be established. Knowledge of synteny can facilitate the lifting
69 over of gene annotations, assist in gene mapping and help to elucidate the genetic basis of
70 fitness variation by identifying genes closely linked to loci responsible for inbreeding
71 depression (Johnston *et al.* 2011; Ekblom and Wolf 2014; Kardos *et al.* 2016).

72 High density SNP markers mapped to a reference genome can furthermore provide insights
73 into processes that shape levels of variation within genomes. For example, the positional
74 information of genomic loci can be used to characterise patterns of linkage disequilibrium (LD).
75 LD is a central concept in population genetics because it is closely associated with factors
76 such as effective population size (N_e), genetic drift, historical fluctuations in population size,

77 population structure, inbreeding and recombination (Slatkin 2008). Understanding the strength
78 and extent of LD can aid in the inference of demographic history and has important
79 implications for identifying genetic variants underlying key fitness traits through genome-wide
80 association analyses or quantitative trait locus mapping (Carlson *et al.* 2004; Miller *et al.* 2015;
81 Kardos *et al.* 2016). Nevertheless, the genomic pattern of LD has only been described in a
82 handful of wild populations. Typically, LD decays within a few tens to hundreds of kilobases
83 (kb) in large and unstructured populations (Poelstra *et al.* 2013; Kawakami *et al.* 2014; Vijay
84 *et al.* 2016), but can extend for several megabases (Mb) in smaller, isolated, heavily
85 bottlenecked and/or inbred populations, such as wolves and sheep (Hagenblad *et al.* 2009;
86 Miller *et al.* 2011).

87 In addition to facilitating the characterisation of genome-wide patterns of variation, dense
88 genomic markers can also be used to describe variation at the population and individual level,
89 even without positional information. For example, studies are increasingly employing
90 approaches such as RAD sequencing to obtain large datasets in order to reliably characterize
91 genetic structure (Malenfant *et al.* 2015; Benestan *et al.* 2015; Younger *et al.* 2017) and many
92 are uncovering patterns that had previously gone undetected (Reitzel *et al.* 2007; Ogden *et*
93 *al.* 2013; Vendrami *et al.* 2017). A precise understanding of population structure is critical for
94 the delineation of management units for conservation (Bowen *et al.* 2005) as well as for
95 avoiding false positives in genome-wide association studies (Johnston *et al.* 2011) but can
96 also be used to determine contemporary and historical barriers to gene flow (McRae *et al.*
97 2005; Hendricks *et al.* 2017) and to elucidate patterns of extinction and recolonization
98 (McCauley 1991).

99 A major topic of interest at the level of the individual is the extent to which inbreeding occurs
100 in natural populations (Kardos *et al.* 2016) and its consequences for fitness variation and
101 population demography (Keller and Waller 2002). Pedigree-based studies, typically of isolated
102 island populations and often involving polygynous species, have uncovered widespread
103 evidence of inbreeding in the wild (Marshall *et al.* 2002; Townsend and Jamieson 2013;
104 Nietlisbach *et al.* 2017). However, the extent of inbreeding in large, continuous and free-
105 ranging populations remains open to question. On the one hand, simulations have suggested
106 that inbreeding will be absent from the vast majority of wild populations with the possible
107 exception of highly polygynous and/or structured populations (Balloux *et al.* 2004). On the
108 other hand, associations between microsatellite heterozygosity and fitness (heterozygosity
109 fitness correlations, HFCs) have been described in hundreds of species (Chapman *et al.* 2009)
110 and it has been argued that these are highly unlikely to arise in the absence of inbreeding
111 (Szulkin *et al.* 2010). Due to the high sampling variance of microsatellites, there has been

112 growing interest in the use of high density SNP data to reliably quantify inbreeding, and recent
113 empirical and simulation studies suggest that this can be achieved with as few as 10,000
114 SNPs (Kardos *et al.* 2015; 2018). Consequently, with approaches like RAD sequencing, it
115 should be possible to quantify the variation in inbreeding in arguably more representative wild
116 populations.

117 The Antarctic fur seal (*Arctocephalus gazella*) is an important marine top predator that has
118 been extensively studied for several decades, yet many fundamental aspects of its biology
119 remain poorly understood. This highly sexually dimorphic pinniped has a circumpolar
120 distribution and breeds on islands across the sub-Antarctic, with 95% of the population
121 concentrated on South Georgia in the South Atlantic (Figure 1). The species was heavily
122 exploited by 18th and 19th Century sealers and was thought to have gone extinct at virtually all
123 of its contemporary breeding sites (Weddell 1825). However, in the 1930s a small breeding
124 population was found at South Georgia (Bonner 1968; Payne 1977), which in the following
125 decades increased to number several million individuals (Boyd 1993). While it is believed that
126 the species former range was recolonised by emigrants from this large and rapidly expanding
127 population (Boyd 1993; Hucke-Gaete *et al.* 2004), one would expect to find little or no
128 population structure under such a scenario. However, a global study using mitochondrial DNA
129 resolved two main island groups (Wynen *et al.* 2000) while microsatellites uncovered
130 significant differences between South Georgia and the nearby South Shetland Islands (Bonin
131 *et al.* 2013), implying that at least two relict populations must have survived sealing.

132 Antarctic fur seals have been intensively studied for several decades at a small breeding
133 colony on Bird Island, South Georgia, where a scaffold walkway provides access to the
134 animals for the collection of detailed life history and genetic data. Genetic studies have
135 confirmed behavioural observations of strong polygyny (Bonner 1968) by showing that a
136 handful of top males father the majority of offspring (Hoffman *et al.* 2003). Furthermore,
137 females exhibit strong natal site fidelity, returning to within a body length of where they were
138 born to breed (Hoffman and Forcada 2012), while adults of both sexes are highly faithful to
139 previously held breeding locations (Hoffman *et al.* 2006). Together these behavioural traits
140 may increase the risk of incestuous matings. In line with this, heterozygosity measured at nine
141 microsatellites has been found to correlate with multiple fitness traits including early survival,
142 body size and reproductive success (Hoffman *et al.* 2004; 2010; Forcada and Hoffman 2014).
143 However, such a small panel of microsatellites cannot provide a very precise estimate of
144 inbreeding (Slate *et al.* 2004; Balloux *et al.* 2004) and therefore high density SNP data are
145 required to provide more detailed insights into the variance in inbreeding in the population.

146 Here, we used PacBio sequencing to improve an existing Antarctic fur seal genome assembly
147 comprising 8,126 scaffolds with an N50 of 3.1 Mb (Humble *et al.* 2016). We additionally RAD
148 sequenced 83 individuals, mainly from South Georgia but also from an additional five
149 populations, to generate a large dataset of mapped genetic markers. The resulting data were
150 then used to investigate synteny with the dog and, within the focal South Georgia population,
151 to characterise the pattern of LD decay as well as variance in inbreeding. Finally, using data
152 from both RAD sequencing and 27 microsatellites, we investigated the strength and pattern
153 of population structure across the species range and compared the ability of the two marker
154 types to resolve genetic differences between island groups. Our hypotheses were as follows:
155 (i) We expected to find strong synteny between the fur seal and dog (*Canis lupus familiaris*),
156 the closest relative with an annotated, chromosome-level genome assembly; (ii) LD might be
157 expected to decay very rapidly given that fur seals are free-ranging with large population sizes.
158 However, the historical bottleneck could potentially have resulted in elevated levels of LD; (iii)
159 We hypothesised that nuclear markers would detect the same two island groups as previously
160 found with mitochondrial DNA as well as possibly resolve finer scale structuring. Furthermore,
161 RAD sequencing should provide greater power to capture genetic differences than
162 microsatellites; (iv) Finally, we expected to find variation in inbreeding consistent with
163 knowledge of the species mating system as well as previous studies documenting HFCs.

164

MATERIALS AND METHODS

165 **Hybrid genome assembly and PacBio DNA library preparation**

166 We first used the program GapCloser v1.12 to fill gaps in the existing fur seal genome v1.02
167 (Humble *et al.* 2016) (NCBI SRA: BioProject PRJNA298406) based on the paired end
168 information of the original Illumina reads. This approach closed 45,852 gaps and reduced the
169 amount of N space in the assembly from 115,235,953 bp to 78,393,057 bp (v1.1, Table 1).
170 Following this, we generated SMRT sequencing data from the DNA used for the original
171 genome assembly (NCBI SRA: BioSample SAMN04159679) following the protocol described
172 in Pendleton *et al.* (2015). First, 10 µg of pure genomic DNA was fragmented to 20 kb using
173 the Hydroshear DNA shearing device (Digilab, Marlborough, MA) and size-selected to 9–50
174 kb using a Blue Pippin according to the standard Pacific Biosciences SMRT bell construction
175 protocol. The library was then sequenced on 64 PacBio RSII SMRT cells using the P6–C4
176 chemistry. This yielded a total of 58 Gb (~19x) of sequencing data contained within 8,101,335
177 subread bases with a mean read length of 7,177 bp (median = 6,705 bp; range = 50–54,622
178 bp). The data have been deposited to the NCBI SRA under accession number XXXX.

179 Next, we used PBJelly v15.8.24 and blasr (<https://github.com/PacificBiosciences/blasr>) with
180 default parameters to align the PacBio sequencing reads to the gap-closed assembly to
181 generate a hybrid genome (v1.2). Lastly, we followed a two-step strategy to remove any indels
182 introduced by single molecule real-time sequencing (Ross *et al.* 2013). We first used Quiver
183 (contained in the SMRT/2.3.0 suite: GenomicConsensus v0.9.2) with the
184 refineDinucleotideRepeats option to perform initial assembly error correction. Due to this step
185 being computationally demanding, we ran it separately for each scaffold. Next, we mapped
186 the original Illumina reads (Humble *et al.* 2016) to the quiver assembly (v1.3) using BWA MEM
187 v0.7.15 (Li 2013) and used Picard tools to sort and mark duplicates. We then used PILON
188 v1.22 (Walker *et al.* 2014) to perform the final error correction step to generate assembly v1.4.
189 The final assembly is available at NCBI under accession number XXXX.

190 **Genome alignment**

191 We aligned the fur seal scaffolds from assembly v1.4 to the dog genome (*Canis lupus*
192 *familiaris* assembly version CanFam3.1, GenBank accession number GCA_000002285.2)
193 using LAST v746 (Kiełbasa *et al.* 2011). First, the dog genome was prepared for alignment
194 using the command lastdb. We then used lastal and last-split in combination with parallel-fastq
195 to align the fur seal scaffolds against the dog genome. Using the program MafFilter, we then
196 processed the resulting multiple alignment format (maf) file and estimated pairwise sequence
197 divergence between the two species (Dutheil *et al.* 2014). Finally, we extracted alignment

198 coordinates from the maf file using bash commands to allow subsequent visualisation with the
199 R package RCircos (Zhang *et al.* 2013).

200 **Sampling and DNA extraction**

201 Tissue samples were collected from 57 Antarctic fur seal individuals from Bird Island, South
202 Georgia. These comprised 24 partially overlapping triads consisting of 24 pups, 16 mothers
203 and 17 fathers. Additional samples were obtained from the main breeding colonies across the
204 species range (Figure 1): Cape Shirreff in the South Shetlands ($n = 6$), Bouvetøya ($n = 5$), îles
205 Kerguelen ($n = 5$), Heard Island ($n = 5$) and Macquarie Island ($n = 5$). Skin samples were
206 collected from the inter-digital margin of the fore-flipper using piglet ear notching pliers and
207 stored in 20% dimethyl sulphoxide saturated with NaCl at -20°C . Skin samples from the South
208 Shetlands were collected using a sterile 2mm biopsy punch and stored in 95% ethanol. Total
209 genomic DNA was extracted using a standard phenol-chloroform protocol (Sambrook *et al.*
210 1989).

211 **Microsatellite genotyping**

212 All samples were genotyped at 27 polymorphic microsatellite loci (see Supplementary table
213 1), previously been found to be in Hardy-Weinberg equilibrium (HWE) in the study population
214 at South Georgia and are unlinked (Stoffel *et al.* 2015; Peters *et al.* 2016). The loci were PCR
215 amplified in three separate multiplexed reactions (see Supplementary Table 1) using a Type
216 It Kit (Qiagen). The following PCR profile was used for all multiplex reactions except for
217 multiplex one: initial denaturation of 5 min at 94°C ; 28 cycles of 30 sec at 94°C , 90 sec at
218 60°C , and 30 sec at 72°C , followed by a final extension of 30 min at 60°C . The PCR profile of
219 multiplex one only differed from this protocol in the annealing temperature used, which was
220 53°C . Fluorescently labelled PCR products were then resolved by electrophoresis on an ABI
221 3730xl capillary sequencer and allele sizes were scored using GeneMarker v1.95. To ensure
222 high genotype quality, all traces were manually inspected and any incorrect calls were
223 adjusted accordingly.

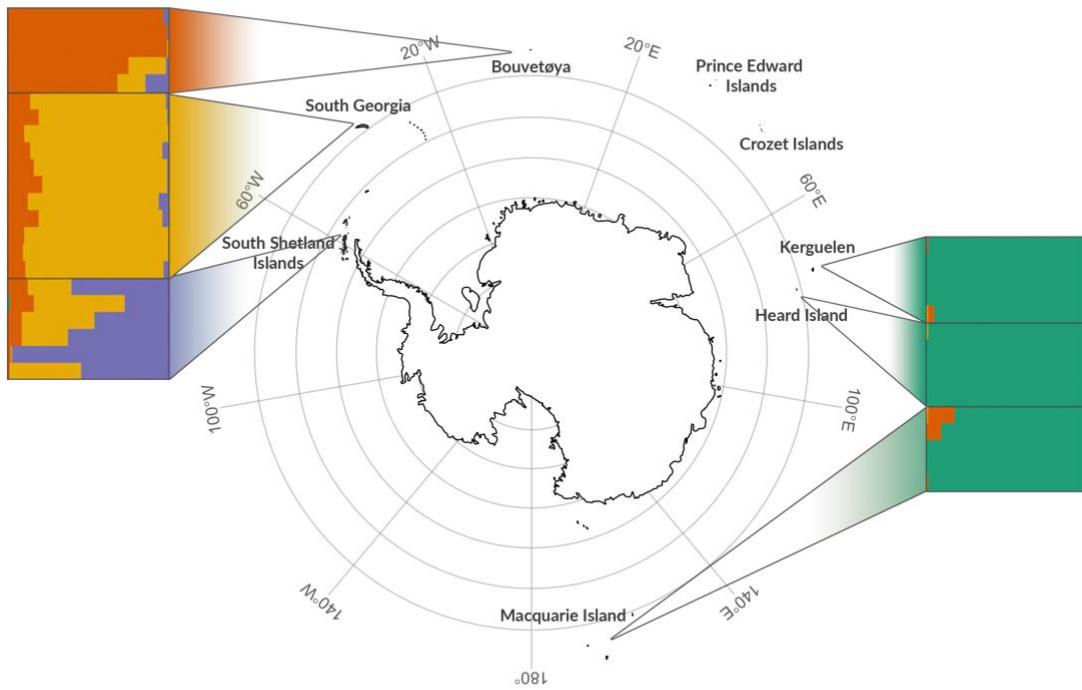


Figure 1. Individual assignment to genetic clusters based on STRUCTURE analysis for $K = 4$ using 28,092 SNPs. Each horizontal bar represents a different individual and the relative proportions of the different colours indicate the probabilities of belonging to each group. Individuals are separated by sampling locations as indicated on the map.

224 **RAD library preparation and sequencing**

225 RAD libraries were prepared using a modified protocol from Etter *et al.* (2011) with minor
226 modifications as described in Hoffman *et al.* (2014). Briefly, 400 ng of genomic DNA from each
227 individual was separately digested with *SbfI* followed by the ligation of P1 adaptors with a
228 unique 6 bp barcode for each individual in a RAD library, allowing the pooling of 16 individuals
229 per library. Libraries were sheared with a Covaris S220 and agarose gel size-selected to 300–
230 700 bp. Following 15–17 cycles of PCR amplification, libraries were further pooled using eight
231 different i5 indices prior to 250 bp paired-end sequencing on two lanes of Illumina HiSeq 1500.
232 The sequences have been deposited in the Short Read Archive (accession no. XXX).

233 **SNP genotyping**

234 Read quality was assessed using FastQC v0.112 and sequences trimmed to 225 bp and
235 demultiplexed using process_radtags in STACKS v1.41 (Catchen *et al.* 2013). We then
236 followed GATK's best practices workflow for variant discovery (Poplin *et al.* 2017). Briefly,
237 individual reads were mapped to the Antarctic fur seal reference genome v1.4 using BWA
238 MEM v0.7.10 (Li 2013) with the default parameters. Any unmapped reads were removed from
239 the SAM alignment files using SAMtools v1.1 (Li 2011). We then used Picard Tools to sort
240 each SAM file, add read groups and remove PCR duplicates. Prior to SNP calling, we
241 performed indel realignment to minimize the number of mismatching bases using the
242 RealignerTargetCreator and IndelRealigner functions in GATK v3.6. Finally, HaplotypeCaller
243 was used to call variants separately for each individual. Genomic VCF files were then passed
244 to GenotypeGVCFs for joint genotyping. The resulting SNP dataset was then filtered to include
245 only biallelic SNPs using BCFtools v1.2 (Li 2011) to obtain a dataset of 677,607 SNPs
246 genotyped in 83 individuals. Subsequently, we applied a variety of filtering steps according to
247 the analysis being performed as shown in figure S1 and described below.

248 **SNP validation**

249 To provide an indication of the quality of our SNP dataset, we attempted to validate a
250 representative subset of loci using Sanger sequencing. First, we randomly selected 50 loci
251 whose 70 bp flanking sequence contained no secondary SNPs and mapped uniquely to the
252 fur seal reference genome and with initial depth of coverage and minor allele frequency (MAF)
253 filters of 5 and 0.05 respectively. We then designed oligonucleotide primers using Primer 3
254 (Untergasser *et al.* 2012) to PCR amplify each putative SNP together with 100–200 bp of
255 flanking sequence. Each locus was PCR amplified in one fur seal individual that had been
256 genotyped as homozygous at that locus and one that had been genotyped as heterozygous.
257 PCRs was carried out using 1.5 μ L of template DNA, 20 mM Tris–HCl (pH 8.3), 100 mM KCl,
258 2 mM MgCl₂, 10x Reactionbuffer Y (Peqlab), 0.25 mM dNTPs, 0.25 mol/L of each primer, and
259 0.5U of Taq DNA polymerase (VWR). The following PCR profile was used: one cycle of 1.5
260 min at between 59° and 62° depending on the primers used (Supplementary Table 2), 60 sec
261 at 72°C; and one final cycle of 7 min at 72°C. 5 μ L of the resulting PCR product was then
262 purified using shrimp alkaline phosphatase and exonuclease I (NEB) following the
263 manufacturer's recommended protocol. All fragments were then sequenced in both directions
264 using the Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher
265 Scientific) and analyzed on an ABI 3730xl capillary sequencer. Forward and reverse reads
266 were aligned using Geneious v10.2.3 (Kearse *et al.* 2012). Heterozygous sites were identified
267 as those with two peaks of roughly equal intensity but with around half the intensity of a
268 homozygote.

269 **Linkage disequilibrium decay**

270 Prior to quantifying linkage disequilibrium, we filtered the SNP dataset as shown in Figure
271 S1A. First, to minimise the occurrence of unreliable genotypes, we removed individual
272 genotypes with a depth of coverage below eight or above 30 using VCFtools (Danecek *et al.*
273 2011). Genotypes with very low depth of coverage have a greater likelihood of being called
274 incorrectly as it can be difficult to distinguish between homozygotes and heterozygotes when
275 very few reads are present. Similarly, genotypes with very high depth of coverage are more
276 likely to be spurious as high coverage can result from misalignment due to the presence of
277 paralogous loci or repeats (Fountain *et al.* 2016). Second, as including SNPs from short
278 scaffolds can downwardly bias LD values, we retained only SNPs located on the longest 100
279 scaffolds of the assembly (min length = 6.6 Mb, max length = 33.1 Mb). Third, as an additional
280 quality filtering step, we used information on known parental relationships to identify loci with
281 Mendelian incompatibilities using the mendel function in PLINK v1.9 and removed these from
282 the dataset. Fourth, to remove any possible confounding effects of population structure, we
283 focussed on the single largest population of South Georgia. Fifth, to provide an informative
284 dataset while further minimising genotyping error, we discarded SNPs with a MAF of less than
285 0.1 and/or called in less than 50% of individuals using PLINK. As a final quality control step,
286 we also removed SNPs that did not conform to Hardy-Weinberg equilibrium (HWE) with a *p*-
287 value threshold < 0.001 using the --hwe function in PLINK.

288 Using the final dataset of 27,347 SNPs genotyped in 57 individuals (Figure S1A), we used the
289 --r2 function in PLINK to quantify pairwise LD between all pairs of SNPs located within 500 kb
290 of each other. We visualised LD decay with distance by fitting a nonlinear regression curve
291 using the nls package in R, where the expected value of r^2 under drift-recombination
292 equilibrium ($E(r^2)$) was expressed according to the Hill and Weir function (Hill and Weir 1988),
293 as implemented by Marroni *et al.* (2011):

$$E(r^2) = \left[\frac{10 + \rho}{(2 + \rho)(11 + \rho)} \right] \left[1 + \frac{(3 + \rho)(12 + 12\rho + \rho^2)}{n(2 + \rho)(11 + \rho)} \right]$$

294 where N_e is the effective population size, c is the recombination fraction between sites, $\rho =$
295 $4N_e c$ and n is the number of scaffolds (Remington *et al.* 2001).

296 **Population structure**

297 Prior to quantifying population structure, we filtered the full SNP dataset as shown in Figure
298 S1B. We did not initially filter the dataset for SNPs with low depth of coverage as for the
299 analysis of population structure we wanted to retain as many SNPs as possible that were
300 genotyped across all the populations. We also did not remove individuals with high levels of
301 missing data in order to maximise the representation of all populations in the final dataset.
302 Nevertheless, because closely related individuals can bias population genetic structure
303 analysis by introducing both Hardy-Weinberg and linkage disequilibrium (Rodriguez-Ramilo
304 and Wang 2012; Wang 2017), we used known parentage information to remove adults and
305 related pups (full and half siblings) from the South Georgia dataset. Second, SNPs with a MAF
306 of less than 0.05 and/or called in less than 99% of individuals were discarded using VCFtools.
307 Third, SNPs were pruned for LD using the --indep function in PLINK. We used a sliding window
308 of 50 SNPs, a step size of 5 SNPs and removed all variants in a window above a variance
309 inflation factor threshold of 2, corresponding to $r^2 = 0.5$. As population structure can lead to
310 deviations from HWE, we did not filter our final dataset for HWE.

311 Using the final dataset of 28,062 SNPs genotyped in 37 individuals (Figure S1B), we first
312 visualised population structure by performing a principal components analysis (PCA) using
313 the R package adegenet (Jombart 2008). We then used a Bayesian clustering algorithm
314 implemented by the program STRUCTURE to identify the number of genetic clusters (K)
315 present in the dataset. We performed STRUCTURE runs for values of K ranging from one to
316 six, with five simulations for each K and a burn-in of 100,000 iterations followed by 1,000,000
317 Markov chain Monte Carlo iterations. We used the admixture and correlated allele frequency
318 models without sampling location information. The R package pophelper (Francis 2017) was
319 then used to analyse the STRUCTURE results, parse the output to CLUMPP for averaging
320 across iterations and for visualising individual assignment probabilities. The optimal K was
321 selected based on the maximum value of the mean estimated \ln probability of the data (\ln
322 $\Pr(X | K)$ as proposed by Pritchard *et al.* (2000) and the ΔK method of Evanno *et al.* (2005).
323 For comparison, we also implemented the above analyses using microsatellite data for the
324 same individuals.

325 **Inbreeding coefficients**

326 Prior to quantifying inbreeding, we filtered the SNP dataset as shown in Figure S1C. First, for
327 the analysis of inbreeding we wanted a dataset with as few gaps as possible so we discarded
328 one individual with more than 90% missing data. Second, we removed individual genotypes
329 with a depth of coverage below eight or above 30 using vcftools. Third, we removed loci with
330 Mendelian incompatibilities, and fourth, we again restricted the dataset to the focal population

331 of South Georgia. Fifth, we discarded SNPs with a MAF of less than 0.05 and/or called in less
332 than 75% of individuals using vcftools. Finally, we filtered the SNPs for HWE as described
333 previously and pruned linked SNPs out of the dataset using the --indep function in PLINK with
334 the parameters shown above.

335 Using the final dataset of 9,853 SNPs genotyped in 56 individuals (Figure S1C), we calculated
336 four genomic estimates of individual inbreeding: standardised multi-locus heterozygosity
337 (sMLH), an estimate based on the variance of additive genotype values (\hat{F}_I), an estimate
338 based on excess homozygosity (\hat{F}_{II}) and an estimate based on the correlation of uniting
339 gametes, which gives more weight to homozygotes of the rare allele at each locus (\hat{F}_{III}). The
340 former was calculated using the sMLH function in the R package inbreedR (Stoffel *et al.* 2016)
341 whilst the latter were calculated in GCTA v1.24.3 (Yang *et al.* 2011). To test for a significant
342 correlation in heterozygosity across marker loci, we quantified identity disequilibrium (ID) using
343 the measure g_2 in the R package inbreedR (Stoffel *et al.* 2016) where significant g_2 values
344 provide support for variance in inbreeding in the population. Finally, we compared the resulting
345 g_2 value with the variance in our inbreeding coefficients to determine the expected correlation
346 between estimated (\hat{f}) and realized (f^*) level of inbreeding (Szulkin *et al.* 2010) given as:

$$r^2(\hat{f}, f^*) = \frac{g_2}{\sigma^2(\hat{f})}$$

347

RESULTS

348 **Hybrid genome assembly**

349 We used PacBio sequencing to improve an existing Antarctic fur seal genome assembly.
350 Using PBJelly, we were able to close a total of 45,394 gaps, resulting in a 40% reduction in
351 overall gap space (assembly v1.2, Table 1). Subsequent assembly correction with Quiver
352 resulted in a total of 11,319,546 modifications to the PBJelly assembly consisting of 291,179
353 insertions, 1,117,226 substitutions and 9,911,141 deletions. Finally, PILON corrected 653,246
354 homozygous insertions (885,794 bp), 87,818 deletions (127,024 bp) and 34,438 single-base
355 substitutions and closed an additional 2,170 gaps in the Quiver assembly. Overall, gap closing
356 and error correction resulted in a hybrid Antarctic fur seal assembly with a total length of 2.3
357 Gb (v1.4, Table 1). The number of scaffolds in the genome was reduced from 8,126 to 6,169
358 such that 50% of the final assembly is now contained within the longest 108 scaffolds (Table
359 1).

360 **Genome synteny**

361 To investigate synteny between the Antarctic fur seal and the dog, we aligned the fur seal
362 scaffolds to the dog genome (CanFam3.1). We estimated overall sequence divergence
363 between the two species to be 13.8%. Visualisation of the full alignment revealed that all of
364 the dog chromosomes are represented in the fur seal assembly (Figure S2). Alignment of the
365 40 longest fur seal scaffolds (min length = 10.7 Mb, max length = 33.1 Mb) revealed strong
366 chromosomal synteny between the two genomes, with the vast majority of the fur seal
367 scaffolds mapping exclusively or mainly to a given dog chromosome (Figure 2). Specifically,
368 for 37 of the scaffolds, over 90% of the total alignment length was to a single dog chromosome,
369 with 26 of those aligning exclusively to a single dog chromosome. Only one scaffold (S4 in
370 Figure 2) aligned in roughly equal portions to two different dog chromosomes (62% to D5 and
371 38% to D26).

Table 1. Genome assembly statistics for successive improvements of the original Antarctic fur seal genome assembly.

| | v1.0.2 ALLPATHS3 | v1.1 GapCloser | v1.2 PBJelly2 | v1.3 Quiver | v1.4 Pilon |
|-----------------------|-----------------------------------|---------------------------------|--------------------------------|------------------------------|-----------------------------|
| Number of scaffolds | 8,126 | 8,126 | 6,170 | 6,170 | 6,169 [†] |
| N90 ^a | 890,836 (768) | 888,912 (768) | 1,624,547 (387) | 1,511,352 (387) | 1,542,705 (387) |
| N50 ^a | 3,169,165 (233) | 3,165,747 (233) | 6,454,664 (108) | 6,076,522 (108) | 6,207,322 (108) |
| N10 ^a | 8,459,351 (25) | 8,458,289 (25) | 17,733,103 (11) | 16,529,571 (11) | 16,861,656 (11) |
| Longest scaffold (bp) | 13,012,173 | 12,999,316 | 34,690,325 | 32,399,786 | 33,062,611 |
| Total size (bp) | 2,405,038,055 | 2,403,626,805 | 2,426,014,533 | 2,268,217,244 | 2,313,485,084 |
| Gaps present (%) | 4.79 | 3.26 | 0.62 | 0.57 | 0.55 |
| Number of gaps | 136,284 | 90,432 | 45,102 | 22,783 | 20,613 |
| Average gap size (bp) | 845.56 | 866.87 | 331.16 | 570.37 | 613.39 |

^a Size in bp (number of scaffolds)

[†] Excluding the mitochondrial genome, which was filtered out by Pilon

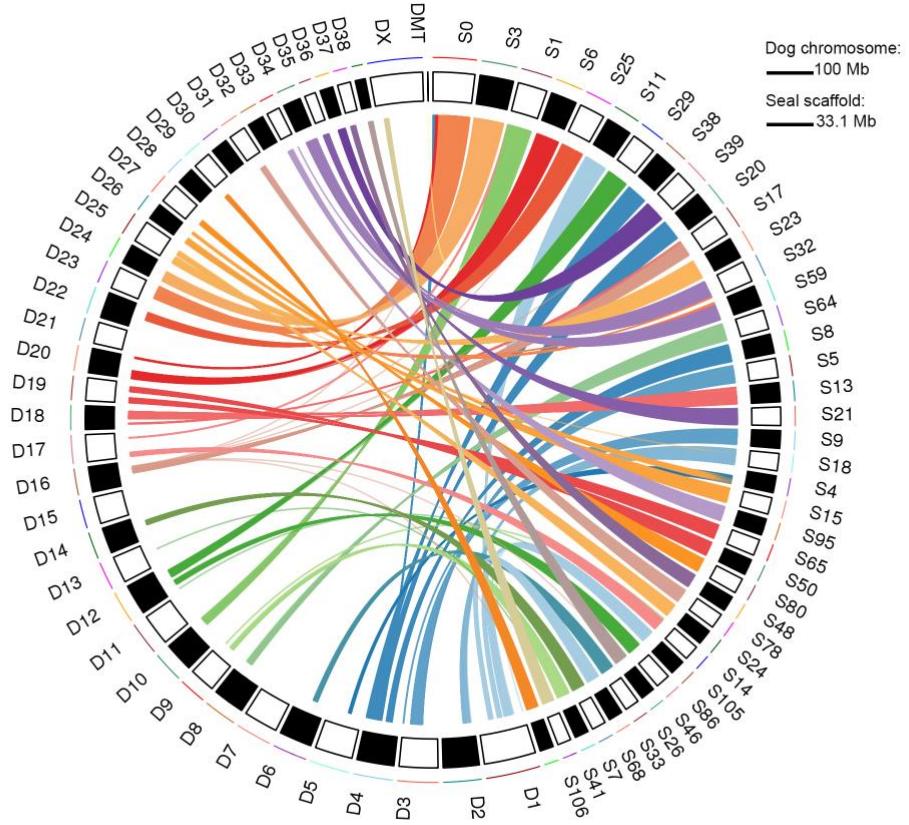


Figure 2. Synteny of the longest 40 Antarctic fur seal scaffolds (10.7-33.1 Mb; right, prefixed S) with dog chromosomes (left, prefixed D). Mapping each fur seal scaffold to the dog genome resulted in multiple alignment blocks (mean = 2.1 kb, range = 0.1–52.8 kb) and alignments over 5 kb are shown.

372 RAD sequencing and SNP discovery

373 RAD sequencing of 83 fur seal individuals generated an average of 5,689,065 250bp paired-
374 end reads per individual. After mapping these reads to the reference genome, a total of
375 677,607 biallelic SNPs were discovered using GATK's best practices workflow for variation
376 discovery (see Materials and methods for details), with an average coverage of 727. We then
377 filtered the dataset in three different ways (Supplementary Figure 1) to generate datasets
378 suitable for the analysis of LD decay, population structure and inbreeding.

379 SNP validation

380 To provide an indication of the quality of our SNP dataset, we used Sanger sequencing to
381 validate 50 randomly selected loci. For each locus, we sequenced a single heterozygote and
382 a single homozygote individual based on the corresponding GATK genotypes. For 40 of these

383 loci, we successfully obtained genotypes for both individuals (Supplementary Table 2).
384 Concordance between the GATK and Sanger genotypes was high, with 76 / 80 genotypes
385 being called identically using both methods, equivalent to a validation rate of 95%. The four
386 discordant genotypes were all initially called as homozygous with GATK but subsequently
387 validated as heterozygous with Sanger sequencing.

388 **LD decay**

389 The pattern of LD decay within South Georgia was quantified based on 27,347 SNPs
390 genotyped in 57 individuals and located on the 100 longest fur seal scaffolds. LD was found
391 to decay rather rapidly, with r^2 reaching the background level (average $r^2 = 0.12$) by around
392 18 kb and decreasing to values approaching zero by around 350 kb (Figure 3). Strong LD (r^2
393 ≥ 0.5) decayed by around 5 kb and moderate LD ($r^2 \geq 0.2$) by around 7 kb.

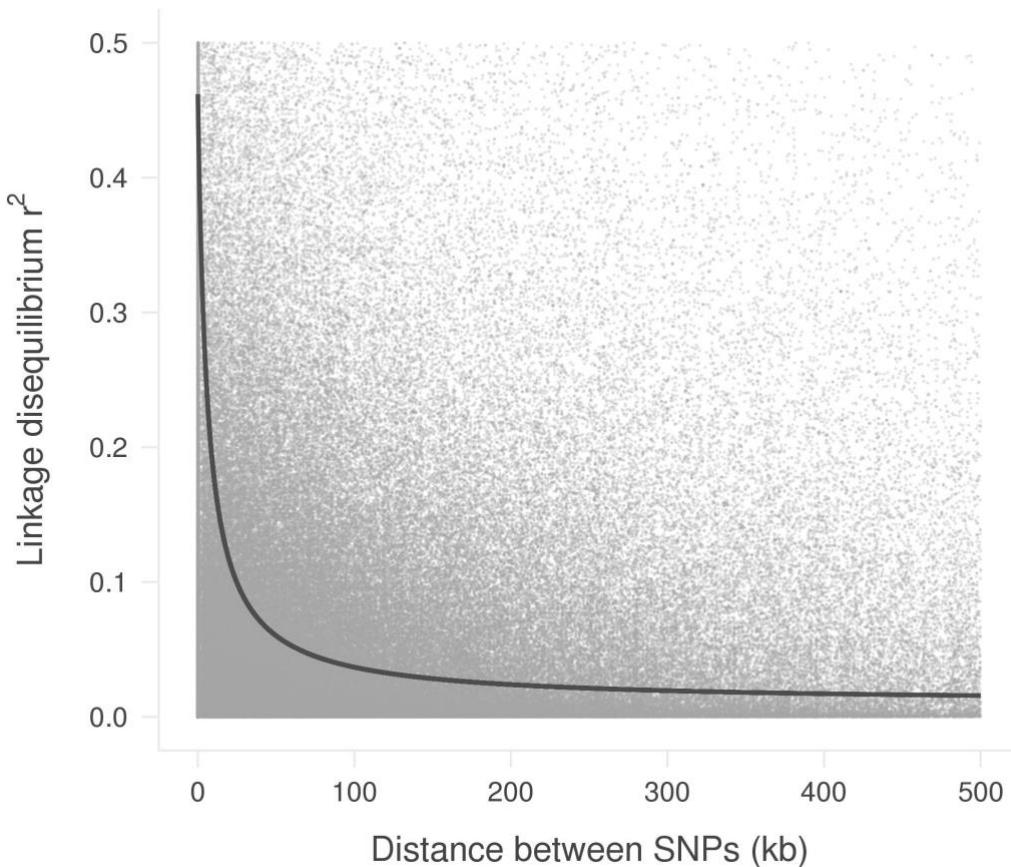


Figure 3. Plot of linkage disequilibrium (r^2) against distance between SNPs in the Antarctic fur seal. LD was calculated using 27,347 filtered RAD SNPs from the 100 largest scaffolds of 57 South Georgia individuals. Grey dots indicate observed pairwise LD. Dark grey curve shows the expected decay of LD in the data estimated by nonlinear regression of r^2 .

394 **Population structure**

395 Finally, we used a dataset of 37 pups genotyped at 27 microsatellites and 28,062 SNPs to
396 quantify the pattern and strength of population structure across the species' circumpolar
397 range. PCA of the microsatellite dataset uncovered weak clustering with South Georgia, the
398 South Shetlands and Bouvetøya tending to separate apart from Kerguelen, Heard and
399 Macquarie Islands along the first PC axis (Figure 4A). However, considerable scatter and no
400 clear pattern of separation was found along either PC2 or PC3 (Figures 4A and 4C). By
401 contrast, population structure was more clearly defined in the PCA of the SNP dataset.
402 Specifically, the first PC axis clearly resolved two distinct island groups, the first comprising
403 South Georgia, the South Shetlands and Bouvetøya and the second comprising Kerguelen,
404 Heard Island and Macquarie Island (Figure 4B). Within the first island group, Bouvetøya
405 clustered apart from South Georgia and the South Shetlands along PC2 (Figure 4B) while all
406 three locations clustered apart from one another along PC3 (Figure 4D).

407

408 To test whether population structure could be detected without prior knowledge of the
409 sampling locations of individuals, we used a Bayesian approach implemented within
410 STRUCTURE (Pritchard *et al.* 2000). This program works by partitioning the data set in such
411 a way that departures from Hardy-Weinberg and linkage equilibrium within the resulting
412 groups are minimized. Separately for the microsatellite and SNP datasets, five replicate runs
413 were conducted for each possible number of groups (K) ranging from one, implying no
414 population differentiation, through to six, which would imply that all of the populations are
415 genetically distinct. For the microsatellite dataset, $\ln \Pr(X | K)$ and ΔK both peaked at 2,
416 indicating support for the presence of two genetically distinct populations (Figure S3A and C).
417 Membership coefficients for the inferred groups are summarized in Figure S4A and indicate
418 the presence of a Western population comprising individuals from South Georgia, the South
419 Shetlands and Bouvetøya, and an Eastern population comprising individuals from Kerguelen,
420 Heard Island and Macquarie Island.

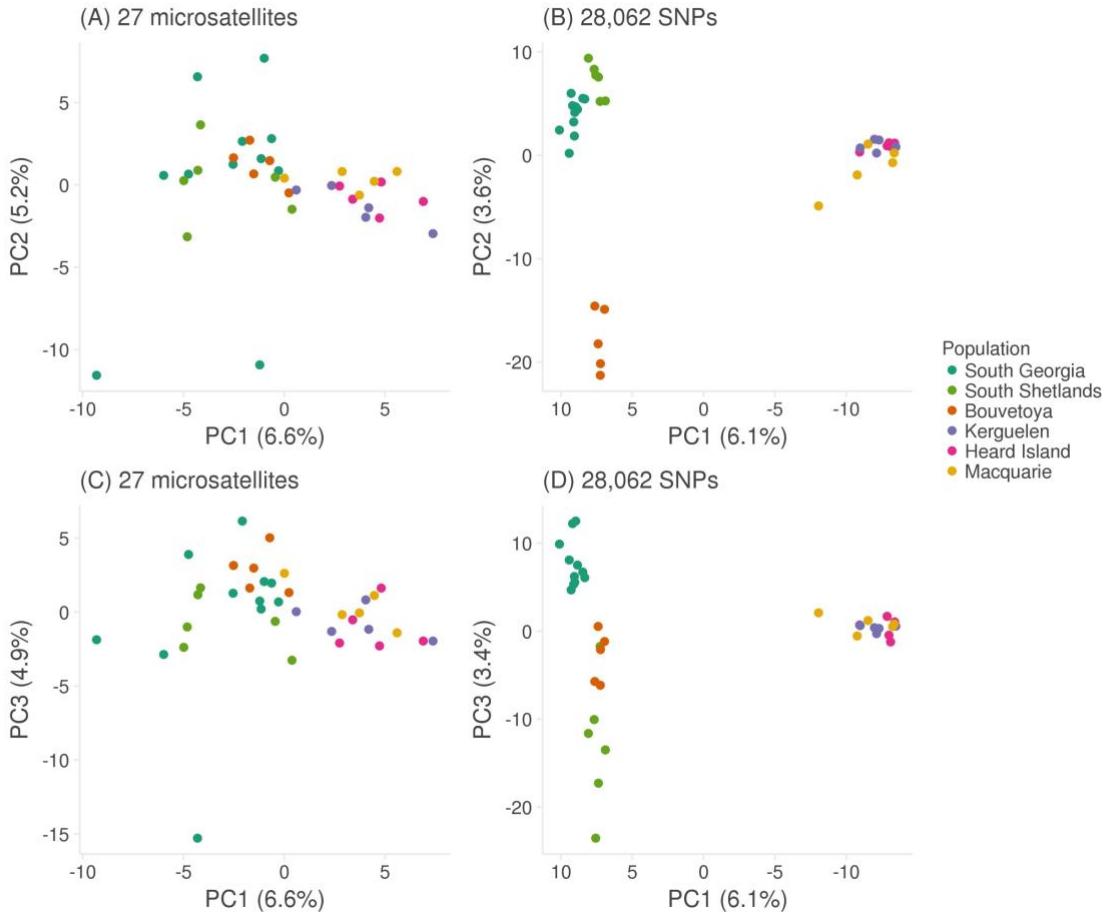


Figure 4. Scatterplots showing individual variation in principal components (PCs) one and two (A and B), and one and three (C and D) derived from a principal component analysis conducted using 27 microsatellites (A and C) and 28,062 SNPs (B and D). Variance explained by each PC is shown in brackets.

421 For the RAD dataset, $\ln \Pr(X | K)$ also peaked at 2 but remained high for $K = 3$ and 4, while
 422 ΔK reached its maximum at $K = 4$ (Figure S3B and D). To explore this further, we plotted
 423 membership coefficients for $K = 2$ to 6 for both the microsatellite and SNP datasets. For the
 424 former, no evidence of population structure was found beyond $K = 2$, with successive
 425 increases in K merely introducing additional admixture (Figure S4A). By contrast for the latter,
 426 plots corresponding to K values greater than 2 clearly resolved further hierarchical structure
 427 (Figure S4B). Results for $K = 4$ are shown in Figure 1, in which Kerguelen, Heard and
 428 Macquarie Islands are resolved as a single population, while South Georgia, the South
 429 Shetlands and Bouvetøya can be readily distinguished based on their corresponding group
 430 membership coefficients.

431 **Inbreeding**

432 Inbreeding in the focal population at South Georgia was investigated using data from 9,853
 433 SNPs genotyped in 56 individuals (Figure 5A). Identity disequilibrium differed significantly from
 434 zero (0.0052; bootstrap 95% confidence interval = 0.0008–0.0091, $p = 0.023$, Figure 5B)
 435 providing evidence for variance in inbreeding within the sample of individuals. Each
 436 individual's level of inbreeding was quantified from the SNP dataset using four different
 437 genomic inbreeding coefficients (sMLH, \hat{F}_I , \hat{F}_II and \hat{F}_{III} , see Materials and methods for details).
 438 All four of these measures were inter-correlated, with correlation coefficients (r) ranging from
 439 0.69 to 0.83. (Figure 5C–E). Furthermore, the variances of \hat{F}_I , \hat{F}_{II} and \hat{F}_{III} fell within the 95%
 440 confidence interval of g_2 , suggesting that the expected correlation between the estimated and
 441 realized level of inbreeding does not differ significantly from one (Figure 5B).

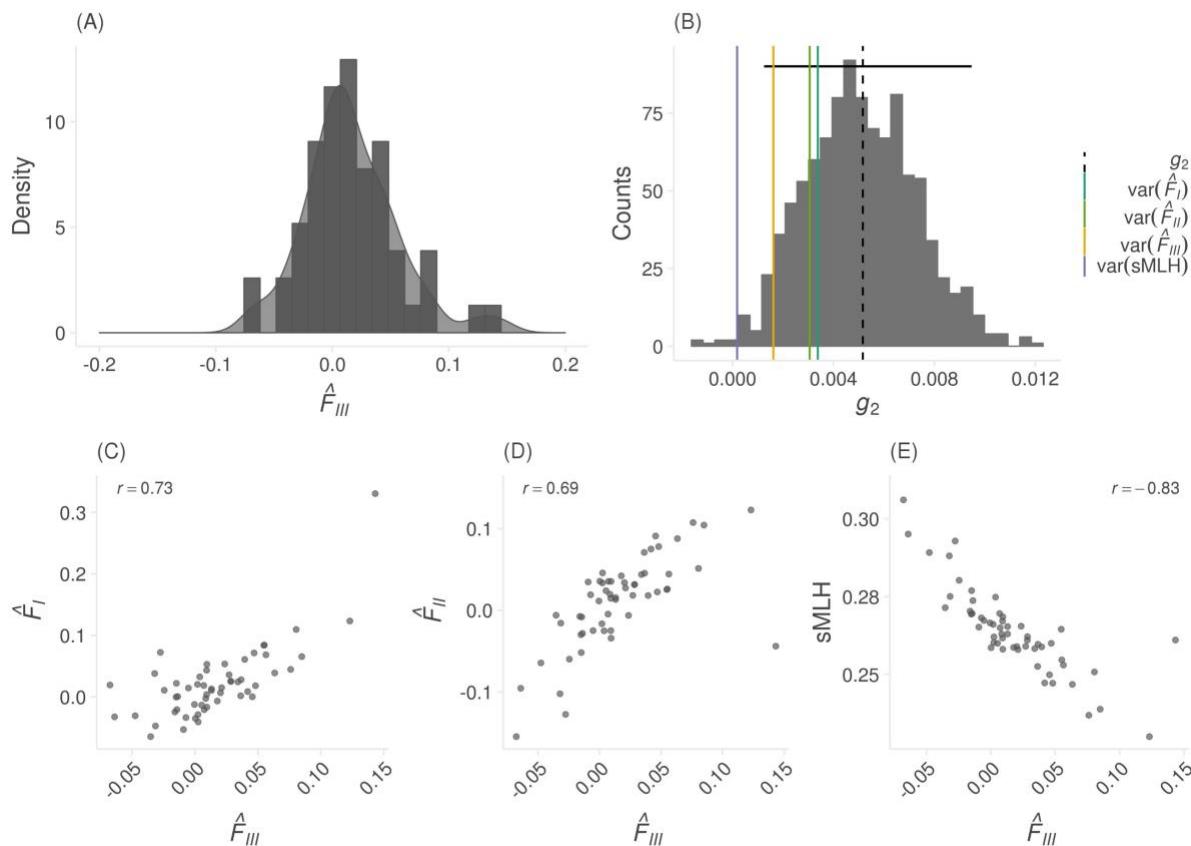


Figure 5. Distribution of inbreeding coefficients (\hat{F}_{III}) for 56 South Georgia individuals (A). Distribution of identity disequilibrium (g_2) estimates from bootstrapping over individuals (B). Horizontal black line shows 95% confidence interval from 1000 bootstrap replications. Vertical dashed line represents empirical g_2 estimate. Vertical coloured lines represent variance in inbreeding coefficients. Pairwise correlation between \hat{F}_{III} and \hat{F}_I , \hat{F}_{II} , and sMLH based on 9,853 SNPs (C, D, E). Pearson's correlation coefficients are shown.

442

DISCUSSION

443 Advances in high throughput sequencing technology have afforded researchers the
444 opportunity to generate genome assemblies and genomic marker datasets for virtually any
445 species for which high quality DNA can be collected. These resources allow a broad range of
446 questions in ecology and evolution to be addressed with greater power and precision than
447 was possible with traditional methods. In this study, we utilised PacBio sequencing to refine
448 an existing Antarctic fur seal genome assembly and combined this with RAD sequencing to
449 characterize synteny with the dog genome, elucidate the rate of LD decay, resolve global
450 population structure and quantify the variance in inbreeding. Our results provide new insights
451 at multiple levels of organisation that enrich our understanding of an important Antarctic
452 marine top predator and indicate the general promise of these and related approaches for
453 tackling broad-reaching questions in population and evolutionary genetics.

454 **Genome alignment**

455 An important outcome of this study is a significantly improved Antarctic fur seal genome
456 assembly. This was achieved through three iterative steps involving gap filling, inclusion of
457 long PacBio reads and assembly error correction respectively. Overall, the number of
458 scaffolds was reduced by around one quarter, while N50 almost doubled to over 6 Mb and the
459 proportion of gaps was reduced by around an order of magnitude to around half a percent.
460 This represents an improvement over existing pinniped assemblies such as the walrus
461 (*Odobenus rosmarus divergens*, GenBank accession number GCA_000321225.1) and
462 Weddell seal (*Leptonychotes weddellii*, GenBank accession number GCA_000349705.1),
463 which both have lower N50 values (2.6 and 0.9 Mb respectively). The improved Antarctic fur
464 seal genome will therefore serve as an important resource for the wider pinniped community.
465 However, there is still considerable room for improvement as a handful of other marine
466 mammal genome assemblies incorporating longer range information show higher levels of
467 contiguity e.g. killer whale, (*Orcinus orca*, N50 = 12.7 Mb) and Hawaiian monk seal
468 (*Neomonachus schauinslandi*, N50 = 22.2 Mb) (Foote *et al.* 2015; Mohr *et al.* 2017).

469 To further quantify genome quality and to explore patterns of synteny, we mapped the
470 scaffolds of our new assembly to the dog genome. The resulting alignment revealed almost
471 complete coverage of the dog chromosomes. This is in line with the observation that the total
472 length of the assembly has not changed appreciably between versions and suggests that the
473 assembly is near-complete, with the exception of the Y-chromosome for which sequence data
474 are currently lacking as the genome individual is a female. In general, carnivore genomes
475 show high levels of synteny (Arnason 1974; Ferguson-Smith and Trifonov 2007), with

476 pinnipeds in particular exhibiting highly conserved karyotypes indicative of slow rates of
477 chromosomal evolution (Beklemisheva *et al.* 2016). By contrast, the domestic dog has an
478 extensively re-arranged karyotype differentiated from the ancestral carnivore karyotype by
479 over 40 separate fission events (Nie *et al.* 2011). To provide insights into the extent of
480 conservation of chromosomal blocks between seals and dogs, we mapped the longest 40 fur
481 seal scaffolds to the dog genome. We found a clear pattern whereby all but one of the scaffolds
482 mapped exclusively or mainly to single chromosomes, indicating the conservation of large
483 genomic tracts often several Mb in length. The remaining scaffold mapped to two dog
484 chromosomes in roughly equal proportions, suggestive of either a fission event in the lineage
485 leading to dogs or a fusion event in the lineage leading to seals. By focusing only on the largest
486 scaffolds, we had little power to detect multiple chromosomal rearrangements, although these
487 are to be expected given a substantial increase in the number of chromosomes in dogs ($2n =$
488 74) relative to the seal ($2n = 36$) (Gustavsson 1964; Arnason 1974)). Nevertheless, the
489 observed high degree of synteny is consistent with previous studies revealing both strong
490 sequence homology and the conservation of polymorphic loci between seals and dogs
491 (Osborne *et al.* 2011; Hoffman *et al.* 2013).

492 **SNP discovery and validation**

493 Our study found a total of 667,607 SNPs in a discovery pool of 83 individuals. These markers
494 will be useful for future studies including the planned development of a high-density SNP array.
495 However, not all SNPs are suitable for every analysis due to differential sensitivity to missing
496 data, low depth of sequencing coverage and the inclusion of low frequency alleles (Shafer *et*
497 *al.* 2017). Similarly, filtering for deviations from HWE and Mendelian incompatibilities should
498 reduce the error rate by reducing the frequency of erroneous genotypes. Yet, as population
499 structure can generate deviations from HWE, stringent filtering may also remove genuine
500 signal. We therefore carefully considered how best to filter our SNP dataset for each of our
501 main analyses. For LD decay, we applied relatively strict filters as we sought a high-quality
502 dataset with consistently high coverage across individuals. For population structure, it was
503 important to have as many SNPs as possible represented in all of the sampling locations, so
504 we did not remove genotypes with low coverage or containing Mendelian incompatibilities but
505 instead filtered to retain SNPs genotyped in at least 99% of individuals. Conversely, for the
506 estimation of inbreeding, we honed in on a reduced subset of higher quality SNPs with greater
507 average depth of coverage, in Hardy-Weinberg and linkage equilibrium, and with no evidence
508 of Mendelian incompatibilities.

509 Even with stringent filtering, it is possible to retain SNPs in a dataset that have been called
510 incorrectly. We therefore attempted to validate 50 randomly selected loci by Sanger

511 sequencing selected individuals with homozygous and heterozygous genotypes as
512 determined from the RAD data. For the 40 loci that we were able to successfully sequence,
513 around 95% of the Sanger genotypes were identical to the RAD genotypes. Although this
514 validation step required additional experimental effort, our results compare favourably with
515 other studies (Cruz *et al.* 2017; Bourgeois *et al.* 2018) and thus give us confidence in the
516 overall quality of our data.

517 **Linkage disequilibrium decay**

518 We used the genomic positions of SNPs mapping to the largest 100 scaffolds to quantify the
519 pattern of LD decay in the focal population of South Georgia. We found that LD decays rapidly,
520 with moderate LD extending less than 10 kb. This is despite the species having experienced
521 a population bottleneck in the 19th century which is expected to increase LD. A direct
522 comparison with other organisms is hindered both by a paucity of data for most species and
523 by the use of different measures for quantifying LD. However, our results are broadly in line
524 with other wild vertebrate populations such as polar bears, Alaskan gray wolves and
525 flycatchers, where moderate LD also extends less than 10 kb (Gray *et al.* 2009; Malenfant *et*
526 *al.* 2015; Kardos, Husby, *et al.* 2016). Extended LD has been documented in a number of
527 species but in most cases this is associated with extreme bottlenecks, such as those
528 experienced during domestication (Harmegnies *et al.* 2006; McKay *et al.* 2007; Meadows *et*
529 *al.* 2008). Although Antarctic fur seals are generally believed to have also experienced a very
530 strong historical bottleneck, a recent Bayesian analysis suggested that this may have been
531 less severe than thought, with the effective population size probably falling to several hundred
532 (Hoffman *et al.* 2011). Furthermore, the population recovered from the bottleneck within a few
533 generations, which could have mitigated the increased genetic drift and inbreeding effects that
534 elevate and maintain strong LD. Additionally, the population is currently estimated to number
535 around 2–3 million individuals (Boyd 1993) and is one of the most genetically diverse
536 pinnipeds (Stoffel *et al.* unpublished results). Therefore, given that LD is a function of both
537 recombination rate and population size (Hill 1981), the rapid decay of LD in this species might
538 also be a reflection of high long-term effective population sizes.

539 **Population structure**

540 To provide further insights into the recovery of Antarctic fur seals globally, we quantified
541 population structure across the species' geographic range. Microsatellite genotypes provided
542 evidence for two major geographic clusters, the first corresponding to South Georgia, the
543 South Shetlands and Bøuvetoya, and the second corresponding to Kerguelen, Heard and
544 Macquarie Island. By contrast, the RAD data uncovered an additional level of hierarchical
545 structure, resolving South Georgia, the South Shetlands and Bøuvetoya as distinct

546 populations. This is consistent with simulation studies suggesting that thousands of SNPs
547 should outperform small panels of microsatellites at resolving population structure (Haasl and
548 Payseur 2011) as well as with more recent empirical studies that have directly compared
549 microsatellites with SNPs (Rašić *et al.* 2014; Vendrami *et al.* 2017). Furthermore, many of our
550 populations had sample sizes of around five individuals yet could still be clearly distinguished
551 from one another. This is in line with a recent simulation study suggesting that sample sizes
552 as small as four individuals may be adequate for resolving population structure when the
553 number of markers is large (Willing *et al.* 2012). Thus, our results have positive implications
554 for studies of threatened species for which extensive sampling can be difficult but where
555 understanding broad as well as fine-scale population structure is of critical importance.

556 It is generally believed that Antarctic fur seals were historically extirpated from virtually all of
557 their contemporary breeding sites across the sub-Antarctic, with the possible exception of
558 Bøuvetoya, where sealing expeditions were more sporadic (Christensen 1935) and around a
559 thousand breeding individuals were sighted just a few decades after the cessation of hunting
560 (Olstad 1928). South Georgia was the first population to stage a major recovery, probably
561 because a number of individuals survived at isolated locations inaccessible to sealers around
562 the South Georgia mainland (Bonner 1968). Consequently, several authors have speculated
563 that emigrant individuals from the expanding South Georgia population may have recolonized
564 the species former range (Boyd 1993; Hucke-Gaete *et al.* 2004). However, Wynen *et al.* (2000)
565 resolved two main island groups with mtDNA, while Bonin *et al.* (2014) found that significant
566 differences between the South Shetland Islands and South Georgia with microsatellites. Our
567 results build on these studies in two ways. First, the two major clusters we resolved using both
568 microsatellites and RAD sequencing are identical to those identified by Wynen *et al.* (2000),
569 suggesting that broad-scale population structure is not simply driven by female philopatry but
570 is also present in the nuclear genome. Second, within the Western part of the species range,
571 we not only found support for the South Shetlands being different from South Georgia, but
572 also Bøuvetoya, suggesting that relict populations probably survived at all three of these
573 locations. By contrast, no sub-structure could be detected within the Eastern part of the
574 species range, which taken at face value might suggest that a single population survived
575 sealing in this region. Consistent with this, historical records suggest that fur seals went locally
576 extinct at Heard and Macquarie islands (Page *et al.* 2003; Goldsworthy *et al.* 2009) and these
577 populations may therefore have been recolonised by surviving populations in the Kerguelen
578 archipelago. Thus, our study highlights the importance of relict populations to species recovery
579 while also providing some evidence for local extinctions having occurred.

580 **Inbreeding**

581 Delving a level deeper, we investigated individual variation in the form of inbreeding. A recent
582 meta-analysis has shown that small panels of microsatellites are almost always underpowered
583 to detect variation in inbreeding (Szulkin *et al.* 2010; Miller *et al.* 2013). By contrast, a handful
584 of recent studies have shown that tens of thousands of SNPs are capable of accurately
585 quantifying inbreeding (Hoffman *et al.* 2014; Huisman *et al.* 2016; Berenos *et al.* 2016; Chen
586 *et al.* 2016; Kardos *et al.* 2018). While empirical studies to date have largely focused on small,
587 isolated populations where inbreeding may be common, it is less clear how prevalent
588 inbreeding could be in larger, free-ranging populations. We found several lines of evidence in
589 support of inbreeding in fur seals. First, g_2 was significantly positive indicating identity
590 disequilibrium within the sample of individuals. Second, the variance of the genomic
591 inbreeding coefficients \hat{F}_I , \hat{F}_{II} and \hat{F}_{III} were found to lie within the 95% confidence intervals of
592 g_2 and therefore we can expect our estimates to reflect the realized level of inbreeding in the
593 population. Third, the genomic inbreeding coefficients were strongly inter-correlated,
594 suggesting that our markers are uncovering consistent information about variation in genome-
595 wide homozygosity caused by inbreeding.

596 Our results are surprising given that Antarctic fur seals number in the millions and are free-
597 ranging and highly vagile. However, the species is also highly polygynous, with a handful of
598 top males fathering the majority of offspring (Hoffman *et al.* 2004) and females exhibiting
599 strong natal site fidelity (Hoffman and Forcada 2012) which could potentially lead to matings
600 between close relatives. As demographic effects can also generate variance in inbreeding
601 *sensu lato*, we also cannot discount the possibility that the historical bottleneck contributed
602 towards the variation we see today. To test this, we would need to quantify the length
603 distribution of runs of homozygosity, which would require denser SNP data.

604 Our work builds upon another recent study that used RAD sequencing to quantify inbreeding
605 in wild harbour seals (Hoffman *et al.* 2014) where a higher estimate of g_2 was found, indicative
606 of a greater variance in inbreeding within the sample. However, the study focused on stranded
607 seals, many of which died of lungworm infection and may therefore have been enriched for
608 unusually inbred individuals. In the current study, pups were sampled at random from within
609 a single breeding colony, together with their parents. Consequently, our sample should be
610 more representative of the underlying distribution of inbreeding within the population. In line
611 with this, our estimate of g_2 is more similar to those obtained in wild populations of other
612 polygynous mammals such as Soay sheep and red deer (Huisman *et al.* 2016; Berenos *et al.*
613 2016).

614 Our results are consistent with previous studies documenting HFCs for numerous traits in the
615 South Georgia population (Hoffman *et al.* 2004; 2010; Forcada and Hoffman 2014) and
616 suggest that these may well reflect inbreeding depression. More generally, literally hundreds
617 of studies have documented HFCs across the animal kingdom (Coltman and Slate 2003) and
618 it has been strongly argued that these HFCs are highly unlikely to occur when there is no
619 variance in inbreeding (Szulkin *et al.* 2010). The fact that we found variation in inbreeding in
620 a large, free-ranging population is consistent with this notion and therefore contributes towards
621 a growing body of evidence suggesting collectively that inbreeding could be more common in
622 wild populations than previously thought.

623 **Conclusion**

624 We have generated an improved genome assembly for an important Antarctic marine top
625 predator and used RAD sequencing to provide diverse insights from the level of the species
626 through the population to the individual. Focusing on the larger South Georgia population, we
627 characterised rapid LD decay and uncovered significant variation in individual inbreeding,
628 while population-level analyses resolved clear differences among island groups that
629 emphasise the importance of relict populations to species recovery. RAD sequencing and
630 related approaches might conceivably be applied to other wild species to characterise patterns
631 of LD decay, elucidate fine scale population structure and uncover the broader prevalence of
632 inbreeding and its importance to wild populations.

633

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647

AUTHOR CONTRIBUTIONS

648 EH and JIH conceived and designed the study. IG and JIH carried out the DNA extractions
649 and microsatellite genotyping. KKD carried out the RAD library preparation. A-CP performed
650 the SNP validation. JF, SG, MG, KKD, JK, JIH and JW contributed materials and funding.
651 AMB assembled the new version of the genome with input from JW. EH carried out the SNP
652 calling and analysed the data. EH and JIH wrote the first version of the manuscript. All of the
653 authors commented on and approved the final manuscript.

654

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