Target journals:

**Nature Communications (10.7)**

http://www.nature.com/ncomms/index.html

http://www.nature.com/ncomms/authors/index.html

up to 12 pages long with <10 display items

main text (not including abstract, methods, references and figure legends) <5000 words

title <15 words

abstract <150 words

**PloS Biology (11.7) / PloS Genetics (8.2)**

http://journals.plos.org/plosbiology/

http://www.plosbiology.org/static/guidelines

encourage **presubmission enquiry**

title <150 characters

referenced (<10) abstract <300 words with background/methodology/principal findings/conclusions/significance but actual abstract not referenced and 1 para

150-200 word author summary + 20-30 word 'blurb'

overall length ?

**Current Biology (10.2)**

http://www.cell.com/current-biology/aims

http://www.cell.com/current-biology/authors

encourage **presubmission enquiry**

Articles: 10 pages, <5000 words main text, <7 display items

Reports: 6 pages, 2500 words main text, <4 display items, results & discussion combined

Title < 150 characters, abstract <250 words

**PNAS**

Molecular Ecology (6.2)

Title:

**xxx**

Short title:

xxx

K. Acevedo-Whitehouse 2

3

Andersen, L. 4

5

6

6

7

8

9

10

J. I. Hoffman 1

1 Department of Animal Behaviour, University of Bielefeld, Postfach 100131, 33501 Bielefeld, Germany

2 Unit for Basic and Applied Microbiology, School of Natural Sciences, Autonomous University of Queretaro, Avenida de las Ciencias S/N, Queretaro 76230, México

3 Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom

4

‡

Elephant seal research group, Via Buonarroti 35, 20145 Milan Milan, Italy

Universidad Autónoma de Querétaro, Campus Aeropuerto, Anillo Vial Fray Junípero Serra Querétaro, Qro. C.P. 76140, Mexico

Keywords: Demographic history, bottleneck, Approximate Bayesian Computation, pinniped, conservation genetics.

Corresponding author:

Joseph I. Hoffman

Department of Animal Behaviour

University of Bielefeld

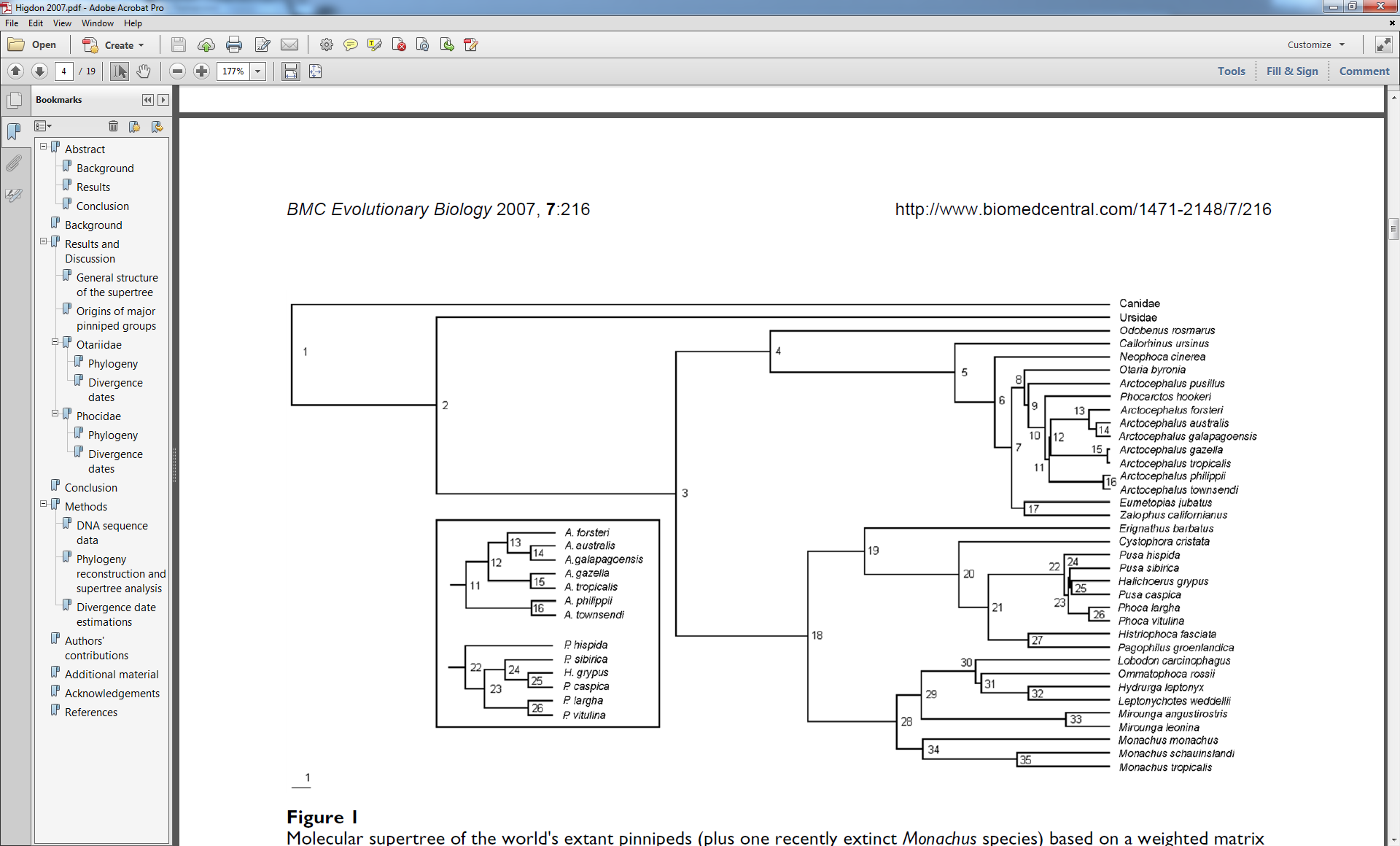
Postfach 100131

33501 Bielefeld

Germany

E-mail: joseph.hoffman@uni-bielefeld.de

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Reference | Number of individuals | Number of loci | Number of loci analysed |
| Antarctic fur seal, *Arctocephalus gazella* | [[1](#_ENREF_1" \o "Hoffman, 2011 #3179)] | 246 | 21 | 21 |
| Galapagos fur seal, *Arctocephalus galapagoensis* | [[2](#_ENREF_2" \o "Lopes, in review #3835)] | 90 | 12 | 12 |
| Steller's sea lion, *Eumetopias jubatus* | [[3](#_ENREF_3" \o "Hoffman, 2006 #2558)] | 668 | 13 | 13 |
| Eastern Atlantic grey seal, *Halichoerus grypus* | [[4](#_ENREF_4" \o "Klimova, 2014 #3836)] | 1254 | 9 | 7 |
| Eastern Atlantic harbour seal, *Phoca vitulina* | [[5](#_ENREF_5" \o "Rijks, 2008 #2945)] | 204 | 27 | 27 |
| Galapagos sea lion, *Zalophus wollebaeki* | Wolf, J.B and Trillmich, F. (unpublished data) | 781 | 22 | 22 |
| South American fur seal, *Arctocephalus australis* | [[6](#_ENREF_6" \o "Rosa de Oliveira, 2008 #3214)] | 226 | 7 | 5 |
| Hooded Seal, *Cystophora cristata* | [[7](#_ENREF_7" \o "Coltman, 2007 #2894)] | 300 | 13 | 13 |
| Mediterranean monk seal, *Monachus monachus* | [[8](#_ENREF_8" \o "Pastor, 2004 #2191)] | 109 | 16 | 14 |
| Hawaiian monk seal, *Monachus schauinslandi* | [[9](#_ENREF_9" \o "Schultz, 2011 #3837)] and Schultz, J. (unpublished data) | 2386 | 18 | 17 |
| Bearded seal, *Erignathus barbatus* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 119 | 13 | 10 |
| Crabeater seal, *Lobodon carcinophagus* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 303 | 9 | 9 |
| Leopard seal, *Hydrurga leptonyx* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 150 | 14 | 12 |
| Arctic ringed seal, *Phoca hispida* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 303 | 10 | 10 |
| Ross Seal, *Ommatophoca rossi* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 90 | 9 | 9 |
| Weddell seal, *Leptonychotes weddelli* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 893 | 15 | 12 |
| Northern fur seal, *Callorhinus ursinus* | [[11](#_ENREF_11" \o "Dickerson, 2010 #3089)] | 492 | 8 | 7 |
| Atlantic walrus, *Odobenus rosmarus rosmarus* | Schafer, A. (unpublished data) | 623 | 10 | 10 |
| Atlantic walrus, *Odobenus rosmarus rosmarus* | Andersen dataset | 555 | 15 | 15 |
| Northern elephant seal, *Mirounga angustrostrus* | Sanvito, S. and Galimberti, F (unpublished data) | 260 | 35 | 32 |
| Southern elephant seal, *Mirounga leonina* | Sanvito, S. and Galimberti, F (unpublished data) | 260 | 13 | 13 |
| California sea lion, *Zalophus californianus* | Acevedo-Whitehouse, K. (unpublished data) | 347 | 13 | 11 |
| South American sea lion, *Otaria flavescens* | Hoffman J.I. (unpublished data) |  |  |  |



**Abstract**

Emerging Bayesian analytical approaches offer increasingly sophisticated means of reconstructing historical population dynamics from genetic data, but have been little applied to scenarios involving demographic bottlenecks. Consequently, we analysed a large mitochondrial and microsatellite dataset from the Antarctic fur seal *Arctocephalus gazella*, a species subjected to one of the most extreme examples of uncontrolled exploitation in history when it was reduced to the brink of extinction by the sealing industry during the late eighteenth and nineteenth centuries. Classical bottleneck tests, which exploit the fact that rare alleles are rapidly lost during demographic reduction, failed to provide convincing evidence for a bottleneck. In contrast, a strong signal of recent demographic decline was detected using both Bayesian skyline plots and approximate Bayesian computing, the latter also allowing derivation of posterior parameter estimates that were remarkably consistent with historical observations. This was achieved using only contemporary samples, further emphasizing the potential of these approaches to address important outstanding problems in conservation and evolutionary biology.

**Author summary**

Severe reductions in population size, termed bottlenecks, can deplete genetic variation and increase extinction risk. This has motivated the development of several genetic approaches for detecting bottlenecks within natural populations. Here, we applied state of the art Bayesian techniques to a well-documented case study, the Antarctic fur seal, which was hunted to the brink of extinction at South Georgia in the late eighteenth and nineteenth centuries. Using only contemporary DNA samples, we found strong evidence for a recent and dramatic reduction in population size, the extent and timing of which was in good agreement with the historical accounts of sealing captains. Our study suggests that Bayesian approaches could prove a powerful tool for conservation biologists interested in reconstructing the recent demographic history of threatened natural populations.

**Introduction**

Many natural populations have experienced severe demographic reductions, or population bottlenecks, due to over-exploitation or anthropogenically induced habitat destruction. This is a major cause of concern to conservation biologists because bottlenecks can lead to the loss of genetic variability, elevated levels of inbreeding and the fixation of mildly deleterious alleles, thereby increasing the risk of extinction and compromising adaptive evolutionary potential [[12](#_ENREF_12),[13](#_ENREF_13),[14](#_ENREF_14),[15](#_ENREF_15),[16](#_ENREF_16)]. Unfortunately however, detecting and measuring the impacts of such changes is not usually possible because patterns of historical abundance are seldom known. Consequently, there has been considerable interest in the development and application of methods for detecting bottleneck signatures using neutral genetic markers such as microsatellites.

One such approach exploits the fact that genetic drift is intensified in small populations, leading to concomitant changes in allele frequencies and in some cases the fixation or loss of particular alleles. This in principle allows variation in the effective population size to be measured when multiple, temporally spaced samples are available [[17](#_ENREF_17),[18](#_ENREF_18)]. However, this approach may underestimate the magnitude of severe bottlenecks because the loss of alleles constrains the extent to which allele frequencies are subsequently able to drift [[19](#_ENREF_19)]. Perhaps more importantly, most investigators are also unable to collect appropriately spaced temporal samples, particularly given that bottleneck testing is usually conducted *post hoc*. Approaches that attempt to elucidate demographic history from a single temporal genetic sample have therefore grown in popularity.

Three classical single-sample methods for detecting population bottlenecks are the heterozygosity excess [[20](#_ENREF_20)], mode-shift [[21](#_ENREF_21)] and *M*-ratio [[22](#_ENREF_22)] tests. The first and arguably most widely used of these is based on the premise that rare alleles are rapidly lost during a bottleneck but their loss only weakly influences heterozygosity. This generates a transient excess of heterozygosity (lasting up to 4 x *Ne* generations, where *Ne* is the bottleneck effective population size) relative to a population at equilibrium with an equivalent number of alleles. The second test [[21](#_ENREF_21)] measures the impact of the loss of rare alleles on the overall allele frequency distribution. The underlying rationale is that large, non-bottlenecked populations should have a high proportion of alleles at low frequency (<0.1), whereas alleles of intermediate frequency (e.g. 0.1-0.2) are expected to become more abundant after a severe bottleneck. Third, the *M*-ratio of Garza and Williamson [[22](#_ENREF_22)], defined as the ratio of the total number of alleles (*k*) to the allelic size range (*r*), may also be informative in respect of bottleneck history. This statistic exploits the fact that the loss of any allele during a bottleneck will reduce *k*, whereas only the loss of alleles at the extremes of the size range will reduce *r*. Consequently, *k* will tend to reduce more quickly than *r* in declining populations, leading to the expectation than *M* will be smaller in recently bottlenecked populations than in those at equilibrium.

Although the mode-shift test has not been extensively evaluated, heterozygosity excess and the *M*-ratio have both been shown to perform well at distinguishing bottlenecked from non-bottlenecked samples when applied to empirical datasets from species or populations with contrasting demographic histories [[20](#_ENREF_20),[22](#_ENREF_22),[23](#_ENREF_23),[24](#_ENREF_24)]. Recent simulations also suggest that these two measures may convey subtly different signals, the *M*-ratio for example being most likely to correctly identify a bottleneck when pre-bottleneck population size was large, the bottleneck lasted several generations or the population subsequently made a demographic recovery [[25](#_ENREF_25)]. However, a major drawback of both approaches is that they require simplifying assumptions to be made about the mutational mechanism of the genetic markers employed. These assumptions if incorrect have the potential to strongly influence equilibrium values of both heterozygosity conditional upon allele number and *M* [[25](#_ENREF_25),[26](#_ENREF_26),[27](#_ENREF_27)].

Fortunately, emerging analytical approaches drawing upon Bayesian methodologies provide a novel avenue for exploring demographic history independently of the bottleneck tests described above. For example, by implementing a flexible demographic model, Bayesian Skyline Plots (BSPs) allow changes in effective population size (*Ne*) over time to be described without the need for assumptions to be made about key demographic parameters [[28](#_ENREF_28)]. Potentially even more powerful is Approximate Bayesian Computation (ABC), an approach that allows for selection of the optimal demographic / evolutionary history and associated parameters among a set of hypothesized models [[29](#_ENREF_29)]. This is achieved by generating alternative simulated datasets based on assumptions about evolutionary and demographic parameters which are then compared to the observed data using summary statistics.

Surprisingly few studies have used ABC to infer the bottleneck histories of natural populations, and these have mostly employed a combination of ancient and contemporary samples to explore demographic events that occurred over time scales of thousands of years ago [[30](#_ENREF_30),[31](#_ENREF_31)]. However, most investigators do not have access to ancient DNA samples, while many are also more interested in relatively recent anthropogenically induced bottlenecks. At the same time, the use of ancient samples has largely restricted studies to using mitochondrial DNA (mtDNA) sequences, whereas the inclusion of multiple unlinked nuclear markers should substantially improve power [[30](#_ENREF_30)]. Finally, previous studies have lacked detailed historical observations with which to parameterize bottleneck scenarios for evaluation within the ABC framework.

The Antarctic fur seal (*Arctocephalus gazella*) provides an excellent opportunity to explore the relative abilities of established and emerging analytical approaches to detect a recent historical bottleneck. This species occurs predominantly to the south of the Antarctic Convergence, with 97% of the extant population breeding on the island of South Georgia (Figure 1)[[32](#_ENREF_32)]. Like most other members of the Arctocephalus genus, Antarctic fur seals were subject to uncontrolled exploitation for their fur and oil during the late eighteenth and nineteenth centuries. Uniquely however, the journals and logbooks of early explorers and sealing captains have been retained and scrutinized (see Supplementary Table 1) allowing reconstruction of the timing and extent of the demographic reduction (Figure 2). Detailed census data are similarly available with which to track the post-exploitation recovery of the population (see Supplementary Table 2). Together, these historical records allow the *a priori* parameterization of a plausible demographic model that can be tested using ABC.

The exploitation of Antarctic fur seals began shortly after the discovery of South Georgia by Captain James Cook in 1775. The most profitable strategy was to take as many seals as it was possible to kill in one season, as Mill [[33](#_ENREF_33)] comments: “Reckless extermination was the only method of seal-hunting resorted to on the islands of South Georgia and the coasts of South America so that the first in the field at a new sealing ground was sure of an immense booty, and late-comers as likely as not would go empty away”. Sealing at South Georgia reached its peak during the 1800-01 season (Figure 2) with thirty-one ships recorded as having been operating there, seventeen of which were responsible for a total catch of 112,000 seals [[34](#_ENREF_34)]. Such uncontrolled harvesting would have greatly depleted the population, probably explaining the brief abatement of sealing activity that followed until a resurgence in 1814. By 1822, Weddell [[35](#_ENREF_35)] estimated that up to 1.2 million seals had been taken at South Georgia, and the almost exterminated population was no longer able to sustain the industry.

After the last commercial catch of 170 fur seals in 1908, very few individuals were sighted until the 1950s. In 1915 a single juvenile male was found and immediately killed [[36](#_ENREF_36)] and in 1911 Larsen [[37](#_ENREF_37)] also reported sighting a group of 30 individuals. A dedicated fur seal survey found 38 animals at Bird Island in 1933 and deduced a total population of 60 [[36](#_ENREF_36)]. The Discovery expedition of 1936 subsequently reported 59 seals, including 12 pups, at the same location [[38](#_ENREF_38)], but the population is thought to have remained at around this level until the 1950s. Rapid population growth ensued in the 1960s and 1970s, and by 1990 fur seal numbers were estimated to have reached 1.5 million [[39](#_ENREF_39)]. The most recent estimate of 3 million was made at the XXIII Antarctic Treaty Consultative Meeting in 1999 (Specially Protected Species in Antarctica. XXIII ATCM / WP24. Agenda Item 7c; Meeting 1999).

Here, we genotyped 246 Antarctic fur seals from South Georgia at a 263-bp region of the hypervariable region 1 (HVR1) of the mitochondrial control region and 21 unlinked highly polymorphic microsatellites. Our aims were to explore the relative abilities of classical bottleneck tests and Bayesian approaches to recover a signal of historical exploitation and to estimate via ABC the distributions of key bottleneck parameters including timing and minimum population size. Our approach differs from previous studies using ABC in two main respects. First, our dataset comprises both mtDNA and microsatellite data, yielding greater genetic resolution as well as bi-parental perspectives. Second, population reduction resulting from harvesting and subsequent recovery are well documented in this species, providing a strong *a priori* demographic model.

**Results**

To test for a genetic signature of a historical population bottleneck, we genotyped 246 Antarctic fur seals from eight rookeries across South Georgia (Table 1) at 21 microsatellite loci and sequenced a 263-bp segment of the hypervariable region 1 (HVR1) of the mitochondrial control region. The microsatellite loci were highly informative, possessing on average 11.3 alleles and with a mean observed heterozygosity of 0.81 (Table 2). Following sequential Bonferroni correction to compensate for multiple statistical tests, none of the loci were found to deviate significantly from Hardy–Weinberg equilibrium and no pairs of loci exhibited significant linkage disequilibrium. A total of 26 mitochondrial haplotypes were found, eleven of which (Genbank accession numbers JF304904-JF304914‏) had not previously been described by Wynen *et al*. [[40](#_ENREF_40)].

Classical bottleneck tests

We first interrogated our microsatellite dataset for evidence of a genetic bottleneck using the heterozygosity excess approach of Luikart et al. [[21](#_ENREF_21)]. The results were highly dependent on the mutational model upon which the predicted relationship between heterozygosity and the number of alleles was based (Table 3). Thus, a significant excess of heterozygosity relative to expectations was detected under the IAM but not the SMM and *P*-values obtained for TPM models scaled positively with the proportion of multi-step mutations specified. This pattern probably reflects the greater power of the IAM to detect a bottleneck [[20](#_ENREF_20)], despite this model being unrealistic for most 'real' microsatellites [[41](#_ENREF_41)]. A mode shift in the allele frequency distribution was not detected.

Recent simulations [[25](#_ENREF_25)] suggest that when pre-bottleneck population size was large or the population made a demographic recovery, the ratio of the number of alleles to allelic size range may be more informative about bottleneck history than heterozygosity excess. Consequently, we also calculated the *M*-ratio of Garza and Williamson [[22](#_ENREF_22)]. The resulting value of 0.798 lies above the 0.7 threshold proposed by Garza and Williamson [[22](#_ENREF_22)], implying a lack of support for a bottleneck. Comparing this value against a null distribution derived from 10,000 theoretical populations in mutation-drift equilibrium, a bottleneck signature was only inferred below *θ* = 1.63, a value that corresponds to an unrealistically low pre-bottleneck *Ne* of 812 assuming a default microsatellite mutation rate of 5x10-4 [[42](#_ENREF_42)].

Approximate Bayesian Computing (ABC) analysis

Evaluation of the two proposed historic models indicated that the one incorporating a population bottleneck based on historical observations most accurately described the genetic data. This model received a posterior probability of between 0.99 and 1, while the model describing a constant population size through time received a posterior probability of between 0 and 0.01. In addition, Type I and Type II error rates for the selection of the bottleneck model were 0.18 and 0.12 respectively. The posterior distribution of selected parameters using values drawn from the 10,000 datasets closest to the observed are shown in Figure 5 and Table 4. These depict a genetic bottleneck that ended approximately 11 generations ago (mean = 12, median = 11, mode = 6 generations ago, 95% CI = 2–27) with an *Ne* at this time of approximately 139 (mean = 164, median = 139, mode = 122, 95% CI = 46–371). No clear posterior estimate of the time parameter associated with *Ne-historical* was recovered (data not shown). The statistical descriptors for contemporary *Ne* were much larger (mean = 744,000, mean = 742,000, mode = 178,000, 95% CI = 66,900–1,420,000) but exhibited a flat posterior distribution. Estimates of historical *Ne* were also comparatively large (mean = 777,000, median = 763,000, mode = 396,000, 95% CI = 155,000–1,430,000), but the lower bound of the CI for historical *Ne* was over twice as large as that estimated for contemporary *Ne*. Moreover, the posterior distribution of historical *Ne* dipped towards lower *Ne* values indicating resolution for estimating the posterior lower bound of this parameter.

With Bayesian analytical approaches, choices of prior distributions can have a large impact on posterior parameter estimates [[30](#_ENREF_30)]. Consequently, we explored the sensitivity of our analysis to a variety of different prior assumptions (see Materials and methods and Table 4 for details). Simulations involving prior parameter adjustments on sex ratio and mitochondrial mutation rate from the initial bottleneck model yielded largely unaltered estimates (Figure 5, Table 4). The main deviation observed was in the estimation of bottleneck and historic *Ne* values from simulations invoking a 1:5 sex ratio. These analyses described a larger bottleneck *Ne* (about double the size) and a historical *Ne* peaking at around a quarter of a million.

For our initial simulations, we chose prior distributions tightly bounded around values based on the available historical data. However, to explore sensitivity to these assumptions we replicated all of the above simulations with wider priors on *Ne* and time (Figure 6, Table 4). The bottleneck model was again highly supported over the constant population size model (posterior probabilities were 1 and 0 respectively), with type I and II error rate estimates being lower than those obtained from the initial simulations (0.08 and 0 respectively). Parameter estimates were almost identical to those obtained in our initial simulations with the exception of bottleneck *Ne*, which was consistently larger at around 700 and 1400 for analyses based on a sex ratio of 1 and 1:5 respectively.

Finally, we tested for any potential differences in the strength of the bottleneck signal contained within the mtDNA and microsatellite datasets by conducting additional simulations separately for each class of marker. For microsatellites, the bottleneck model was supported over the constant population size model regardless of *Ne* and time priors (Figures 5 and 6, Table 4). Posterior support values were also closely comparable to those reported above (e.g. bottleneck model = 0.94–0.96 versus constant population size model = 0.04–0.05 for simulations using the initial priors on *Ne* and time). In contrast, the mtDNA dataset was marginally better supported by the constant population size model than the bottleneck model (bottleneck model = 0.24–0.27; constant population size model = 0.72–0.75).

**Discussion**

xxx

Conclusions

Although detailed historical records document a reduction in population size in the Antarctic fur seal down to 30 individuals or fewer, it seems likely that larger numbers may have escaped sealing, either by breeding at remote or inaccessible locations such as the Willis Islands [[40](#_ENREF_40)] or perhaps by remaining out at sea. Our results suggest that a dramatic reduction in population size did indeed take place, but that this may not have been substantial or long-lasting enough to have appreciably reduced levels of genetic diversity. Similar insights from other bottlenecked species offer to substantially improve our understanding of how historical demographic reductions influence contemporary genetic diversity, with important implications for the conservation and management of threatened natural populations.

**Materials and methods**

##### Tissue sample collection

xx

Tests for population substructure

To check for the presence of cryptic population structure within South Georgia, we calculated pairwise *F*st values among all of the sampling locations for both classes of marker. *F*st was calculated for microsatellites using Fstat version 2.9.3 [[47](#_ENREF_47)] and for mitochondrial DNA using Arlequin version 2.0 [[48](#_ENREF_48)]. Only three of the 72 resulting values were individually significant at *P* < 0.05, none of which remained so following sequential Bonferroni correction for multiple tests. Moreover, the results of subsequent data analyses were qualitatively very similar regardless of whether the full dataset was used or analyses were restricted to the study colony at Bird Island (data not shown).

Microsatellite-based bottleneck tests

To test for evidence of a genetic bottleneck, we first used the heterozygosity excess method of Luikart et al. [[21](#_ENREF_21)] implemented within the program Bottleneck v 1.2.02 [[49](#_ENREF_49)]. One potential drawback of this approach is that, although microsatellites evolve mainly by gaining or losing a single repeat unit (the Stepwise Mutation Model, SMM [[50](#_ENREF_50)]), occasional larger ‘jump’ mutations of several repeat units are also common [[41](#_ENREF_41),[51](#_ENREF_51)]. Consequently, Bottleneck allows the user to specify a range of mutation models, from the strict SMM through two-phase models (TPMs) with varying proportions of multi-step mutations to the infinite alleles model (IAM) where every new mutation is novel. For our analysis, four TPM models were evaluated with 1%, 5%, 10% and 30% multi-step mutations respectively and a default variance of 30. For each of the mutational models, the heterozygosity of each locus expected under mutation-drift equilibrium given the observed number of alleles (Hexp) was determined using 10, 000 simulations and then compared against observed heterozygosity (Ho). We then recorded the number of loci for which Ho was greater than Hexp and determined whether the overall set of deviations was statistically significant using sign, standardized differences and Wilcoxon signed ranks tests. Bottlenecked populations are also expected to exhibit a characteristic ‘mode shift’ in the frequency distribution of alleles away from the L-shaped distribution expected under mutation-drift equilibrium [[21](#_ENREF_21)]. Consequently, Bottleneck was also used to generate a qualitative descriptor of whether the observed allele frequencies at each locus deviate from such a distribution.

As an alternative test for a population bottleneck, we also calculated Garza and Williamson's *M*-ratio for our dataset using the program M\_P\_Val [[22](#_ENREF_22)]. The significance of the resulting value was determined by comparison against a distribution of *M* values calculated from 10,000 theoretical populations in mutation-drift equilibrium. Using conventional criteria, a significant reduction in population size is inferred if fewer than 5% of the replicates fall below the observed value of *M*. The program allows the user to modify three parameters that approximate the mutation process in natural populations: the proportion of mutations that are larger than a single step (*pg*), the average size of non-single-step mutations (Δ*g*) and *θ* = 4 *Neµ* (where *Ne* is the effective pre-bottleneck population size at equilibrium and *µ* is the mutation rate). We used the default settings of *pg* = 0.1 and Δ*g* = 3.5, and varied *θ* between 1 and 1000, the latter corresponding to an effective pre-bottleneck population size of 500,000 assuming a commonly used estimate of the dinucleotide microsatellite mutation rate of 5x10-4 mutants per gamete per generation [[42](#_ENREF_42)] as suggested by Garza and Williamson [[22](#_ENREF_22)].

Approximate Bayesian Computing (ABC) analysis

Statistical support for alternative historical scenarios that either included or excluded a bottleneck was tested within an ABC framework. This allowed us not only to determine whether or not a bottleneck is likely to have occurred, but also to estimate values of key parameters of interest. We initially simulated two primary historical models. The first incorporated a recent population bottleneck, with prior distributions broadly surrounding values derived from historical records (Figure 2). The model described an ancestral *Ne* uniformly distributed between 1 and 1.5 x 106, the upper bound encompassing Weddell's [[35](#_ENREF_35)] estimate of 1.2 million seals having been taken during the initial bout of harvesting. The occurrence of the bottleneck was described by two uniform distributions representing the time parameter associated with *Ne-historical* (15-100 generations ago) and the time associated with the end of the bottleneck (i.e. with the lowest value of *Ne-bottleneck*, 1-30 generations ago). The bottleneck model was constrained such that changes in effective population size occur sumultaneously. The prior on *Ne-bottleneck* was bounded between 1 and 1000, values that generously surround the 1911 census population of 30 individuals [[37](#_ENREF_37)]. *Ne-contemporary* was uniformly distributed between 1 and 1.5 x 106. For comparison, a null model of no population bottleneck was also defined, in which *Ne* was uniformly distributed through time (1-100 generations) and bounded between 1 and 1.5 x 106 individuals. For both models, the HVR1 mutation rate was defined by a uniform distribution with lower and upper bounds of 5.74 × 10-7 [[11](#_ENREF_11)] and 2.71 × 10-6 [[65](#_ENREF_65)] substitutions per site per generation respectively. The HKY + I + G (I = 0.57, G = 0.50) mutation model defined for HVR1 was determined using the hierarchical likelihood ratio test and Akaike information criterion, as implemented in ModelTest v 3.7 [[67](#_ENREF_67)]. For microsatellites, the Generalized Stepwise Mutation model [[68](#_ENREF_68)] was implemented with a mean rate uniformly distributed between 1.00 × 10-4 and 1.00 × 10-3 substitutions/generation. These simulations were performed assuming a 1:1 sex ratio.

To explore the influence of prior assumptions on posterior conclusions using ABC we next initiated a series of simulations incorporating specific prior parameter adjustments. Specifically, we examined the influence of assumptions on the prior distributions of the mitochondrial mutation rate and sex ratio on the posterior distributions of parameters of interest. This was important because the assumed value of *µmitochondrial* describes the source of genetic diversity for simulated populations while sex ratio influences the diversity of bi-parentally inherited markers. The effect of HRV1 mutation rate prior was explored by evoking a liberal prior bounded by 5.74 × 10-7 and 3.65 × 10-5. The effect of assuming a 1:1 sex ratio was assessed through additional simulations based on a sex ratio of 1 male to each 5 females. This sex ratio was drawn from field observations and it is unclear how accurately this value reflects the true genomic contributions of the two sexes.

In addition to the above simulations, all models were simulated a second time, but with broadened uniform priors on all parameters relating to *Ne* and time. Specifically, contemporary and historic *Ne* were distributed between 1 and 6 x 106, bottleneck *Ne* was distributed between 1-2000, timing of the start of the bottleneck was distributed between 1-500, and timing of the end of the bottleneck distributed between 1-50. Prior distributions of parameters for all analyses are described in Table 4. Finally, to assess whether the strength of the bottleneck signal differs between the mtDNA and microsatellite datasets, additional simulations were performed on each data type independently. These were carried out twice, once with the initially defined priors on *Ne* and time, and also following the broadened priors on these parameters.

For each model, one million genetic datasets were simulated with the defined demographic and marker parameters. Four summary statistics were then generated for the observed and simulated datasets: mean pairwise difference and Tajima’s *D* [[60](#_ENREF_60)] for HVR1, and mean heterozygosity and the mean number of alleles for microsatellites. Normalized Euclidean distances were calculated between the observed dataset and each of the simulated datasets using the local linear regression method of Beaumont et al. [[29](#_ENREF_29)]. The ten thousand datasets with the smallest Euclidean distances were then retained to build posterior parameter distributions, which were smooth weighted using the Locfit function within R version 2.9.1 [[69](#_ENREF_69)]. The posterior probabilities of each scenario were estimated using a logistic regression approach, providing both point estimates and 95% confidence intervals [[70](#_ENREF_70),[71](#_ENREF_71)]. Statistical measures of performance and Type I and Type II error rates were also calculated as a means of model checking [[72](#_ENREF_72)]. All of the above analyses were implemented within the DiyAbc v1 software package [[70](#_ENREF_70),[73](#_ENREF_73)].

Ethics statement

Tissue samples were collected and retained under permits issued by the Department for Environment, Food and Rural Affairs (License number AHZ/2024A/2005/1) and in accordance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora. All field procedures were approved by the British Antarctic Survey (reference PEA 6).

**Acknowledgements**

We would like to thank xxx.

**Author contributions**

Conceived and designed the study: JH. Collated and analyzed the data: JH CP JF. Wrote the paper: JH CP

**Funding**

This work was funded by xxx. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**References**

1. Hoffman JI, Grant SM, Forcada J, Phillips CD (2011) Bayesian inference of a historical genetic bottleneck in a heavily exploited marine mammal. Molecular Ecology 20: 3989–4008.

2. Lopes FRV, Hoffman JI, Valati VH, Bonatto S, Wolf JBW, et al. (in review) Fine-scale matrilineal population structure in the Galápagos fur seal and its implications for conservation management. Conservation Genetics.

3. Hoffman JI, Matson C, Amos W, Loughlin TR, Bickham JW (2006) Deep genetic subdivision within a continuously distributed and highly vagile marine mammal, the Steller's sea lion *Eumetopias jubatus*. Molecular Ecology 15: 2821–2832.

4. Klimova A, Fietz K, Olsen MT, Harwood J, Amos W, et al. (2014) Global population structure and demographic history of the grey seal. Molecular Ecology 23: 3999–4017.

5. Rijks JM, Hoffman JI, Kuiken T, Osterhaus ADME, Amos W (2008) Heteroygosity and lungworm burden in harbour seals (*Phoca vitulina*). Heredity 100: 587–593.

6. Rosa de Oliveira L, Hoffman JI, Hingst-Zaher E, Majluf P, Muelbert MMC, et al. (2008) Morphological and genetic evidence for two Evolutionary Significant Units (ESUs) in the South American fur seal *Arctocephalus australis*. Conservation Genetics 9: 1451–1466.

7. Coltman DW, Stenson G, Hammill MO, Haug T, Davis CS, et al. (2007) Panmictic population structure in the hooded seal (*Cystophora cristata*). Molecular Ecology 16: 1639-1648.

8. Pastor T, Garza JC, Allen P, Amos W, Aguilar A (2004) Low genetic variability in the highly endangered Mediterranean monk seal. Journal of Heredity 95: 291-300.

9. Schultz JK, Baker JD, Toonen RJ, Harting AL, Bowen BW (2011) Range-Wide Genetic Connectivity of the Hawaiian Monk Seal and Implications for Translocation. Conservation Biology 25: 124-132.

10. Davis CS, Stirling I, Strobeck C, Coltman DW (2008) Population structure of ice-breeding seals. Molecular Ecology.

11. Dickerson BR, Ream RR, Vignieri SN, Bentzen P (2010) Population structure as revealed by mtDNA and microsatellites in Northern fur seals, *Callorhinus ursinus*, throughout their range. PLoS One 5: e10671.

12. Frankham R, Lees K, Montgomery M, England PR, Lowe EH, et al. (1999) Do population size bottlenecks reduce evolutionary potential? Animal Conservation 2: 255-260.

13. Lande R (1994) Risk of population extinction from fixation of new deleterious mutations. Evolution 48: 1460-1469.

14. Lynch M, Conery J, Burger R (1995) Mutation accumulation and the extinction of small populations. American Naturalist 146: 489-518.

15. Mills LS, Smouse PE (1994) Demographic consequences of inbreeding in remnant populations. American Naturalist 144: 412-431.

16. Hedrick PW, Miller PS (1992) Conservation genetics: techniques and fundamentals. Ecological Applications 2: 30-46.

17. Waples RS (1989) A generalised approach for estimating effective population size from temporal changes in allele frequency. Genetics 121: 379-391.

18. Luikart G, Cornuet JM, Allendorf FW (1999) Temporal changes in allele frequencies provide estimates of population bottleneck size. Conservation Biology 13: 523-530.

19. Richards C, Leberg PL (1996) Temporal changes in allele frequencies and a population's history of severe bottlenecks. Conservation Biology 10: 832–839.

20. Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001-2014.

21. Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89: 238-247.

22. Garza JC, Williamson EG (2001) Detection of reduced population size using data from microsatellite loci. Molecular Ecology 10: 305-318.

23. Beebee T, Rowe G (2001) Application of genetic bottleneck testing to the investigation of Amphibian declines: a case study with Natterjack Toads. Conservation Biology 15: 266-270.

24. Spencer CC, Neigel JE, Leberg PL (2000) Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. Molecular Ecology 9: 1517-1528.

25. Williamson-Natesan EG (2005) Comparison of methods for detecting bottlenecks from mirosatellite loci. Conservation Genetics 6: 551-562.

26. Guinand B, Scribner KT (2003) Evaluation of methodology for detection of genetic bottlenecks: inferences from temporally replicated lake trout populations. Comptus Rendus Biologies 326: S61-S67.

27. Busch JD, Waser PM, DeWoody JA (2007) Recent demographic bottlenecks are not accompanied by a genetic signature in banner-tailed kangaroo rats (*Dipodomys spectabilis*). Molecular Ecology 16: 2450-2462.

28. Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular Biology and Evolution 22: 1185-1192.

29. Beaumont MA, Zhang W, Balding DJ (2002) Approximate Bayesian Computation in Population Genetics. Genetics 162: 2025–2035.

30. Chan YL, Anderson CNK, Hadley EA (2006) Bayesian estimation of the timing and severity of a population bottleneck from ancient DNA. PLoS Genetics 2: e59.

31. Thornton K, Andolfatto P (2006) Approximate Bayesian Inference reveals evidence for a recent, severe bottleneck in a Netherlands population of *Drosophila melanogaster*. Genetics 172: 1607-1619.

32. Boyd IL, Roberts JP (1993) Tooth growth in male Antarctic fur seals (*Arctocephalus gazella*) from South Georgia - an indicator of long-term growth history. Journal of Zoology 229: 177-190.

33. Mill HR (1905) The seige of the South Pole: the story of Antarctic exploration. London: Alston Rivers.

34. Fanning E (1833) Voyages and Discoveries in the South Seas 1792-1832. Salem, Massachusetts: Marine Research Society.

35. Weddell J (1825) A voyage towards the south pole performed in the years 1822-1824. London: Longman, Hurst, Rees, Orme, Brown and Green.

36. Bonner WN. Population increase in the fur seal *Arctocephalus gazella* at South Georgia. In: Carrick R, Prevost J, Holdgate MW, editors; 1964; Paris. Hermann. pp. 433-443.

37. Larson CA (1920) Report of the Interdepartmental Committee on Research and Development in the Dependencies of the Falkland Islands. London: His Majesty's Stationary Office. Command 657 p.

38. Payne MR (1977) Growth of a fur seal population. Philosophical Transactions of the Royal Society of London, B 279: 67-79.

39. Boyd IL (1993) Pup production and distribution of breeding Antarctic fur seals (*Arctocephalus gazella*) at South Georgia. Antarctic Science 5: 17-24.

40. Wynen LP, Goldsworthy SD, Guinet C, Bester MN, Boyd IL, et al. (2000) Postsealing genetic variation and population structure of two species of fur seal (*Arctocephalus gazella* and *A. tropicalis*). Molecular Ecology 9: 299-314.

41. Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M (1994) Mutational processes of simple sequence repeat loci in human populations. Proceedings of the National Academy of Sciences of the United States of America 91: 3166-3170.

42. Weber JL, Wong C (1993) Mutation of human short tandem repeats. Human Molecular Genetics 2: 1123-1128.

43. Doidge DW, Croxall JP, Baker JR (1984) Density-dependent pup mortality in the Antarctic fur seal *Arctocephalus gazella* at South Georgia. Journal of Zoology (London) 202: 449-460.

44. Hoffman JI, Amos W (2005) Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology 14: 599–612.

45. Raymond M, Rousset F (1995) Genepop (Version 1.2) - population genetics software for exact tests of ecumenicism. Journal of Heredity 86: 248–249.

46. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

47. Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. Journal of Heredity 86: 485-486.

48. Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN ver 2.000: a software for population genetics data analysis. Geneva, Switzerland: Genetics and Biometry Laboratory, Department of Anthropology and Ecology, University of Geneva.

49. Piry S, Luikart G, Cornuet J-M (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90: 502-503.

50. Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. Proceedings of the National Academy of Sciences of the United States of America 75: 2868-2872.

51. Schlötterer C, Ritter R, Harr B, Brem GHmroalmaiDmpefa-smrMBE (1998) High mutation rate of a long microsatellite allele in *Drosophila melanogaster* provides evidence for allele-specific mutation rates. Molecular Biology and Evolution 15: 1269-1274.

52. Bandelt H-J, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37–48.

53. Excoffier L, Smouse PE (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. Genetics 136: 343–359.

54. Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic asociations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132: 619-633.

55. Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657-1659.

56. Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129: 555-562.

57. Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9: 552–569.

58. Excoffier L, Schneider S (1999) Why hunter-gatherer populations do not show signs of Pleistocene demographic expansions. Proceedings of the National Academy of Sciences of the United States of America 96: 10597–10602.

59. Harpending H (1994) Signature of an ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human Biology 66: 591-600.

60. Tajima F (1989) Statistical method for testing the neutral mutation hypothesisby DNA polymorphism. Genetics: 585-595.

61. Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915-925.

62. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214.

63. Caswell H (2001) Matrix population models. Sunderland, Massachusetts: Sinauer Associates.

64. Forcada J, Trathan PN, Murphy EJ (2008) Life history buffering in Antarctic mammals and birds against changing patterns of climate and environmental variation. Global Change Biology 14: 2473–2488.

65. Phillips CD, Trujillo RG, Gelatt TS, smolen MJ, Matson CW, et al. (2009) Assessing substitution patterns, rates and homoplasy at HVRI of Steller sea lions, *Eumetopias jubatus*. Molecular Ecology 18: 3379–3393.

66. Higdon JW, Bininda-Edmonds ORP, Beck RMD, Ferguson SH (2007) Phylogeny and divergence of the pinnipeds (Carnivora: Mammalia) assessed using a multigene dataset. BMC Evolutionary Biology 7: 216.

67. Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818.

68. Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. Molecular Ecology 11: 1591-1604.

69. Team RDC (2005) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

70. Cornuet JM, Santo F, Beaumont MA, Robert CP, Marin J-M, et al. (2008) Inferring population history with DIYABC: a user-friendly approach to Approximate Bayesian Computations. Bioinformatics 24: 2713–2719.

71. Fagundes NJR, Ray N, Beaumont MA, Neuenschwander S, Salanzo FM, et al. (2007) Statistical evaluation of alternative models of human evolution. Proceedings of the National Acadamy of Sciences 104: 17614-17619.

72. Excoffier L, Estoup A, Cornuet JM (2005) Bayesian analysis of an admixture model with mutations and arbitrarily linked markers. Genetics 169: 1727-1738.

73. Cornuet JM, Ravigne V, Estoup A (2010) Inference on population history and model checking using DNA sequence and microsatellite data with the software DIYABC (v1.0). BMC Bioinformatics 11: 104.

74. Forcada J, Staniland IJ (2009) Antarctic fur seal *Arctocephalus gazella*.

75. Headland RK (1989) Chronology of Antarctic expeditions and related historical events. Cambridge: Cambridge University Press.

76. Gemmell NJ, Allen PJ, Goodman SJ, Reed JZ (1997) Interspecific microsatellite markers for the study of pinniped populations. Molecular Ecology 6: 661-666.

77. Hoffman JI, Dasmahapatra KK, Nichols HJ (2008) Ten novel polymorphic dinucleotide microsatellite loci cloned from the Antarctic fur seal *Arctocephalus gazella*. Molecular Ecology Resources 8: 459–461.

78. Hoffman JI (2009) A panel of new microsatellite loci for genetic studies of Antarctic fur seals and other otariids. Conservation Genetics 10: 989–992.

79. Allen PJ, Amos W, Pomeroy PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. Molecular Ecology 4: 653–662.

80. Davis CS, Gelatt TS, Siniff D, Strobeck C (2002) Dinucleotide microsatellite markers from the Antarctic seals and their use in other pinnipeds. Molecular Ecology Notes 2: 203-208.

81. Hoelzel AR, LeBoeuf BJ, Reiter J, Campagna C (1999) Alpha-male paternity in elephant seals. Behavioral Ecology and Sociobiology 46: 298-306.

82. Buchanan FC, Maiers LD, Thue TD, DeMarch BGE, Stewart REA (1998) Microsatellites from the Atlantic walrus *Odobenus rosmarus rosmarus*. Molecular Ecology 7: 1083-1085.

83. Coltman DW, Bowen WD, Wright JM (1996) PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in other pinniped species. Molecular Ecology 5: 161-163.

84. Hoffman JI, Steinfartz S, Wolf JBW (2007) Ten novel dinucleotide microsatellite loci cloned from the Galápagos sea lion (*Zalophus californianus wollebaeki)* are polymorphic in other pinniped species. Moleular Ecology Notes 7: 103–105.

**Figure Legends**

Figure 1 xx.

Figure 2 xx.

Figure 3 xx.

Figure 4 xx.

**Figure 5** Posterior density curves of model parameters based on 10,000 accepted values from 1x106 iterations of the initial bottleneck model (see Materials and methods for details). Continuous, large-dashed and short-dashed lines represent posterior density curves obtained for simulations with a sex ratio of 1, a sex ratio of 1:5 and with expanded priors on mitochondrial mutation rate respectively. Dotted lines represent results obtained for the microsatellite dataset only. Data from simulations using only mtDNA are not included due to the bottleneck model not being supported.

**Figure 6** Posterior density curves of model parameters based on 10,000 accepted values from 1x106 iterations of the bottleneck model with expanded priors on *Ne* and time (see Materials and methods for details). Continuous, large-dashed and short-dashed lines represent posterior density curves obtained for simulations with a sex ratio of 1, a sex ratio of 1:5 and with expanded priors on mitochondrial mutation rate respectively. Dotted lines represent results obtained for the microsatellite dataset only. Data from simulations using only mtDNA are not included due to the bottleneck model not being supported.

**Tables**

**Table 1** Numbers of Antarctic fur seals genotyped at the mitochondrial HVR1 and 21 microsatellite loci. For a map of the sampling locations within South Georgia, see Figure S1.

|  |  |  |
| --- | --- | --- |
| Location | Sampling site | Number of samples genotyped |
| Willis Islands | Main Island | 16 |
| Bird Island | Study colony | 142 |
| Freshwater beach | 25 |
| South Georgia | Prince Olav Harbour | 12 |
| Leith Harbour | 1 |
| Husvik | 12 |
| Cooper Bay | 14 |
| Annenkov Island | 15 |
| Wilson Harbour | 9 |
|  |  | 246 |



**Table 2** The number of loci with heterozygosity excess and test probabilities obtained using a range of mutational models (see methods for details) within the program Bottleneck [[49](#_ENREF_49)].The mode test revealed normal L-shaped distributions under all of the scenarios tested. *P*-values significant at α < 0.05 without correction for multiple statistical tests are highlighted in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Mutational model | No. of loci with heterozygosity excess | Sign test  *P*-value | Standardized differences test  *P*-value | Wilcoxon test  *P*-value |
| IAM | 21 | **<0.0001** | **<0.0001** | **<0.0001** |
| TPM70 | 20 | **<0.001** | **<0.0001** | **<0.0001** |
| TPM90 | 17 | **0.008** | **0.002** | **<0.001** |
| TPM95 | 16 | 0.075 | **0.018** | **0.008** |
| TPM99 | 16 | 0.076 | 0.326 | 0.320 |
| SMM | 12 | 0.531 | 0.373 | 0.919 |

Table 3 Prior uniform distributions, mean, median, mode, quantiles, and estimates of bias and precision (MRB = mean relative bias, RMSE = root mean square error) for the posteriors of parameters calculated from simulations of historic models that differed in prior bounds of specific parameters.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Model | Prior | Mean | Median | Mode | 5% | 95% | MRB | RMSE |
| *Ne-contemporary* | **Initial** | 1–1.5×106 | 7.44×105 | 7.42×105 | 1.78×105 | 6.69×104 | 1.42×106 | 6.50 | 98.68 |
| Sex ratio = 1:5 |  | 7.44×105 | 7.45×105 | 2.24×105 | 7.53×104 | 1.42×106 | 2.36 | 18.19 |
| Broadened *µ*seq |  | 7.38×105 | 7.33×105 | 4.32×105 | 7.00×104 | 1.43×106 | 5.76 | 58.36 |
| Microsatellites only |  | 7.45×105 | 7.44×105 | 1.94e×105 | 7.28×104 | 1.42×106 | 4.49 | 40.78 |
| **Broadened** | 1–6×106 | 2.98×106 | 2.97×106 | 6.98×105 | 2.79×105 | 5.72×106 | 2.13 | 11.90 |
| Sex ratio = 1:5 |  | 2.95×106 | 2.92×106 | 2.74×105 | 2.73×105 | 5.67×106 | 6.10 | 60.50 |
| Broadened *µ*seq |  | 3.01×106 | 3.04×106 | 5.17×106 | 2.85×105 | 5.71×106 | 7.80 | 105.60 |
| Microsatellites only |  | 3.00×106 | 2.98×106 | 2.72×106 | 2.93×105 | 5.70×106 | 2.83 | 19.90 |
| *Ne-bottleneck* | **Initial** | 1–1000 | 164 | 139 | 122 | 46 | 371 | 0.62 | 1.85 |
| Sex ratio = 1:5 |  | 356 | 313 | 215 | 92 | 821 | 0.77 | 2.70 |
| Broadened *µ*seq |  | 169 | 140 | 133 | 48 | 429 | 0.60 | 2.69 |
| Microsatellites only |  | 188 | 153 | 153 | 53 | 563 | 0.68 | 2.97 |
| **Broadened** | 1–2000 | 658 | 662 | 688 | 217 | 1070 | 0.43 | 1.49 |
| Sex ratio = 1:5 |  | 1200 | 1250 | 1360 | 469 | 1810 | 0.48 | 3.64 |
| Broadened *µ*seq |  | 660 | 667 | 678 | 228 | 1090 | 0.42 | 1.30 |
| Microsatellites only |  | 665 | 655 | 593 | 216 | 1100 | 0.33 | 0.98 |
| *Ne-historical* | **Initial** | 1–1.5×106 | 7.77×105 | 7.63×105 | 3.96×105 | 1.55×105 | 1.43×106 | 1.56 | 22.52 |
| Sex ratio = 1:5 |  | 3.60×105 | 2.42×105 | 1.17×105 | 6.39×104 | 1.10×106 | 3.29 | 33.13 |
| Broadened *µ*seq |  | 7.48×105 | 7.20×105 | 3.59×105 | 1.44×105 | 1.42×106 | 1.07 | 8.64 |
| Microsatellites only |  | 6.31×105 | 5.94×105 | 9.56×103 | 1.19×104 | 1.41×106 | 2.90 | 35.65 |
| **Broadened** | 1–6×106 | 2.41×106 | 2.05×106 | 7.40×105 | 3.20×105 | 5.49×106 | 0.83 | 3.52 |
| Sex ratio = 1:5 |  | 9.07×105 | 4.35×105 | 9.88×104 | 6.79×104 | 3.67×106 | 2.03 | 20.00 |
| Broadened *µ*seq |  | 2.29×106 | 1.92×106 | 5.74×105 | 2.87×105 | 5.42×106 | 1.31 | 6.57 |
| Microsatellites only |  | 2.79×106 | 2.76×106 | 1.13×104 | 1.23×105 | 5.67×106 | 9.04 | 133.37 |

**Table 3 continued**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Model | Prior | Mean | Median | Mode | 5% | 95% | MRB | RMSE |
| *τbottleneck-end* | **Initial** | 1–30 | 12 | 11 | 6 | 2 | 27 | 0.77 | 2.49 |
|  | Sex ratio = 1:5 |  | 13 | 12 | 6 | 2 | 27 | 0.75 | 2.31 |
|  | Broadened *µ*seq |  | 12 | 11 | 5 | 2 | 27 | 0.81 | 2.49 |
|  | Microsatellites only |  | 13 | 12 | 7 | 3 | 28 | 1.05 | 2.867 |
|  | **Broadened** | 1–50 | 17 | 13 | 5 | 2 | 42 | 0.97 | 3.08 |
|  | Sex ratio = 1:5 |  | 17 | 13 | 4 | 2 | 43 | 0.67 | 2.38 |
|  | Broadened *µ*seq |  | 17 | 13 | 7 | 2 | 43 | 1.00 | 3.56 |
|  | Microsatellites only |  | 18 | 14 | 1 | 2 | 44 | 0.95 | 2.93 |
| *τbottleneck-start* | **Initial** | 15–100 | 70 | 74 | 100 | 32 | 98 | 0.18 | 0.59 |
|  | Sex ratio = 1:5 |  | 69 | 72 | 96 | 29 | 98 | 0.16 | 0.57 |
|  | Broadened *µ*seq |  | 71 | 74 | 99 | 32 | 98 | 0.14 | 0.56 |
|  | Microsatellites only |  | 71 | 74 | 94 | 31 | 98 | 0.19 | 0.59 |
|  | **Broadened** | 1–2000 | 316 | 329 | 490 | 104 | 485 | 2.5 | 17.88 |
|  | Sex ratio = 1:5 |  | 284 | 287 | 279 | 77.0 | 475 | 1.60 | 14.18 |
|  | Broadened *µ*seq |  | 316 | 327 | 466 | 105 | 484 | 3.62 | 3.56 |
|  | Microsatellites only |  | 320 | 333 | 497 | 108 | 486 | 2.83 | 23.33 |
| *µmitochondrial* | **Initial** | 5.78×10-7–2.74×10-6 | 1.67×10-6 | 1.67×10-6 | 6.27×10-7 | 6.80×10-7 | 2.64×10-6 | 0.20 | 0.61 |
|  | Sex ratio = 1:5 |  | 1.64×10-6 | 1.65×10-6 | 2.70×10-6 | 6.43×10-7 | 2.62×10-6 | 0.15 | 0.58 |
|  | Broadened *µ*seq |  | 1.84×10-5 | 1.84×10-5 | 7.52×10-6 | 2.37×10-6 | 3.47×10-5 | 0.98 | 3.14 |
|  | **Broadened** |  | 1.65×10-6 | 1.65×10-6 | 5.78×10-7 | 6.67×10-7 | 2.65×10-6 | 0.21 | 0.65 |
|  | Sex ratio = 1:5 |  | 1.57×10-6 | 1.52×10-6 | 5.78×10-7 | 6.31×10-7 | 2.65×10-6 | 0.22 | 0.68 |
|  | Broadened *µ*seq | 5.78×10-7–3.65×10-5 | 1.85×10-5 | 1.85×10-5 | 1.21×10-6 | 2.22×10-6 | 3.47×10-5 | 1.29 | 4.18 |
| *µmicrosatellite* | **Initial** | 1.00×10-4–1.00×10-3 | 4.69×10-4 | 4.31×10-4 | 2.66×10-4 | 1.34×10-4 | 9.12×10-4 | 0.29 | 0.92 |
|  | Sex ratio = 1:5 |  | 4.85×10-4 | 4.53×10-4 | 2.49×10-4 | 1.35×10-4 | 9.27×10-4 | 0.26 | 0.84 |
|  | Broadened *µ*seq |  | 4.75×10-4 | 4.35×10-4 | 2.98×10-4 | 1.35×10-4 | 9.23×10-4 | 0.33 | 0.93 |
|  | Microsatellites only |  | 5.24×10-4 | 5.00×10-4 | 4.12×10-4 | 1.56×10-4 | 9.38×10-4 | 0.32 | 0.91 |
|  | **Broadened** |  | 3.16×10-4 | 2.46×10-4 | 1.44×10-4 | 1.18×104 | 7.68×10-4 | 0.25 | 0.78 |
|  | Sex ratio = 1:5 |  | 3.90×10-4 | 3.26×10-4 | 1.78×10-4 | 1.37×104 | 8.61×10-4 | 0.24 | 0.68 |
|  | Broadened *µ*seq |  | 3.12×10-4 | 2.45×10-4 | 1.38×10-4 | 1.17×104 | 7.59×10-4 | 0.18 | 0.73 |
|  | Microsatellites only |  | 3.53×10-4 | 2.85×10-4 | 1.83×10-4 | 1.18×104 | 8.17×10-4 | 0.28 | 0.79 |

**Supplementary table 1** Details of the pinniped microsatellite datasets used for this study. All

Shown are the number of individuals and microsatellite loci genotyped for each species. The number of loci analysed refers to the number of microsatellite loci that did not deviate significantly from HWE after FDR correction and hence which were retained for analysis (See Materials and methods for details).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Reference | Number of individuals | Number of loci | Number of loci analysed |
| Antarctic fur seal, *Arctocephalus gazella* | [[1](#_ENREF_1" \o "Hoffman, 2011 #3179)] | 246 | 21 | 21 |
| Galapagos fur seal, *Arctocephalus galapagoensis* | [[2](#_ENREF_2" \o "Lopes, in review #3835)] | 90 | 12 | 12 |
| Steller's sea lion, *Eumetopias jubatus* | [[3](#_ENREF_3" \o "Hoffman, 2006 #2558)] | 668 | 13 | 13 |
| Eastern Atlantic grey seal, *Halichoerus grypus* | [[4](#_ENREF_4" \o "Klimova, 2014 #3836)] | 1254 | 9 | 7 |
| Eastern Atlantic harbour seal, *Phoca vitulina* | [[5](#_ENREF_5" \o "Rijks, 2008 #2945)] | 204 | 27 | 27 |
| Galapagos sea lion, *Zalophus wollebaeki* | Wolf, J.B and Trillmich, F. (unpublished data) | 781 | 22 | 22 |
| South American fur seal, *Arctocephalus australis* | [[6](#_ENREF_6" \o "Rosa de Oliveira, 2008 #3214)] | 226 | 7 | 5 |
| Hooded Seal, *Cystophora cristata* | [[7](#_ENREF_7" \o "Coltman, 2007 #2894)] | 300 | 13 | 13 |
| Mediterranean monk seal, *Monachus monachus* | [[8](#_ENREF_8" \o "Pastor, 2004 #2191)] | 109 | 16 | 14 |
| Hawaiian monk seal, *Monachus schauinslandi* | [[9](#_ENREF_9" \o "Schultz, 2011 #3837)] and Schultz, J. (unpublished data) | 2386 | 18 | 17 |
| Bearded seal, *Erignathus barbatus* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 119 | 13 | 10 |
| Crabeater seal, *Lobodon carcinophagus* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 303 | 9 | 9 |
| Leopard seal, *Hydrurga leptonyx* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 150 | 14 | 12 |
| Arctic ringed seal, *Phoca hispida* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 303 | 10 | 10 |
| Ross Seal, *Ommatophoca rossi* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 90 | 9 | 9 |
| Weddell seal, *Leptonychotes weddelli* | [[10](#_ENREF_10" \o "Davis, 2008 #2911)] | 893 | 15 | 12 |
| Northern fur seal, *Callorhinus ursinus* | [[11](#_ENREF_11" \o "Dickerson, 2010 #3089)] | 492 | 8 | 7 |
| Atlantic walrus, *Odobenus rosmarus rosmarus* | Schafer, A. (unpublished data) | 623 | 10 | 10 |
| Atlantic walrus, *Odobenus rosmarus rosmarus* | Andersen dataset | 555 | 15 | 15 |
| Northern elephant seal, *Mirounga angustrostrus* | Sanvito, S. and Galimberti, F (unpublished data) | 260 | 35 | 32 |
| Southern elephant seal, *Mirounga leonina* | Sanvito, S. and Galimberti, F (unpublished data) | 260 | 13 | 13 |
| California sea lion, *Zalophus californianus* | Acevedo-Whitehouse, K. (unpublished data) | 347 | 13 | 11 |
| South American sea lion, *Otaria flavescens* | Hoffman J.I. (unpublished data) |  |  |  |