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Patterns of adaptive and purifying selection in the genomes of phocid seals

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Abstract

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Modern genomic sequencing technologies provide the opportunity to address long-standing questions in molecular evolution with empirical data. In this dissertation, I combine this new technology with advances in statistical population genetics to describe how deleterious mutations and adaptive evolution have shaped the genomic evolution of phocid seals.

In Chapter 1, I model historical demographic processes using whole genome sequences of eight seal taxa: the Hawaiian monk seal, the Mediterranean monk seal, the northern elephant seal, the southern elephant seal, the Weddell seal, the grey seal, the Baltic ringed seal, and the Saimaa ringed seal. Through this, I establish that the endangered monk seal species have long-term small population sizes, as do grey seals. On the other hand, the elephant seals, Weddell seal, and ringed seals had much larger populations in the distant past. Notably, the most recent glaciation (c. 12,000-120,000 years ago) appeared to have a dramatic effect on phocid populations throughout the world. With this knowledge of historical population sizes, I test a fundamental premise of molecular evolution: that the rate of mutation accumulation will be higher in smaller populations due to less efficient purifying selection. I show that there is not a higher substitution rate or overall rate of mutation accumulation in the long-term small populations of monk seals compared to other seal species. On the contrary, overall rates of mutation accumulation appear to be lower in monk seals and grey seals, both of which show smaller long-term population sizes

compared to the other species. This suggests either that the distribution of fitness effects may differ across seal species in a way that depends on population size and history.

In Chapter 2, I use population genomic data and a newly developed statistical model to detect positive selection in the protein coding genes of phocid seals (monk seals, elephant seals, Weddell seals, grey seals, and ringed seals). In addition, I use a phylogenetic framework to detect parallel evolution across multiple lineages of seals, relating to traits such as polar adaptations, hypoxia tolerance during long dives, and mating behavior. I develop a new bioinformatic tool to process raw BAM files and transform them into useable input for MASS-PRF, a tool to detect selection from polymorphism and divergence data. Through these analyses, I identify thousands of genes that show positive selection across multiple seal lineages. Genes associated with immune function, sperm competition, and blubber composition show positive selection in all lineages, highlighting how complex and important these traits are in seals. In the deep-diving elephant seals, the list of positively selected genes was enriched for genes relating to cardiac muscle development and function, providing important insight into how adaptive protein evolution has helped allow these seals to survive sustained bradycardia during dives that last over an hour. Weddell seals, on the other hand, showed enrichment for genes relating to neuronal development, which may relate to molecular adaptations that allow their neurons to survive hypoxic conditions during long dives. Because MASS-PRF allows for site-specific tests of selection, I am able to show how parallel evolution in the same genes across lineages sometimes may or may not involve positive selection at the same genic site.

In Chapter 3, I use the population genomic data from Chapter 2 to model the distribution of fitness effects (DFE) of segregating alleles in each population. Due to

sample size issues, only parameters for the Hawaiian monk seal were confidently estimated. Using the site frequency spectrum of synonymous sites, I show that the Hawaiian monk seal has had a long-term effective population size below 5000, in agreement with the results from Chapter 1. In addition, I should that after the arrival of humans in Hawaii, the monk seal experienced a 95% decline in effective population size, in line with the current census size of fewer than 1500 individuals. Conditioning the model on the Hawaiian monk seal demographic parameters, I am able to estimate the shape of DFE in Hawaiian monk seals using the site frequency spectrum of nonsynonymous sites. I estimate a DFE for the Hawaiian monk seal that is nearly identical to the one estimated in humans. This DFE, however, is different than the one estimated for mouse, with the seal and human DFEs having a higher proportion of more strongly deleterious alleles. This pattern cannot be explained by phylogenetic relatedness or differences in phenotypic complexity, but instead is likely related to differences in effective population size. I discuss how the geometric model of evolution predicts such a shift in DFE in response to the epistatic effect of fixed deleterious mutations in smaller populations.

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GENERAL DISCUSSION

GENERAL INTRODUCTION

Population genetics and molecular evolution may be the fields of biology with the strongest mathematical foundations. Produced through decades of theoretical research, this robust mathematical framework offers a range of tools from reconstructing phylogenetic relationships to modelling the effects of natural selection. Only with recent genomic sequencing tools has it become possible to fully integrate empirical molecular genetic data into these theoretical frameworks. This integration presents countless opportunities to test hypotheses about molecular evolution, the results of which can be used to understand how molecular evolution plays out in natural populations. With technological barriers lifted, the largest burden for modern geneticists is therefore choosing the proper study systems in which to address pressing questions in molecular evolution. In this dissertation, I establish phocid seals as an excellent natural system to address two core topics in molecular evolution: the effect of population size on genome evolution and the role of molecular changes in phenotypic adaptations.

Phocid seals (family Phocidae) are marine mammals that rely on both the ocean and land. This clade split from Otarioidea (i.e. fur seals, sea lions, and walruses) around 26.9 Ma (Paterson *et al.* 2020) with the crown group inhabiting the warm waters of the central Atlantic basic (Fulton & Strobeck 2010; Berta *et al.* 2018). Within this family are 18 extant and one recently extinction species, which form two subfamilies: Monachinae and Phocinae. Across this family are species that range from small, endangered populations like the Hawaiian monk seal (*Neomonachus schauinslandi*) and Mediterranean monk seal (*Monachus monachus*) to the extremely abundant Weddell seal (*Leptonychotes weddellii*) and ringed seal (*Pusa hispida*). Other species, like the Northern elephant seal, were once

on the verge of extinction but have since recovered to large population sizes (Hoelzel *et al.* 2002).

These extreme differences in population size across the phocid phylogeny make these species an excellent natural system to test how population size affects patterns of molecular evolution. This relationship has been a central question in the field, with increased attention coming from the development of the nearly neutral theory (Ohta 1972, 1973). This theory proposed slightly deleterious mutations would drift to high frequencies at a rate inversely proportional to the effective population size (N_e). Early empirical studies of molecular substitution rates and molecular clocks attempted to broadly characterize this pattern (e.g. Yang & Nielsen 1998), while theoretical population genetics work suggested that slightly deleterious mutation accumulation could be a significant concern for endangered species (Lynch & Lande 1998, Lande 2003). However, many empirical studies have been unable to disentangle the effects of population size from other variables such as generation time, body size, and phylogenetic signal (e.g. Martin & Palumbi 1993, Welch *et al.* 2008, Bromham 2009) and other studies conflate long-term population size with recent bottleneck (e.g Abascal *et al.* 2016).

In addition, alternative theoretical work has suggested evolutionary models in which mutation accumulation would have little or no dependence on population size (Cherry 1998, Gillespie 2001, Goldstein 2013). These alternative models center around the idea that the distribution of fitness effects (DFE) of new mutations may correlate with population size. For example, the concave fitness function proposed by Cherry (1998) suggests that smaller populations may have lower fitness, but that new mutations are expected to have much larger effects. Labar and Adami (2017) explored a similar idea in

simulations that showed how smaller populations could drift to plateaued fitness peaks, again shifting the predicted effects of new mutations. In these cases, the mathematical framework of nearly neutral theory would still hold but purifying selection would still be able to act in smaller populations given the larger selection coefficients ($|s|$). Unfortunately, there is still very little empirical measurement or modelling of the DFE, so these models cannot be properly parameterized (Whitlock *et al.* 2003). The extreme differences in population size among closely related phocid seal species, however, offers the opportunity to test hypotheses of the relationship between population size and both mutation accumulation and DFE.

In addition to purifying selection, there are open questions about the role protein evolution plays in phenotypic adaptation. From the early days of molecular genetics, there has been a debate about the relative contributions of protein-coding vs. regulatory changes to phenotypic evolution (Lynch & Wagner 2008). This is particularly true for complex traits, the evolution of which may be affected by multiple loci in the genome (Glazier *et al.* 2002). Fortunately, theoretical population genetics has devised statistical frameworks that can be used to detect adaptive changes in protein coding genes (Vitti *et al.* 2013). This originates with the idea that nonsynonymous changes are fixed through positive selection while synonymous mutations fix through drift at a rate equal to the neutral mutation rate (Hurst 2002). This idea was further developed in the Poisson Random Field (PRF) theory (Sawyer & Hartl 1992). This statistical framework derives the expected number of polymorphic or fixed sites that are synonymous or nonsynonymous in two sister taxa under a given selection regime. With the observed number of polymorphic and fixed sites available from molecular sequence data, these expectation equations can be solved to

estimate the scaled selection coefficient for a gene or even an individual amino acid site (Zhao *et al.* 2017).

Adaptive evolution in marine mammals is a particularly interesting area of evolutionary biology for two reasons. First, marine mammals have evolved numerous morphological and physiological adaptations that allow them to inhabit the marine environment, giving them a suite of unique derived traits compared to terrestrial mammals. Second, marine mammals—and especially pinnipeds—have evolved adaptations to extreme conditions. These include extreme environments, such as polar oceans and extreme depths, as well as extreme life history traits, such as months of fasting and extreme sexual dimorphism (Berta *et al.* 2015). In many of these cases, the morphological or physiological adaptations have been identified but the underlying molecular adaptation is not understood (Foote *et al.* 2015).

In this dissertation, I generate dozens of whole genome sequences across multiple phocid seal species and use this clade to study fundamental aspects of purifying and positive selection in mammalian evolution. In Chapter 1, I start by using whole genomes to reconstruct the demographic history of eight seal taxa. After showing that these taxa have dramatic differences in long-term effective population size, I test how purifying selection has acted on the genomic evolution of these species with long-term differences in population size. Then in Chapter 2, I sequence additional genomes to generate polymorphism data for each taxon. I develop a program to transform medium-coverage genome sequencing data into a format that can be processed in MASS-PRF to detect sites under positive selection. With these tools, I identify genes that help to explain the molecular underpinnings of adaptive evolution in various seal lineages. Finally in Chapter

3, I use this polymorphism data to return to the question of purifying selection, this time by explicitly modelling the distribution of fitness effects using a population genetics approach. I compare the DFE in seals with those that have been modelled in other mammalian species to gain a better understanding of how the shape of the DFE evolves across mammals. In addressing these questions in this dissertation, I also provide valuable insights into the history and biology of these species, which will inform the future conservation and management of these marine mammals.

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CHAPTER ONE:

No evidence that purifying selection is less efficient in long-term small populations of seals

Abstract

A widely accepted principle of population genetics is that the efficiency of purifying selection is inversely proportional to population size. Given that most new mutations are assumed to be slightly deleterious, many have proposed that amino acid substitution rates, or mutation accumulation rates more broadly, will be higher in smaller populations. Comparative genomics allows a way to empirically measure these rates in natural populations, but the species comparisons must be done in a way that does not inadvertently bias the result. Here we compare population genomic evolution across six species and two subspecies of phocid seals. These species are all closely related (maximum divergence 15 million years), but we should that they have dramatically different long-term effective population sizes and demographic histories. We use a statistic that is insensitive to demography to measure differences in the rate of mutation accumulation across species, thereby testing for differences in the long-term efficacy of purifying selection. Contrary to expectations, we find no evidence that purifying selection has acted less efficiently in long-term small populations of seals, many of which are currently endangered. This result presents a surprisingly optimistic outlook for the genetic health of these species, while simultaneously requiring a re-examination of commonly held assumptions in the field of population genetics.

Introduction

From carrying capacity to the probability of allele fixation to an IUCN species assessment, population size is one of the most important parameters in ecology, evolution, and conservation biology. In population genetics, population size is usually dealt with in its idealized form: effective population size (N_e). This parameter, first proposed by Sewall Wright (1931), represents the number of randomly mating individuals in an idealized population that would produce a given population genetic pattern (e.g. genetic drift, diversity, coalescence). As such, N_e has played a central role in everything from mathematical population genetics to conservation management decisions (e.g. Nei & Tajima 1981, Soulé 1985, Charlesworth 2009). Importantly, N_e is thought to directly relate to the interplay of selection and drift in the molecular evolution of natural populations (Charlesworth 2009).

The fields of population genetics and molecular evolution have widely embraced the mathematical premise of nearly neutral theory: that the probability of fixation of a slightly deleterious allele increases with decreasing effective population size (Ohta 1972, 1973). Nearly neutral theory has been championed in all areas of molecular evolution, from phylogenetic substitution rates (Nabholz *et al.* 2013) to the evolution of genome architecture and mutation rates (Lynch & Conery 2003, Lynch 2007) to conservation genetics (Lynch *et al.* 1995, Lynch & Lande 1998, Yoder *et al.* 2018).

Theoretical and experimental evidence suggests that most new nonsynonymous mutations are expected to be either lethal or slightly deleterious (Ohta 1992, Eyre-Walker & Keightley 2007). If this is the case, we should expect smaller populations to have higher amino acid substitution rates compared to larger populations (Ohta 1972), although in

empirical data the pattern could be confounded by differences in mutation rate, generation time, linkage, and the prevalence of positive or balancing selection (Nei & Graur 1984, Gillespie 2001, Woolfit 2009, Nabholz *et al.* 2013).

On the other hand, a few evolutionary models have proposed that substitution rate and mutation accumulation may be independent of population size for reasons other than simple confounding factors. Theoretical population genetic (Cherry 1998) and protein evolution (Goldstein 2013) models that allow for concave fitness functions and epistasis show that the distribution of fitness effects (DFE) is dependent on population fitness and, relatedly, population size. In this framework, the substitution rate is nearly insensitive to absolute population size; instead, sudden bursts of substitutions are expected when population size changes. Notably, these studies do not contradict the main premise of nearly neutral theory because they predict that the selection coefficients of new mutations change in a way that is correlated with population size. More recently, Labar and Adami (2017) showed how substitution patterns and shifts in the DFE could occur through the evolution of "drift robustness" in small populations. Their work raises the possibility that smaller populations can accumulate fewer large-effect mutations by landing on flatter fitness peaks.

A few empirical studies have attempted to find support for these various evolutionary models, but the results have been mixed. In humans, there is little evidence for differences in substitution rate or mutational load across populations (Henn *et al.* 2015). In other species, studies often compare very distantly related taxa (Kosiol *et al.* 2008, Huber *et al.* 2017), which are substantially confounded by generation time, metabolic rates, phylogenetic signal, and genomic differentiation. Other studies have examined island-

mainland pairs (Johnson & Seger 2001, Woolfit & Bromham 2005, Charlesworth & Eyre-Walker 2007, Kutschera *et al.* 2020) or recently bottlenecked endangered populations/species (e.g. Robinson *et al.* 2016, Rogers & Slatkin 2017, van der Valk *et al.* 2019). As Goldstein (2013) points out, however, such comparisons conflate changes in N_e with small N_e. As described above, such population size changes may in themselves produce different patterns of substitutions, making these poor systems in which to test the impact of N_e on molecular evolution. In addition, most studies that have found differences among populations rely on analyses of polymorphic sites, such as the ratio of polymorphic nonsynonymous sites to polymorphic synonymous sites (pN/pS) or homozygosity rates (Lohmueller *et al.* 2008, Loire *et al.* 2013, Marsden *et al.* 2016, Robinson *et al.* 2016). Through theory and simulations, however, Simons and Sella (2016) demonstrated how these metrics are strongly influenced by demography and are inappropriate measures of the efficiency of selection.

In this study, we attempt to overcome these obstacles in two important ways. First, our comparative genomics approach examines a set of closely related seal species. The eight phocid species in our study are separated by less than 15 Ma (Fulton & Strobeck 2010), yet they have census population sizes that differ by orders of magnitude. The Hawaiian (*Neomonachus schauinslandi*) and Mediterranean (*Monachus monachus*) monk seals, for example, are endangered tropical species that sister taxa to the incredibly abundant Antarctic Weddell seal (*Leptonychotes weddellii*). The northern elephant seal (*Mirounga angustirostris*) experienced a population bottleneck that was much more extreme than the one faced by the southern elephant seal (*M. leonina*) (Stoffel *et al.* 2018). Finally, the common grey seal (*Halichoerus grypus*) is closely related to the extremely

abundant arctic ringed seal (*Pusa hispida*), although the Baltic (*P. h. botnica*) and Saimaa (*P. h. saimensis*) subspecies have both experienced recent declines, the latter of which is one of the most endangered marine mammals in the world (Valtonen *et al.* 2012). With our whole-genome data set, we are able to reconstruct the demographic histories of these species, in many cases confirming long-term differences in population size.

These extreme differences in demography create a natural experiment to test how population size affects the rate of amino acid substitution and mutation accumulation in closely related taxa. To measure this, we use multiple statistical approaches that have been shown to reflect patterns of selection rather than demographic changes (Simons *et al.* 2014, Do *et al.* 2015, Simons & Sella 2016, Pedersen *et al.* 2017). Through this framework, we provide detailed empirical evidence for how population size affects patterns of molecular evolution in natural populations.

Methods

Samples and sequencing

We generated whole genome sequence data for five samples: 2 Hawaiian monk seals, 2 Mediterranean monk seals, and 1 southern elephant seal. The additional monk seal genomes were used to check for the effect of individual samples in our downstream analyses. All sequencing was paired-end and done on an Illumina HiSeq 2500 or Illumina HiSeq X.

In addition, we downloaded publicly available sequence data from the NCBI Sequence Read Archive (SRA) for six other samples (NCBI BioSample ID in parentheses): 1 northern elephant seal (SAMN13072016), 1 Weddell seal (SAMN00672463), 1 grey seal

(SAMEA104712343), 1 Baltic ringed seal (SAMEA104712315), 1 Saimaa ringed seal (SAMEA104712221), and 1 Steller sea lion (SAMN09402722). Raw reads were downloaded in FASTQ format from SRA.

Reference genomes, read mapping and filtering

We made use of two different high-quality reference genomes for this study: the Steller sea lion (ASM402803v1) and the Hawaiian monk seal (ASM220157v1). All species in our study are phylogenetically most closely related to the Hawaiian monk seal, making this reference genome the most appropriate reference for demographic modelling through MSMC and hPSMC. However, we were concerned that using an in-group reference genome may introduce a bias in our mutation accumulation analyses because the study species would vary in similarity to the reference. Because the Steller sea lion is an outgroup to the phocid seals included in our study, the genomes of our study species should share the same level of similarity to the Steller sea lion genome. We therefore decided to use the annotated Steller sea lion genome as the reference genome for the mutation accumulation and variant annotation portion of the study. The same pipeline and quality filters were applied in both cases, unless otherwise specified.

Raw reads were trimmed for quality and adapter removal using TrimGalore v0.4.2 (https://github.com/FelixKrueger/TrimGalore). Trimmed reads were then mapped to the reference genome using BWA mem (Li 2013). PCR duplicates were removed and depth of coverage was assessed with Picard (Van der Auwera *et al.* 2013).

Heterozygosity, MSMC, and hPSMC

Genome-wide heterozygosity was calculated in ANGSD (Korneliussen *et al.* 2014), using BAM files of reads mapped to the Hawaiian monk seal reference genome. All scaffolds greater than 1Mb and not thought to belong to sex-chromosomes (see supplemental material) were included in the analysis. For each genome, heterozygosity was estimated as the single sample SFS across non-overlapping blocks of 20Mb, which were allowed to span more than one scaffold. Genome-wide heterozygosity for each sample was calculated by taking the arithmetic mean of heterozygosity across all segments. The standard deviation (SD) and coefficient of variation (CV) were also calculated. Genome-wide heterozygosities were compared to those calculated for other species in Robinson *et al.* (2016), Westbury *et al.* (2018), Westbury *et al.* (2019), and Morin *et al.* (2020).

Sequentially Markovian Coalescent (SMC) models are a way of using the distribution of heterozygous sites across a genome to reconstruct deep demographic histories (typically on the order of 1000-100,000 generations before the present) and coalescent divergence times. To reconstruct demographic histories, we used MSMC2 (Schiffels & Durbin 2014) and following the protocol provided by Schiffels and Wang (2020). This included generating a reference genome "mappability" mask file through the SNPable pipeline (http://lh3lh3.users.sourceforge.net/snpable.shtml), as well as an individual sample mask file based on the specific depth of coverage of each sample. The `bcftools mpileup + call` pipeline (Li 2011) was then used to call variants, filtering out reads with a map quality of less than 20, bases with base quality less than 20, and the `-C50` flag to adjust map quality of reads with excessive mismatches.

hPSMC performs the same SMC model on a pseudo-diploid genome created by combining haploid versions of genomes from two individuals from different populations.

In unphased genome data like ours, this can be done by either using the X-chromosome from two males (as described by Li and Durbin (2011) or by randomly selecting one allele at heterozygous sites throughout the genome of two individuals (Cahill *et al.* 2016). PSMC (Li & Durbin 2011) is then run on the resulting pseudo-diploid genome. At times before the two populations split, the model recovers realistic ancestral population trends. After divergence, however, the model fails to recover coalescent events and therefore estimates near infinite population sizes. Divergence can be qualitatively assessed by noting when the population trend line deviates from a reasonable population size towards an infinite size. Because both pseudo-diploidization methods have limitations, we ran hPSMC on both the X-chromosome pseudo-diploid genomes and the random allele pseudo-diploid genomes for all species pairs.

Both MSMC and hPSMC require user-specified values for generation time and mutation rate. In the supplementary material we discuss our choices for these parameters.

Variant annotation and load statistics

To avoid biases from using an in-group reference genome, for the mutation accumulation analyses we used reads mapped to the Steller sea lion reference genome and annotation (Kwan *et al.* 2019). The `bcftools mpileup + call` pipeline was again used to call variants, with stricter minimum quality filters of 25 map quality and 25 base quality. The resulting VCF was then filtered to include only bi-allelic sites that intersect with autosomal protein coding sequence (CDS) from the Steller sea lion genome annotation file. We created a custom effect annotation database for the Steller sea lion with SNPeff (Cingolani *et al.* 2012), with which we annotated the variants in each genome. In addition,

we did a custom annotation of nonsynonymous variants to assign a Grantham distance score to each derived allele. The Grantham distance score (D), which ranges from 5 to 215, describes how different the derived amino acid is from the ancestral amino acid based on biophysical properties like polarity and volume (Grantham 1974). Following the classification scheme from Li *et al.* (1984), we categorized amino acid changes as conservative ($D < 50$), moderately conservative ($50 \leq D < 100$), moderately radical ($100 \leq D < 150$), or radical ($D \geq 150$), with the expectation that radical amino acid changes have more significant fitness effects than conservative changes (Huzurbazar *et al.* 2010).We wrote a custom python script to parse the annotated VCF file, assign Grantham scores, determine homozygous derived sites, and compute a number of statistics that have been proposed to measure mutational load.

The measures we use rely on identifying the ancestral and derived alleles for any variant site. We considered the Steller sea lion allele to be ancestral, and did not include any sites that were heterozygous in the Steller sea lion sample. While this method of defining ancestral alleles by an outgroup sample can occasionally identify the wrong allele as ancestral, we expect that error to be unbiased in our analyses.

The first method we used was proposed by Simons *et al*. (2014). This method simply counts the number of derived alleles of each type in a single diploid genome. Genomes with higher counts of derived nonsynonymous alleles should have higher mutational loads under the assumption that most nonsynonymous alleles are slightly deleterious and additive. This method also avoids the demographic signal that is known to confound statistics that consider homozygous and heterozygous sites separately.

However, in our study most species share significant phylogenetic history between the ancestral split (22.5 Ma) and target species splits (100 ka to 15 Ma). The counting method cannot distinguish between derived alleles that are shared between lineages and those that occur after the splits between lineages. To avoid this issue, Do *et al.* (2015) proposed a set of *R*-statistics that count derived alleles while controlling for shared derived alleles, making this statistic more sensitive to mutation accumulation differences between species. At each variable site *i*, d_X^i is the number of derived alleles [0, 1, 2] in the diploid genome of sample X and d_Y^i is the number of derived alleles [0, 1, 2] in the diploid genome of sample Y, such that the number of derived alleles found in the genome of sample X but not sample Y is

$$L_{X,not\ Y} = \sum_i (\frac{d_X^i}{2})(1 - \frac{d_Y^i}{2}),$$

and the number of derived alleles found in the genome of sample Y but not sample X is

$$L_{Y,not\ X} = \sum_i (\frac{d_Y^i}{2})(1 - \frac{d_X^i}{2}).$$

From these counts, a ratio of mutation accumulation in the two genomes can be calculated as

$$R_{X/Y} = L_{X,not\ Y} / L_{Y,not\ X}.$$

When this ratio is greater than 1, the rate of mutation accumulation is higher in sample X, and if it is less than 1 the rate is higher in sample Y.

However, when comparing distantly related species differences in effective mutation rate (due to differences in molecular mutation rate, generation time, or other factors) may confound signals of mutational load. Do *et al.* (2015) created an additional statistic, called $R'_{X/Y}$, that normalizes the rate from a target class (e.g. nonsynonymous) with that of a neutral class (e.g. synonymous), as

$$R'^{class}_{X/Y} = \left(\frac{L^{class}_{X,not\,Y}}{L^{class}_{Y,not\,X}}\right) / \left(\frac{L^{normalization}_{X,not\,Y}}{L^{normalizations}_{Y,not\,X}}\right) = R^{class}_{X/Y} / R^{normalization}_{X/Y}.$$

In this way, $R'^{nonsyn}_{X/Y}$ gives a measure of nonsynonymous mutation accumulation while accounting for non-selective forces. In addition, we use this framework to explore other aspects of the data, such as

$$R'^{rad}_{X/Y} = R^{radical}_{X/Y} / R^{synonymous}_{X/Y},$$

which shows the rate of the radical amino acid mutation accumulation normalized against neutral synonymous alleles and

$$R'^{stop}_{X/Y} = R^{stop}_{X/Y} / R^{synonymous}_{X/Y},$$

which likewise shows the rate of premature stop codon accumulation normalized against neutral synonymous alleles. Additionally, we use

$$R'^{rad/con}_{X/Y} = R^{radical}_{X/Y} / R^{conservative}_{X/Y}$$

to compare the accumulation rates of radical and conservative amino acid mutation accumulation.

Although previous work by Simons and Sella (2015) showed that measures based exclusively on homozygous or heterozygous sites do not accurately measure mutational load, others (e.g. Pedersen *et al.* 2017) have argued that counting derived homozygous genotypes in the genome better characterizes recessive, rather than additive, variation. In addition, we wanted to investigate whether differences in heterozygosity levels *per se* were affecting our results. We therefore re-analyzed all above statistics using only homozygous sites, in which each site is counted as either a homozygous derived genotype [1] or not [0].

Both Do *et al.* (2015) and Simons and Sella (2015) have pointed out that assessing significance is not straightforward for these analyses. We followed their general suggestion to perform resampling across segments of the genome. To do this, we divided our annotated VCF file into 1000 segments, each with an equal number of contiguous variant sites. We then performed bootstrapping by resampling with replacement, and calculated all statistics on the resampled VCF. This bootstrapping was repeated 1000 times, which allowed us to create 95% confidence intervals for each statistic. *R* statistics were considered significant when the confidence interval did not include the null expectation of 1.00.

We felt that using the full set of Steller sea lion annotated genes was most appropriate for our analyses. To check that including all genes did not bias our results, we repeated the analysis on a VCF that was filtered for only known one-to-one orthologs in mammals, which is a more evolutionarily conservative set of genes. To do this, we downloaded a set of 14,507 genes from OrthoMam v10 (Scornavacca *et al*. 2019), and used bedtools (Quinlan *et al*. 2010) to intersect the CDS of those one-to-one orthologs with our annotated VCF.

Results

Heterozygosity, demographic histories, and divergence times

All genomes included in this study had an average depth of coverage of at least 20X after PCR duplicates were removed (Table S1). Among the seal species we analyzed, genome-wide heterozygosity spanned more than an order of magnitude, from 0.000099 in the Hawaiian monk seal to 0.002500 in the Baltic ringed seal. As shown in Figure 1, when

viewed in the context of other species, our species data set appears to be a representative sampling of heterozygosities from other mammalian orders.

The localized patterns of heterozygosity within a genome can also be used to model the demographic history of a population using SMC methods. Below we report in detail the historical population trends for each species of seal and coalescent divergence times for the most recently diverged species.

Compared to most other species in our analysis, both species of monk seal show population trends that are small and declining, especially in the last 100,000 years (Fig. 2). Notably, the trend lines for these species end considerably earlier than the lines for most other seal species, which is due to complete coalescence occurring earlier in these genomes with low heterozygosity. The long-term effective population sizes are 7,109 for the Hawaiian monk seal and 13,777 for the Mediterranean monk seal (Table 1). Over just the last 100,000 years, the N_e is below 5,000 for both species. We recover a split between the northern elephant seal and southern elephant seal to be ~700-800ka (Supplemental Fig. S6). After their split, the southern elephant seal population stays relatively steady (mean N_e: 70,474) while the northern elephant seal population decreases and remains comparatively smaller (mean N_e: 14,562). Compared to the other species, the Weddell seal shows a moderate N_e (mean: 43,748), with a significant increase over the last 100,000 years (Fig. 2).

The ringed and grey seals are estimated to have split around 2 million years ago (Fulton & Strobeck 2010). The grey seal population is notably smaller than the ringed seal populations after their split (Fig. 2). The two ringed seal subspecies, Baltic and Saimaa, are estimated to have split around 100ka (supplementary figure S7). This split is also evident

in the full MSMC plot (Fig. 2), which shows the Saimaa and Baltic ringed seal population curves aligned before about ~200kya but following separate trends after that time. For at least one million years before their split, the ringed seal subspecies had larger N_e than the other seal species examined (Fig. 2).

Mutational load

Through multiple measures, we quantified the rate of mutation accumulation and proxies for mutational load. The R_{xy} and R'_{xy} statistics we use were developed by Do *et al.* 2015 and capture the relative rates of derived mutation accumulation across lineages. Briefly, the R_{xy} statistic counts the number of derived alleles in the genome of species *X* that are not found in species *Y*, and compare that to the number of derived alleles in the genome of species *Y* that are not found in Species *X*. To account for the potential variation in mutation rate and generation time across lineages, we focus on the R'_{xy} statistic. This statistic normalizes the R_{xy} of certain functional classes (e.g. nonsynonymous, radical) with the R_{xy} of a different functional class (e.g. synonymous, conservative), which is presumed to be evolving neutrally or near-neutrally. In this way, the ratio can be interpreted similarly to *dN/dS* ratios. Significance can be determined by bootstrapping across contiguous regions of the genome. The R_{xy} results are presented and discussed in the supplementary material.

Given that R'_{xy} is a pairwise statistic, we focused on pairs of species that showed clear differences in current population size and/or long-term N_e. For example, our MSMC analysis showed that the endangered Mediterranean monk seal and Hawaiian monk seal have had N_e that are smaller than those of the Weddell seal and southern elephant seal for thousands or tens of thousands of generations (Table 1, Fig. 2). Contrary to expectations,

our analysis shows that R'_{xy} is lower in both monk seal species compared to the Weddell seal and southern elephant seal (Table 2).

This same pattern emerges in phocine seals. Since the lineages split, the grey seal has consistently had a smaller population size than the Baltic ringed seal (Table 1, Fig. 2). The R'_{xy} is significantly lower in the grey seal compared to the Baltic ringed seal, suggesting that mutations have accumulated faster in the larger ringed seal population. Notably, the grey seal and monk seal species, which appear to have historical population trends of similar magnitude and shape, have little or no significant difference in R'_{xy} when compared to each other.

On the other hand, the northern elephant seal appears to have had a smaller N_e than the southern elephant seal since their split (Fig. 2), and in this case the R'_{xy} shows higher mutation accumulation in the northern elephant seal compared to the southern elephant seal. Notably, though, both elephant seals show significantly higher mutation accumulation when compared to every other species. In addition, comparisons of species with apparently similar demographic histories (e.g. Hawaiian monk seal and Mediterranean monk seal, or Mediterranean monk seal and northern elephant seal) show significantly different rates of mutation accumulation.

Besides analyzing the rates of nonsynonymous and synonymous mutation accumulation, we also annotated nonsynonymous variants for how different the derived amino acid was compared to the ancestral amino acid based on biophysical properties (Grantham 1974). Following Li *et al.* 1984, we categorized amino acid changes as conservative, moderately conservative, moderately radical, or radical, with the expectation that radical amino acid changes have more significant fitness effects than conservative

changes (Huzurbazar *et al.* 2010). When we calculated the R'_{xy} statistic with radical amino acid changes as the functional (nonsynonymous) class and synonymous changes as the normalizing class, we obtained qualitatively similar results to the overall patterns observed when the statistic is calculated with all nonsynonymous alleles.

However, a distinct pattern emerges when we calculate R'_{xy} with radical amino acid changes as the functional class (numerator) and conservative amino acid changes as the normalizing class (denominator). In this case, many of the pairwise patterns described are inverted. The taxon with a lower rate of overall nonsynonymous mutation accumulation instead shows a higher ratio of radical-to-conservative amino acid changes (Table 3). Notably, this does not happen in all cases (e.g. there is no significant difference between the northern elephant seal and southern elephant seal, and the pattern does not invert for the comparison of grey seal and Weddell seal).

To test that heterozygous sites *per se* were not biasing our analyses, we calculated all statistics using only homozygous variant sites and found the same qualitative pattern (Supplemental table S8). In addition, we confirmed these patterns through counts of derived alleles (Supplemental material). These counts show qualitatively the same pattern, though to a lesser degree because all shared derived alleles are also included in the counts.

Discussion

A strong relationship between historical climate change and population size across phocid seals

The Hawaiian monk seal and Mediterranean monk seal are unique among living phocids in that they inhabit tropical or sub-tropical waters (Alava 2017). Both of these endangered seal species show long-term small population sizes (i.e. N_e<10,000 for much of their reconstructed history). Furthermore, we reconstruct both monk seal species as having effective population sizes of only a few thousand in the most recent time periods (i.e. the past three thousand generations). This finding is consistent with previous hypotheses, based on ecological estimates and microsatellite diversity, that the historical Hawaiian monk seal population never exceeded a few thousand (Schultz *et al.* 2009, 2010). Thus, our results confirm that the Hawaiian monk seal was already in low numbers well before the arrival of Polynesian settlers on the islands less than 1500 years ago (Kirch 2011). This finding fits with archeological evidence and the biocultural knowledge of native Hawaiians (Kittinger *et al.* 2011).

On the other hand, the historical abundance of the Mediterranean monk seal is more controversial. There are few historical records commenting on the number of seals in the Mediterranean region, although some authors note that references to seals in Classical European literature serve as evidence of their abundance (Johnson & Lavigne 1999). Our results suggest that the species was not extremely abundant prior to human contact, although it is possible that strong population subdivision in this species (Karamanlidis *et al.* 2016b) affects our reconstruction of past population size for this species. Despite a distribution throughout the Mediterranean Sea, factors such as low ecosystem productivity (Stambler 2014) or limited suitable pupping habitat (e.g. shoreline caves and protected beaches, Dendrinos *et al.* (2007) may have kept the overall abundance of seals low. As in the case of the Hawaiian monk seal, our results suggest that the Mediterranean monk seal

population was already small and vulnerable to extinction prior to the extensive human colonization of the Mediterranean coast in the last 10,000 years. Both monk seal species experienced well documented recent population declines due to habitat disruption and hunting (Johnson & Lavigne 1999, Kittinger *et al.* 2011, Karamanlidis *et al.* 2016a), but these declines are too recent to be captured in our MSMC analysis. Future studies with genomes from more individuals will help to elucidate the patterns of recent population decline directly caused by human activities (Terhorst *et al.* 2017).

The northern elephant seal and the grey seal both inhabit temperate waters for all or part of the year (Ferguson & Higdon 2006). Both of these species show population sizes in the recent past that are on the order of those reconstructed for the tropical monk seals. The northern elephant seal and grey seal were both subject to recent anthropogenic bottlenecks (Stoffel *et al.* 2018). The case of the northern elephant seal is especially notable: from an estimated base population of 100,000 individuals, the species was intensively hunted for oil from the 1840s to 1860s, after which it was thought to be possibly extinct (Busch 1985). A remnant population was able to grow over the following century and now the species numbers many hundreds of thousands. While these bottlenecks are too recent to recover in our MSMC analysis, our reconstruction gives important insight into the long-term population size of these species that have recovered from recent bottlenecks, clarifying uncertainties from previous microsatellite-based work (Hoelzel *et al.* 1993, 2002, Hedrick 1995, Abadía-Cardoso *et al.* 2017, Stoffel *et al.* 2018).

Contrary to the demographic patterns of warm- and temperate-water species, the three polar seal species (Weddell seal, southern elephant seal, and ringed seal) all show significantly larger populations over at least the last million years. The Weddell seal is

known to be extremely abundant, with around 200,000–1,000,000 individuals (Southwell *et al.* 2012)) around the coast of Antarctica, and was never subject to extensive hunting or disturbance from humans (Busch 1985). Previous genetic studies found an increase in population size for the Weddell seal coinciding with the glaciation of Antarctica around 81,000 years ago and a current effective population size of around 150,000 (Curtis *et al.* 2009, 2011). Our MSMC analysis is closely aligned with this timing and magnitude (Figure 2), giving independent support to this analysis.

The southern elephant seal, on the other hand, was recorded as very abundant before it became extensively hunted for oil in the 1800s (Busch 1985). However, the southern elephant seal population never experienced the extreme bottleneck of the northern species, and has since recovered to many hundreds of thousands of individuals (Busch 1985). Our analysis shows this population was relatively stable throughout the distant past, with an effective population size similar to previous estimates using microsatellites (Slade *et al.* 1998) and RAD-loci (Peart *et al.* 2020). As noted above, the genetic impact of industrial sealing, as described elsewhere (Stoffel *et al.* 2018), is too recent to be recovered in our analysis.

Ringed seals are extremely abundant, with a circumarctic distribution of over 1 million individuals (Reeves 1998). The two subspecies analyzed here (Baltic ringed seal and Saimaa ringed seal) have smaller populations limited to around 11,000 individuals in the Baltic Sea and 200 individuals in Lake Saimaa, respectively (Kokko *et al.* 1999, Nyman *et al.* 2014). Our MSMC analysis shows historically large effective population sizes for both of these subspecies, but also precipitous declines starting around 100,000 years ago in the case of the Saimaa ringed seal and 30,000 years ago in the Baltic ringed seal (Figure

2). This timing suggests that extensive Arctic ice cover during the last glaciation may have caused a subdivision of and decline in the global ringed seal population. Our results agree with previous microsatellite studies which have suggested that heterozygosity in the Baltic ringed seal was at least two times greater than in the Saimaa ringed seal (Nyman *et al.* 2014), and that the long-term N_e of the Baltic ringed seal was about 1.5X larger than the grey seal (Palo *et al.* 2001).

Looking across these species, one clear picture emerges: historical climate change significantly impacted many phocid seal populations. This finding is not surprising, given that these coastal marine mammals may be reliant on coastal terrestrial habitats, coastal ecosystem productivity, ice cover and distribution, and regional storm patterns. On the other hand, we argue that these specific responses of each species are in fact idiosyncratic and cannot directly be used for future predictions about a species' response to climate change. For example, the warm-water monk seal species both experienced declines during the last glacial period. However, we cannot say whether this decline is explained by a cooling climate or rather by a dramatic change in climate. If warm-water carrying capacities are affected by perturbations to coastal ecosystems, then a rapidly warming climate could also affect the abundance of these species. Likewise, in the ice-breeding seal species we see that expanding ice around Antarctica coincides with a dramatically increasing Weddell seal population, while expanding Arctic ice cover eventually leads to a subdivision and presumed range restriction of ringed seals. Future in depth modelling is required to better understand how each of these species may respond to current and future climate change, but our historical population reconstructions offer a grave warning that

seal species are very susceptible to dramatic changes in climate (O'Corry-Crowe 2008, Kovacs *et al.* 2012).

Divergence times of species and subspecies

We tested two approaches to dating divergent times through an extension of PSMC. Both approaches take a haploid genome from two populations and combine them into a pseudo-diploid genome, on which PSMC is run. The effective population size estimated by the model runs off to infinity at the time when gene flow stops between the two populations, but the model gives realistic estimates of N_e prior to the split. For each comparison, we ran PSMC separately on a pseudo-diploid genome generated by combining the X-chromosome sequence of two male individuals and on a pseudo-diploid genome generated by randomly selecting a basepair from the full genomes of each of the two individuals. In every case, the results from the two methods agreed, which is expected in cases such as ours when most segregating variation is not shared between the populations.

While most of our divergence time estimates align well with previous estimates based on fossil-calibrated phylogenies, our analysis provides novel insights into the divergence of two groups of taxa. First, we find that the two elephant seal species diverged around 700–800 ka. This estimate is much younger than most phylogenetic studies of these species (e.g. Fyler *et al.* 2005, Fulton & Strobeck 2010) but matches closely with an earlier estimate from Slade *et al.* 1998. Biogeography based on fossils, however, have supported a later colonization of the North Pacific, during the Early (2500–770 ka) or Middle (770–126 ka) Pleistocene (Boessenecker & Churchill 2016). In fact, a fossil from the Middle or Late Pleistocene was discovered in northern Chile (Valenzuela-Toro *et al.* 2015), which

could represent the time when the species diverged or when the two species still had a close enough range to have persistent gene flow. Because pseudo-diploid PSMC is thought to indicate the cessation of gene flow between populations, it is possible that our younger split time reflects the end of gene flow even if the species had begun to split substantially before this time. In our full MSMC graph (Figure 2), at around this time the northern elephant seal curve appears to start aligning with the SES curve, as would be expected at a time before the population split. However, this happens to be the same time period that the north elephant seal genome reaches coalescence and therefore we cannot reconstruct the alignment of the curves prior to this point.

We also are able to shed light on the previously contentious demographic history of the Saimaa ringed seal, the world's most endangered pinniped. It has commonly been assumed that this subspecies became isolated from other ringed seal populations when Lake Saimaa was formed around 9500 years ago (Valtonen *et al.* 2012, Nyman *et al.* 2014, Savriama *et al.* 2018), and previous studies have used this data as a strict prior. For example, a study of mtDNA diversity in these subspecies (Valtonen *et al*. 2012) used simulations to show that under commonly assumed mammalian mutation rates, the Saimaa and Baltic ringed seal subspecies separated 95,000 years ago, which the authors rejected as impossible to reconcile with geological data. The authors suggested that mutational hotspots in ringed seals may explain this older date. However, our MSMC whole-genome analysis (Figure 2) and the pseudo-diploid PSMC analysis (supplementary figure S7) show that these subspecies split around 100,000 years ago. In addition, we find no evidence that genome-wide synonymous mutation rates are higher in ringed seals than other seals (supplementary table S4). Our results suggest that the evolutionary history of ringed seals

is more complex than a single geographic isolation caused by the formation of Lake Saimaa. More generally, this demonstrates the usefulness of nonparameterized modelling from genome sequence data compared to more user-defined demographic model testing.

Mutation accumulation

Through this analysis, we detected statistically significant differences in mutation accumulation and the rates of molecular evolution in almost all of our pairwise comparisons. Surprisingly, many of these signals were in the opposite direction as would be predicted by a classical interpretation of nearly neutral theory. To make sense of this, we first consider methodological errors/artefacts that could give rise to these results. After rejecting those, we consider biological explanations and make suggestions for future empirical studies to further explore these explanations.

One possible explanation is that insufficient time has passed for differences to accumulate in these lineages. Theory predicts that these processes should play out over time scales related to fixation time ($4N$ generations) and mutational input ($1/2Nu$ generations, Simons *et al.* 2014). In our species, we expect this to be equivalent to tens to hundreds of thousands of years. Indeed, the fact that we detect no statistically significant differences between the Baltic ringed seal and the Saimaa ringed seal may be attributable to the relatively shallow divergence (100–200 ka) and more recent difference in population size of these species. However, all of our other species split well before 100ka, and we detect statistically significant differences in the more recently split species (e.g. northern and southern elephant seals; grey and ringed seals). Therefore, although our results support

the idea that differences in mutation accumulation are long-term processes, our sample of species clearly allows sufficient evolutionary time for these patterns to emerge.

Another possibility is that our demographic reconstructions are deeply flawed and that the actual historical population sizes were much different than what we reconstruct. Indeed, much has been written on the dangers of misinterpreting SMC results (Mazet *et al.* 2016) as well as the limitations of the method itself (Beichman *et al.* 2017, 2018). Our primary concern would be if current small population sizes biased historical estimates (e.g. through the recent loss of genetic diversity). However, other studies of recently bottlenecked species have still reconstructed large historical population sizes (e.g. Der Sarkissian *et al.* 2015, Osada *et al.* 2015, Abascal *et al.* 2016). Through our analysis of the Saimaa and Baltic ringed seals, we also show that this is unlikely to be a problem. The Saimaa ringed seal has lost significant genetic diversity through a well-documented bottleneck, yet we successfully recover a large historical population size that matches perfectly with the curve of the Baltic ringed seal before the taxa split. While it is possible that recent demographic events could still have affected the most recent time periods of the model, our ringed seal results suggest that we can accurately estimate deeper demographic history even from genomes of severely bottlenecked species. In addition, the patterns we recover generally match those described in previous genetic studies of these species (e.g. Schultz *et al.* 2010, Curtis *et al.* 2011, Peart *et al.* 2020).

Finally, it could be that the statistics we use are not measuring mutation accumulation or are otherwise confounded by artefacts in our data. As mentioned above, population genetic theory and simulations have shown that comparisons based solely on polymorphisms (e.g. pN/pS) are strongly influenced by demography because the site

frequency spectra of synonymous and nonsynonymous alleles respond differently to demographic changes (Simons & Sella 2015). Though using the presence of homozygous derived alleles has also been rejected (Simons & Sella 2015), we recover the same patterns in our data when using only homozygous sites (supplementary material). This finding is expected given that in species that are separated by millions of years, the vast majority of derived alleles are expected to be fixed or lost. Interpreting our results also requires many assumptions (e.g. that most mutations are deleterious, that most fixed alleles are not the result of positive selection, and that mutation is a random process). However, the basic population genetic theories being tested here make these same assumptions.

Assuming, then, that our results are not artefacts of our study design, we turn to possible biological explanations for the patterns we observe. The first possibility is that the population sizes during the time period we have reconstructed (i.e. 10 ka to 1 Ma) are very different from the sizes further back in time (e.g. 3–6Ma). To explain our observed pattern, this would require the species that have been smaller population sizes for recent time (<1 Ma) in fact had much larger populations than the recently large populations. It is not obvious why this should be the case, though it is biologically plausible, especially if species distributions, climate, and productivity were different in the distant past. In order for this first biological explanation to be true, our reconstructions would have to be wrong and the patterns of large and small populations would have had to be swapped for many species. Such a perfect combination of events seems unlikely.

Another explanation is that positive selection could be a much more pervasive force than is typically assumed in molecular evolution. If many or most new mutations are beneficial, then molecular evolution is predicted to occur at a higher rate in larger

populations, as is observed in our study. The prevalence of positive selection in molecular evolution has been widely debated (Boyko *et al.* 2008, Keightley & Eyre-Walker 2010, Huber *et al.* 2017), and we acknowledge that this may play a role in the signal we observe. Still, to explain our observed pattern the signal from positive selection would have to strongly outweigh the reduced efficiency of purifying selection predicted by nearly neutral theory. This pattern on a genome-wide scale would be surprisingly inconsistent with fundamental assumptions about patterns of selection and the fitness effects of new mutations.

In the study of small populations, purging of deleterious alleles is often proposed as mechanism to prevent the fixation of deleterious alleles. In our case, this seems an unsatisfactory explanation for a number of reasons. The first is that the pattern we observe is genome-wide. Purging requires that alleles act in a Mendelian recessive way, rather than an additive one (Fuller *et al.* 2019). However, data and theory in molecular population genetics suggest that additive effect loci make up a substantial portion of the genome (Hill *et al.* 2008). Second, if purging were the dominant explanation then we would expect the highest-effect mutations to be purged most easily in the small populations, leading to a pattern of lower rates of mutation accumulation for this class of alleles. In fact, the pattern we observe is the opposite, with relative rates of presumably large-effect mutations (e.g. premature stop codons and radical amino acid changes) being higher in smaller populations. Therefore, widespread purging does not fit with the overall pattern we observe.

Instead, we propose two plausible biological explanations that seem to fit the observed patterns in this study. The first is that rates of molecular evolution and patterns

of mutation accumulation are not affected by population size, or are much more strongly affected by other factors. For example, shared phylogenetic history, life history traits, molecular phenotypes, or other biological or ecological factors could strongly affect molecular evolution in mammals (e.g. Smith & Eyre-Walker 2003, Welch *et al.* 2008, Bromham 2011). Although we have tried to minimize these factors by focusing on a single mammalian family (Phocidae) and looking for repeated patterns across clades within that family, relevant outlier traits still remain. For example, the elephant seal species have an extremely polygynous mating system, with some of the most extreme sexual dimorphism observed in any mammal (Le Boeuf & Laws 1994). Compared to other closely related seal species, the two species of elephant seals have elevated rates of mutation accumulation of almost every functional class (Table 2, Supplementary Table S4). It is possible that the extreme life history traits of this species are causally related to their increased mutation accumulation. We note, however, that that the only other moderately polygynous species in our data set (i.e. grey seal) apparently exhibits opposite patterns of molecular evolution. Notably, we also see clear phylogenetic signal in our analyses, though we cannot tease apart the proportion of this due to ancestral signal and that relating to lineage characteristics.

The second plausible explanation is that—contrary to assumptions in the literature—the distribution of fitness effects (DFE) of new mutations may not be the same across populations. In fact, the DFE itself may not be independent of population size or evolutionary history. If this is the case, it would have profound implications for the patterns we would expect to see in empirical systems, and as well as how we interpret those patterns.

Some theoretical frameworks, themselves consistent with the mathematics of nearly neutral theory, predict that mutation accumulation patterns should be mostly independent of population size due to a feedback between molecular evolution and the DFE of new mutations. These proposals include theoretical models (Cherry 1998), population genetic simulations (Labar and Adami 2017), protein model simulation (Goldstein 2013), and empirical studies of DFE across species (Huber *et al*. 2017). As Silander *et al*. (2007) argue based on mutation accumulation experiments, mutational effects are dynamic, depend on the genetic context in which they arise, and can shift over short timescales. Under these frameworks, smaller populations have lower fitness than larger populations, but this in turn makes the smaller populations less tolerant of new mutations. In other words, the evolutionary history of a population influences the DFE of new mutations.

While not conclusive, we believe that our results may provide empirical support for this theoretical framework. We find that smaller populations (monk seals, grey seals) have lower rates of mutation accumulation compared to larger populations. Notably, our larger populations have gone through recent expansions (Weddell seal, ringed seals) or declines (elephant seals, ringed seals). This observed pattern appears to be in line with quantitative genetic and protein evolution models (Cherry 1998, Goldstein 2013) that predict an elevated rate of molecular evolution when populations change in size, but not from population size *per se*.

On the other hand, our smaller populations show higher ratios of radical-to-conservative amino acid changes. This surprising difference could have a number of explanations. These ratios could suggest a difference in DFE, leading to smaller populations fixing a greater proportion of large effect mutations. Notably, as predicted by

Labar and Adami (2017), our results show that our smaller populations have fewer nonsynonymous changes overall and yet a greater proportion of large-effect changes. Another possibility is that the higher radical-to-conservative ratio is indicative of positive selection in these populations, as has been proposed at the gene level (Hughes & Hughes 1993, Zhang 2000). Huzurbazar *et al*. (2010), however, used a simulation framework to show that selection coefficients decreased with an increasing Grantham score, which suggests that fixed radical changes may actually be more neutral. This result is similar to a theoretical argument made by Simons and Sella (2015) that the effect of an amino-acid change may be unexpectedly low conditional upon that allele reaching a high frequency in the population. In general, though, we believe that the theoretical (Simons and Sella 2015) and empirical (Henn *et al*. 2015, Huber *et al*. 2020) evidence suggests we should be skeptical of annotating classes of nonsynonymous variants, especially in non-model species.

Conservation implications

Our study provides important insight in the evolutionary history of some of the world's most endangered marine mammals. As discussed above, demographic reconstructions indicate that both species of monk seal had relatively small and declining populations prior to any human interactions. This result suggests that the Mediterranean and Hawaiian monk seal species were already vulnerable to extinction when human populations expanded across the Mediterranean region around 5000 years ago and when Polynesians arrived in Hawaii around 1000 years ago. While both ancient and modern humans have undoubtedly had an impact on these species, other biological and ecological

factors likely have played a role in their vulnerability to extinction and slow recovery. Our study will help to establish realistic recovery goals for these species based on historical population sizes.

The Saimaa ringed seal is another endangered pinniped, with fewer than 200 mature individuals remaining. Our analysis suggests that the split between Saimaa ringed seals and other ringed seal subspecies is much older than previously proposed. This divergence estimate suggests that the evolutionary history of ringed seals, and perhaps other Arctic pinnipeds, is complex, with unappreciated geological and climatic phenomena likely playing a role in species' distribution and evolution. Importantly, this finding also highlights the evolutionary uniqueness of the Saimaa ringed seal and may warrant a taxonomic re-examination of this critically endangered taxon.

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Figures and Tables

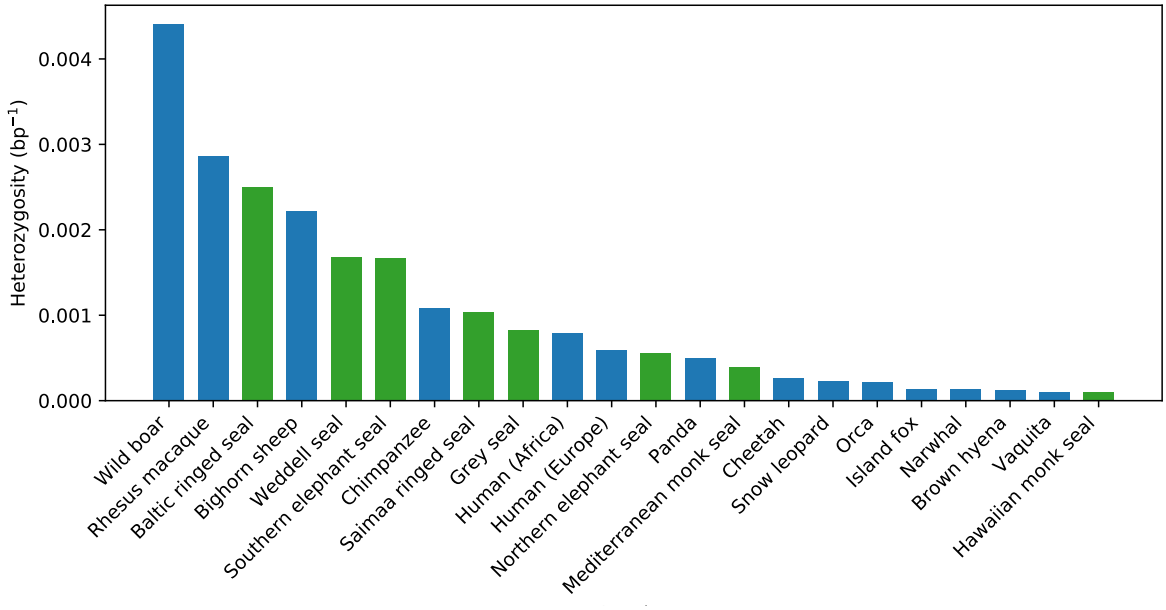

Figure 1. Genome-wide heterozygosity (bp^{-1}) for 25 species of seals (green) and other mammal species (blue). Data and citations can be found in Table S3.

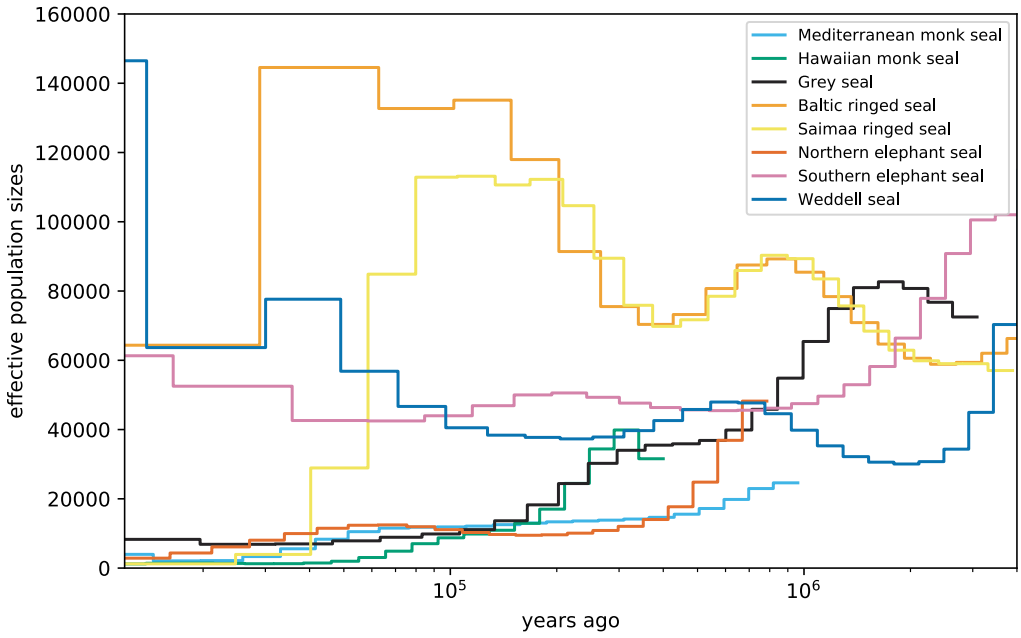

Figure 2. Reconstructed historical population sizes with MSMC from a single diploid genome of each species or subspecies.

Table 1. Taxa in this study, with respective heterozygosity and population size estimates.

Species	Census population size (N_c)	Genome-wide heterozygosity	Harmonic Mean of N_e	Harmonic Mean of N_e (last 100 ka)
Hawaiian monk seal (HMS)	632	0.000099	7,109	2,098
Mediterranean monk seal (MMS)	400	0.000396	13,777	4,267
Northern elephant seal (NES)	110,000	0.000552	14,562	5,944
Southern elephant seal (SES)	325,000	0.001664	70,474	56,232
Weddell seal (WED)	300,000	0.001676	43,748	82,621
Grey seal (GRS)	316,000	0.000821	46,286	7,440
Baltic ringed seal (BRS)	11,500	0.002500	70,497	64,354
Saimaa ringed seal (SRS)	150	0.001035	52,701	3,508

Table 2. R'_{xy} values in which the column is species X and the row is species Y. R'_{xy} values > 1.000 show elevated rates of amino-acid mutation accumulation in species X, and R'_{xy} values < 1.000 show lower rates of amino-acid mutation accumulation in species X.

	HMS	MMS	NES	SES	WED	GRS	BRS	SRS
HMS	-							
MMS	1.045	-						
NES	0.925	0.888	-					
SES	0.941	0.903	1.068	-				
WED	0.971	0.933	1.047	1.027	-			
GRS	1.017	NS	1.072	1.059	1.035	-		
BRS	NS	0.980	1.060	1.047	1.023	0.958	-	
SRS	NS	0.979	1.059	1.045	1.022	0.953	NS	-

Table 3. R'_{xy} values with radical amino acid changes as the functional class and conservative amino acid changes as the normalizing class. The column is species X and the row is species Y. R'_{xy} values > 1.000 show elevated rates of amino acid mutation accumulation in species X, and R'_{xy} values < 1.000 show lower rates of amino acid mutation accumulation in species X.

	HMS	MMS	NES	SES	WED	GRS	BRS	SRS
HMS	-							
MMS	NS	-						
NES	1.080	1.096	-					
SES	1.099	1.115	NS	-				
WED	NS	NS	0.920	0.902	-			
GRS	1.087	1.089	NS	NS	1.082	-		
BRS	1.117	1.121	1.070	1.055	1.113	1.088	-	
SRS	1.100	1.104	NS	NS	1.096	NS	NS	-

Supplemental Material

I. Genome coverage statistics

Table S1. Sample ID and genome coverage statistics for each sample examined in this study.

Species	Sample ID	Median depth	SD depth
Hawaiian monk seal[1]	PJ22	22X	8.2
Hawaiian monk seal[1]	YE37	21X	8.0
Mediterranean monk seal[1]	114	57X	17.6
Mediterranean monk seal[1]	195	20X	8.2
Southern elephant seal[1]	612	37X	13.6
Northern elephant seal[2]	NES	81X	23.3
Weddell seal[3]	WED	65X	21.1
Baltic ringed seal[4]	PHB03	16X	8.5
Saimaa ringed seal[4]	PHS1983	16X	9.0
Grey seal[4]	HG01	21X	15.0

[1]This study; [2]https://www.dnazoo.org/assemblies/Mirounga_angustirostris; [3]https://www.ncbi.nlm.nih.gov/assembly/GCF_000349705.1/; [4]Savriama *et al.* 2018

II. Annotating probable sex chromosomes

For many of our analyses, we wanted to include only autosomes or only haploid X-chromosomes. Because our reference genomes were not chromosome-level assemblies, we needed to create a list of scaffolds from our assembly that likely belonged on the seal X and Y chromosomes. As an X chromosome reference, we used the X chromosome from the dog (*Canis familiaris*) reference genome (CanFam3.1), which is a high-quality chromosome-level assembly. We used the Genbank SRY reference sequence for the Hawaiian monk seal (AY424654.1) as a Y-chromosome reference. Using BLAST, we were able to find scaffolds in the reference genomes that are likely compromise the sex chromosomes.

In mammals, the Y chromosome is repetitive and mostly lacking protein coding sequence, making it difficult to assemble in most genome assembly pipelines. We made the reasonable assumption that no Y chromosome scaffolds were above 1 Mb, and

therefore were not at risk in being included in our demographic analyses. Still, we were able to identify that the SRY gene, which is the hallmark Y chromosome gene in mammals, is located on scaffold NW_018730440.1 in the Hawaiian monk seal reference genome. The Steller sea lion reference genome is assembled from a female seal and therefore does not contain Y chromosome sequences.

The X chromosome, on the other hand, is a large chromosome in most mammal species, including many protein-coding sequences. We were able to identify many scaffolds in our assemblies that likely correspond to the X chromosome and were therefore excluded in our MSMC and mutation accumulation analyses. The following scaffolds from the Hawaiian monk seal reference genome mapped to the dog X chromosome:

NW_018726532.1, NW_018726533.1, NW_018726534.1, NW_018726535.1, NW_018726536.1, NW_018726537.1, NW_018726538.1, NW_018726539.1, NW_018726540.1, NW_018726541.1, NW_018726542.1, NW_018726543.1, NW_018726544.1, NW_018726545.1, NW_018726546.1, NW_018726547.1, NW_018726548.1, NW_018726549.1, NW_018726550.1, NW_018726551.1, NW_018726552.1, NW_018726553.1, NW_018727768.1, NW_018729375.1, NW_018729545.1, NW_018729664.1, NW_018729739.1, NW_018729752.1, NW_018729761.1, NW_018729802.1, NW_018729916.1, NW_018730077.1, NW_018730097.1, and NW_018731547.1.

For the Steller sea lion reference genome, the following scaffolds mapped to the dog X chromosome:

NW_020998626.1, NW_020998672.1, NW_020998679.1, NW_020998694.1, NW_020998717.1, NW_020998719.1, NW_020998726.1, NW_020998728.1, NW_020998745.1, NW_020998747.1, NW_020998749.1, NW_020998756.1, NW_020998762.1, NW_020998765.1, NW_020998777.1, NW_020998785.1, NW_020998787.1, NW_020998794.1, NW_020998795.1, NW_020998797.1, NW_020998799.1, NW_020998802.1, NW_020998806.1, NW_020998807.1, NW_020998811.1, NW_020998814.1, NW_020998821.1, NW_020998824.1, NW_020998825.1, NW_020998827.1, NW_020998829.1, NW_020998834.1, NW_020998835.1, NW_020998838.1, NW_020998843.1, NW_020998851.1, NW_020998853.1, NW_020998858.1, and NW_020998859.1

III. MSMC supplement

A. Scaffolds, mutation rate and generation time

To run MSMC, only scaffolds from the Hawaiian monk seal reference genome that were larger than 1Mb were used. Scaffolds that mapped to the dog X chromosome were excluded. In addition, one scaffold (NW_018730126.1) appeared to be monomorphic in many samples, which could indicate that it is a poorly assembled scaffold or that it is part of the X chromosome in seals. In either case, this scaffold was removed from the analysis. In total, 128 scaffolds over 1Mb were used for the MSMC analysis.

MSMC analyses output results that need to be scaled by generation time and mutation rate in order to be interpretable as N_e and *years before present*. As others have noted (Nadachowska-Brzyska *et al*. 2016, Mather *et al*. 2020), errors in these parameters change the scale of the MSMC plots but do not qualitatively change the shape of the plots. For non-model species, knowing either of these parameters with certainty is currently impossible. However, a recent study estimated the mutation rate in seals from the neutral substitution rate, obtaining a per generation mutation rate estimate of ~7×10^{-9} bp^{-1} (Peart *et al.* 2020). We used this as the mutation rate for this study.

Estimates of generation times differ substantially between species and between studies of the same species. We used generation times from previously published molecular work to make our study comparable to previous genetic studies. Errors in the generation time estimates, as well as inherent biological issues such as overlapping generations and changes to generation time during the evolution of a lineage, undoubtedly introduce error into our timing estimates. Table SXX shows the generation times used for each species.

Table S2. Generation-time estimate and reference for each species.

Species	Generation time	Reference
Hawaiian monk seal	13 years	Schultz *et al.* 2010
Mediterranean monk seal	11 years	Karamanlidis & Dendrinos 2015
Northern/Southern elephant seal	8 years	Slade *et al.* 1998
Weddell seal	9 years	Curtis *et al.* 2009
Baltic/Saimaa ringed seal	11 years	Palo *et al.* 2001
Grey seal	14 years	Kilmova *et al.* 2014

B. In depth plots

A number of our species share similar habitats, life histories, or IUCN status. Below are various plots that show the same MSMC demographic reconstructions as in Main Figure 2, but here grouped into subsets (e.g. clade, climate, threat status) and adjusted to show time periods and scales of interest.

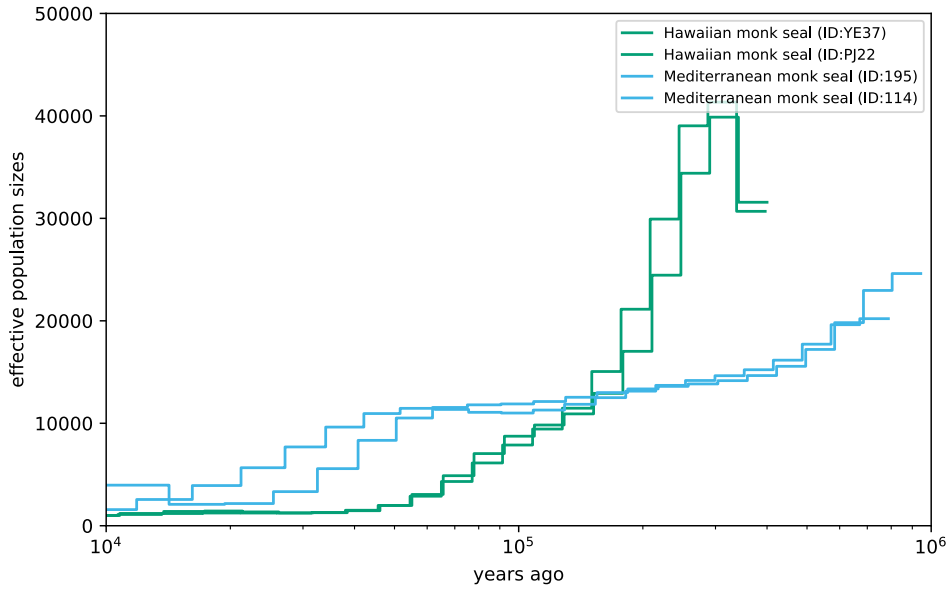

Figure S1a. Reconstructed historical population sizes with MSMC from the genomes of two Hawaiian monk seals (ID: YE37 and PJ22) and two Mediterranean monk seals (ID: 114 and 195). The similar trajectories for samples of the same species show that sample choice does not appear to introduce bias into our demographic analyses.

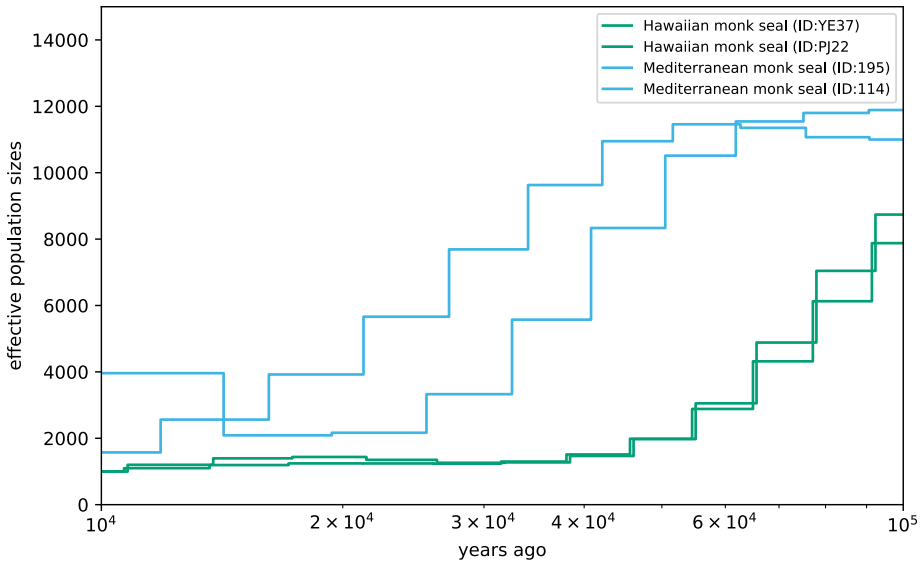

Figure S1b. The same reconstructed demographic histories as in Figure S1a, but focused on the interval between 10,000 and 100,000 years ago (i.e. the last glacial period).

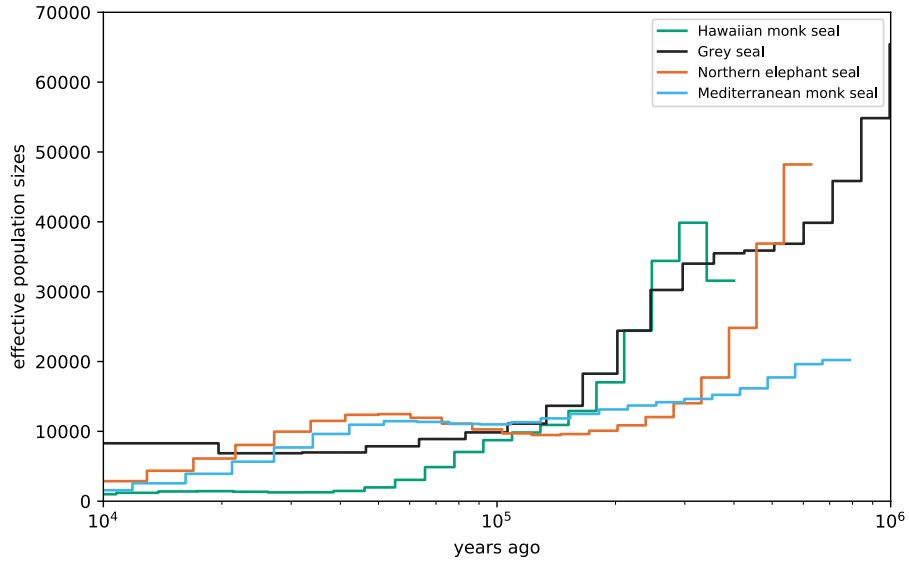

Figure S2a. Reconstructed historical population sizes with MSMC from the genomes of tropical and temperate water species (Hawaiian monk seal, Mediterranean monk seal, northern elephant seal, and grey seal).

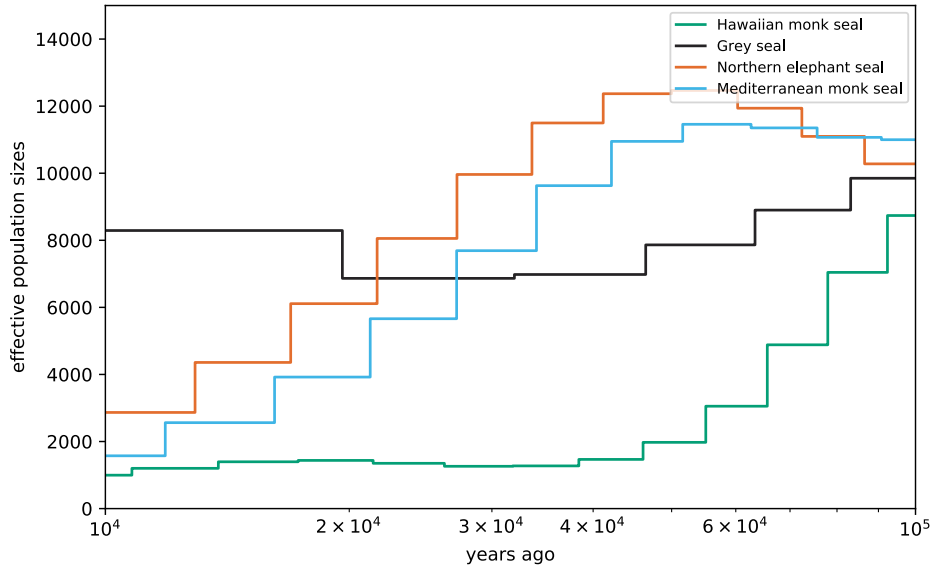

Figure S2b. The same reconstructed demographic histories as in Figure S2a, but focused on the interval between 10,000 and 100,000 years ago (i.e. the last glacial period).

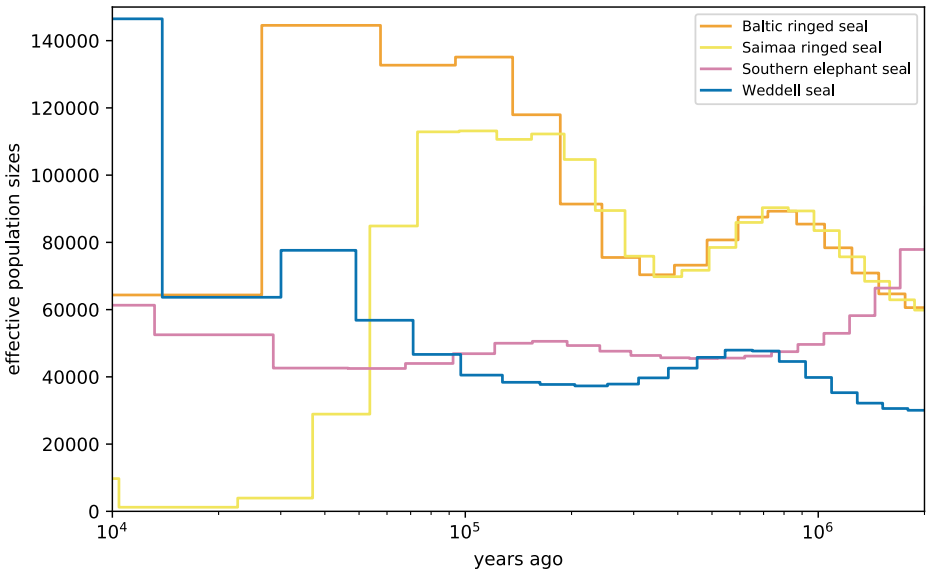

Figure S3a. Reconstructed historical population sizes with MSMC from the genomes of polar water species (Weddell seal, southern elephant seal, ringed seals).

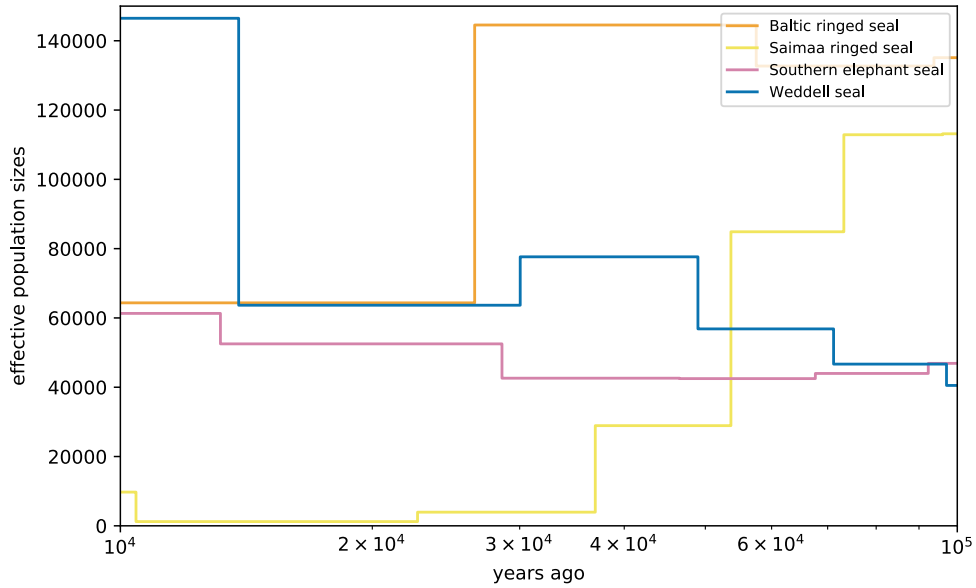

Figure S3b. The same reconstructed demographic histories as in Figure S3a, but focused on the interval between 10,000 and 100,000 years ago (i.e. the last glacial period).

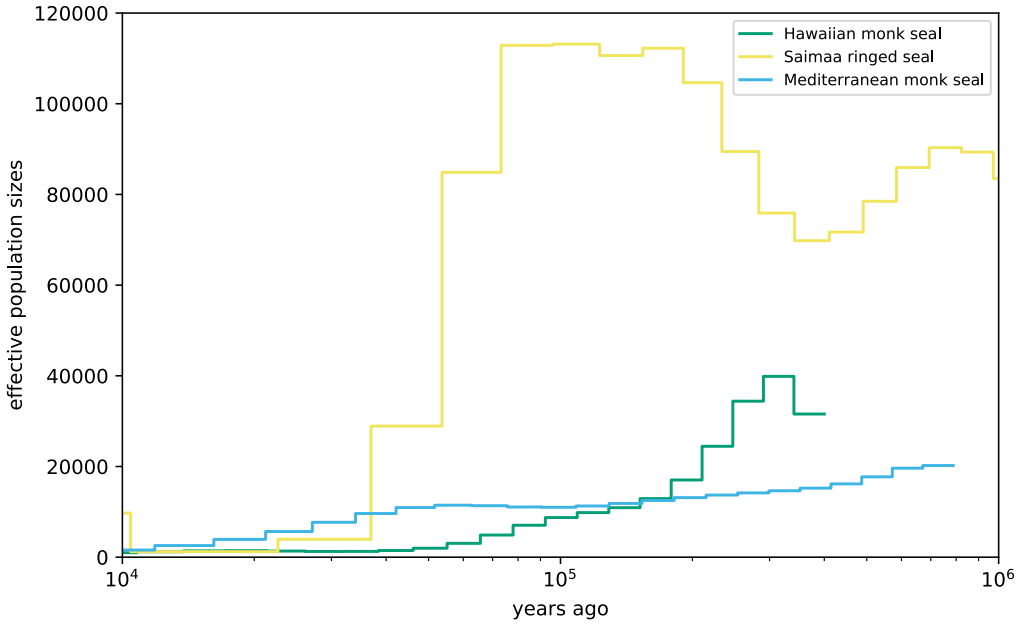

Figure S4a. Reconstructed historical population sizes with MSMC from the genomes of species listed as Endangered by the IUCN (Hawaiian monk seal, Mediterranean monk seal, Saimaa ringed seal).

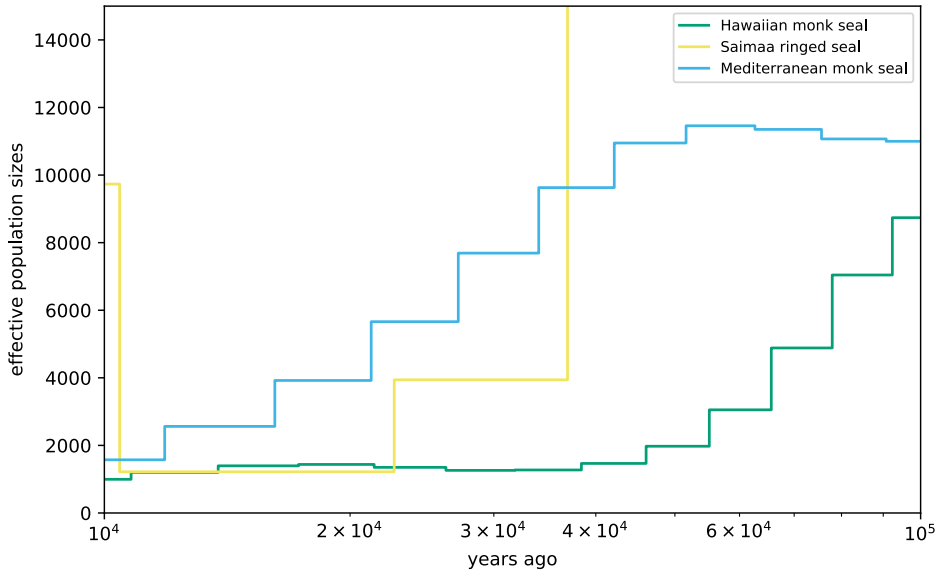

Figure S4b. The same reconstructed demographic histories as in Figure S4a, but focused on the interval between 10,000 and 100,000 years ago (i.e. the last glacial period).

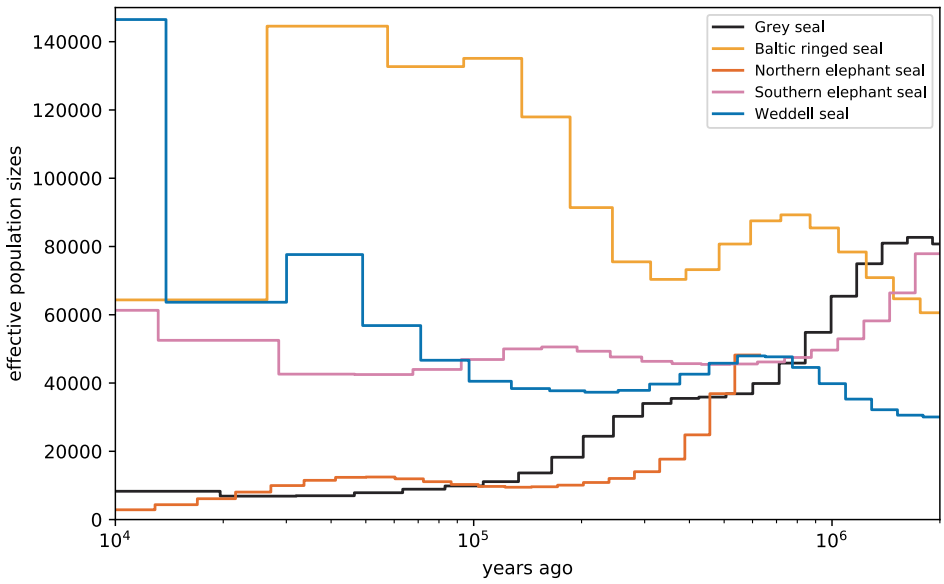

Figure S5a. Reconstructed historical population sizes with MSMC from the genomes of species listed as Least Concern by the IUCN (grey seal, Baltic ringed seal, northern elephant seal, southern elephant seal, Weddell seal).

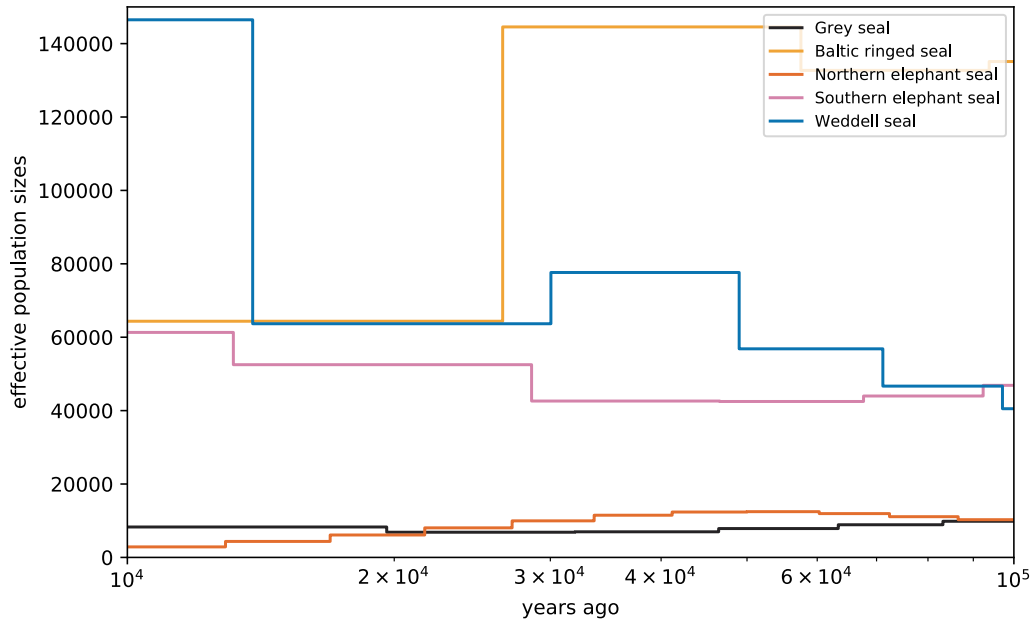

Figure S5b. The same reconstructed demographic histories as in Figure S5a, but focused on the interval between 10,000 and 100,000 years ago (i.e. the last glacial period).

IV. hPSMC and divergence estimates

A. Detailed methods

From its creation, the pairwise sequentially Markovian coalescent (PSMC) model has been shown to recover divergence times when applied to pseudo-diploid chromosomes created by combining haploid chromosomes from two populations or species. With unphased genomes, Li and Durbin (2011) showed this pseudo-diploid chromosome could be created by combining the haploid X chromosomes from two male samples. Cahill *et al.* (2016) alternatively proposed an hPSMC pipeline that creates a full pseudo-diploid genome by randomly selecting an allele for every site in each sample.

On the one hand, using only X chromosomes is expected to create some error because the amount of sequence is relatively short and because the X chromosome may have a different demographic and mutational history than the autosomes. On the other, the hPSMC method introduces some error through the random selection of alleles. This error is expected to be exacerbated in recently-diverged populations that share many of the same segregating alleles. In light of this, we applied both methods and compared the resulting divergence estimates.

We used angsd -doFasta 2 to create a FASTA file from each sample's bam file. This method selects the most common base at a site to be included in the FASTA file, which decreases the likelihood of selecting bases that are due to sequencing error. In addition, we required a minimum base quality of 35, a minimum MapQ of 20, and a maximum depth of 3X the median sample depth. We discarded reads that had more than one best mapping hit (-uniqueOnly 1), and applied the -C50 flag to adjust the map quality of reads with excessive mismatches. We then applied the hPSMC pipeline to combine the haploidized X chromosomes or full genomes from different populations and ran PSMC on these pseudo-diploid X chromosomes or genomes. To avoid saturation of heterozygous sites, we binned sites by 10 rather than the PSMC standard of 100. Because the main northern elephant seal sample we used in this study was female, we performed additional illumina paired-end sequencing on a male northern elephant seal sample (ID:3747), resulting in a genome with a median depth of 38X (SD: 18.1). We used this sample for our haploid X chromosome, but the main northern elephant seal sample used in the rest of the study was used for the pseudo-diploid genome hPSMC. For plotting, the X chromosome effective population sizes were scaled by 4/3 to adjust for the expected 3/4 ratio of

autosomal to X chromosome N_e. In addition, the X chromosome mutation rate was scaled by 0.9, as recommended by Li and Durbin (2011), to account for the ratio of male-to-female mutation rates.

As seen in Figure S6, both the X-chromosome and pseudo-diploid genome hPSMC analyses show that gene flow ceased between the northern and southern elephant seal about 700–800 ka. Prior to divergence, the hPSMC results broadly follow the southern elephant seal MSMC curve in both magnitude and shape. Figure S7 shows the same analyses from the ringed seal subspecies. In this case, divergence appears to happen between 100–200 ka. Again, prior to divergence the hPSMC results appear to generally track the whole genome MSMC results. We show that in both the elephant seal and ringed seal example, the two hPSMC methods (X-chromosome or pseudo-diploid genome) are in strong agreement. This agreement suggests that our estimates are not being strongly influenced by the above-mentioned sources of error.

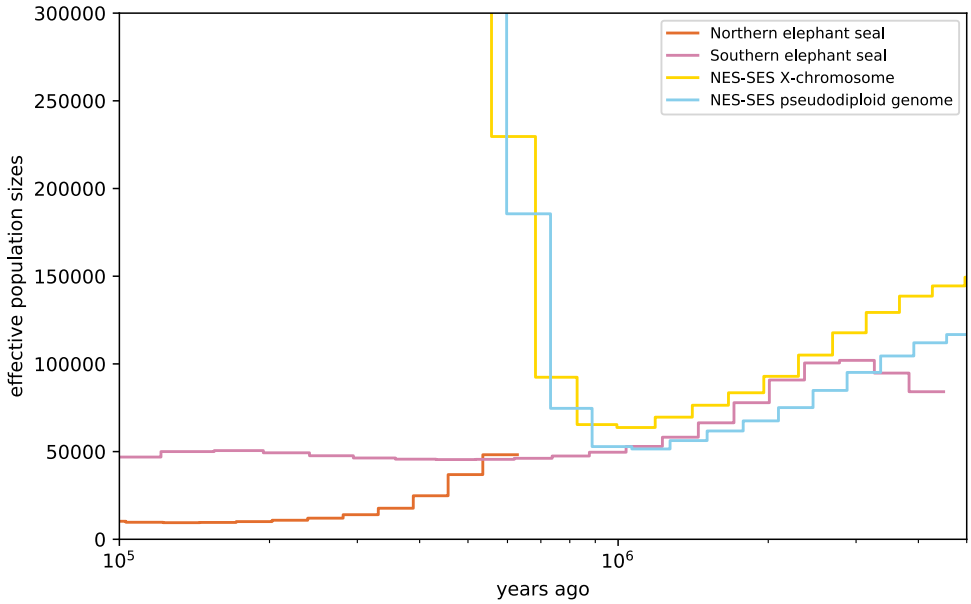

Figure S6. Reconstructed demographic histories from the whole-genome northern elephant seal (red) and southern elephant seal (purple) MSMC results, as well as the divergence hPSMC results derived from X-chromosome (gold) or genome pseudo-diploidization (blue).

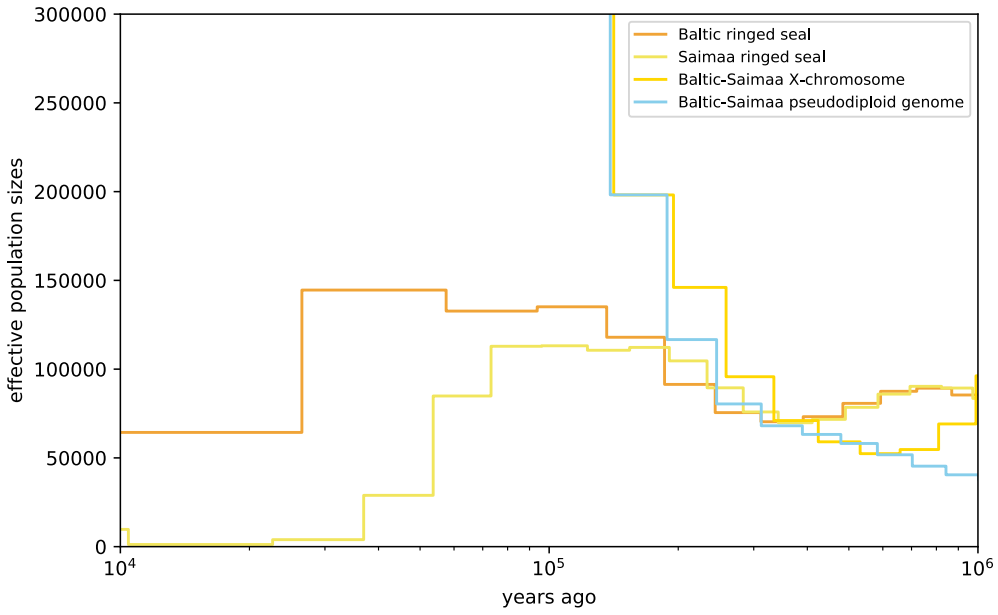

Figure S7. Reconstructed demographic histories from the whole-genome Baltic ringed seal (orange) and Saimaa ringed seal (yellow) MSMC results, as well as the divergence hPSMC results derived from X-chromosome (gold) or genome pseudo-diploidization (blue).

V. Comparative heterozygosity across species

Table S3. Genome-wide heterozygosity (bp^{-1}) for a number of species. Standard deviations, and therefore coefficients of variation, were only available from this study and Westbury *et al.* 2018.

Species	Heterozygosity (bp^{-1})	CV	SD
Hawaiian monk seal[1]	0.000099	0.2657	0.000026
Mediterranean monk seal[1]	0.000396	0.1780	0.000071
Northern elephant seal[1]	0.000552	0.1525	0.000084
Southern elephant seal[1]	0.001664	0.1388	0.000231
Weddell seal[1]	0.001676	0.0937	0.000157
Saimaa ringed seal[1]	0.001035	0.4320	0.000447
Baltic ringed seal[1]	0.002500	0.1157	0.000289
Grey seal[1]	0.000821	0.1410	0.000116
Chimpanzee[2]	0.00108	0.1667	0.000180
Human (Africa)[2]	0.000791	0.2351	0.000186
Human (Europe)[2]	0.000595	0.2857	0.000170
Panda[2]	0.000497	0.7787	0.000387
Cheetah[2]	0.000269	0.1673	0.000045
Orca[2]	0.000214	0.1916	0.000041
San Miguel Island fox[2]	0.000139	0.5899	0.000082
Brown hyena[2]	0.000121	0.1900	0.000023
Snow leopard[3,4]	0.000231	NA	NA
Big horn sheep[3,5]	0.002218	NA	NA
Rhesus macaque[3,5]	0.002867	NA	NA
Wild boar[3,5]	0.004408	NA	NA
Vaquita[6]	0.000105	NA	NA
Narwhal[7]	0.000138	NA	NA

[1]This study; [2]Westbury *et al.* 2018; [3]Robinson *et al.* 2016; [4]Cho et al. 2013; [5]Corbett-Detig *et al.* 2015; [6]Morin *et al.* 2020; [7]Westbury *et al.* 2019

VI. Additional mutation accumulation results and discussion

Table S4. R_{XY} statistic for synonymous sites across phocid species.

	HMS	MMS	NES	SES	WED	GRS	BRS	SRS
HMS	-							
MMS	0.959	-						
NES	0.789	0.819	-					
SES	0.795	0.826	1.036	-				
WED	NS	1.046	1.317	1.305	-			
GRS	1.109	1.135	1.292	1.285	1.104	-		
BRS	1.114	1.142	1.300	1.294	1.110	NS	-	
SRS	1.122	1.149	1.309	1.302	1.117	1.038	1.033	-

Table S5. R_{XY} for nonsynonymous sites across phocid species.

	HMS	MMS	NES	SES	WED	GRS	BRS	SRS
HMS	-							
MMS	NS	-						
NES	0.737	0.731	-					
SES	0.752	0.746	1.090	-				
WED	NS	0.981	1.381	1.348	-			
GRS	1.136	1.132	1.388	1.368	1.144	-		
BRS	1.129	1.125	1.381	1.361	1.137	0.978	-	
SRS	1.134	1.130	1.388	1.368	1.143	NS	NS	-

The pattern of premature stop codons is hard to interpret, given that the reference genome is from a species about 30 million years diverged, and loss of function alleles may have unknown effects. We should be careful about making assumptions about the distribution of selection coefficients of LOF alleles: given that they are observed as high frequency or fixed in a population, we might expect that compared to amino acid-changing alleles, LOF alleles are likely to have $s = 0$ (i.e. gene become not important to fitness before LOF allele occurred) or $s > 0$ (i.e. adaptive). In comparisons of closely related populations, however, patterns of LOF alleles are often used to assess mutational load. For comparative purposes, we also assessed *R* statistics for LOF alleles and found significant differences across species (Tables S6-8).

Table S6. R_{XY} for premature stop codons across phocid species.

	HMS	MMS	NES	SES	WED	GRS	BRS	SRS
HMS	-							
MMS	NS	-						
NES	NS	NS	-					
SES	NS	NS	1.280	-				
WED	NS	NS	NS	NS	-			
GRS	1.431	1.484	1.501	1.376	1.420	-		
BRS	1.593	1.652	1.669	1.531	1.572	1.242	-	
SRS	1.508	1.570	1.587	1.453	1.499	NS	NS	-

Table S7. *R'XY* for premature stop codons normalized against synonymous sites.

	HMS	MMS	NES	SES	WED	GRS	BRS	SRS
HMS	-							
MMS	NS	-						
NES	1.193	1.211	-					
SES	1.319	1.339	1.235	-				
WED	NS	NS	0.816	0.733	-			
GRS	1.291	1.307	1.162	NS	1.285	-		
BRS	1.429	1.447	1.284	1.183	1.416	1.221	-	
SRS	1.344	1.366	1.212	1.116	1.342	NS	0.853	-

Table S8. Rxy for homozygous premature stop codons across phocid species.

	HMS	MMS	NES	SES	WED	GRS	BRS	SRS
HMS	-							
MMS	1.055	-						
NES	0.932	0.887	-					
SES	0.916	0.872	NS	-				
WED	NS	0.940	1.054	1.077	-			
GRS	1.044	NS	1.092	1.104	1.054	-		
BRS	NS	NS	1.062	1.074	1.025	0.876	-	
SRS	1.033	NS	1.080	1.092	1.043	0.959	1.138	-

CHAPTER TWO:

Molecular evolution in phocid seals shows adaptations to aquatic life, polar environments, and hypoxia

Abstract

Pinnipeds are one of the few mammalian lineages that have evolved to rely entirely on the sea to survive. Seals share many morphological and physiological traits that are adaptations to marine life, and some features have evolved in parallel as species have independently colonized similar habitats and evolved similar behaviors and life histories. In this study, we use population-genomic data sets, a statistically powerful method of detecting positive selection, and a phylogenetic framework to find positively-selected genes underlying physiological adaptations in eight seal lineages. We find that all lineages show positive selection in genes associated with a thick, thermos-insulating blubber layer, with collagen genes being especially overrepresented in the set of positively selected genes. This ubiquitous signal suggests that the repurposing of mammalian collagen genes in the blubber layer is an ongoing and complex adaptive walk. Genes relating to sperm flagellar development and male fertility also show positive selection across all seal lineages, including in the strongly polygynous elephant seals. Weddell seals and elephant seals, both of which perform long, deep underwater dives, show an enrichment of positively selected genes relating to neuronal development and cardiac muscle function, respectively. Cellular and physiological changes in the heart and brain have been proposed as important adaptations to hypoxia in deep-diving seals, and our results suggest that these changes are driven by a suite of amino-acid adaptations in multiple genes. This in-depth study of

molecular adaptations in seals provides novel insight into the process of parallel molecular evolution in closely related mammalian lineages.

Introduction

Pinnipeds represent one of the few tetrapod lineages that have evolved to rely on marine habitats (Berta *et al.* 2015). This extreme shift in habitat was accompanied by a suite of adaptations. Some gross anatomical adaptations, such as the evolution of hydrodynamic flippers and the development of an insulating blubber layer, are physically obvious. More complex traits, such as tolerance of prolonged fasting and of hypoxia during long dives, likely have numerous underlying adaptive changes at the genetic level.

The main phylogenic relationships of pinniped species have been well-resolved, with agreement among morphological, mitochondrial, and genomic data sets (Fulton & Strobeck 2010, Paterson *et al.* 2020). In addition, a fairly robust fossil record helps to solve the broad historical biogeography of this clade. For phocids, fossil evidence suggests that stem phocids inhabited the central Atlantic basin around 15 Ma (Berta *et al.* 2018). The warm-water affinities of the ancestors to all modern phocids (Berta *et al.* 2018) is in contrast to the current distribution of phocids, in which only the monk seals inhabit tropical and sub-tropical waters while other phocids have anti-tropical distributions (Ferguson & Higdon 2006). This pattern suggests both that adaptations to warmer water are ancestral, and that tolerance of polar waters has evolved in parallel in multiple lineages. For example, the stem phocine seals, a group that includes ringed seals and grey seals, colonized Arctic polar waters, while the ancestors of Weddell seals and elephant seals diverged in the central Atlantic basin and independently colonized the Southern Ocean around Antarctica (Fulton & Strobeck 2010). There are other examples of parallel evolution in phocids, such as the

evolution of extreme polygyny (elephant seals and grey seals) and likely the evolution of extreme hypoxia (Weddell seals and elephant seals). Individual lineages also have likely had their own unique adaptations.

Previous comparative genomic studies of terrestrial and marine mammals have attempted to identify positively selected genes in marine mammals (Foote *et al.* 2015, Chikina *et al.* 2016, Thomas *et al.* 2017). These analyses, which focused on convergent substitution rates across marine mammals, found numerous candidate genes that were positively selected in marine mammals, but were also troubled by a high false positive rate from methodological artefacts (Thomas *et al.* 2017). In addition, these analyses assumed that molecular evolution was taking place in parallel (i.e. acting on the same genes) rather than convergently (i.e. achieving the same adaptation through distinct molecular changes) across marine mammal lineages. In this study, we narrow our focus to only phocid seals, and use a more sensitive and robust statistical framework to detect positive selection in these lineages.

Detecting selection using population genetic concepts in a phylogenetic framework

Numerous statistical tests have been developed to identify signals of selection in genetic and genomic data. These methods range from phylogenetic-based tests that detect historical selection by fixed differences in protein-coding genes (dN/dS) or conserved non-coding sequences (Sackton *et al.* 2019) to population genetic statistics that detect very recent selective sweeps (Sabeti *et al.* 2006, Vitti *et al.* 2013). In between these extremes are a set of tests that incorporate polymorphism and divergence data to detect selection in protein-coding genes. Originally developed by McDonald and Kreitman (1991), this

mathematical framework was expanded by Sawyer and Hartl (1992) in the Poisson Random Field framework. More recently, Zhao *et al.* (2017) created the Model-Averaged Site Selection with Poisson Random Field (MASS-PRF) tool, which uses model averaging and a site clustering algorithm to estimate the selection intensity (scaled selection coefficient, or γ) at every site in a gene.

Using this MASS-PRF method on individual species or lineages can give statistically robust insights into historical and ongoing molecular adaptation, which is of particular interest in seals given their unique adaptations among mammals to extreme environments and life history strategies. To extend this framework further, we hypothesized that in these closely related seal species, the genes underlying independent adaptations to similar selective pressures—such as polar environments, deep diving, and income breeding—would show up across multiple lineages as showing signals of positive selection but would not show positive selection in lineages that do not share the same selective pressures. By searching for these signals of convergent adaptive evolution, we present a conservative framework for identifying genes and molecular pathways that drive adaptive evolution.

The unique anatomical and physiological traits of marine mammals have been of particular interest to the study of adaptation in evolutionary biology (Foote *et al.* 2015). Seals offer an additional layer of interesting adaptations because of their radiation of different environments, extreme physiological conditions, and divergent life histories. Besides being of interest to evolutionary biologists, comparative results of positive selection across species can be helpful in understanding gene function in mammals and may even have implications for human health and disease.

Methods

Sequencing, alignment

We generated low-to-medium coverage shotgun sequence data for Hawaiian monk seals (n = 15), Mediterranean monk seal (n = 3), Weddell seals (n = 9), northern elephant seals (n = 10), southern elephant seal (n = 1). In addition, we included publicly available sequencing data for grey seal (n = 10), Baltic ringed seal (n = 9), and Saimaa ringed seal (n = 12), and one additional Weddell seal (n = 1).

Shotgun sequencing reads were trimmed using TrimGalore (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and aligned to the Hawaiian monk seal reference genome (Mohr *et al.* 2017) using BWA mem (Li 2013). Duplicates were removed from bam files using Picard Tools (Van der Auwera *et al.* 2013).

Identification of polymorphic sites

Polymorphic sites were identified using the Minor Allele Frequency function (-doMaf 1) in ANGSD (Korneliussen *et al.* 2014). This assesses the probability that a given site is polymorphic at a P value threshold of 10^{-6}. We fixed the reference allele as the major allele (-doMajorMinor 4), which allowed for the downstream reconstruction of the ancestral sequences across different seal clades. Minimum map quality (-minMapQ) and basepair quality (-minQ) were both set to 30 so that only high quality sites were considered, since the MKT framework can be sensitive to error. Only biallelic sites were kept.

Phylogenetic framework

We used well-resolved phylogenies from Fulton and Strobeck (2010) to guide our comparisons across lineages. We reasoned that because MASS-PRF does not rely on allele frequencies, the program can be run on any branch of a tree in which one or more species, forming a monophyletic clade, for which there is a sister species. If ancestral sequence reconstruction is used, then an additional outgroup to the two sister taxa is required (Figure 1). Because we were interested in adaptive selection in multiple species and across multiple branches, we designed a framework combining data from various species that allowed us to examine adaptation throughout the history of seals. Because some traits have evolved convergently (e.g. diving, polar adaptations, polygyny, dimorphism), we can also search for genes that show positive selection in lineages where the adaptation has occurred independently (Figure 1 and Table 1 for specifics).

Pre-massprf and Massprf

We used the Model-Averaged Site Selection with Poisson Random Field (MASS-PRF) statistical tool to detect regions of protein coding genes that showed significant positive selection ($\gamma > 4$ and lower bound of γ confidence interval above 0). To prepare MASS-PRF input gene files from raw genomic BAM files, we created two tools: *mafs2vcf* and *premassprf*. Briefly, this pipeline translates an ANGSD .mafs file into a pseudo-VCF file, which encodes population polymorphisms but not sample genotypes. The pseudo-VCF is then used to 1) identify polymorphic sites within a the target population 2) reconstruct the ancestral sequence for each gene through simple parsimony 3) identify fixed (divergent) sites between the target population and the reconstructed ancestral sequence.

Full documentation for these tools can be found at https://github.com/sjgaughran/massprf-pipeline.

We then used the resulting polymorphism and divergence files to run MASS-PRF with the following command:

```
massprf -p polymorphism.txt -d ancestral_divergence.txt -o 1 -SCL 1 -a 900 \
-ic 1 -ci_m 1 -sn {sample_size} -s 1 -m 0 -NI 1 -mn 30000 -ssd -n 0
```

Processing results

We used custom scripts to identify sites in each gene that showed significant positive ($\gamma > 4$, lower CI > 0) or negative ($\gamma < 0$, upper CI < 0) selection, and to plot the estimated γ and CI for every site across a gene. We compiled a list of 13,599 one-to-one mammalian orthologs from the OrthoMamV1.0 database (Scornavacca *et al.* 2019) to decrease the probability of paralogous genes biasing our results. Only one-to-one orthologs were kept for downstream interpretation.

We checked the list of positively selected genes for GO term enrichment using the PANTHER algorithm with a false discovery rate (FDR) set to $P < 0.05$ (Ashburner *et al.* 2000, Mi *et al.* 2019). We also intersected each list of positively selected genes with curated lists of genes with functions that were relevant to the phocid phenotypes (e.g. immunity, sperm motility, hypoxia).

Results

Out of 13,599 mammalian orthologs examined in each of eight lineages, our analyses recovered a total of 2,169 genes that showed statistically significant signs of

positive selection across the lineages, or 1.99% of possible genes. The number of genes with positive selection in an individual lineage varied from 74 in the Baltic ringed seal to 798 in elephant seals.

Monachini

In total, 109 genes showed positive selection in the monk seal lineage (Table XX). This set of genes was enriched for two GO terms: homophilic cell adhesion via plasma membrane adhesion molecules and anatomical structure morphogenesis. Two collagen genes (COL6A1 and COL6A3) showed positive selection in monk seals, as did one gene proposed to be associated with diving and hypoxia (NOTCH1). Despite being a tropical lineage, the monk seals also showed positive selection for 16 genes that have been proposed to be associated with polar adaptation (COL6A1, PCNT, TG, ABL1, HIVEP1, MADD, ALPK2, DNAH11, AHCTF1, URB1, ATP7B, ZDBF2, KIAA1671, ACAN, POM121L2, APOBR). The monk seals also showed signals of positive selection in three genes considered to be related to immunity (ITGAL, CD5, IL4R). Finally, this lineage showed positive selection in three genes thought to be associated with sperm motility and competition in mammals (DNAH11, NPHP4, ASH1L). The monk seal lineage also had 115 genes that showed negative selection. Of the genes analyzed, 87 showed only neutral evolution throughout the entire gene.

Miroungini

We examined patterns of selection in three separate parts of the Miroungini lineage: the ancestral branch of elephant seals + Weddell seals, the ancestral branch of northern elephant seals + southern elephant seals, and Weddell seals.

In the ancestral Miroungini branch, 192 genes showed signals of positive selection (Table XX). However, this set of genes was not enriched for any particular GO category. Six collagen genes (COL3A1, COL5A2, COL6A3, COL6A5, COL17A1, COL18A1) showed positive selection in this lineage, as did a gene associated with collagen secretion in blubber (MIA3). One gene associated with diving and hypoxia (LOXHD1) showed positive selection, although this particular gene likely has many other functions. Despite this lineage not living in the tropics, 23 genes that are associated with polar adaptations were found to have positive selection (PCNT, DNMBP, BOD1L1, RFWD3, LAMA2, MADD, CUL9, AHCTF1, DNAH9, ATP7B, ROS1, CEP250, MKI67, AKAP13, DCHS2, MYO15A, ALPK3, DISP1, PKHD1L1, AKNA, POM121L2, APOBR, PARP14). Three genes associated with immune function showed positive selection (JAK3, PTPRJ, MUC1). Four genes associated with sperm motility (ATP1A4, QRICH2, CFAP44, DNAH17) also showed positive selection. 95 genes showed negative selection, and 78 genes showed only neutral evolution.

The elephant seal branch had the greatest number of genes with positive selection (798). This set of genes was significantly enriched for biological function relating to heart function (e.g. membrane depolarization during AV node cell action potential; regulation of cardiac muscle cell contraction; regulation of heart rate by cardiac conduction), extracellular matrix organization (Negative regulation of supramolecular fiber organization, Extracellular matrix organization, Supramolecular fiber organization),

microtubule structure (Regulation of microtubule polymerization, Negative regulation of microtubule polymerization, Regulation of protein depolymerization, Microtubule cytoskeleton organization, Tube morphogenesis) cell surface and cell junctions (Homophilic cell adhesion via plasma membrane adhesion molecules, Calcium ion transmembrane transport, Calcium ion transport, Cell junction organization, Regulation of plasma membrane bounded cell projection organization, Phosphatidylinositol metabolic process), cell movement (Cilium assembly, Cilium movement, Ameboidal-type cell migration), and developmental processes (Cell morphogenesis involving differentiation, Anatomical structure formation involved in morphogenesis, Cell cycle, Tissue development, Nervous system development, Animal organ development).

Nine collagen genes showed positive selection (COL1A2, COL4A2, COL4A3, COL4A6, COL6A1, COL6A6, COL7A1, COL18A1, COL26A1), as did three other genes associated with blubber in marine mammals (MIA3, ADAMTS16, DPYSL4). 11 genes previously associated with diving and hypoxia showed positive selection (LIMD1, TOX1, ICAM1, DUOX1, MPO, FMN2, CA9, NOS1, MYH7B, ANPEP, PINK1), three of which have GO associations with reactive oxygen species processing (DUOX1, NOS1, MPO). Elephant seals also showed positive selection in two genes associated with metabolism and fasting (CEL and LEPR), both of which are related to lipid metabolism. 21 genes with positive selection were associated with immune response (CSF3R, ITGA6, SEMA4D, ICAM1, IGF2R, TCF3, MPO, SLC4A1, IL3RA, PTPRJ, PTPRC, PDGFRB, BLM, LTF, C5, THBD, CD38, CD96, ANPEP, IL16, IL17RE). 69 genes associated with polar adaptations showed positive selection. Eight genes associated with sperm motility showed positive selection (CACNA1I, QRICH2, GAPDHS, DNAH11, VPS13A, CCDC40,

CFAP61, CFAP65). 327 genes showed negative selection, and 606 showed evolution under neutrality.

The Weddell seal branch showed the second highest number of genes with positive selection (613). This set of genes was significantly enriched for biological function relating to neurological function (Neuronal action potential, Neuron projection development, Cell morphogenesis involved in neuron differentiation), extracellular matrix organization (extracellular matrix organization, supramolecular fiber organization), cell surface and cell junctions (cell-cell adhesion, cell junction organization, cell matrix adhesion), cell movement (Cilium movement, Non-motile cilium assembly, Regulation of cell migration), and cellular and developmental processes (Epithelial cell morphogenesis, Animal organ morphogenesis, Chemical homeostasis, Regulation of organelle organization, Positive regulation of cellular component organization, Positive regulation of transport, Centriole replication, Actin cytoskeleton organization, Regulation of Ras protein signal transduction, Phospholipid translocation).

Eight collagen genes (COL5A3, COL6A3, COL6A5, COL6A6, COL15A1, COL20A1, COL27A1, COL28A1) and three other blubber-associated genes (RAB3GAP2, MIA3 and ADAMTS16) showed positive selection in Weddell seals. Six genes associated with deep diving showed positive selection (NOTCH1, DUOX2, MYH7B, VASN, HYOU1, ANPEP). One gene associated with fasting (CEL) showed positive selection. 18 genes showing positive selection were associated with immune response (IL12RB2, ITGA6, IRAK3, IGF2R, CD177, ITGAX, IL3RA, PTPRJ, ITGAL, NFATC1, IL1R1, DCLRE1C, LTF, C5, ITGA1, HRH4, ANPEP, ITGA2, IL4R), many of which are integrin-related genes. 68 genes related to polar adaptations showed positive selection. Eleven genes

associated with sperm motility showed positive selection (CATSPER2, ATP1A4, CACNA1I, QRICH2, DNAH8, DNAH11, DNAH17, NPHP4, ASH1L, SPEF2, CFAP65). 180 genes showed negative selection, and 621 were evolving under neutrality.

Phocini

We examined in four lineages of phocini seals: grey seals, ringed seals as a species (Baltic ringed seals + Saimaa ringed seals), Baltic ringed seal subspecies and Saimaa ringed seal subspecies.

Grey seals showed positive selection in 124 genes. This set of genes was enriched for GO terms relating to epithelial cell morphogenesis, cell adhesion, microtubule-based process, and extracellular matrix organization. Three collagen genes (COL10A1, COL15A1, and COL18A1) showed positive selection, as did 10 other genes associated with the extracellular matrix, though none of these have previously been shown to be active components of seal blubber. One gene associated with diving and hypoxia (NOS2) showed positive selection. Two genes associated with immune response (SEMA4D and CXCL16) showed positive selection. 26 genes relating to polar adaptation showed positive selection. Two genes relating to sperm motility showed positive selection (QRICH2, CFAP65). 48 genes showed negative selection, and 179 were evolving under neutrality.

Ringed seals showed positive selection in 172 genes. This set of genes was significantly enriched for two GO terms: gland morphogenesis and branching morphogenesis of an epithelial tube. Four collagen genes (COL5A2, COL6A5, COL27A1, COL15A1) and two other blubber-associated genes (RAB3GAP2 and MIA3) showed positive selection. One gene associated with deep diving and hypoxia (USP19) showed

positive selection. Six genes associated with immune response showed positive selection (MST1R, MARCO, PTPRJ, NOD2, INSR, ITGA1). Ringed seals showed positive selection in 15 genes relating to polar adaptations. Two genes relating to sperm motility (QRICH2 and VPS13A) showed positive selection. 72 genes showed negative selection, and 105 were evolving neutrally.

Baltic ringed seals showed positive selection in 74 genes, but they were not significantly enriched for any GO category. Two collagen genes (COL6A3 and COL6A6) showed positive selection. Three genes relating to immune response (MST1R, SH2B2, and A2M) showed positive selection. 13 genes relating to polar adaptation showed positive selection. Two genes relating to sperm motility (QRICH2, DNAH17) showed positive selection. 34 genes showed negative selection, and 38 were evolving neutrally.

Saimaa ringed seals showed positive selection in 87 genes, but they were not significantly enriched for any GO category. Five collagen genes (COL4A3, COL6A6, COL10A1, COL12A1, COL15A1) and one other blubber-associated gene (MIA3) showed positive selection. Three genes related to immune function (ITGAL, TLR4, TLR5) showed positive selection. 18 genes associated with polar adaptation showed positive selection. Three genes related to sperm motility (CACNA1I, DNAH11, VPS13A) showed positive selection. 25 genes showed negative selection, and 106 were evolving neutrally.

Phylogenetic comparisons

The ice-breeding Weddell seal and ringed seal both showed positive selection in an overlapping set of 41 genes. Of those, 16 were found to be under selection in these polar species but not in monk seals, elephant seals, or grey seals. Conversely, there were 37 genes

that only showed positive selection in the tropical monk seal lineage and not the other cold-water lineages. All species outside of the tropics showed independent positive selection in only three genes that did not have positive selection in monk seals.

The grey seal and elephant seals are polygynous species that showed positive selection in an overlapping set of 62 genes. Of those, 22 showed positive selection in these lineages and not the others.

The deep-diving Weddell seal and elephant seals independently showed positive selection in the same 209 genes. Of these, 146 showed positive selection in these lineages and not others.

Seals in the phocini tribe (grey seals and ringed seals) showed positive selection in 17 genes in common. Of those, only three did not show positive selection in other lineages (ADAMTS13, ADAMTS7, KIAA1211). Both subspecies of ringed seal independently showed positive selection in eight genes. However, none were under selection in only the ringed seal subspecies.

One gene (TNN) showed positive selection in every lineage except WED-ES. Two other genes (APOBR and TEX15) showed positive selection in all lineages except the most recent PHB-PHS divergence. Within TNN, the signals of significant positive selection all fall within nucleotide positions 1500–2500, suggesting that this specific region of TNN is under strong, ongoing selection. In APOBR and TEX15, on the other hand, there are signals of positive selection in different parts of the genes in different lineages.

Discussion

Population genetics offers multiple strategies for detecting signals of positive selection in genomic data. The framework we use in this study, the Poisson Random Field as implemented in MASS-PRF, provides a robust statistical framework for detecting historical positive selection at individual regions or sites of a gene. Through this, we identify hundreds of genes across multiple phocid seal lineages that show signs of significant positive selection.

Phocid-specific and marine mammal-specific genes

We used a number of approaches to identify genes that could be generally related to marine mammal or phocid adaptive evolution. First, we identified three genes that were under selection in all phocid lineages examined. A single gene, TNN, showed significant positive selection in every comparison, including in the short time period of divergence (~100,000 years) between the Baltic and Saimaa ringed seal subspecies. The exact nucleotide position of significant selection differed across lineages, but all showed significant positive selection between positions 1500–2500 of this gene. TNN encodes for Tenascin N protein, which is associated with many molecular and cellular functions. Most notably, this protein is associated with collagen-containing extracellular matrix. Given the apparent importance of the collagen extracellular matrix in the molecular evolution of phocids, this association suggests that TNN may play a central role in the evolution of efficient blubber.

APOBR shows positive selection in every lineage except the two ringed seal subspecies, although it did show positive selection in the ringed seal species overall (Figure 2). APOBR encodes for the apolipoprotein B receptor, and is associated with lipid uptake

in cells. In humans, variants in this gene are associated with increased hypercholesterolemia (Fujita *et al.* 2005) and obesity (Volckmar *et al.* 2015). Expression studies in mice and humans have shown upregulation of these gene when individuals are given a high-fat diet (Brown *et al.* 2002, Varela *et al.* 2013). Phocids have extremely high body fat percentages compared to other mammals due to the thick blubber layer. Persistent positive selection in APOBR could be a key aspect of how phocid physiology has evolved to regulate and store lipids.

TEX15 also shows positive selection in every lineage except the two ringed seal subspecies. This gene encodes the testis expressed 15 protein, which is only expressed in the testis. Studies in mice have shown this protein to be associated with meiosis in male mice, and is especially relevant in recombination and DNA break repair (Yang *et al.* 2008). Given the strong evidence we uncover for adaptation relating to sperm competition in phocids, TEX15 may be an important gene regulating sperm quality, under consistent positive selection to keep up with the evolutionary arms race of sperm competition.

We also compared our signals of positive selection against a curated list of genes that were proposed by Foote *et al*. (2015) and Chikina *et al.* (2016) to be under positive selection in marine mammal lineages. Surprisingly, we found little overlap between this list of genes and those we recover in phocids. However, the few overlapping genes are likely evolutionarily informative. Some important examples were DSP in elephant seals, Weddell seals, and grey seals; ANPEP in elephant seals and Weddell seals (Figure 2a–b); ZNF582 in elephant seals and Weddell seals; MYH7B in elephant seals and Weddell seal (Figure 2c–d); GRIN2C in monk seals and Baltic ringed seals; DUSP27 in elephant seals; and MUC1 in the ancestral Miroungini lineage. As discussed below, ANPEP, DSP, and

MYH7B may be related to adaptations to deep diving. ZNF582 is a tumor suppressor gene, and may play an anti-cancer role in large body size evolution of marine mammals. Notably, this gene shows positive selection in the two largest seal lineages examined here (elephant seals and the Weddell seal). The other genes have less clear associations in marine mammals, but relate to mucin production (MUC1), energy metabolism (DUSP27), and synaptic transmission (GRIN2C). Chikina *et al.* (2016) also proposed a number of GO terms that were enriched in genes under positive selection in marine mammals (especially related to lung function and muscle contraction), but none of these terms were enriched for in our results.

Blubber, metabolism, and fasting

Genes involved in blubber composition and lipid processing show some of the most consistent signals of molecular evolution across the phocid lineages examined here. Blubber is a specialized, derived trait present in all marine mammals. It consists mainly of adipocytes (fat cells) embedded in an extracellular matrix of collagen fibers. It serves primarily as thermal insulation, but is also likely an important source of energy during fasts.

Many lineages showed GO term enrichment for extracellular matrix organization and cell-cell adhesion, both of which may relate to the evolution of a thick, collagen-rich blubber layer in seals. There are more than two dozen collagen genes in mammals, and collagens are the most abundant proteins in mammalian bodies. In evolving blubber layers, marine mammals appear to have evolved at least some of these collagen genes under adaptive evolution. Every phocid lineage examined here, including the ringed seal

subspecies, showed signatures of positive selection in collagen genes, with COL6A1, COL6A3, and COL6A6, which encode for collagen type VI (ColVI), apparently showing the most consistent positive selection across lineages (Figure 5). ColVI is found in many tissues, including bone, muscle, nervous, cartilaginous, skin, and adipose tissue (Cescon *et al.* 2015). As others have suggested, it is also possible that changes in collagen in marine mammals could be related to adaptive changes in bone mass (Zhou *et al.* 2018). As discussed above, TNN, which shows positive selection in all lineages, encodes for a protein common in collagen-containing extracellular matrices like blubber.

Previous studies have identified other genes that are actively transcribed in seal blubber tissue. This includes MIA3, which is involved in collagen secretion and shows positive selection in the ancestral Miroungini lineage, elephant seals, Weddell seal, ringed seals, and the Saimaa ringed seal. ADAMTS16, which is associated with regulating blood pressure (Gopalakrishnan *et al.* 2012) but is actively transcribed in stressed elephant seal blubber (Deyarmin *et al.* 2019), also showed positive selection in the elephant seal and Weddell seal. Grey seals showed an additional ten genes with positive selection associated with the extracellular matrix, though not ones that have been previously shown to be expressed in seal blubber. Because prior transcriptomic and proteomic studies on seal blubber relied on elephant seals, though, it is possible that these other extracellular matrix protein genes have been more important in the evolution of blubber in the phocini lineage.

All seal species endure some period of fasting, usually during the winter season when food is scarce (e.g. ringed seals), as adult males during mating and pupping seasons (e.g. elephant seals, grey seals), as adult females while lactating and feeding young (e.g. elephant seals, monk seals, gray seals, Weddell seal), as pups after weaning (e.g. monk

seals, elephant seals), and during molts (e.g. elephant seal, monk seals). These fasts can last weeks or months, depending on the species and life stage, during which the individuals rely entirely on fat stores. Individuals can lose between 20–40% of their total mass, which is almost entirely accounted for by lipid loss (Champagne *et al.* 2013).

However, the vast majority of the genes observed by Martinez *et al.* (2018) and Khudyakov *et al.* (2019) to be differentially regulated in the blubber of seals do not show signals of positive selection in our results. A few of these candidate genes, however, do show positive selection. DPYSL4, which shows positive selection in elephant seals, is upregulated in the blubber of fasting elephant seal pups (Martinez *et al.* 2018), and is thought to act as a tumor suppressor by regulating energy metabolism (Nagano *et al.* 2018). RAB3GAP2, a GTPase, shows positive selection in ringed seals and the Weddell seal, and was also observed to be upregulated in the blubber of fasting northern elephant seal pups (Martinez *et al.* 2018).

Other studies have assessed molecular changes in fasting seals through proteomic and enzymatic analyses (Fowler *et al.* 2014) and through comparative physiology to discover pathways that are important to survival during fasting (Fowler *et al.* 2018). Again, our list of candidate genes curated from these physiological approaches showed very little overlap with positively selected genes in seals. Elephant seals, which experience long fasts during multiple life stages, did show positive selection in the leptin receptor gene (LEPR) and a lipase gene (CEL), both of which could be involved in lipid storage and metabolism. Weddell seals also showed positive selection in CEL. Other lineages (monk seals, grey seals, ringed seals), however, did not have positive selection in any of the fasting candidate genes. As discussed above, though, APOBR does show positive selection in all seal

lineages except the ringed seal subspecies. This gene could play a role in lipid storage and metabolism, although it was not in our *a priori* candidate list of genes involved in fasting.

Taken together, these results show that numerous genes putatively relating to blubber structure and composition show signs of positive selection across seal lineages. In many cases, this positive selection appears to be ongoing and independently occurring in different species. On the other hand, very few candidate genes relating to fasting appear to show evidence of positive selection, despite how extreme fasting can be in seal (Champagne *et al.* 2013). This difference most likely results from different physiological needs and constraints in the two cases. Structural adaptations in blubber may adapt through molecular changes to extracellular matrix proteins, like collagen, that allow the blubber layer to be constructed for greater thermal efficiency. Physiological changes, like fasting tolerance and metabolic changes, might be expected to adapt through regulatory changes, rather than amino acid substitutions, especially given the complexity of metabolism and the variability in fasting across life stages.

Sperm competition

Sperm competition is a potentially ubiquitous phenomenon among mammals, and occurs when sperm from two males compete to fertilize the ova of a female (Wigby & Chapman 2004). Genomic scans for positive selection in other species often recover genes relating to sperm competition (e.g. Clark & Swanson 2005, Dean *et al.* 2017). It is thought to be an especially strong selective pressure in species where females mate non-monogamously, and less strong in monogamous or polygynous mating systems (Dapper & Wade 2016). Given that all seal species examined here range from slightly (i.e. ringed

seals) to extremely (i.e. elephant seals) polygynous, we expected there to be little evidence of sperm competition. To test this, we curated a list of genes involved in sperm motility and male fertility based on gene ontologies and reviews (Xu *et al.* 2020, Huang *et al.* 2020), and compared those candidate genes to our results.

Surprisingly, we found evidence of positive selection in sperm-related genes across seal lineages. As mentioned above, a testis-specific gene (TEX15) relating to meiotic recombination showed positive selection in every lineage except the ringed seal subspecies, suggesting that this gene has been under consistent positive selection in phocids. In addition, many lineages showed positive selection in the DNAH gene family, which control axoneme development in sperm flagella (Huang *et al.* 2020). All lineages except monk seals and Saimaa ringed seals showed positive selection in QRICH2, a gene that is crucial in sperm flagellum development and male fertility (Shen *et al.* 2019). Likewise, many lineages (Miroungini ancestors, Weddell seal, elephant seals, grey seals) showed positive selection in members of the CFAP family, which are also critical to male fertility and flagellar development (Huang *et al.* 2020).

It is especially surprising that elephant seals show positive selection in such a large number of genes relating to sperm development. Northern and southern elephant seals have the most polygynous mating system of mammals, with a single male thought to mate with a harem consisting of dozens of females (Leboeuf 1972). In such a system, sperm competition should be low as male-male competition should be exclusively pre-copulatory. There are two plausible explanations. One is that the signal of positive selection we pick up is driven by selection in the elephant seal lineage that occurred prior to the evolution of extreme polygyny. The second is that female elephant seals may in fact mate with males at

sea, away from their breeding beaches where single males dominate. There is behavioral data from southern elephant seals suggesting that more than half of females may adopt alternative mating strategies at least some of the time (de Bruyn *et al.* 2011), which would allow for continued sperm competition within this supposedly polygynous mating system.

Immunity

Genes involved in immune response are also widely assumed to be under positive selection in most species (Vallender & Lahn 2004, Sabeti *et al.* 2006, Van Der Lee *et al.* 2017). New mutations in host immune genes may be selected by continuously evolving pathogens in a Red Queen dynamic, or novel pathogens may exert strong selective pressure on standing variation (Sabeti *et al.* 2002, Papkou *et al.* 2019). We used a curated list of immune gene orthologs, deemed the "immunome" (Rannikko *et al.* 2007), to identify genes with immune function in our results. As expected, every lineage showed positive selection in some genes related to immune function. Interestingly, however, none of the lineages had sets of positively selected genes that were enriched for GO terms relating to immune response.

Multiple lineages showed positive selection in genes from the integrin (ITGA) gene family, although these genes serve many other functions other than immune response. Many lineages also showed positive selection in interleukin (IL) and interleukin receptor (ILR) genes, but the exact member of the gene family differed across lineages. There were also many signals of positive selection in cluster-of-differentiation proteins (CD), though again they differed across lineages.

Immunological studies in Weddell seals and northern elephant seals have shown that these species show remarkably low innate immune responses to lipopolysaccharide (LPS) exposure compared to humans, and in fact their serum may be actively anti-inflammatory (Bagchi *et al.* 2018). Intriguingly, we find that important innate immune response genes involved in LPS recognition, like TLR5 and TNFAIP2, appear to be evolving entirely neutrally in the Weddell seal. On the other hand, the Saimaa ringed seal showed positive selection in both TLR4 and TLR5 (Figure 4), both of which are important in activating the innate immune response in response to bacterial pathogens (van der Aar *et al.* 2007). One possible selective scenario is that the isolation of the Saimaa ringed seal subspecies in a freshwater lake led to a shift in the bacterial pathogen burden on this subspecies, providing a strong selective pressure on genes that detect LPS. Future immunological studies of the Saimaa ringed seal may help to reveal functional changes of these selected TLR genes, which could provide important information about the threat of bacterial pathogens to this critically endangered subspecies.

Deep diving and hypoxia

One of the most remarkable adaptations in phocids is their ability to tolerate hypoxia during long and deep dives. The extent of diving patterns differs across species, with Weddell seals and elephant seals providing the more extreme cases of deep dives of long duration. We compiled a list of candidate genes associated with deep diving in marine mammals from a number of studies of physiological responses to diving in seals (Tift & Ponganis 2019, Hindle 2020) and hypoxia in humans (Crawford *et al.* 2017).

Genes from this list that showed up as positively selected included NOS2 in grey seals, NOTCH1 in monk seals, and USP19 in ringed seals. More hypoxia-associated genes were positively selected for in the deep-diving Weddell seal (six genes) and elephant seals (eleven). Among these were ANPEP and MYH7B, which had previously been discussed by Foote *et al*. (2015) and Thomas *et al*. (2017) as being involved in adaptation to deep diving in marine mammals. Foote *et al*. (2015) described ANPEP as a glutathione metabolism pathway gene that could serve an antioxidant capacity and reduce damage from reactive oxygen species (ROS) in hypoxic conditions. Notably, though, Chikina *et al*. (2016) found that positive selection in ANPEP is also found in terrestrial mammal lineages. MYH7B is involved in cardiac muscle development, and has strong evidence for being positively selected in deep diving cetaceans (Foot *et al.* 2015, Chikina *et al*. 2016, Thomas *et al.* 2017).

In addition, our study provides a good starting point to identify new genes that are putatively related to deep-diving adaptations in pinnipeds. For example, deep dives of long duration are common in elephant seals and Weddell seals, but not the other lineages examined here. We found 146 genes that show positive selection exclusively in Weddell seals and elephant seals, which suggests some of these genes could be involved in the parallel molecular evolution of adaptations to hypoxia. Notably, many of the genes under positive selection in both elephant seals and Weddell seals relate to cardiac function and cardiac tissue (e.g. DSP, DSG2, SCN5A, MYH7B). In addition, the subset of genes showing positive selection in elephant seals was enriched for multiple GO terms relating to cardiac function (i.e. membrane depolarization during AV node cell action potential; regulation of cardiac muscle cell contraction; regulation of heart rate by cardiac

conduction). Diving seals are known to experience bradycardia, or low heart rate, with rates in diving elephant seals recorded to be as low as 2-3 beats per minute (Andrews *et al.* 1997). Our GO enrichment results suggest that the elephant seal lineage has experienced significant molecular adaptation in genes relating to cardiac muscle development and contraction, which allow its heart muscles to sustain the strain of regular extended bradycardia.

Besides inflicting extreme physiological stress on the heart, hypoxic dives also should present a danger to the integrity of the seal nervous system. Thought multiple physiological and anatomical studies have been done to study potential differences between seal brain physiology and those of non-diving mammals (reviewed in Blix (2018)), no clear patterns have emerged to explain how seal nervous systems cope with deep dives. Intriguingly, though, our study suggests that there may be significant molecular adaptation involved in neuronal cell development that could be involved in protecting Weddell seal brain cells from hypoxia. The set of genes under positive selection in Weddell seals was significantly enriched for multiple GO terms related to neuron development (neuronal action potential, neuron projection development, cell morphogenesis involved in neuron differentiation).

As mentioned above, the serum of Weddell seals and elephant seals has also been shown to have extremely low inflammatory responses, and may in fact be anti-inflammatory (Bagchi *et al.* 2018). Notably, two of the genes showing parallel positive selection in Weddell and elephant seals (CARD6 and CARD14, Figure2e–h) are important regulators of inflammatory response (Martinon *et al.* 2002). Adaptive changes to these cell-

surface proteins may be involved in regulating the innate inflammatory response in these deep-diving seals.

Polar vs tropical adaptations

Because the seal lineages we examine inhabit tropical, temperate, and polar waters, we attempted to identify molecular adaptations that correlate with living in these climates. First, we attempted to use a list of candidate genes compiled by Yudin *et al.* (2017) that show apparent positive selection in polar-adapted mammals. However, we found that this list was relatively uninformative, with many of the genes showing positive selection in the tropical-water monk seals and temperate-water grey seals. Given that many of the taxa examined by Yudin *et al*. (2017) were terrestrial, this could suggest that many of these genes relate to counteracting heat loss, which is relevant even to tropical marine mammals given the thermodynamic properties of water. Alternatively, this may be a sign that the criteria used by Yudin *et al*. (2017) were not strict enough, resulting in genes unrelated to polar adaptation being included in their list.

Instead, we took a comparative phylogenetic approach. First, we identified genes that were only positively selected in monk seals but not the other lineages, and identified 37 such genes. These genes were not enriched for any particular GO category, nor did any provide obvious connections to tropical adaptations such as defenses against UV radiation damage.

Conversely, we identified genes that were only positively selected in the polar polar, ice-dependent species (Weddell seal and ringed seals), but not in monk seals, grey seals, or elephant seals. There were 16 such genes. Notably, two of these genes are collagen

proteins (COL27A1 and COL6A5) and another is an extracellular matrix protein (TNC), suggesting that genes relating to blubber development may be under particularly strong selection in polar phocids.

Mating systems and sexual dimorphism

We examined a signal of positive selection driving molecular evolution of genes related to polygynous mating systems. Although all seals appear to be non-monogamous, two species in our data set have especially strong polygyny and sexual dimorphism: elephant seals and grey seals (Ferguson & Higdon 2006). We searched for genes that showed positive selection in these species but not the less polygynous monk seals, Weddell seals, and ringed seals. There were 22 genes that showed positive selection in only the polygynous species. Interestingly, two of these (DNAH7, DNAH9) are expressed in sperm flagella. As discussed above, the signal of positive selection relating to sperm competition could either reflect selection prior to the emergence of a polygynous mating system (i.e. that polygyny released the lineage from previously intense sperm competition), or that sperm competition may be ongoing if females are mating outside of their polygynous harem structures. We see no genes that would obviously relate to the evolution of sexual dimorphism, such as cancer susceptibility (due to larger male body size). This lack of signal is likely because the genetic basis of sexual dimorphism is dominated by differential gene regulation rather than changes to protein structure (Naqvi *et al.* 2019).

General patterns of adaptive molecular evolution

Finally, the thousands of examples of positive selection in this study make it possible to make broad assessments of how adaptive molecular evolution occurs in mammalian genomes, and how molecular adaptation occurs in parallel across lineages. As discussed above, we identified many genes that showed independent positive selection in across seal lineages. This suggests that certain traits (e.g. adaptation to a polar environment, tolerance of hypoxia during long dives) exhibit parallel evolution at both the phenotypic and molecular levels. On the one hand, this result may be expected given how closely related these species are. On the other, such clear parallel evolution of protein coding genes is surprising given that the adaptive traits examined here are complex physiological traits with presumably complex developmental and regulatory pathways. Indeed, when compared to the full results of each lineage, genes showing adaptive parallel evolution make up a small proportion of positively-selected genes.

Interestingly, we also find that parallel molecular evolution at the gene level does not necessarily involve parallel molecular evolution at the amino acid level. For example, some genes have signals of positive selection throughout the gene (e.g. ANPEP, Figure3a–b). Others, such as MYH7B, show very localized signals of positive selection, but at different sites in different species (Figure 3c–d). Finally some, like APOBR, show positive selection in the same region of the gene across lineages (Figure 2). In general, these patterns support the idea that parallel evolution is not common at a convergent amino acid level (Foote *et al*. 2015).

Conclusion

In this study, we show how multiple genes and molecular pathways have been subject to positive selection in phocid seal lineages. Many of these results have obvious connections to anatomical and physiological adaptations, such as positive selection on many collagen proteins found in blubber and positive selection on genes involved in sperm motility. Others, such as an enrichment for cardiac-related genes in elephant seals and neuronal development genes in Weddell seals, provide intriguing support for the role of adaptive molecular evolution in protecting cardiac muscle cells and neurons during deep dives, but do not provide clear mechanistic explanations for how these adaptations work. Future studies exploring the role of these positively selected genes in seal cardiac and neuron cells may finally help explain how molecular and cellular adaptations in seals play a role in the evolution of their extreme phenotypes.

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Figures and Tables

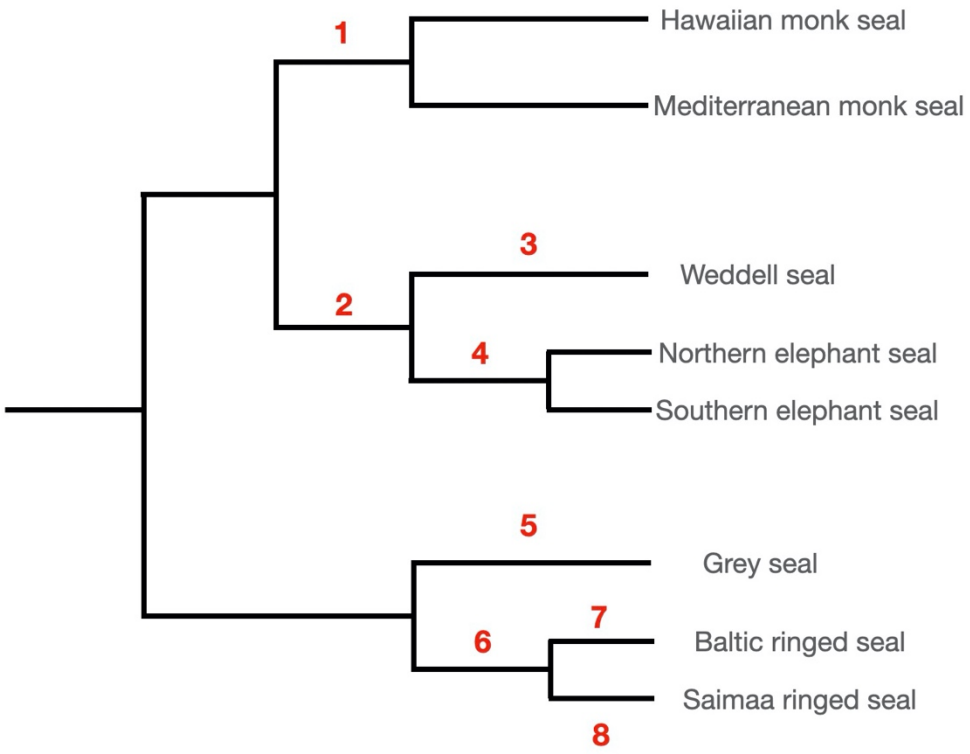
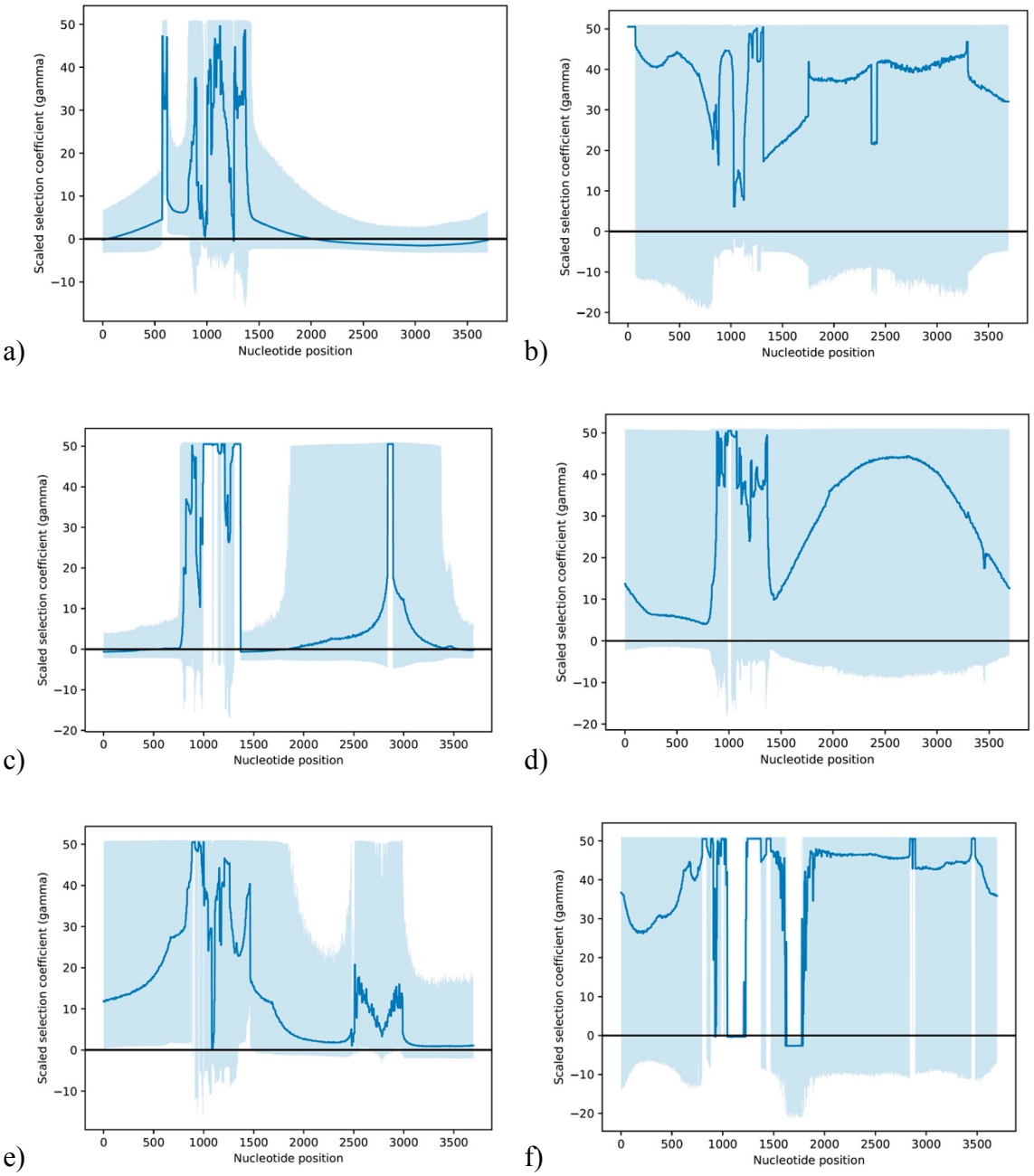
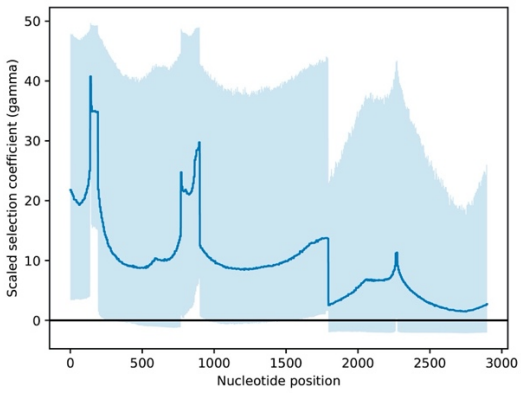


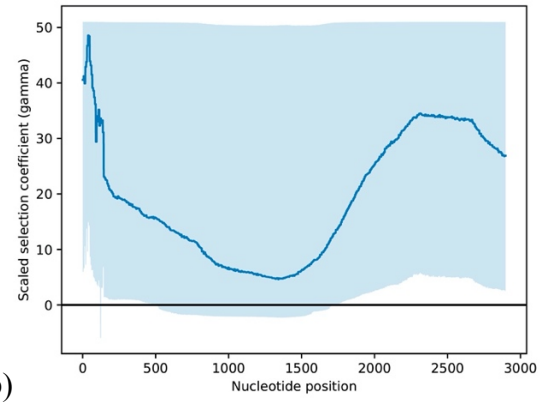
Figure 1. Phylogeny of phocid seals included in this study, reproduced from Fulton and Strobeck (2010). Branches examined in this study are labeled 1–8 and described in Table 1.



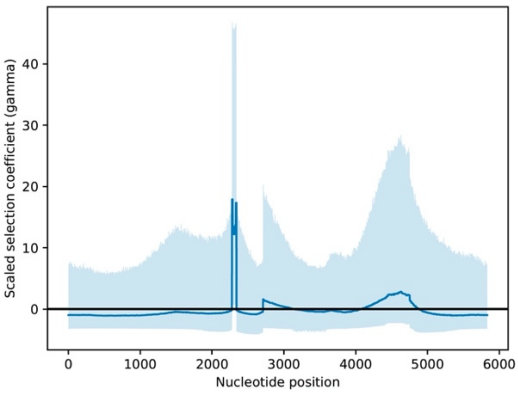
Figured 2. Estimate selection intensity (γ, dark blue) and 95% confidence interval (light blue) across all nucleotides in the APOBR gene in a) grey seal b) monk seals c) elephant seals d) ringed seals e) Weddell seal f) Miroungini ancestor. The horizontal black line shows neutrality (i.e. $\gamma = 0$). Selection intensities and confidence intervals were estimated through model-averaging in MASS-PRF.


Scaled selection coefficient (gamma)
Nucleotide position

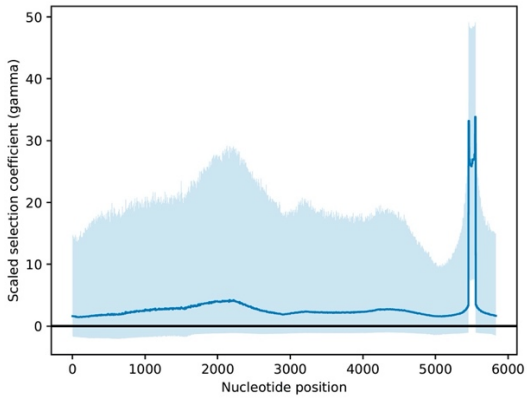
a)


Scaled selection coefficient (gamma)
Nucleotide position

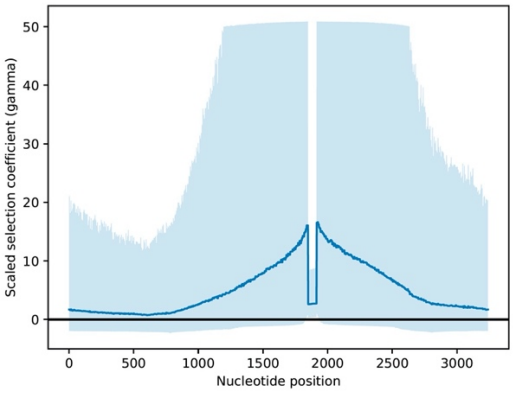
b)


Scaled selection coefficient (gamma)
Nucleotide position

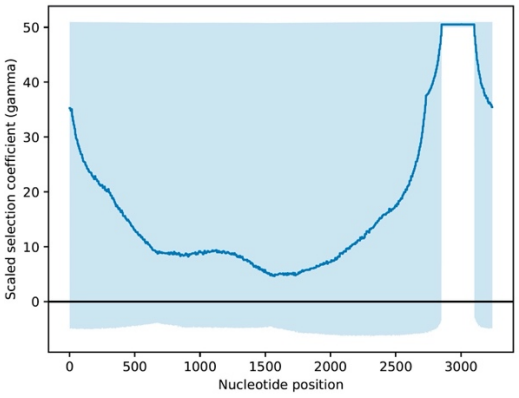
c)


Scaled selection coefficient (gamma)
Nucleotide position

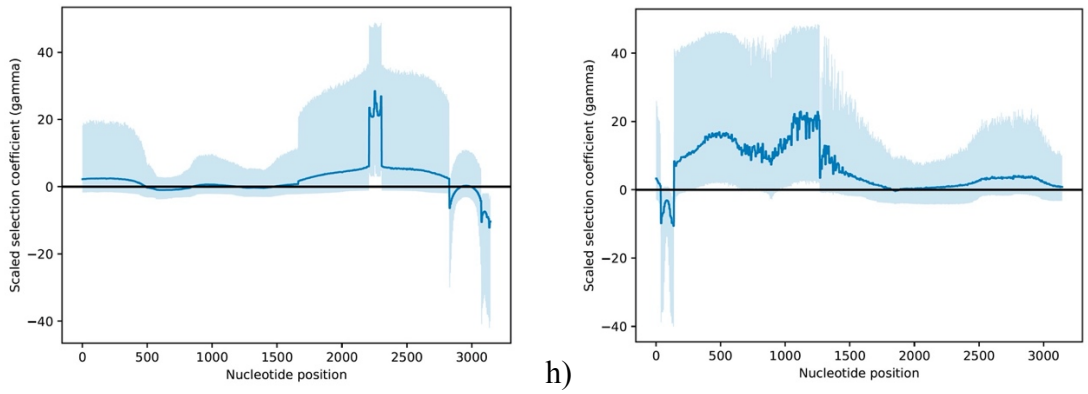
d)


Scaled selection coefficient (gamma)
Nucleotide position

e)

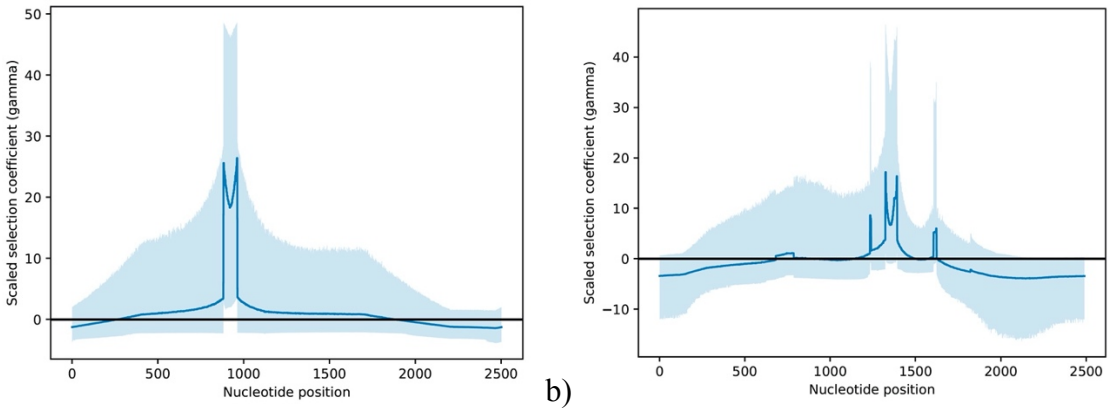

Scaled selection coefficient (gamma)
Nucleotide position

f)



g) h)

Figure 3. Estimate selection intensity (γ, dark blue) and 95% confidence interval (light blue) across the ANPEP gene in a) elephant seals b) Weddell seal; the MYH7B gene in c) elephant seals d) Weddell seal; the CARD6 in e) elephant seals and f) Weddell seal; and the CARD14 in g) elephant seals and h) Weddell seal. The horizontal black line shows neutrality (i.e. $\gamma = 0$). Selection intensities and confidence intervals were estimated through model-averaging in MASS-PRF.



a) b)

Figure 4. Estimate selection intensity (γ, dark blue) and 95% confidence interval (light blue) across the a) TLR4 and b) TLR5 genes in the Saimaa ringed seal. The horizontal black line shows neutrality (i.e. $\gamma = 0$). Selection intensities and confidence intervals were estimated through model-averaging in MASS-PRF.

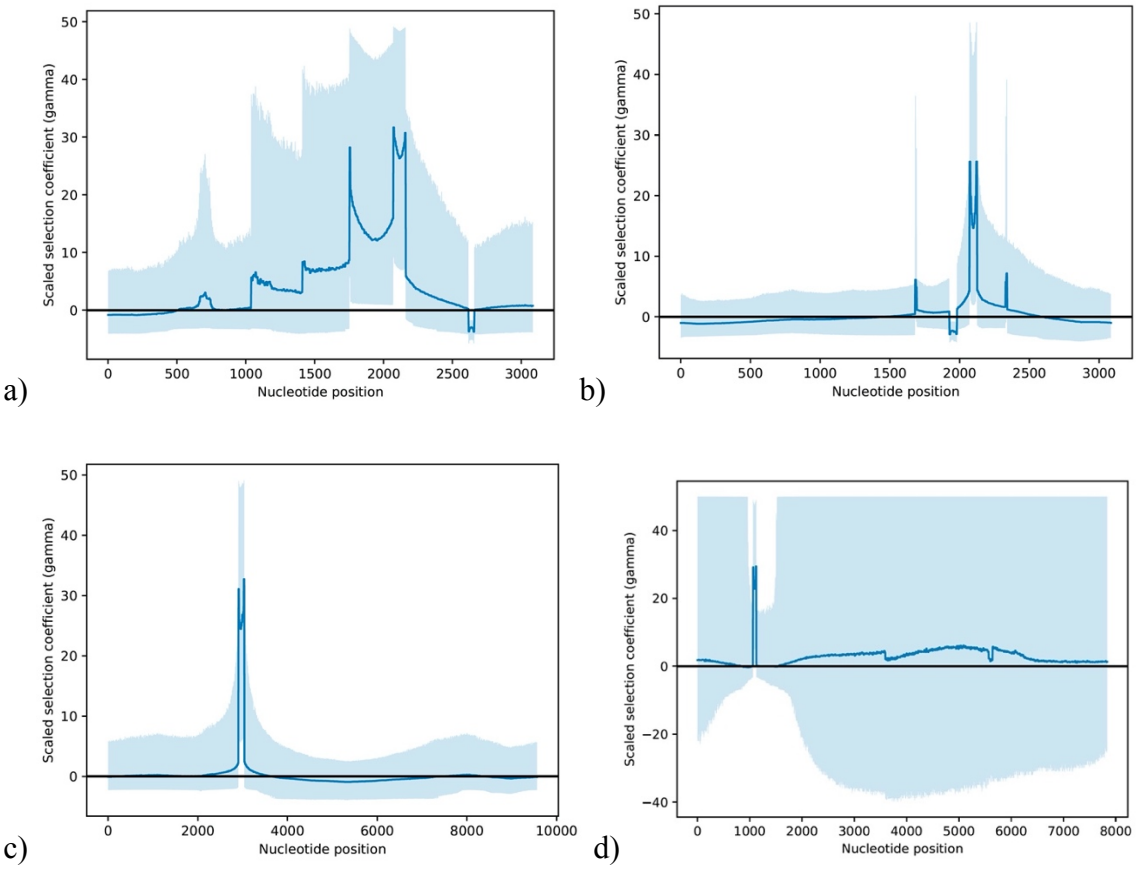


Figure 5. Estimate selection intensity (γ, dark blue) and 95% confidence interval (light blue) across selected collagen genes in phocid seals. a) COL6A1 in monk seals b) COL6A1 in elephant seals c) COL6A3 in the Weddell seal d) COL6A5 in ringed seals. The horizontal black line shows neutrality (i.e. $\gamma = 0$). Selection intensities and confidence intervals were estimated through model-averaging in MASS-PRF.

Branch number (Fig. 1)	Taxon name	Estimated length of branch in years
1	Monk seals	1.7 million years
2	Miroungini ancestor	1.2 million years
3	Weddell seal	6.88 million years
4	Elephant seals	6.88 million years
5	Grey seal	2.0 million years
6	Ringed seal (species)	2.0 million years
7	Baltic ringed seal (ssp.)	100,000 years
8	Saimaa ringed seal (ssp.)	100,000 years

Table 1. Lineages examined in this study, as shown in Figure 1. Branch lengths are taken from Fulton & Strobeck (2010) (monk seals, Miroungini, Weddell seal, elephant seals, grey seal, ringed seals) or from Chapter 1 of this dissertation (Baltic ringed seal, Saimaa ringed seal).

Adaptation	Lineages	Excluded lineages	Overlapping genes (excluding outgroup)
Polar environment	Weddell seal Ringed seals	Elephant seals Monk seals Grey seal	ALX4, ARHGEF5, COL27A1, COL6A5, CRYBG2, EGF, ELP1, IRS2, ITGA1, KMT2E, MAML2, MFSD9, MYO15A, NOC4L, TNC, WDR27
Warm water	Monk seals	Weddell seal Elephant seal Grey seal Ringed seals	ALDH1B1, CCDC30, CD5, CDH19, DNAH14, DVL3, FAM135B, GGT6, GRIN2C, IGSF9, JAG2, KIF26B, LAMC2, LATS2, N4BP2L2, NFKB1, NLRP5, NOBOX, OAS3, PDGFD, PPIP5K1, PPP6R1, PRAG1, PRR14, RNF114, RPS23, SCN10A, SLC6A18, SNAP23, TFB2M, TOP3A, UGGT2, UTRN, ZBTB44, ZIC3, ZNF318, ZNF451
Polygyny and sexual dimorphism	Elephant seals Grey seal	Weddell seal Monk seal Ringed seals	ADAMTS18, CELSR3, CNTNAP4, COL18A1, DNAH7, DNAH9, DOPEY2, F5, FAM186A, GPR132, GPR179, HEG1, MDC1, PEX1, PKHD1L1, PTPN21, SEC16B, SEMA4D, SLX4, SVIL, TTLL4, ZBED4
Deep diving	Weddell seals Elephant seals	Monk seals Grey seals Ringed seals	ABCA7, ABCB4, ADAMTS16, AGRN, ANPEP, ARMCX4, ASB10, ATP13A2, BDP1, BOD1L1, BRAT1, C5, CACNA1I, CARD14, CARD6, CARMIL3, CCDC114, CDH1, CDON, CEL, CELSR2, CEMIP, CNGB1, COL6A6, CORIN, CUL7, CWF19L2, DIAPH1, DIAPH3, DIDO1, DNAH6, DSEL, DSG3, EFCAB8, EIF2AK4, ERMP1, ESPL1, EVPL, EXPH5, FAM198A, FASN, FAT1, FBF1, FBXL13, FMN1, GUCY2D, HELZ2, HERC6, HJURP, HPX, IGF2R, IL3RA,

			INPP5F, IQGAP3, ITGA6, KANK1, KIAA0753, KIAA1549, KIAA1549L, KIF13A, KIF26A, KMT2A, LNX1, LTF, MCM9, MCPH1, MROH1, MTUS2, MYH7B, NCKAP5, NFASC, NWD1, OTOL1, PAPPA2, PARP14, PATJ, PER2, PLEKHG1, PLEKHH2, PLXNB2, PML, POLE, POLR1A, POLRMT, PPP1R9A, PPP6R2, PTPN13, PTPN23, PTPRN2, QSOX2, RAB11FIP5, RAD51AP2, RELN, REV3L, REXO1, RIF1, RNH1, ROBO4, RRBP1, RTL9, SALL3, SCN5A, SEL1L3, SEMA4B, SH3TC1, SI, SIPA1L3, SLIT3, SNAPC4, SPPL2B, SREBF1, STK36, STRC, SYNM, TBCD, TCHHL1, TDRD1, TEP1, THAP3, TIAM1, TJP2, TOGARAM1, TOGARAM2, TRIM66, TRPC3, TRPM6, TSHZ2, TSHZ3, TTBK1, TTC3, TTI2, USP16, USP42, UTP20, VCAN, WDR81, WNK1, ZBTB24, ZFPM1, ZNF316, ZNF462, ZNF541, ZNF582, ZNF592, ZNF646, ZNF804A
Out-of-the-tropics	Weddell seal Elephant seals Grey seal Ringed seals	Monk seals	ADGRG4, MKI67, QRICH2
Phocid-specific trait	Monk seals Weddell seal Elephant seals Grey seal Ringed seals	None	APOBR, TEX15, TNN

Table 2. Adaptive traits or conditions that are found in some branches of the phocid phylogeny but not others. Genes showing positive selection in the lineage(s) with the trait but not showing positive selection in other lineages.

Supplemental Material

Monachini	ZNF318, COL6A1, DOCK1, NOTCH1, MAMLD1, PCNT, LAMC2, SDK1, ZIC3, CDH19, CCDC30, PAPLN, ANKRD24, COL6A3, TMC5, TEX15, PLCH2, WDR97, TG, UGGT2, IQSEC3, PRAG1, PPIP5K1, ABL1, SNAP23, FHOD3, FAT3, HIVEP1, N4BP2L2, NISCH, MADD, RPS23, ABTB2, MYT1, UTRN, GAK, ALPK2, DNAH11, FAM135B, ITGAL, DVL3, NPHP4, CD5, AHCTF1, NRDE2, PDGFD, CEP290, SLC6A18, NOTCH4, URB1, NLRP5, PPM1F, IGSF9, STAB1, DNAH14, ATP7B, PKD1, OAS3, ERICH3, PRR14, NOBOX, FYCO1, SCN10A, ZDBF2, ZBTB44, COBLL1, RPGRIP1, GGT6, ZCCHC6, TNN, KIAA1671, PRUNE2, OTOG, ZNF407, FAM83G, PPP6R1, ADAMTS12, KNL1, ABCA13, KIAA2012, NPC1, ALDH1B1, DSG2, ASH1L, SEC16A, PKDREJ, ZAN, JAG2, GRIN2C, ACAN, WNK2, SDK2, RIPOR1, ADAD2, LATS2, FHDC1, TFB2M, TOP3A, MISP, ZNF451, KIF26B, NFKB1, ASXL3, POM121L2, ZNF236, CEP170B, RNF114, APOBR, IL4R
Miroungini ancestor	ZC3H4, TMEM132C, GPBAR1, FGA, SORBS1, PCNT, SPEG, PIGR, ATP1A4, NEDD4, SDK1, TACC2, DNMBP, MYO18B, URGCP, MYO15B, ZNF592, AK9, NHSL2, SNED1, AATK, DDX58, TMEM131, COL6A3, GLI2, GFY, BOD1L1, MROH1, SEC23IP, SLC12A8, TEX15, DEGS2, MYO16, HSPG2, TMC6, KIF24, FAM111A, ARHGEF11, TRPM7, FMN1, NWD1, PKD1L3, RFWD3, ANKLE2, ANK3, IMPG1, LAMA2, ARHGAP39, SNAP23, QRICH2, ZHX3, FHOD3, RUSC2, SLX4, WDR62, KRT15, PBXIP1, KIAA1549, MADD, TMEM235, ABCA7, ESYT1, MAP3K19, COL5A2, JAK3, PATJ, COL3A1, CASKIN2, PPFIA4, MGAT1, PTPRJ, TBRG4, HELZ2, ZCCHC14, MAML3, CUL9, LOXHD1, AHCTF1, CRYBG2, KIAA1211L, MUC1, ABI3BP, AKAP11, FRMD8, CEP290, CDH23, TTLL4, PLEKHG3, PCM1, UMODL1, NHSL1, POLRMT, MIA3, NOM1, ADAR, SPG11, DNAH9, PCNX2, ARMCX4, FNDC1, IQSEC1, FN1, VWDE, NYNRIN, RTTN, KIAA1551, ATP7B, PCDH12, SETD2, MRGPRG, ICE2, ROS1, CEP250, MROH2A, MKI67, MEGF6, AKAP6, PRUNE2, AKAP13, FBLN2, OTOG, ZNF407, CELSR1, GOLGA3, MPDZ, DCHS2, CACNA1H, CUX2, KIAA0556, CFAP44, ADAMTS13, ARHGAP29, PDE3B, COL6A5, WDFY4, PALB2, MYO15A, ALPK3, DISP1, DNAH17, PCLO, TEP1, SEC16A, PKDREJ, PKHD1L1, TICRR, SPPL2C, PTPRZ1, SV2C, GPR149, ABCA2, PALM3, KMT2C, WNK2, COL17A1, AKNA, WDR27, FASTKD3, USP35, SERPINF2, TMC3, ASPM, TRPM4, KMT5B, KANK4, TLDC1, TNS1, PLIN4, POM121L2, DNHD1, ZNF236, CEP170B, DNAH6, CARD14, MTMR3, MYOM1, RBM44, ABCB11, GLI3, CACNA1E, APOBR, PARP14, MROH2B, ICE1, PTPRN2, COL18A1, SLC44A4, TBC1D9B, TDRD6, ZNF804A, LRRIQ1, OTOL1
Weddell	CATSPER2, PPP1R9A, IRS4, HJURP, NES, EPHA1, CUL7, FGA, APOH, P3H1, ADRA1A, VPS13C, USP36, MAP4, DOCK1, NOTCH1, KCNT1, IRS1, PLEKHG1, POLE, SNAPC4, SORBS1, FRAS1, PTPRB, MAMLD1, TBC1D2, ZSCAN26, NUP210L, RIMBP2, RAB11FIP5, PCNT, MAML2, CCDC129, ELP1, ESPL1, CTBP2, CDH17, NFE2L3, RTN4, SLC22A1, SPEG, IL12RB2, RIN3, ITGA6, BSN, TBC1D2B, GTDC1, ALDH9A1, ATP1A4, MAP2, ASIC4, SIPA1L3, SBNO2, TTC3, PLEKHM2, TMPRSS9, TACC2, ABCC2, KRBA1, GNPTAB, UBA7, FRYL, SFI1, CHRNA3, LNX1, SUN1, TP73, ZNF592, WNK3, HPX, ZNF582, SRRM2, RBBP8NL, PLEKHH2, KIF26A, RELN, CCDC13, STK36, DUOX2, TUSC5, PHF3, ZBTB24, EPHX2, BDP1, TMPRSS2, ADCY6, ALS2, SREBF1, BBS10, NLRP6, ATP13A4, POLR1A, ZFPM1, ROBO3, SLC37A2, BRAT1, ANGPTL8, COL6A3, TRIM68, GLI2, BOD1L1, KLHDC7A, MROH1, CDON, SULF1, IRAK3, MCPH1, FAM161B, SGPP2, TEX15, FKBP15, CCDC151, OTOA, ATP8B1, AFF1, HSPG2, THSD7A, RBBP6, P2RX7, FREM2, ROBO4, RMI1, FANCA, GRASP, KMT2E, PYGL, SHROOM4, MPHOSPH9, PLA2G6, NCKAP5, SH3TC1, VWA5B2, FMN1, NWD1, WDR81, IQSEC3, CC2D2B, TIAM1, GUCY2D, TTBK1, ZFHX2, TTF2, CSMD2, FAT1, ANK3, SNTG2, CACNA1I, MYH14, SEC24A, SYTL3, PTPRF, IGF2R, PHLDB2, WDR6, BRCA1, ARAP1, MMP8, GPR20, ASB10, TSPAN1, ZNF536, ALOX15B, LRBA, RTL9, FAT2, CDH1, VWA7, FBRSL1, DIAPH1, QRICH2, COL20A1, GPRIN2, FBXO18, THBS2,

	HIVEP1, QSOX2, PROSER3, WDR62, LRP4, SYNJ2, THAP3, NLRP3, GPR35, KRT15, NRK, CENPE, ANO7, MZF1, WDR19, SLC16A1, FLNA, DCDC2C, PBXIP1, MYBBP1A, TTI2, KIAA1549, RSF1, COL28A1, NISCH, MADD, NEK1, PER3, SLC45A4, EMC1, TBCD, CCDC57, PCDH15, ABCA7, CD177, DISP3, FER1L6, PTPN23, PLEKHA7, DSG3, DNER, SPPL2B, LMO7, ITGAX, KIAA0753, ZNF541, EXPH5, ZC3H3, MEI1, PATJ, PPL, INPP5F, LAMB3, ADAMTSL3, DNAH11, USP43, WRN, DNAJC6, ZNF608, KIAA1549L, MLXIP, IL3RA, PTPRJ, TBRG4, SLC14A2, HELZ2, TARBP1, MCTP2, FAAP100, IRS2, TDRD1, LTBP3, COL5A3, MFSD9, GRK4, MAML3, HS1BP3, ITGAL, ARHGEF5, SEC24B, CCDC177, AEN, NPHP4, CUL9, DSEL, TRPA1, SYCP2L, AHCTF1, NOC4L, PAPPA, NLRC5, CRYBG2, CEP295, ECM2, ALDH3A1, SEMA5A, CARMIL3, CEL, CERKL, NRDE2, BUB1, SRRD, OSGIN1, CEP290, CCDC88C, NFATC1, NOTCH4, TOGARAM2, AP3B2, BCL9L, WFS1, RAD51AP2, SAMD15, SI, FASN, URB1, PCM1, AGRN, ACACB, ADCY10, ATP8B3, RAB44, SSC5D, LRP1B, TRPM6, COL27A1, POLRMT, CCDC8, MIA3, NOM1, SPG11, PCSK5, LAMA5, ST14, COL6A6, RIF1, PRRC2A, PPM1F, EAF1, ARMCX4, RNF180, ADGRL2, PDE3A, IQSEC1, ASPSCR1, ITGA11, STAB1, EIF2AK4, VWA3B, NYNRIN, RTTN, TNIP1, KIAA1755, FNIP2, GPRIN1, ATP7B, ARMCX2, SLC38A10, CYLC1, PPP1R13B, NLRP14, IQGAP3, TOGARAM1, HENMT1, PUM3, AP5B1, ADCY4, MFSD6L, USPL1, ATP2A3, RRBP1, FHAD1, MRGPRG, PLCG2, SEL1L3, FHOD1, CORIN, GGN, SETD1B, HERC6, BAIAP2L2, HTRA4, CNGB1, TJP2, HRC, ERMP1, INSRR, FAM196A, SCN4A, BRD4, SLIT3, LAMB1, IL1R1, HEATR5A, FYCO1, CEMIP, PPP6R2, DDX51, EFCAB5, FLNB, MKI67, DCLRE1C, LTF, SYCP2, H6PD, CIZ1, MEGF6, ABCA1, ADAMTS16, COL15A1, SCN9A, FAM198A, CASS4, IGSF5, SCRIB, COBLL1, CORO2A, TSPAN15, ZNF462, RPGRIP1, ZBTB40, FBF1, HFE, TRIM66, ZCCHC6, FAM234B, TNN, LTBP4, KIAA1671, STRC, PRUNE2, AKAP13, CHRNE, PAPPA2, FUT4, NCAN, OTOG, RNF207, ZNF407, ADGRG4, FAM83G, USF3, BOC, MPDZ, DCHS2, CARD6, CACNA1H, RP1, CDH15, C5, RAI1, ANK2, EFCAB6, BPTF, ELP2, ADAMTS12, TEX14, KNL1, ABCA13, PLD4, USP42, PIK3C2G, ARHGAP29, ADGRF1, USP16, PER2, KIAA2012, PLXNB2, CAMSAP1, ADGRF3, GNAS, COL6A5, NPC1, PCNX1, RBSN, TENM1, HEATR1, CHD7, ANKAR, PRRT4, TTC24, KIF13A, DOCK4, MYO15A, BCAN, NID2, RRP1B, CWF19L2, ITGA1, SLC26A6, MCM9, DISP1, MTUS2, ABCC8, TAS1R3, DNAH17, ADCY1, SLC39A12, KMT2A, PCLO, INPPL1, DSG2, DSP, TRPC3, ASH1L, THSD4, TEP1, CDC42BPG, SEC16A, C2CD3, ZAN, NBEAL2, FAM193A, AMOTL2, TCHHL1, SPEF2, ANO2, PLEKHG2, MYH7B, SEMA4B, EFCAB8, GRIN3A, HRH4, PIMREG, REV3L, PTPRZ1, DIDO1, PTPN13, ACAN, PLEKHA6, MYBPC3, PALM3, WNK2, CLCA2, MYO9B, DDI1, ZNF646, ABCB4, THSD1, EGFLAM, RNH1, ESYT3, ZNF316, PLCE1, SDK2, RNF19B, ANLN, FRMPD1, RIPOR1, VWA8, PRX, CENPJ, WDR27, TMEM132E, CHRNA4, ATP8B2, TUBGCP6, UTP20, NAT10, EVPL, SETX, VASN, ACOX3, AGT, FHDC1, DIAPH3, TENM2, KIF18B, TECPR1, STARD13, HYOU1, TSHZ3, SYNM, HIPK3, WNK1, SCEL, PLB1, MYH15, ALX4, EGF, FAM114A1, MICALL1, ZHX2, TEX45, ANPEP, TNC, CC2D1A, ATP13A2, WFIKKN2, AP5Z1, MTTP, POM121L2, KANK1, CCDC114, LPIN3, ARHGEF10L, PNPLA7, CDSN, TRIP11, DNHD1, ZNF236, CEP162, CEP170B, TTC6, SALL3, TPBG, VCAN, DNAH6, SLC24A2, PML, LAD1, CARD14, NFASC, RBM20, ZFYVE16, THEMIS2, CFAP65, ITGA2, FBXL13, TMTC1, APOBR, PARP14, AP3D1, CELSR2, ZNF786, PTPRN2, RHPN1, TSHZ2, KIAA1217, MMP21, TELO2, ZNF804A, WHAMM, SCN5A, REXO1, IL4R, SGSM2, AKAP12, OTOL1
Elephant seals	ZC3H4, VRTN, PPP1R9A, CDK5RAP2, USP24, FRMPD3, ADAMTS14, TOP2A, YBX3, HJURP, ACOX2, CUL7, CSF3R, ATG2A, GRTP1, SYNPO, FAM110C, COL6A1, SEC31B, LIMD1, PLEKHG1, CACNA1F, SHANK2, POLE, ADGRG7, SNAPC4, SLC23A3, FAM71A, TJP3, DCLRE1A, DACT2, RAB11FIP5, NRXN2, HDAC10, PNPLA1, ESPL1, MKL2, PEX10, STOX1, LRRN4, FER1L5, ITGA6, BSN, TBC1D2B, ZFHX3, DOPEY2, IPO4, CADPS2, CDC42BPB, NPHS1, MYLK2, DHX57, SASH1, SIPA1L3, TTC3, ZNF532, TANC1, PYROXD2, LAYN, MYO18B,

	CFAP54, WWP1, DOCK8, LAT, MYO15B, EPHA10, CPD, GPR132, LNX1, SEMA4D, XPC, ZNF592, CNTRL, FOXRED1, MEGF8, NHSL2, USP26, HPX, ZNF582, SRRM2, MROH6, ELMO3, PLEKHH2, KIF26A, ALKBH8, GTSE1, RELN, PAPLN, ANKRD24, HIP1R, SHANK1, DSC3, ANKRD12, STK36, ZBTB24, BDP1, DGKZ, PLEKHH1, PIEZO1, DMRT3, SPECC1, USP47, MTMR10, TTC28, AATK, AGL, WDFY3, ARHGAP22, SREBF1, PTPN14, TSC22D1, CDCP2, ARHGAP21, GPR50, LAMC1, POLR1A, PTPRU, ZFPM1, BRAT1, IFT172, DLG5, SHROOM3, PROSER1, TMC5, BOD1L1, CDH3, MROH1, CDON, TNIP3, KLF11, MYO19, MCPH1, DGKI, TONSL, TEX15, FAM186A, HSPG2, TBRG1, PLCH2, GPR39, COBL, PRAM1, ATP11A, PPP1R3A, THSD7A, F5, ZCCHC7, ROBO4, KIF24, WDR97, FAM111A, FKBP4, DOT1L, DRC1, PAXIP1, LMF2, NCKAP5, RALGAPA2, ADAMTS5, SH3TC1, CFAP57, FMN1, NWD1, WDR81, SPHKAP, CAMTA1, MAN2A1, TIAM1, MYRFL, GUCY2D, CARMIL1, TTBK1, LTBP1, ABCC4, RBM19, ZFR2, FAT1, CELSR3, CACNA1I, DLK2, ICAM1, PNPLA5, IGF2R, CCDC185, GALNT6, CRAT, IAH1, CEP104, ARHGAP17, ASB10, ABL1, TCOF1, TCIRG1, CACNA1S, FSD2, RTL9, PEX1, PAXX, LSS, TCF3, CDH1, TGM5, HELQ, TRIO, TDRD5, TBC1D4, DIAPH1, QRICH2, NKAPL, SPRTN, SAMD9L, ZHX3, GARNL3, FAT3, ZNF292, TDRD12, HIVEP1, SLX4, QSOX2, PPP1R32, TRAPPC12, AGAP1, WDR62, GPR179, SYNJ2, DUSP27, THAP3, KCNV2, DUOX1, ABCC6, PITPNM1, PBXIP1, ITGB7, MYBBP1A, MORN1, TTI2, GAPDHS, MAPKBP1, KIAA1549, MPO, NINL, AEBP1, CAMSAP2, SLC4A1, TRMT44, PER3, CDC20B, TBCD, SLC24A1, ABCA7, CNTNAP4, ZNF518B, ADGRE5, ARID5B, ELMSAN1, FER1L6, PHLDA1, PTPN23, INPP5J, ZSCAN12, HIVEP3, DSG3, DSE, SPPL2B, FAM35A, ARSJ, SWAP70, MYT1, DCLK3, PRRT3, ZNF598, ABCG5, ALDH5A1, FAM83F, KIAA0753, ZNF541, RECQL4, EXPH5, TNKS1BP1, FAM71B, ZC3H3, ALPK2, HPS1, TLE6, PATJ, APC2, TTC23, FARP2, INPP5F, CLUH, AMOTL1, LAMB3, CASKIN2, MAMDC4, DNAH11, FAM83A, KAT6A, KIAA1549L, TTLL11, ARFGEF3, NRDC, FMN2, HELB, IRGQ, HECW1, COL1A2, HEG1, IL3RA, PTPRJ, HTR3B, HELZ2, FBXO34, ZCCHC14, PDIA4, CCDC80, KIAA2026, TDRD1, ADGRA3, KRT84, NCKIPSD, MAML3, BNC1, AMIGO3, MAP3K6, APBA1, NAV3, PHF20, ABCA12, PTPRC, CAGE1, PADI4, RPAP1, MAP1A, CUL9, KIF15, DSEL, NDUFAF7, KIAA0319, RREB1, TECTA, DNAAF1, PHRF1, DOCK11, NUP214, FSTL5, ERCC6L, TET2, SIK3, VLDLR, NR1I2, LARS2, TNFRSF25, PDGFRB, CEP131, ARMC5, RAD51D, AKAP11, FAM221B, SORCS1, CA9, SVEP1, CARMIL3, CEL, NRDE2, CHRNB1, URB2, XDH, NWD2, MN1, CRTC2, AMBRA1, CCDC88C, SHH, ACAP3, CDH23, ZC3H12D, MTR, TOGARAM2, PDZD3, TTLL4, FIGNL1, WFS1, RAD51AP2, F2RL1, PLCB3, MTCL1, TMEM94, SI, TDRD9, FASN, PLEKHG3, AKAP9, AGRN, ATP8B3, PHLPP2, RPAIN, ARAP3, TRPM6, UMODL1, CACNB2, NHSL1, POLRMT, TNRC6C, FAP, PIK3R1, ZADH2, MIA3, SPG7, ADAR, COL4A6, DNAH9, LAMA5, PCNX2, COL6A6, SVIL, ADGRA1, GOLIM4, RIF1, ARMCX4, AHRR, FNDC1, ANKMY1, ACADVL, EPB41L2, ARHGEF17, EIF2AK4, BLM, NYNRIN, RTTN, ATP2A1, KIF20B, LRPPRC, MYOF, PNPLA3, PCDH12, TGM7, PKD1, AFAP1L2, CDHR3, TMEM161A, FAM171B, IQGAP3, TOGARAM1, BRIP1, ZNF831, BPIFB2, ZFAT, MFSD6L, PLIN3, MED13L, VPS13A, CSMD3, RRBP1, SNCAIP, PLXNB1, POLI, FPGS, SEL1L3, KNTC1, FAM160A1, ERICH3, TTI1, AVIL, PARG, CORIN, HERC6, BICRA, TGM2, CNGB1, TJP2, ACOT12, LCMT2, GPLD1, ERMP1, CPN2, AFF3, SLIT3, CNTLN, KIF3C, FYCO1, MAST1, CEMIP, PPP6R2, DDX51, EFCAB5, OTUD7A, MAVS, ZDBF2, MKI67, GGACT, STK11IP, LTF, JCAD, DHX34, KDM6B, MEGF6, TTBK2, ZFP3, ADAMTS16, FAM198A, PSD4, FNBP4, COBLL1, TSPOAP1, ADGRD1, APOA5, ZNF462, RPGRIP1, ZMYND8, FBF1, RB1CC1, TTF1, TRIM66, NXPH2, ADNP2, TNN, KIAA1671, AKAP6, STRC, SYDE2, AKAP13, SLC12A7, ZNF142, PLA2G4F, PAPPA2, NFAT5, SPINT1, POMT2, AKAP1, TTC21B, CEP126, SCMH1, COL7A1, CASKIN1, ZNF407, ADAMTS18, VWF, ADGRG4, CELSR1, FAM83G, PRRC2B, ZBED4, BOC, WEE2, OAS2, CARD6, ZSCAN22, C5, PLXND1, DLGAP2, KCNG4, COL4A2, N4BP1, TMEM132A, IQCA1, LRCH3, KANK2, CHRM3, KNL1, ABCA13,

	USP42, PER1, COL26A1, THBD, USP16, MYOCD, PER2, SUSD1, MELTF, PLXNB2, CAMSAP1, PDE3B, HCN4, MDC1, PITPNM2, TGFBRAP1, PLCB2, EPS8L3, NPC1, LAMA4, WDFY4, NOS1, ECM1, RPH3A, KIF13A, RTL1, CES5A, VWCE, CWF19L2, CCDC155, MAPK8IP1, SETD6, MCM9, MTUS2, MYBPC2, RTN1, ADCY1, HAUS5, PCDH18, SLC10A3, KMT2A, PODXL, BRDT, CFAP46, METTL22, MLH1, CNTROB, TICAM1, DNAH7, DSG2, CCDC116, MRS2, DSP, TRPC3, NUP153, KIAA1683, TEP1, PASK, SEC16A, LEMD3, PIGM, MYOM3, CEP164, NUP62, PKDREJ, ZAN, PKHD1L1, DHX33, NBEAL2, STAB2, MYO7A, ZNF628, APBB1IP, CGN, TCHHL1, SCAF11, MYH7B, SEMA4B, EFCAB8, TMEM131L, EFL1, KRT2, RADIL, HAS1, GUCY2F, REV3L, DIDO1, PTPN13, ACAN, F11R, LMTK2, WNK2, HGS, ZNF646, ABCB4, DPYSL4, ACIN1, CERS4, RNH1, ZNF316, KCNA10, INVS, SDK2, KRT12, SLC26A1, MYT1L, PRX, LEPR, PPARGC1B, OBSL1, ZNF473, TMEM184B, KLK5, VIPR2, UTP20, TRPS1, HECTD4, ERAP1, EVPL, ADAD2, TRANK1, PLA2G4E, FHDC1, CHD6, DIAPH3, FAM155A, GAA, CIC, COL4A3, CDT1, TSHZ3, CD38, FAM208B, MISP, CD96, MAGEB16, SYNM, ADAMTS20, WNK1, PIGN, TMC3, ZC3HAV1, SNX19, TRPM4, OTOP1, FAM13A, ZNRF4, TUBGCP2, ZNF277, CCDC88B, KANK4, ANKRD11, TET3, PLCH1, ANPEP, CBARP, VWA3A, NIN, PTPN21, ATP13A2, PINK1, ATP2B2, CACNA1A, CAMSAP3, EVC2, POM121L2, CCDC40, SEC16B, CACNA1G, KANK1, CCDC114, DENND2A, SPAG17, NYAP2, MRPL21, TMC4, DNHD1, ZNF236, NOLC1, ASXL2, RBPJL, SALL3, TRIM14, ANKRD35, ST6GALNAC1, VCAN, DNAH6, GPR158, IL16, PML, IL17RE, CARD14, DICER1, NFASC, MYOM1, CLNK, MAP7D1, ATP9B, CCDC136, CFAP65, CRYBG3, PLA2G4D, CFAP47, FBXL13, CFAP61, LRRC14B, TMEM132D, PTCD2, NEK4, TECPR2, APOBR, PARP14, TMX4, CELSR2, GALC, SCUBE2, EXOSC10, PTPRN2, TSHZ2, COL18A1, TBC1D9B, GALNT12, TDRD6, WDR55, TMTC4, WDR72, RALGDS, ZNF804A, TTC17, SCN5A, REXO1, ZDHHC1, RINL, SCAPER, PJA2, TRDN, KIAA1210, OTOL1,
Grey seal	CFTR, IQCH, GREB1, BSN, DOPEY2, SPTB, SDK1, TACC2, WDR11, ABCC2, KIAA1211, GPR132, SEMA4D, SRRM2, PHF3, AATK, ADAMTS7, SHROOM3, LYST, TEX15, FAM186A, AFF1, MYO16, HSPG2, PRG4, F5, FREM2, NOP14, TG, ZFHX2, CELSR3, PEX1, QRICH2, TBC1D31, FHOD3, HIVEP1, SLX4, GPATCH4, NOS2, WDR62, GPR179, SYNJ2, MYBBP1A, CNTNAP4, FER1L6, PDCD11, GAK, LAMB3, ADAMTSL3, HEG1, LRIG1, ARHGAP32, VPS13B, MMEL1, CEP295, PIGT, CXCL16, CCDC88C, TTLL4, DPH7, ATP8B3, CDHR2, DNAH9, LAMA5, SVIL, FNDC1, NYNRIN, COL10A1, ERICH3, CAPN8, HRC, FYCO1, DDX51, EFCAB5, MKI67, MEGF6, COL15A1, TNN, PRUNE2, AKAP13, FUT4, ADAMTS18, SALL2, ADGRG4, CELSR1, ZBED4, BOC, DCHS2, EFCAB6, ADAMTS13, MDC1, PCNX1, WDR60, CROCC, DISP1, DNAH7, DSP, MMP17, PKDREJ, PKHD1L1, NBEAL2, LTBP2, ACAN, KMT2C, WNK2, FRMPD1, PRX, TUBGCP6, TRANK1, DISC1, ASXL3, PTPN21, POM121L2, SEC16B, ZNF236, CEP170B, PKHD1, CFAP65, APOBR, ICE1, COL18A1, KMT2B, KIAA1614, SGSM2
Ringed seal	RAB3GAP2, TBX3, AQP8, MAML2, ELP1, WDR1, TMEM121, GTF3C1, BCR, TBC1D2B, TRHDE, SNTB1, MTUS1, STAG3, DNMBP, PHLPP1, RRP12, MYO15B, KIAA1211, BASP1, SHANK1, PGBD1, NEFH, PIEZO1, AATK, ADAMTS7, SHROOM3, PROSER1, KCNMA1, TEX15, MNX1, RYR1, THSD7A, DSG4, KMT2E, NHLRC1, TMEM229A, MST1R, VRK3, ATOH1, B3GNT6, CAMTA2, ASCC3, LACTBL1, CCDC171, QRICH2, TDRD12, CHST11, GLIS1, EXD2, PBXIP1, PER3, ABTB2, INPP5J, COL5A2, HIVEP3, PGR, RECQL4, AGBL5, ZC3H3, MARCO, KCNG1, USP19, HECW1, PTPRJ, IRS2, MFSD9, MAML3, ARHGEF5, RNF150, CUL9, KIAA0319, NOC4L, CRYBG2, MCM5, CHRNB2, CEP350, WFS1, CPA1, COL27A1, FAM83H, KCTD14, MIA3, WWC2, ADAR, MOCOS, FAM181B, PCDH17, FNDC1, VWDE, RTTN, LRPPRC, DCAF5, ZNF831, SETD2, MFSD6L, VPS13A, PLXNB1, NOD2, KCNC2, DISP2, BICRA, MKI67, GRID2IP, FBXL7, COL15A1, INSR, MAST2, ZNF648, TNN, PRUNE2, ZNF217, CDH6, ZNF407, ADGRG4, CELSR1, FCRLB, JAG1, HUWE1, CHRM3, CDCA2, ADAMTS13, PEG3,

	CAMSAP1, PDE3B, COL6A5, BMP3, LAMA1, MYO15A, UBN2, ITGA1, ZBTB42, GDF7, ADCY1, CRYBG1, TICAM1, KIAA1683, PIGM, PKDREJ, ZAN, RXFP3, TBX2, DLGAP1, STAB2, ZNF628, ABCA2, B3GLCT, WDR27, SPAG1, CMPK2, TRANK1, CHD6, SOX9, FAM208B, IRX2, ALX4, EGF, SLIT2, ANKRD11, SOX11, TNC, BTBD11, DNHD1, SORL1, ARHGEF40, CFAP47, ABCB11, APOBR, ABCC12, CSPG4, TBC1D30, KIAA1210
Baltic ringed seal	XIRP2, DACT2, SPEG, MYO18B, KIAA1211, ZC3H14, DSC3, AATK, ADAMTS7, GPR50, BRAT1, COL6A3, SHROOM3, BOD1L1, MROH1, TONSL, FAM186A, FREM2, KIF24, CASZ1, TG, MST1R, SNAP23, FAT2, QRICH2, MYBBP1A, NKX2-6, TMEM126A, EXPH5, ALPK2, APC2, LAMC3, CRYBG2, IGSF10, ATP8B3, LAMA5, PCNX2, COL6A6, HTATSF1, ZNF831, FAM160A1, SCN10A, MKI67, JCAD, TNN, PRUNE2, NTN5, ZNF142, SH2B2, ZNF407, BOC, CACNA1H, SLC25A43, PPP6R1, KNL1, ABCA13, MDC1, CDC16, LAMA1, ALPK3, DNAH17, CRYBG1, PCLO, ZAN, GRIN2C, PLEKHA6, FSCN2, SPAG1, A2M, CEP170B, ROR2, ICE1, PTPRN2, KIAA1210
Saimaa ringed seal	SNAPC4, ARHGEF10, TACC2, DNMBP, KRBA1, MYO18B, MYO15B, AK9, EMILIN2, SRRM2, CUBN, SHROOM3, LYST, AFF1, F5, WDR97, NOP14, ARHGEF11, CACNA1I, DSCAM, SPOCD1, DIAPH1, PER3, ZNF804B, ESYT1, DENND3, EXPH5, FAM71B, LAMB3, MAMDC4, DNAH11, KIAA1549L, HELZ2, IRS2, MAML3, ITGAL, TECTA, CEP131, SEMA5A, CDH23, DPH7, URB1, SSC5D, MIA3, COL6A6, COL10A1, IQGAP3, TIAM2, VPS13A, FHAD1, BICRA, CNGB1, FYCO1, MKI67, SYCP2, MEGF6, TAS2R40, COL15A1, TNN, KIAA1671, FBLN2, NPHP3, RAI1, EFCAB6, HCN4, CROCC, PCLO, C2CD3, ZAN, PKHD1L1, NBEAL2, PLEKHG2, TICRR, TLR4, COL12A1, REV3L, AKNA, ERAP1, COL4A3, TLR5, CACNA1G, KANK1, SALL3, MAP3K5, PARP14, CELSR2, HLCS

Table S1. All genes with signals of positive selection in each lineage. Gene symbols correspond to nomenclature used in the Hawaiian monk seal reference annotation.

Monachini	SMUG1, COL6A1, NOTCH1, FRAS1, PCNT, ACSF3, HDAC10, ATP1A4, SDK1, ZIC3, PYROXD2, XKR5, CCDC30, COL6A3, TMC5, BOD1L1, CDH3, KRT4, HSPG2, ARHGAP45, KIF24, NOP14, VRK3, PHLDB2, LAMA2, BPI, LSS, HIVEP1, FGG, USHBP1, MADD, RPS23, ABCA7, ABTB2, GTF3C5, UTRN, XRCC3, EFCAB12, DNAH11, KIAA1549L, DVL3, CD5, LAMC3, TECTA, KLHL33, PDGFD, SLC6A18, NKD2, URB1, TTLL2, LAMA5, PPM1F, STAB1, NYNRIN, DNAH14, PKD1, PARP3, PRR14, FYCO1, SCN10A, MAVS, ZDBF2, EVC, H6PD, TOR4A, TNN, PRUNE2, OASL, AKAP13, OTOG, CEP126, ZNF407, VWF, ADGRG4, ZSCAN22, MAP3K21, EME1, KNL1, TPO, ADGRF3, KIAA1024, ADCY1, CCR6, ALDH1B1, CCDC116, ASH1L, KIAA1683, SEC16A, PKDREJ, ZAN, GPRASP1, JAG2, GRIN2C, FUT7, ACAN, IL18R1, CTU2, TACC3, TOP3A, PIK3R5, SLC15A1, MISP, ZNF451, WNK1, ASXL3, KNOP1, ANKRD34C, CC2D1A, PLIN4, POM121L2, ZNF236, CEP170B, FAM120B, TUBB1, HEMGN
Miroungini ancestor	GPBAR1, FGA, SORBS1, PCNT, PIGR, ATP1A4, NEDD4, DNMBP, MYO18B, DDX58, COL6A3, GLI2, GFY, BOD1L1, MROH1, SEC23IP, SLC12A8, TEX15, TMC6, KIF24, FAM111A, FMN1, NWD1, PKD1L3, RFWD3, ANK3, IMPG1, LAMA2, SNAP23, ZHX3, FHOD3, WDR62, KRT15, PBXIP1, MGAT1, TBRG4, AHCTF1, CRYBG2, KIAA1211L, MUC1, AKAP11, CEP290, CDH23, PLEKHG3, PCM1, UMODL1, MIA3, DNAH9, ARMCX4, FNDC1, IQSEC1, RTTN, ICE2, MKI67, AKAP6, AKAP13, FBLN2, OTOG, ZNF407, DCHS2, CACNA1H, CUX2, PDE3B, COL6A5, MYO15A, DNAH17, PCLO, PKDREJ, PKHD1L1, SPPL2C, PTPRZ1, ABCA2, PALM3, KMT2C, WNK2, WDR27, FASTKD3, ASPM, KMT5B, KANK4, TLDC1, TNS1, PLIN4, POM121L2, DNHD1, CARD14, RBM44, GLI3, APOBR, PARP14, COL18A1, SLC44A4, TDRD6, ZNF804A, LRRIQ1

Weddell	CATSPER2, IRS4, HJURP, EPHA1, APOH, VPS13C, USP36, DOCK1, KCNT1, MAMLD1, ZSCAN26, NUP210L, RAB11FIP5, MAML2, SLC22A1, RIN3, BSN, TBC1D2B, ALDH9A1, ATP1A4, SBNO2, PLEKHM2, TMPRSS9, TP73, HPX, RBBP8NL, CCDC13, ZBTB24, ADCY6, ZFPM1, TRIM68, SGPP2, OTOA, AFF1, RMI1, FANCA, GRASP, KMT2E, PLA2G6, FMN1, CSMD2, FAT1, SNTG2, PTPRF, GPR20, ALOX15B, FBRSL1, GPRIN2, HIVEP1, QSOX2, PROSER3, THAP3, GPR35, KRT15, MZF1, NISCH, NEK1, PER3, TBCD, PCDH15, EXPH5, ADAMTSL3, DNAH11, DNAJC6, ZNF608, MLXIP, TARBP1, FAAP100, TDRD1, COL5A3, MAML3, SEC24B, CCDC177, NOC4L, ECM2, ALDH3A1, SEMA5A, SRRD, NOTCH4, TOGARAM2, AP3B2, SAMD15, AGRN, LRP1B, TRPM6, POLRMT, CCDC8, MIA3, EAF1, RNF180, ADGRL2, PDE3A, IQSEC1, FNIP2, GPRIN1, SLC38A10, IQGAP3, HENMT1, AP5B1, MFSD6L, RRBP1, MRGPRG, PLCG2, CORIN, GGN, HTRA4, CNGB1, HRC, INSRR, LAMB1, IL1R1, MKI67, MEGF6, ABCA1, ZNF462, FBF1, HFE, ADGRG4, CDH15, BPTF, ELP2, KNL1, PLD4, ARHGAP29, USP16, KIAA2012, ADGRF3, GNAS, PCNX1, RBSN, HEATR1, CHD7, TTC24, CWF19L2, TAS1R3, ADCY1, SLC39A12, KMT2A, INPPL1, ASH1L, THSD4, TEP1, ZAN, ANO2, SEMA4B, PTPRZ1, DIDO1, PTPN13, PALM3, WNK2, CLCA2, ZNF316, RNF19B, FRMPD1, UTP20, ACOX3, FHDC1, DIAPH3, KIF18B, TECPR1, WNK1, TNC, CC2D1A, AP5Z1, MTTP, KANK1, ARHGEF10L, CDSN, DNHD1, TTC6, SALL3, TPBG, DNAH6, LAD1, CARD14, CELSR2, TSHZ2, WHAMM, SCN5A, REXO1
Elephant seals	VRTN, CDK5RAP2, ADAMTS14, TOP2A, ATG2A, GRTP1, COL6A1, CACNA1F, SNAPC4, FAM71A, TJP3, DCLRE1A, DACT2, RAB11FIP5, NRXN2, LRRN4, BSN, TBC1D2B, ZFHX3, CDC42BPB, MYLK2, DHX57, SASH1, SIPA1L3, PYROXD2, LAYN, MYO18B, CFAP54, WWP1, DOCK8, LAT, MYO15B, CPD, SEMA4D, CNTRL, FOXRED1, MEGF8, KIF26A, ALKBH8, HIP1R, SHANK1, ANKRD12, ZBTB24, DMRT3, SPECC1, TTC28, ARHGAP22, TSC22D1, BRAT1, PROSER1, BOD1L1, CDH3, MROH1, KLF11, MCPH1, TEX15, HSPG2, COBL, PRAM1, THSD7A, F5, ROBO4, KIF24, PAXIP1, WDR81, SPHKAP, CAMTA1, GUCY2D, TTBK1, RBM19, ZFR2, CELSR3, DLK2, ICAM1, CCDC185, GALNT6, CRAT, CEP104, TCIRG1, CACNA1S, FSD2, RTL9, PEX1, PAXX, LSS, TGM5, TRIO, TBC1D4, DIAPH1, NKAPL, TDRD12, HIVEP1, QSOX2, AGAP1, WDR62, GPR179, SYNJ2, THAP3, KCNV2, MORN1, KIAA1549, CAMSAP2, TRMT44, PER3, ZNF518B, ADGRE5, ARID5B, FER1L6, PHLDA1, ZSCAN12, DSG3, SPPL2B, FAM35A, SWAP70, ZNF598, ABCG5, EXPH5, FAM71B, ZC3H3, ALPK2, HPS1, PATJ, FARP2, INPP5F, CLUH, CASKIN2, FAM83A, KAT6A, ARFGEF3, NRDC, IRGQ, HECW1, ZCCHC14, PDIA4, CCDC80, KIAA2026, KRT84, BNC1, APBA1, PHF20, CAGE1, MAP1A, CUL9, KIF15, NDUFAF7, KIAA0319, DNAAF1, FSTL5, SIK3, VLDLR, AKAP11, FAM221B, CA9, SVEP1, CARMIL3, CEL, URB2, NWD2, CRTC2, AMBRA1, SHH, ZC3H12D, TOGARAM2, RAD51AP2, PLCB3, TDRD9, FASN, PLEKHG3, AGRN, PHLPP2, CACNB2, FAP, ZADH2, DNAH9, LAMA5, PCNX2, ARMCX4, FNDC1, ARHGEF17, RTTN, MYOF, PNPLA3, TGM7, TOGARAM1, ZNF831, MFSD6L, PLIN3, VPS13A, CSMD3, SNCAIP, FAM160A1, AVIL, BICRA, TJP2, ERMP1, KIF3C, PPP6R2, DDX51, EFCAB5, ZDBF2, MKI67, GGACT, STK11IP, JCAD, KDM6B, TTBK2, FAM198A, PSD4, FNBP4, COBLL1, ZNF462, RB1CC1, TTF1, NXPH2, TNN, KIAA1671, AKAP6, AKAP13, PAPPA2, NFAT5, AKAP1, SCMH1, COL7A1, CASKIN1, ADGRG4, CELSR1, PRRC2B, BOC, ZSCAN22, C5, PLXND1, KCNG4, N4BP1, TMEM132A, IQCA1, USP42, USP16, MYOCD, HCN4, TGFBRAP1, RPH3A, KIF13A, CES5A, CCDC155, MAPK8IP1, SETD6, MTUS2, ADCY1, SLC10A3, KMT2A, PODXL, BRDT, TICAM1, TEP1, SEC16A, LEMD3, CEP164, ZAN, PKHD1L1, DHX33, NBEAL2, STAB2, ZNF628, EFCAB8, TMEM131L, HAS1, DIDO1, PTPN13, WNK2, HGS, ABCB4, DPYSL4, CERS4, KRT12, MYT1L, LEPR, PPARGC1B, KLK5, VIPR2, UTP20, TRPS1, HECTD4, ERAP1, TRANK1, FAM155A, CIC, COL4A3, MISP, CD96, WNK1, PIGN, ZC3HAV1, SNX19, TRPM4, VWA3A, PINK1, CACNA1A, CAMSAP3, POM121L2, NYAP2, DNHD1, ASXL2, SALL3, ANKRD35, ST6GALNAC1, VCAN, DNAH6,

	CARD14, DICER1, MYOM1, ATP9B, CCDC136, CFAP65, CRYBG3, LRRC14B, NEK4, CELSR2, GALC, EXOSC10, TSHZ2, TDRD6, TMTC4, RALGDS, ZNF804A, TTC17, ZDHHC1, KIAA1210,
Grey seal	CFTR, KIAA1211, PHF3, FAM186A, HSPG2, PRG4, NOP14, TG, QRICH2, NOS2, CNTNAP4, LAMB3, ARHGAP32, CEP295, CCDC88C, DPH7, CDHR2, LAMA5, SVIL, NYNRIN, COL10A1, ERICH3, DLEC1, CAPN8, HRC, DDX51, MEGF6, COL15A1, TNN, FUT4, ADGRG4, ZBED4, EFCAB6, WDR60, CROCC, DNAH7, PKDREJ, PKHD1L1, LTBP2, KMT2C, PRX, ZNF473, DISC1, POM121L2, CEP170B, ICE1, KMT2B, SGSM2
Ringed seal	RAB3GAP2, GTF3C1, TBC1D2B, TRHDE, MTUS1, PHLPP1, MYO15B, PGBD1, NEFH, PIEZO1, SHROOM3, PROSER1, KMT2E, TMEM229A, ATOH1, LACTBL1, CHST11, GLIS1, EXD2, PBXIP1, ABTB2, INPP5J, PGR, AGBL5, MARCO, KCNG1, PTPRJ, IRS2, MFSD9, CUL9, KIAA0319, MCM5, MIA3, MOCOS, FAM181B, PCDH17, MFSD6L, KCNC2, MKI67, FBXL7, ZNF648, ZNF217, CDH6, ADGRG4, CELSR1, FCRLB, CDCA2, BMP3, LAMA1, ZBTB42, ADCY1, TICAM1, PIGM, ZAN, RXFP3, TBX2, DLGAP1, STAB2, ZNF628, SPAG1, TRANK1, CHD6, IRX2, ALX4, ANKRD11, SOX11, TNC, BTBD11, DNHD1, ABCB11, CSPG4, TBC1D30
Baltic ringed seal	XIRP2, DACT2, ZC3H14, ADAMTS7, BRAT1, COL6A3, SHROOM3, FREM2, CASZ1, TG, SNAP23, FAT2, QRICH2, NKX2-6, EXPH5, LAMA5, PCNX2, HTATSF1, FAM160A1, SCN10A, MKI67, PRUNE2, SH2B2, ZNF407, CACNA1H, SLC25A43, CDC16, DNAH17, CRYBG1, PCLO, GRIN2C, FSCN2, SPAG1, A2M
Saimaa ringed seal	ARHGEF10, TACC2, SRRM2, SHROOM3, F5, WDR97, NOP14, DIAPH1, EXPH5, LAMB3, DNAH11, IRS2, COL10A1, MKI67, MEGF6, TNN, FBLN2, RAI1, HCN4, PCLO, C2CD3, PKHD1L1, COL4A3, TLR5, CACNA1G

Table S2. All genes with signals of negative selection in each lineage. Gene symbols correspond to nomenclature used in the Hawaiian monk seal reference annotation.

Monachini	CFTR, C1QB, SLC4A11, TMPRSS9, KRBA1, MYO18B, SNED1, PIEZO1, ZNF205, OSBPL7, PLA2R1, SCARF1, CDON, FAM186A, FREM2, LMF2, NWD1, POLR1C, FAT1, CEP104, ADCY7, LSS, ZC3H13, FBXO18, FHOD3, SLX4, GPR179, DUOX1, F8, DOK7, MYBBP1A, KIAA1549, NINL, PDCD11, SYNPO2, TNKS1BP1, DHX37, PATJ, LAMB3, E2F2, HELZ2, ITGAL, AHCTF1, CEP295, SLC9A3, BPIFB1, PRKDC, AGRN, TTC34, CDHR2, UMODL1, PCSK5, SLC22A15, CEP250, MROH2A, BAIAP2L2, SCN4A, CEMIP, MEGF6, DCHS2, FAM151A, HTT, DLGAP2, PRSS16, PLXNB2, NPC1, CFAP46, C2CD3, ZNF628, COL12A1, FUT7, PTPN13, SLC26A1, TMEM132E, MYOM2, CAMKK2, HYOU1, TIGIT, SYNM, TNC, CC2D1A, ARHGEF10L, SORL1, KCP, CD300LG, GPRIN3, TMEM132B
Miroungini ancestor	CACNA1F, GREB1, BTBD6, NEDD4, GLYATL3, MTUS1, ADAMTSL1, CFAP54, PHF3, DMXL1, ZNF275, LYST, REN, C7, GDF9, HS3ST1, FOCAD, ASCC3, GJB3, CBFA2T3, GPR179, GPR35, IKBKE, ZNF541, CAND2, SLC19A1, KIAA1549L, RHBDF2, SCYL1, NPHP4, LAMC3, ERBIN, PHRF1, AHCTF1, ERCC6L, CEP295, SEMA5A, FOLR2, COL27A1, NEXMIF, COL4A6, FN1, ACADVL, VWA3B, DNAH14, FHOD1, CNGB1, INSRR, CNTLN, ADGB, OTUD7A, JCAD, KDM6B, FBF1, TAF3, KIAA1671, ADGRG4, USF3, ZKSCAN8, PER2, LAMA1, ITGA1, SLC41A3, DISP1, GPRASP1, OTOF, ABCB4, SLC26A1, TLN1, NCOR1, SETX, DENND4B, TMPRSS7, IQGAP2, NIN, ABCA9, OTUD4, KIAA1210
Weddell	TMEM163, TMEM132C, CDK5RAP2, ADAMTS14, MYLK3, RAB3GAP2, ST18, NCKAP5L, CCDC18, PIK3CG, NAV2, CSF3R, ZNF318, POLN, SYNPO, FAM110C, SEC31B, NOTCH1, FDFT1, ULK1, CACNA1F, CNKSR1, KIF21A, DDX27, ENTPD8, WDR46, PUS1, FAM71A, DCLRE1A, DACT2, EIF2D, MYO1H, STOX1, IL12RB2, TTC21A, MYRF, WDHD1, ZFHX3, MTO1, DOPEY2, CSPP1, CCDC157, TRIM67, MAP2, NEDD4, SBF1, PIK3AP1, TTC3, COL14A1, C2CD2, TANC1, ANKRD53, MTUS1, NCOA6, GNPTAB, PHLPP1, PPP1R26, PYROXD2, FRY,

	EIF4ENIF1, CFAP54, SGSM3, USP54, RRP12, PIGZ, MAB21L3, MYO15B, RIC1, KIAA1211, TEKT5, ZNF335, ATM, SUN1, COL4A4, EMILIN2, NHSL2, NCOR2, AHDC1, SIDT1, MROH6, BBS12, PAPLN, ALDH3A2, HIP1R, SECISBP2, MINPP1, PKN3, SNED1, CFI, GPC5, ABCA4, SORCS3, MYO3B, BANK1, WHRN, WDFY3, EPN3, OSBPL5, SREBF1, ASTN1, CUBN, LAMC1, HABP2, TYK2, EFHB, TMEM131, SLC29A3, TRAK1, SOX13, ZW10, MSH3, FBP1, LYST, MLXIPL, MROH1, FTSJ3, KIAA0100, ATF7IP, TONSL, FNDC7, ACTN3, COBL, CAPN13, CNTN5, ATP11A, TMPRSS15, NRIP1, ZNF438, MEP1A, PRRC2C, PRR14L, MASTL, FAM111A, NCSTN, CASZ1, PHACTR3, EPSTI1, ARHGEF11, ZNF518A, DCST1, CFAP57, FMN1, PIPOX, PKD1L3, UGGT2, CIPC, FREM1, SPHKAP, TIAM1, SEC24C, CARMIL1, ELK4, KIAA1109, GCFC2, NAALADL1, ANKLE2, CELSR3, JAML, SOWAHB, F13A1, ITIH1, ZNF672, ASCC3, KIDINS220, FBXO24, SLC8B1, SLC45A3, MAP1S, HEPHL1, NOXA1, TNS3, TCOF1, SLC9B2, ZNF710, TPCN2, HELQ, GLT1D1, GPR31, CARMIL2, ADAM8, NVL, NLRC3, TBC1D31, IGSF21, RUSC2, POLD1, TDRD12, KIF14, SLX4, TNFRSF11A, GPR179, SPINK5, AIRE, KIFC2, F8, ABCC6, ERBB3, MICAL2, N4BP2L2, SPICE1, ITGB7, PPARGC1A, AHSG, THADA, TMEM235, CCDC180, BAZ2A, EMC1, HEATR4, TTLL5, TRPV6, CNTNAP4, PRSS57, ZNF804B, FAM189A1, SLFN14, PTK7, ELMSAN1, SLC5A5, DSG3, KIF7, LRSAM1, TBC1D32, SORBS2, NUP155, ARSJ, DENND3, DSC2, C8B, GAK, ALPK2, DHX37, TLE6, SEZ6L, PCDH7, SCN11A, MARCO, JMY, LAMB3, MAMDC4, ARFGEF3, VPS13D, HELB, RHBDF2, HEG1, MAP3K15, TNRC18, ZSCAN20, ESPN, PDIA4, HCAR1, CHRND, KRT84, ADPGK, ITIH5, ADGRE1, NCKIPSD, KLB, ZNF609, ACOT11, ABCA12, FGD6, ARHGAP32, CASP8AP2, LRRK1, EPG5, PADI4, ABL2, RPAP1, LAMC3, VPS13B, LOXHD1, KIAA0319, RREB1, TECTA, DNAAF1, HOXB13, NUP214, SIK3, KRT20, TNFRSF25, DMXL2, KIF9, FAM129A, PIEZO2, DYSF, KLHL33, TMEM245, VPS16, COL16A1, XDH, DZIP1, CEP350, MTR, AP3B2, TOMM34, VARS2, DNAH3, NCOA3, ERCC6, NKD2, PLEKHG3, GSN, AKAP9, PCM1, NLRP5, PRKDC, IGSF10, TTC34, NOD1, PHLPP2, GRM6, SIK2, TNFAIP2, UMODL1, FAM83H, MMP9, HIVEP2, MIA3, CCDC73, EHBP1L1, NUP210, CRB1, WDR49, IGSF9, ADGRG6, TXLNB, TXNDC16, FNDC1, ANKMY1, ADD1, DNAH8, VWDE, ADGRG3, TNIP1, BFSP1, TROAP, MYOF, TGM7, LONP1, PKD1, CHTF18, ADAM15, CD109, CIT, ZNF687, OAS3, PUM3, TIAM2, DMRT2, ECD, PLIN3, DLC1, MED13L, PARP3, PLXNB1, ABCC3, ROS1, FAM160A1, POLG, CEP250, ERICH3, TTI1, TEX2, NOD2, HHLA1, DISP2, MROH2A, HERC6, RNASEL, TGM2, TTYH2, MYSM1, BRD4, FAM83C, FBN2, CDH5, ADGB, EPS8L1, SCN10A, EMILIN3, ESX1, EML5, EVC, JCAD, DHX34, MEGF6, UNC80, SNX29, GDPD4, COBLL1, TSPOAP1, DRC7, GBGT1, FYB2, KIF1C, ZMYND8, CABIN1, TTF1, MAP9, ZCCHC6, ADNP2, ADORA3, KCTD8, ACOT6, TTC37, HASPIN, SNX1, TDRP, SLC4A5, SIGLEC1, AKAP1, DSC1, IARS, PCNX4, ABCC10, ATXN7, CRACR2B, VWF, SCTR, PRRC2B, MARCH10, KY, ZBED4, ENAM, SCLY, OAS2, DLGAP2, MRC2, COL4A2, N4BP1, RASEF, TLR1, CFAP44, SMPDL3B, ADAMTS13, PER1, RMND1, PEG3, TPO, PABPC1L, NBEAL1, MDC1, IRX4, KIF6, EPS8L3, NPC1, LAMA4, ASPRV1, WDFY4, ATP2C2, PALB2, ATP10A, WDR60, ALPK3, MYO7B, PRTG, UHRF1BP1, KIF13B, PDE6C, MYBPC2, SKOR1, ASIC5, CHPF2, BPIFB4, TICAM1, DNAH7, JMJD4, MMRN1, HGFAC, MMP17, RAB20, NUP153, BAHCC1, KIAA1683, AMBN, PASK, MYOM3, NFAM1, CABS1, TTLL8, PLXNA2, PKHD1L1, STAB2, DCHS1, MEGF11, MYO7A, ZNF628, CGN, SCAF11, TMEM131L, CGNL1, LTBP2, RADIL, COL12A1, SPPL2C, GHDC, GHR, OTOF, CDCP1, EFHC2, RAD9B, FLT1, AKNA, RNH1, B3GALT5, ESYT3, PAPSS2, INVS, TNS2, ATP6V0A4, NRAP, TTLL10, TMPRSS6, TBC1D12, CHRNA4, MYOM2, PPARGC1B, LRIT2, GPAA1, ZNF169, NID1, OSMR, MAPK15, IRX3, TRANK1, SOWAHA, CHD6, GCNT1, CIC, ANKFY1, COL4A3, SLFNL1, GPATCH3, DOCK5, TLR5, SAMD7, MISP, CD96, KIF26B, LMNTD2, WNK1, SCEL, NLRX1, TMC3, FLT4, STAP2, CTC1, TRPM4, FAM13A, TMPRSS7, PLCZ1, ZNRF4, CCDC88B, DMRTB1, FSTL4, TET3, SLC7A5, TNS1, LRGUK, PLCH1, CRTC3, VWA3A, GPR108, NIN, EPN2, LARGE2, CCSER2,

	IGSF11, EVC2, CCDC40, CACNA1G, LNPEP, KANK1, DENND2A, AHI1, SPAG17, PNPLA7, CTSF, FASTKD1, MMP15, MS4A14, TDRD7, FAM135A, NCAPD2, ANKRD35, FMO1, HSH2D, ZFYVE26, CLNK, RBM44, PTGDR, CFAP61, LRRC14B, POMGNT2, GPRIN3, MYPN, TECPR2, WRAP53, ZCCHC4, ABCC12, MROH2B, DHX58, COL18A1, RASAL3, CSPG4, FAM166A, MAGI2, TBC1D9B, ZNF366, LIMCH1, TDRD6, WDR72, RALGDS, DENND1C, LRRIQ1, CCDC33, REXO1, KIAA1614, IL4R, LARP6
Elephant seals	CHRDL2, PAM, COL19A1, ATP10B, TRPV2, GLCCI1, NES, EPHA1, CFTR, DENND4C, SEC31B, NOTCH1, ITIH4, CLSPN, CNKSR1, UNC13C, CRHR2, TRPV4, ANKZF1, SORBS1, CALHM1, TRPC7, ARHGEF10, USP31, RAB11FIP5, PCNT, MAML2, SPTA1, ACSF3, SEPT8, RIMS2, IL12RB2, LAMC2, LRRC24, DAP3, HTR1A, RPS6KL1, ADGRA2, CSPP1, PLK4, CCRL2, BOP1, MRPL38, SPTB, ATRNL1, SDK1, ANKRD53, MTUS1, LRP11, NCAPG, DNMBP, KRBA1, PHLDB1, PHLPP1, PPP1R26, CHRNA5, RRNAD1, FRY, TSHR, SFI1, TRMT1L, RRP12, GMIP, ST6GALNAC2, KIAA1211, SUN1, XPC, COL4A4, EMILIN2, WNK3, FOXRED1, SRRM2, MROH6, F7, CPXM1, RNPEPL1, GBA, ALDH3A2, KIF17, COL11A2, SH3TC2, SECISBP2, PKN3, PHF3, EPHX2, SNED1, LRRC66, MYO3B, KRT24, SHE, RASAL1, OSBPL5, SREBF1, PTPN14, PIGO, TIMELESS, CUBN, RUBCN, TYK2, GAL, WDR4, GLI2, DLG5, MSH3, MKKS, LIFR, MYO19, FKBP15, OTOA, FNDC7, TMCC2, AFF1, CHRNA2, CNTN5, RNPEP, NEO1, NRIP1, TMC6, PRRC2C, LIG3, KCNH8, CARF, RNF17, NOP14, FMO2, ARHGEF11, CEP68, LMF2, PADI6, FOXJ2, TG, NDUFA10, FARS2, FREM1, IQSEC3, CC2D2B, DCST2, GCFC2, ABCC4, TTF2, IARS2, MSTO1, CSMD2, SNTG2, CACTIN, FOCAD, TULP1, MILR1, MYH14, MMP20, EPRS, CTSO, WDR6, LAMA2, BRCA1, VIT, ARAP1, BAZ2B, MMP8, CEP104, TNS3, ZNF536, RGS22, LRBA, RABL6, LRRIQ4, CCDC171, TPCN2, AP4E1, VWA7, DENND2C, ADAM8, TDRD5, AK8, NVL, PFAS, COL20A1, ZC3H13, FBXO18, FHOD3, ZNF106, DDX54, RUSC2, KLHDC4, NKX1-2, CILP, SIAE, LRP4, ENGASE, SPINK5, GIPR, FBXO40, KIFC2, STK10, INF2, KCNH6, TRPM5, ITGA7, TSPYL5, ITGB7, USHBP1, NUBP2, NCAPG2, LSG1, MADD, PM20D1, PCDH15, PLEKHG6, TRPV6, FBXO43, CNTNAP4, ZNF804B, ATCAY, MAP3K19, ARID5B, MAML1, GRAMD1C, CARD10, PDCD11, GTF3C5, KIF7, SPPL2B, TBC1D32, LMO7, CNST, LCT, DENND3, SLC44A3, ALDH5A1, JAK3, GAK, DRC3, MEI1, DHX37, ANAPC1, APC2, TPRN, SLC39A4, COL3A1, FMO4, GNL2, DNAJC6, SLC2A4, TBRG4, TARBP1, NCL, IRS2, ITIH5, ADGRE1, COL5A3, KLB, PDZD7, ARHGAP32, TRMT1, AEN, LAMC3, ERBIN, PDE4A, KIF11, CHST9, AHCTF1, FGFBP3, PAPPA, PALLD, NLRC5, SLCO2B1, CRYBG2, CEP295, ABI3BP, FAM129A, OLFML1, MICAL1, KIAA0232, DYSF, COL16A1, SERPINE1, TANGO6, DZIP1, ACAP1, SLC6A18, GRIN2B, OCSTAMP, PLEKHS1, CEP350, SLCO4A1, DNAH3, ADAM33, PLOD1, SLF2, VMO1, FANCF, ZNF446, GSN, INTU, IGSF10, ACACB, ADCY10, RAB44, PARP4, PROB1, LRP1B, COL27A1, FAM83H, KLHL31, SBF2, CDC25B, NSUN2, FCHO2, EHBP1L1, SPG11, NUP210, PTPN22, MANSC1, CRB1, WDR49, RMDN3, TXLNB, PDE3A, IQSEC1, CEP120, TOPBP1, ADCK5, KDM4B, GIMAP8, FAM124B, UACA, RETSAT, TRMT12, KIAA1755, FNIP2, GPRIN1, GPR45, C5AR1, RUFY4, AFAP1L2, CHTF18, MYBPC1, COL13A1, NUTM1, PCSK1, ADAMDEC1, CD109, UVSSA, DCBLD2, PPP1R15B, AFF4, ECD, USPL1, HPS4, ATG16L2, MLIP, FHAD1, ROS1, ADGRG5, FHOD1, CEP250, CD6, KIF27, VWA2, CFAP69, OPLAH, AMER2, NUP88, ASPDH, DISP2, ZNF408, BUD13, BRD4, PRSS56, HEATR5A, FLNB, FAM129C, WDR66, TNR, GRID2IP, TRIM29, CIZ1, MEGF6, CC2D2A, ABCA1, IGHMBP2, COL15A1, NCKAP1L, CASS4, NBAS, PAK4, FBF1, ATAD5, CABIN1, UCP1, GALNT5, TTLL6, GGT6, MARVELD3, TTC37, AKAP8, FBLN2, MPP4, PLA2G4F, FBXO46, TDRP, ITGA8, SIGLEC1, NCAN, IARS, RNF207, ABCC10, CTSZ, PTCH2, SALL2, OLFML2A, PRRC2B, MARCH10, FAM124A, ENAM, SCLY, FGF21, CACNA1H, KLK15, CDH15, ANO8, EFCAB6, ADAMTS12, WRAP73, CCDC110, GEMIN4, CFAP44, ERCC4, SMPDL3B, ADAMTS13, CD68, ADGRF1, ZXDC, NBEAL1, KIAA2012, MAN2A2, SRMS, RUBCNL, PDLIM3, NPC1, ERCC6L2, ENPP1,

	QSOX1, MYO15A, OGDHL, AIP, TCF7L2, WDR60, RRP1B, VSIG10L, ITGA1, CROCC, ADAMTSL4, KIF13B, DISP1, SLC41A1, CRYBG1, GADL1, IL18RAP, ALDH1B1, PDE1C, PRDM8, SREBF2, ADGRF5, MAP6, FANCI, PPRC1, CDC42BPG, FAM53A, LEMD3, LUZP1, UMPS, NFAM1, ZFP57, CFAP70, MAMDC2, DCHS1, PLEKHG2, GRIN2C, PREX1, TICRR, DTX3L, MET, LTBP2, P2RY6, PIMREG, IP6K3, COL12A1, SPPL2C, ZKSCAN2, ZBP1, OTOF, CDCP1, NKAIN4, PDE6A, FSCN2, MYO5C, MAPT, RAD9B, CTU2, COL17A1, AKNA, SIPA1L2, ATAD2, PLCE1, TACC3, ABT1, SLC35G1, TMEM63A, CERCAM, CCDC141, VWA8, CTTNBP2NL, TLN1, MYOM2, ASTN2, ZFP64, SLC26A2, NCOR1, SYNE3, VASN, PLA2G4E, TRMT5, MORC1, PANX2, GPAT2, SH3RF1, LAP3, PIK3R5, PRG3, GAS2L3, AMDHD1, SORBS3, TOM1L1, GCC2, KIF26B, LRRFIP1, NLRX1, RELT, PALD1, CLSTN2, EGF, EPB42, CUZD1, FSTL4, DUT, TNS1, CLMN, LRGUK, PCK1, EDN3, ZSWIM3, SLC6A20, CC2D1A, LARGE2, ERN2, RAB11FIP1, DAGLB, PRDM16, CCT8L2, CACNA1G, PLIN5, ABCA9, RGMB, SQLE, ZC3H18, CEP162, TMPRSS4, NCAPD2, TPCN1, GALNT10, MYBPH, GRIP2, ARHGEF40, LIG1, KCP, LRRN4CL, PHC1, ANO1, ITGA2, DAPK1, GLI3, REPS2, MYPN, GOLGA4, MAN2C1, ALPK1, PODXL2, EREG, TRAF5, TLL2, PGM2, CEP152, TP53BP2, TSHZ1, LIMCH1, SLC2A9, DHX29, AFM, N4BP2, SCN5A, ZMYND15, KMT2B, IL4R, CEP97, ZBTB4
Grey seal	FRMPD3, MAP4, CACNA1F, FRAS1, ARHGEF10, SGSM1, RTN4, STARD8, SPEG, ZFHX3, DOPEY2, ITGA10, MYO15B, TAF1C, NCOR2, SRRM2, KIF26A, PAPLN, HIP1R, SHANK1, NEFH, LRRC66, PIEZO1, SREBF1, CUBN, GLI2, COBL, AGBL1, RYR1, FANCA, KIF24, WDR97, FAM111A, CASZ1, NCKAP5, NWD1, MINDY4, ZFR2, FAT1, ANK3, VRK3, TJP1, TYSND1, TCOF1, FAT2, ABCC6, KIAA1549, USHBP1, MYCT1, ABCA7, CACNA1B, SLC5A5, UTRN, KIAA0753, ZNF541, ALPK2, APC2, MARCO, TBRG4, FAAP100, TDRD1, ITGAL, CUL9, LAMC3, RREB1, BCO2, KIAA0586, PHRF1, NLRC5, SIK3, PRR22, NRDE2, DYSF, ABCG8, DNAH3, FASN, URB1, AKAP9, DOCK6, LRP1B, TRPM6, UMODL1, POLRMT, MIA3, SPG11, ARMCX4, STAB1, MMRN2, DNAH14, SLC38A10, CHTF18, NLRP14, IL12RB1, MYO1G, KNTC1, TTI1, NOD2, ZBTB49, DISP2, SETD1B, TGM2, FYCO1, FLNB, MAVS, ZDBF2, EVC, JCAD, H6PD, CIZ1, IGHMBP2, SCRIB, NBAS, FBF1, TRIM66, PLEKHG4, OTOG, SALL2, FAM83G, USF3, MARCH10, HTT, COL22A1, CACNA1H, MRC2, KIAA0556, TEX14, USP42, CAMSAP1, GNAS, COL6A5, LAMA4, MYO15A, RTL1, ITGA1, MYO7B, MTUS2, DNAH17, PIK3C2A, DSP, BAHCC1, KIAA1683, TEP1, SMTNL2, ZAN, MYO7A, PLEKHG2, TICRR, LTBP2, RADIL, OTOF, COL17A1, NRAP, MYOM2, OBSL1, UTP20, OSMR, LDLR, FHDC1, INTS1, XIRP1, SYNM, YEATS2, ASPM, MYH15, CCDC88B, ANKRD11, ANPEP, KANK1, IL16, ZFYVE26, CRYBG3, CFAP47, PARP14, COL18A1, CSPG4, LIMCH1, SCN5A, AADAC, KIAA1210
Ringed seal	S100PBP, VPS13C, PCNT, LAMC2, GREB1, CHST3, ZNF592, SRRM2, NPAS3, ANAPC2, DUOX2, BDP1, BARD1, MROH1, COBL, PRR14L, USP53, DOT1L, IGSF1, TENM4, ZFHX2, VRK3, SNTG2, FOCAD, TIGD5, TRIO, DUOX1, FANCD2, PBXIP1, MFSD3, MYCT1, CCSER1, SLC15A4, SYNPO2, CNST, GATA5, PCDH7, COL3A1, DNAH11, LRIG1, FAM84B, NAV3, ERBIN, AHCTF1, NUP214, CRYBG2, CCDC105, AKAP11, MN1, MOV10L1, ACACB, POLRMT, PIK3R1, COL4A6, PCSK5, OGFR, DNAH14, DCAF5, HID1, SPATA18, ARHGEF19, PRDM1, ICE2, KNTC1, CEP250, MAVS, ZDBF2, TOR4A, TRIM66, KCTD8, AKAP13, RASGRF1, USF3, COL22A1, NOTCH2, ABCA13, THBD, HEATR1, ENPP1, MTUS2, CRYBG1, HMGCS2, CEP164, REV3L, WNK2, ANLN, NAT10, EVPL, OSMR, FLT4, OTOP1, MYH15, IQGAP2, ASXL3, POM121L2, FOXI2, DNAH6, DACT1, MAP3K5, EXO1, TMEM132D, ICE1, FLVCR1, COL18A1, TDRD6
Baltic ringed seal	NES, LRP2, C8A, CFAP54, MROH1, RALGAPA2, ENTPD2, ARAP1, TDRD12, GPR179, DUOX1, DCDC2C, SLC27A3, ZNF541, MAML3, KIAA0586, KIAA1211L, DYSF, TRIM17, MOV10L1, PRKDC, PARP4, COL4A6, NOL8, ARHGEF17, ETV5, CFAP46, COL12A1, PTPRZ1, PRX, TAP1, CIC, FAM208B, ASXL3, ANKRD11, ZNF236, CRYBG3, REXO1

Saimaa ringed seal	CDK5RAP2, SNAPC4, TTLL12, ZNF605, SPTA1, GTF3C1, ZFHX3, DNMBP, FLNC, CFAP54, ATM, TAF1C, ADAMTS7, TSC22D1, ARHGAP21, LRRD1, ACTN3, SH3TC1, ITPR3, TJP1, MERTK, LAMA2, BRCA1, TNS3, TCOF1, DUOX1, SLC4A1, PER3, ABCA7, MAP3K19, FER1L6, LCT, JAK3, DSC2, TNKS1BP1, GAK, PATJ, HEG1, PTPRJ, FAM135B, CEP295, GPATCH8, CEP350, DNAH3, TDRD9, NLRP5, LRP1B, FAM83H, EHBP1L1, RIF1, KIAA1755, MYOF, FNIP2, DNAH14, BRCA2, GPRC6A, KNTC1, ROS1, ARHGAP31, TNR, H6PD, IGHMBP2, CASS4, PRUNE2, AKAP13, TREML1, COL7A1, FAM83G, COL22A1, SERAC1, DLGAP2, ANK2, ADGRF3, WDFY4, NID2, MYO7B, CROCC, UHRF1BP1, KMT2A, DNAH7, ANKK1, SEC16A, NBEAL2, LTBP2, COL12A1, PTPRZ1, WNK2, COL17A1, WDR27, MCM3AP, OSMR, TRANK1, CHD6, RELT, ASPM, ASXL3, MICALL1, ANPEP, CSF1R, EVC2, KANK1, ARHGEF10L, CFAP47, TLR8, CSPG4, TDRD6

Table S3. All genes that show only neutral evolution in each lineage. Gene symbols correspond to nomenclature used in the Hawaiian monk seal reference annotation.

CHAPTER THREE:

Evidence that the distribution of fitness effects of new mutations evolves in response to long-term effective population size in mammals

Abstract

The distribution of fitness effects (DFE) is arguably one of the most important parameters in understanding population genetic dynamics and molecular evolution. Despite this, there is still relatively little data that provide a framework for parameterizing DFE, and even less information on how and why the DFE varies across species. In this chapter, I use a large population genomic data set to model the DFE for multiple species of phocid seal, which differ dramatically in their long-term population sizes and demographic histories. I find that in the species with the largest data set (Hawaiian monk seal, $n = 14$), I am able to accurately estimate the recent demographic history as well as the shape and scale parameters for a gamma-distributed DFE. A model of the synonymous site frequency spectrum for the Hawaiian monk seal shows that this species had a small ancestral effective population size ($N_e = 4343$) but that the population has further contracted by 95% since the arrival of humans in Hawaii. The DFE that I model for the Hawaiian monk seal is very similar in shape and scale to the DFE previously estimated for humans, but different from the one estimated for mouse. Although I could not estimate the DFE for other seal species, the comparison of mammal species shows that evolution of the DFE is closely correlated with long-term effective population size and not with phylogenetic signal or organismal complexity.

Introduction

New mutations can have one of three effects on the fitness of an organism: positive, deleterious, or neutral. The proportion of mutations in each category, known as the distribution of fitness effects (DFE), must play a critical role in population genetics and molecular evolution. For decades, theoretical and empirical research in population genetics has worked to describe the DFE (Eyre-Walker & Keightley 2007), and the effect different distribution shapes and parameters would have on molecular evolution (Whitlock 2000, Orr 2003, Lourenço *et al.* 2011, Huber *et al.* 2017).

For example, if most new mutations are truly neutral then much of molecular evolution and population genetic dynamics should follow the predictions of the neutral theory (Kimura 1968, 1977). However, decades of molecular genetic studies show a preponderance of evidence that the neutral theory cannot adequately explain broad patterns of molecular evolution (Kreitman & Akashi 1995; Kreitman 1996; Hahn 2008; Thomas *et al.* 2017; Kern & Hahn 2018; Yoder *et al.* 2018). Nearly neutral theory was developed instead, which proposed that most new mutations had a slightly deleterious effect but segregated and became fixed as if they were neutral (Ohta 1973, Ohta & Gillespie 1996). This theory of molecular evolution has become widely accepted and forms the basis of much of modern theory in population genetics and molecular evolution (Lynch 2007).

Importantly, however, nearly neutral theory only establishes the mathematical framework through which the fixation probability of a mutation can be assessed, but does not directly propose the parameters of the DFE, aside from the proposal that is gamma- or exponentially-distributed, with a mean selection coefficient close to zero (Ohta 1992, Keightley 1998). Many assume that the DFE is a biological universal, with the same

distribution across species (Galtier & Rousselle 2020). Other models, however, predict that the DFE will shift in response to factors like genome complexity, epistasis, modularity, and population size (Cherry 1998, Gillespie 2001, Goldstein 2013, Tenaillon 2014).

Among these, the Geometric Model (GM) has received attention recently for its simplicity and applicability to population genomic data sets. Originally developed by R.A. Fisher in 1931, this model predicts a DFE of new mutations based on a few simple parameters like phenotypic complexity and distance of the organism from the fitness optimum (Tenaillon 2014). Notably, this model provides acceptable solutions to paradoxes such as the long-term survival of large-bodied mammalian populations with small effective population sizes (Lynch & Lande 1998; Poon & Otto 2000; Whitlock 2000).

Until recently, the main source of empirical data on the DFE came from mutation accumulation experiments that directly measured fitness effects in viruses (Sanjuán *et al.* 2004), bacteria (Couce *et al.* 2017), *Arabidopsis* (Schultz *et al.* 1999), and *Caenorhabditis elegans* (Estes *et al.* 2004). Recent advances in population genetics and genomic sequencing have now made it possible to model the fitness effects of segregating alleles from population genomic data. This class of methods, including DFEalpha (Keightley & Eyre-Walker 2007), polyDFE (Tataru & Bataillon 2019), and *fitδaδi* (Kim *et al.* 2017a). All of these methods use the site frequency spectrum (SFS) of nonsynonymous to model the parameters of the DFE that would produce such an SFS.

With these computational advances, it is now possible to estimate the DFE for natural populations. Studies of closely related species have found apparent differences in the DFE between island and mainland species of corvids (Kutschera *et al.* 2020) but similarly shaped DFE for all great apes (Castellano *et al.* 2019). In more distantly related

taxa, Huber *et al.* (2017) found substantially different DFE for yeast, *Drosophila*, mouse, and humans, with the average selection coefficient increasing in that order. The authors proposed that this pattern fit with the Geometric Model, but attributed the difference to increasing phenotypic complexity.

In this study, I model the DFE in six taxa of phocid seals for which population genomic sequence data were available. As described in chapters 1 and 2 of this dissertation, these taxa are all closely related but have dramatically different long-term population sizes. These range from the Hawaiian monk seal, which is only found in the Hawaiian archipelago, to the Weddell seal, which has a circumpolar distribution around Antarctica. Importantly, these taxa do not suffer from the same population artefacts as island and mainland taxa pairs (Goldstein 2013), and show much greater variation in population size compared to great apes (Castellano *et al.* 2019). As such, these species offer an excellent way to study the evolution of DFE in response to long-term population size.

Methods

Samples, sequencing, and alignment

In this study, I attempted to analyze six different populations: Hawaiian monk seals ($n = 14$), Weddell seals ($n = 10$), northern elephant seals ($n = 10$), grey seals ($n = 10$), Baltic ringed seals ($n = 9$), and Saimaa ringed seals ($n = 12$). As described in Chapter 2 of this dissertation, I generated the data for the Hawaiian monk seals, Weddell seals, and elephant seals, while the data for other species came from publicly available datasets. In addition, to test for the effect of sample size I down-sampled the Hawaiian monk seal data set to 10

medium-coverage individuals. Details of sampling and sequencing depth can be found in Chapter 2.

Variant calling and site frequency spectra

I used the `mpileup + call` pipeline in BCFtools (Li 2011), including only basepairs with a quality above 25 and a mapping quality above 25, excluding indels, and including only variants that were within the coding region of autosomal mammalian orthologs (see Chapter 2 Methods for ortholog selection). I then used VCFtools (Danecek *et al.* 2011) to filter the resulting variants for Hardy-Weinberg equilibrium (`--hwe 0.001`), a minimum genotype quality of 40 (`--minQ 40`), and only biallelic variants. I used custom SnpEff (Cingolani *et al.* 2012) database built from the Hawaiian monk seal reference genome to annotate synonymous and nonsynonymous sites, and split the resulting VCF into one VCF with only synonymous sites and one VCF with only nonsynonymous sites. I used easySFS (https://github.com/isaacovercast/easySFS) to generate an SFS for each functional class.

Principle component analysis (PCA)

I performed a principal components analysis (PCA) on each taxon using the VCF of synonymous sites. I used Plink v1.9 (Purcell *et al.* 2007) to perform the PCA, and plotted the resulting eigenvectors with custom scripts in *R*.

δaδi neutral synonymous demographic models

The first step in fitting a DFE to population genetic data is to fit a demographic model to the synonymous SFS, which reflects how neutral processes have affected the shape of the SFS. I used *δaδi* (Gutenkunst *et al.* 2009) to fit a two epoch demographic model, which describes a single historical change in population size, to each population's SFS (Figure 1). The model consisted of two parameters: *nu* (the ratio of current effective population size to ancestral effective population size) and *T* (the time ago that population size changed, measured in $2N_{e,Ancestral}$ generations). *Nu* was constrained to be between 0.0001 and 10, and T was constrained to be between 0.0001 and 5. Starting parameters were randomly perturbed in *δaδi* and each model run was started from at least 10 different starting parameters to ensure that the model was not finding a local optimum from a particular starting point. The SFS generated by the model was then compared to the SFS from the data, and the residuals were assessed with simple Poisson residuals as:

$$residual = \frac{(model - data)}{\sqrt{model}}.$$

Fitting a DFE with fitδaδi

Once the neutral demographic model of the population has been obtained, *fitδaδi* (Kim *et al.* 2017b) can be used to fit a model incorporating selection to the nonsynonymous SFS, conditioned upon the established demographic parameters. I attempted to fit two different models incorporating selection. The first is a simple two epoch model that fixes the demographic parameters from the neutral model (nu, T), and estimates the shape (α) and scale (β) parameters for a gamma distributed DFE. In this case, the β parameter is scaled by $2N_{e,Ancestral}$. α was constrained between 0.001 and 1. β was constrained between 0.001 and a maximum that was calculated as ($2N_{e\text{-}Ancestral} * s_{max}$), where s_{max} was the maximum unscaled selection coefficient (i.e. 0.5).

Models were assessed by their Poisson likelihoods, as implemented in *δaδi*. Poisson residuals comparing the SFS of the model and real data were assessed.

Evaluation of distributions

To evaluate the proportion of segregating alleles in each of different selection coefficients, I applied the shape (α) and scale (β) parameter estimates for each species in the formula for a probability density function of a gamma distribution:

$$f(x;\alpha,\beta) = \frac{\beta^{-\alpha}x^{\alpha-1}e^{-\frac{x}{\beta}}}{\Gamma(\alpha)}$$

and solved the definite integral for the corresponding range of selection coefficients (e.g. 0, 10^{-4}). This was done for both the scaled and de-scaled parameter in order to describe the distribution of scaled ($\gamma = |2Ns|$) and unscaled ($|s|$) selection coefficients. The expected value of a gamma distribution is calculated as:

$$E[X] = \alpha\beta,$$

And the variance as:

$$Var[X] = \alpha\beta^2 .$$

Results

Population structure among samples

The PCA analyses showed very little population structure for any of the taxa examined. In the Hawaiian monk seal, samples were collected from throughout the Hawaiian archipelago. There appears to be some clusters of samples in the Main Hawaiian Islands, as well as a possible geographic gradient along the Northwest Hawaiian Islands

(Figure 2a). In the Weddell seal, all samples from Erebus Bay cluster together, with the reference sample of unknown origin showing a separation along PC1 (Figure 2b). There is no clear clustering of samples in the northern elephant seal (Figure 2c), all of which came from animals that stranded in northern California.

There is no apparent structure in this sampling of grey seals (Figure 2d), Baltic ringed seals (1e), or Saimaa ringed seals (Figure 2f). In the grey seal and Baltic ringed seal, however, there are outlier samples that correspond to samples with much higher depth of sequencing.

Neutral demographic models

Two-epoch demographic change models successfully converged in the grey seal, Hawaiian monk seal, down-sampled Hawaiian monk seal, northern elephant seal, Weddell seal, and Baltic ringed seal. In the Saimaa ringed seal, model fitting consistently pushed the parameters to their lower bounds, with unrealistically small values of *nu* and *T*.

Grey seals, Hawaiian monk seals, and northern elephant seals all showed population contractions ($nu < 1.0$) in the relatively recent past ($T < 0.05$). When scaled by synonymous sequence length, mutation rate, and generation time, the grey seal had an ancestral N_e of 23,676, a current N_e of 4541, and a change in N_e at 17,814 years ago (Table 1). The northern elephant seal had a larger ancestral N_e of 55,824, but a smaller current N_e of 798, with the change in size occurring around 41,131 years ago (Table 1). The Hawaiian monk seal had the smallest estimates of N_e (ancestral N_e of 4343 and current N_e of 202), with the most recent size change (1512 years ago). When the Hawaiian monk seal data set

was down-sampled to 10 individuals, ancestral N_e was 5745 and the current N_e was 576, with the size change taking place 5432 years ago (Table 1).

Conversely, Baltic ringed seals and Weddell seals both showed population expansions ($nu > 1.0$) in the more distant past ($T > 0.5$). When scaled, the Baltic ringed seal had an ancestral N_e of 36,625 and a current N_e of 73,716, with the size change dating to 2.522 million years ago. Weddell seals had an ancestral N_e of 32,576 and a current N_e of 76,957, with the size change occurring 476,452 years ago (Table 1).

Selection models

Using their respective demographic parameters, I fit models of selection to the nonsynonymous SFS of each species, in which selection coefficients of the DFE were gamma-distributed with freely varying shape (α) and scale (β) parameters. Models for the grey seal, Hawaiian monk seal, down-sampled Hawaiian monk seal, and Weddell seal all converged (Table 2). Models for the northern elephant seal and the Baltic ringed seal did not converge, with both consistently hugging the upper bound of β, which represents an unrealistic value of $|s| > 0.5$. I was not able to run the selection model for the Saimaa ringed seal because the demographic model did not converge.

The estimates of the shape parameter (α) were similar across populations, ranging from 0.161077 to 0.18232 (Table 2). The estimates of scale parameters (β) were much more variable, ranging from 576.01 in the Hawaiian monk seal to 39016.04 in the grey seal. Even when the scale parameters were de-scaled by diving by $2N_{e\text{-}Ancestral}$ of the species, β ranged widely from 0.06631 in Hawaiian monk seal to 0.65916 in the grey seal.

Reflective of these differences in shape and scale parameters, E[$|s|$], E[$|S|$], and the proportion of alleles in each range of selection coefficients differ across species (Table 3).

E[|*s*|] are larger in the Weddell seals and grey seal than in the Hawaiian monk seal. Var[|*s*|] and Var[|*S*|] were also much larger in the Weddell and grey seal compared to the Hawaiian monk seal (Table 3). Notably, the proportion of alleles with a scaled selection coefficient of less than 1 (i.e. neutral) is higher in the Hawaiian monk seal than in the grey seal and Weddell seal.

The parameter estimates of the Hawaiian monk seal data set consisting of only 10 individuals, however, differed substantially from those estimated from the full Hawaiian monk seal data set. The estimate of α was much smaller (0.112), while β was much larger (1.288) (Table 2). This resulted in a much higher E[|*s*|], E[|*S*|], Var[|*s*|] and Var[|*S*|] for the down-sampled Hawaiian monk seal data set (Table 3). However, the proportion of alleles with $|2Ns| < 1$ was nearly identical between the full and down-sampled data set.

Discussion

The effect of sample size

All parameter estimates for the Hawaiian monk seal differed between the full data set (15 individuals) and the down-sampled data set (10 individuals). The demographic parameters (*nu*, *T*) were of the same order of magnitude, suggesting that a demographic scenario can be described relatively well from 10 individuals. This is in agreement with Robinson *et al.* (2014), who found that recent bottlenecks could accurately be described by genomic SNP data sets consisting of ten or more individuals. Robinson et al. (2014) also note, however, that larger sample sizes are required to accurately describe more recent events, and especially recent expansions. This may explain why a more recent change in population size was detected in the Hawaiian monk seal (i.e. around 1500 years ago) compared to in the taxa with smaller sample sizes (> 15,000 years ago). As discussed

below, the parameter estimates from the full data set better align with the known history of this species, suggesting that increasing the number of individual does increase the accuracy of the parameter estimates, especially if those individuals are associated with higher quality genome sequencing.

On the other hand, the estimates for the shape and scale parameters of the gamma distribution in the selection model are dramatically different between the full and down-sampled data sets. The scale parameter in particular differs by two orders of magnitude. Because the scale parameter describes the spread of the distribution, the larger scale parameter in the down-sampled data set shifts a greater proportion of the distribution to larger selection coefficients (Table 3a and 3b). Interestingly, the proportion of nearly neutral mutations ($|2Ns| < 1$) is nearly identical in both sample sizes. This may be coincidental. However, it is also possible that this portion of the distribution is easier to accurately describe with a reduced sample size because nearly neutral mutations segregate at higher frequencies than more strongly deleterious mutations. Kim *et al.* (2017) found a similar pattern in down-sampling a human population genomic data set to 12 individuals, although they argued that this sample size allowed them to estimate the parameters of the gamma distribution with relative accuracy. My results here suggest that further down-sampling to 10 individuals may introduce too much error, or that the necessary sample size is dependent on the particular SFS and DFE of the population. This latter reason may explain why the selection models for some species (i.e. northern elephant seal and Baltic ringed seal) did not converge, despite having 9-10 individuals.

A number of recent studies that model the DFE through the site frequency spectrum use much smaller sample sizes than 10 individuals, although they fit the model with

methods other than *fitδaδi*. In a simulation study using DFEalpha (Eyre-Walker & Keightley 2007), Keightley and Eyre-Walker (2010) attempted to find the optimum sequencing effort that would result in reliable parameter estimation for the DFE and concluded that 10 alleles (i.e. five diploid individuals) would be the optimum for genomic data sets. This clearly stands in contrast to the empirical sampling results I found here, which suggest more than 10 diploid individuals are necessary for accurate estimation. Notably, the two methods of DFE estimation (DFEalpha vs. *fitδaδi*) differ in their approach to model fitting, and in fact have found conflicting estimates of the DFE in Drosophila and humans (see Eyre-Walker & Keightley (2007) vs. Huber et al. (2017)). Given the empirical results here, a more thorough comparison of these modelling methods and their required sample sizes is warranted.

For now, this conflict makes it difficult to compare my results to studies that use different estimation methods given the differences in both modelling and sample size. For example, Kutschera *et al.* (2020) compare the DFE across corvid species living on islands and mainland using only 4 samples per taxon (implemented in DFEalpha). Galtier and Rousselle (2020) examine the DFE in 28 animal taxa with sample sizes as low as 5 per taxon, again using the DFEalpha but with a new "Gamma+Lethal" distribution model. Castellano *et al.* (2019) use yet another method, polyDFE (Tataru & Bataillon 2019), to compare DFE shape and purifying selection efficiency across primates. Comparisons of the actual shape of the DFE, rather than simply the proportion of nearly neutral mutations, are therefore likely unreliable across methods and with small sample sizes. Because of this, I restrict my in-depth comparisons to the few studies that have also used *fitδaδi* to model the DFE.

In addition, the difference I found in parameter estimates between the full data set and down-sampled data set of the Hawaiian monk seal suggests that the results from the taxa with 10 or fewer individuals (i.e. grey seal, northern elephant seal, Baltic ringed seal, Saimaa ringed seal, Weddell seal) should be interpreted cautiously. Specifically, the parameter estimates of the demographic model may be accurate, but the parameters of the selection model should be viewed as inaccurate. The one exception is the estimated proportion of nearly neutral mutations, which apparently can be accurately estimated even from a smaller sample size. For the remainder of the discussion, I will therefore mainly address the models from the full Hawaiian monk seal data set given the uncertainty around the parameter estimates from the other species.

Population structure and recent demography of Hawaiian monk seals

Using synonymous SNPs, I found only weak evidence of population structure among monk seals in the Hawaiian Islands. This is in agreement with previous studies using microsatellite markers, which found inconclusive (Kretzmann *et al.* 2001) or no structure (Schultz *et al.* 2009) in this species. Rather than a true structuring among the islands of the archipelago, my results suggest a weak isolation-by-distance that forms a gradient along the length of the Northwest Hawaiian Islands. Notably, the seals from the Main Hawaiian Islands do not fall along this continuous gradient, which may be evidence of a re-colonization of the Main Hawaiian Islands.

This weak structure is somewhat surprising, given that the Hawaiian archipelago spans 2400 kilometers, and the related Mediterranean monk seal shows population structure at a relatively small scale (Karamanlidis *et al.* 2016b). In recent years, some

Hawaiian monk seal pups have been translocated from Papahānaumokuākea Marine National Monument to the Main Hawaiian Islands (Baker *et al.* 2020), though there were concerns that this may disrupt natural population structure in the islands (Kretzmann *et al.* 2001). With only a few seals translocated every year in a population of 1400, the translocated seals themselves could not have produced the pattern I recover in this analysis. My results show little meaningful population structure exists in Hawaiian monk seals, and translocations can continue without concerns of disrupting local adaptations.

The results of the demographic modelling in *δaδi* also offer interesting insight into the history of this endangered species. The scaled parameters suggest that prior to around 1500 years ago, this species had an ancestral N_e of 4343. This is very similar to my results from Chapter 1, in which I used pairwise coalescent modelling (MSMC, Schiffels & Durbin 2014) from a single Hawaiian monk seal genome to estimate that the species had declining population leading to an N_e of around 2000 as recently as 10,000 years ago. Because the model I fit in this analysis contains only two time period, the ancestral N_e is more accurately viewed as an average of the N_e during the older time period, which likely explains why the estimate from *δaδi* is slightly higher than the final estimate from MSMC.

Around 1500 years ago, the *δaδi* demographic model recovers dramatic bottleneck of 95% to an N_e of 202. The timing of this bottleneck is remarkably in line with when Hawaiian monk seals first interacted with humans. Polynesians most likely settled the Hawaiian archipelago between 1500-1000 years ago (Kirch 2011), mainly settling in the Main Hawaiian Islands. Though physical evidence is scarce, archeological evidence suggests that seals were effectively extirpated from the Main Hawaiian Islands early on (Kittinger *et al.* 2011), although European colonizers noted them in the Northwest

Hawaiian Islands (NWHI), with one ship (the *Gambia*) reportedly collecting 1,500 seal skins from the NWHI in 1859 (Schultz *et al.* 2010).

Taken together, the historical and genetic evidence paint a clear picture of the history of this endangered species. As an island species reliant on coastal waters in an isolated archipelago, the Hawaiian monk seal had a naturally low carrying capacity in the Hawaiian islands. When humans first arrived in the islands, they hunted the species and restricted its territory to the NWHI. Finally, significant European seal hunts during the 19th century further decreased the population of seals in the NWHI. This combination of long-term small population size and more recent bottlenecks has left the species with the lowest genetic diversity of any naturally occurring mammalian population.

The evolution of the DFE in mammals

Using the same two epoch demographic and gamma-distributed selection models in *fitδaδi*, Huber *et al.* (2017) found that the DFE for humans (ancestral N_e = 7070) could be modeled as a gamma distribution with a shape parameter (α) of 0.19 and a scale parameter (β) of 0.074. They also found that the DFE for mouse (ancestral N_e = 282,800) was gamma-distributed with $\alpha = 0.22$ and $\beta = 0.016$. The parameters describing the human DFE are nearly identical to those I found for the DFE of the Hawaiian monk seal. In fact, the Poisson likelihood of the Hawaiian monk seal model with the human-derived scale and shape parameters is marginally higher than the likelihood of the model with the parameters optimized from the Hawaiian monk seal SFS (–69.7782 and –69.83949, respectively). On the other hand, the likelihood of the Hawaiian monk seal model using the mouse parameters is slightly lower (–70.0061). Accordingly, the mean selection coefficient for the Hawaiian

monk seal ($|s|$ = 0.01209) is nearly the same as in humans ($|s|$ = 0.01406), both of which are 3–4X smaller in the mouse ($|s|$ = 0.00352).

When alleles are binned into ranges of selection coefficients, the similarity between the human and Hawaiian monk seal DFEs is also clear. Humans and the Hawaiian monk seal have fewer mutations of small effect ($|s| < 10^{-4}$) and many more of large effect ($|s| > 10^{-2}$) compared to the mouse. In the distribution of scaled selection coefficients, humans and Hawaiian monk seal have many more nearly neutral alleles ($|2Ns| < 1$), while the mouse has many more alleles with large scaled selection coefficients ($|2Ns| > 100$).

In their study, Huber *et al*. (2017) noted the DFE in mouse is shifted toward smaller selection coefficients compared to in humans. In addition, they noted that in Drosophila and yeast the DFE was even more dramatically towards smaller selection coefficients. They consider four evolutionary theory frameworks that could explain this pattern: a functional importance model, a protein stability model, a back-mutation model, a mutational-robustness model, and the geometric model. Through their analysis, they conclude that the geometric model (Tenaillon 2014) best fits the observed pattern.

My results similarly support the geometric model, with one crucial difference in interpretation from Huber *et al*. (2017). The geometric model has two main parameters that affect the DFE: the distance a population is from a fitness optimum (d), and the phenotypic complexity (n). Tenaillon *et al.* (2007) showed that if the distance (d) is determined by the fixation of slightly deleterious alleles through genetic drift, the equilibrium drift load in the geometric model can be approximated as:

$$-\frac{n}{2QN},$$

where n is phenotypic complexity, Q is an epistasis parameter, and N is the effective population size.

Huber *et al*. (2017) argue that phenotypic complexity (n) increases from yeast to Drosophila to mouse to human. Complexity is a fraught term in evolutionary biology, which lacks a strong definition (Adami *et al.* 2000, Tenaillon *et al.* 2007). Intuitive, but taxonomically biased, views of complexity may invoke aspects such as morphological diversity, multicellularity, and tissue differentiation in assessments of diversity. Even if this view of complexity is used, it is difficult to defend the position that a human is more complex than a mouse when the two are compared to all other forms of biodiversity. Furthermore, to align this hypothesis with my estimate of the DFE in seals, one would have to argue that Hawaiian monk seals are more complex than mice and about as complex as humans. In light of my results, invoking complexity is a weak biological argument. In addition, increasing n is predicted to lead to a smaller proportion of beneficial alleles (P_{ben}) in the geometric model, which conflicts with pattern of P_{ben} across species as described by Huber et al. (2017).

As the drift load formula shows, however, distance from the fitness optimum is also be affected by N. Notably, the effective population sizes of these species decrease from yeast to drosophila to mouse to humans, with the effective population size of humans being roughly equivalent to that in Hawaiian monk seals. Effective population size therefore appears to be a more biologically plausible explanation when the DFE results from the Hawaiian monk seal are included. As Huber *et al*. (2017) note, though, the difference in mean selection coefficient between humans (or Hawaiian monk seal) and mouse is about an order of magnitude smaller than the difference between their effective population sizes.

While long-term effective population size appears to be an important determinant of the DFE, other factors are likely to affect this distribution as well.

Conclusion

In summary, I find further evidence that the Hawaiian monk seal population has always been relatively small, but I add important context by showing that the species went through a 95% reduction in population size after the arrival of humans in Hawaii. By modelling the DFE, I show that the shape of the DFE in Hawaiian monk seals is similar to that in humans, but both are different from the DFE in mouse. Given that mice are phylogenetically closer to humans than to seals, this cannot be due to phylogenetic signal. There is also not an obvious biological case that seals are more phenotypically complex than mice. The obvious explanation is that humans and Hawaiian monk seals have extremely similar long-term effective population sizes, while the long term effective population size of mice is much larger. The geometric model predicts that the DFE would evolve in response to population size if smaller populations are less fit through the accumulation of slightly deleterious mutations.

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Tables and Figures

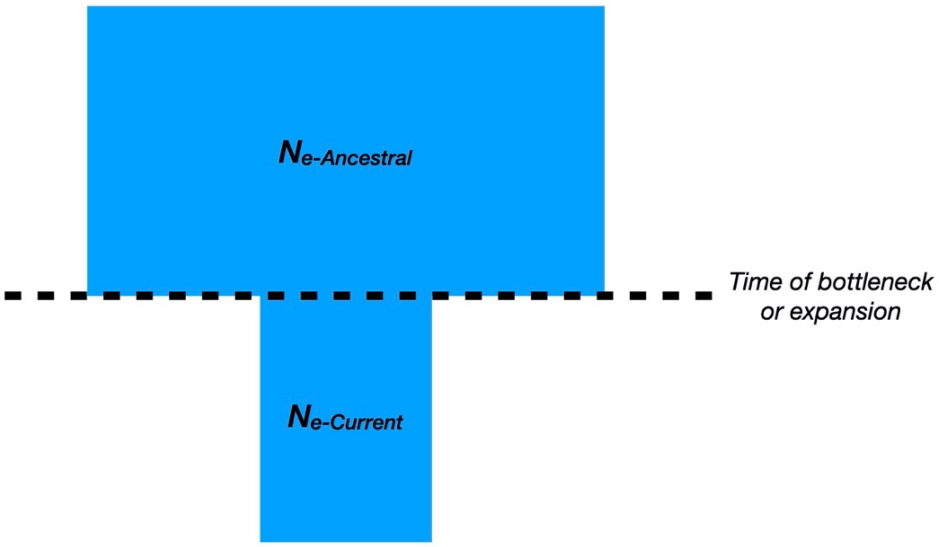


Figure 1. Schematic of two-epoch demographic model fit to each taxon in *δaδi*. Parameters for ancestral and current N_e, as well as timing of bottleneck or expansion, are allowed to vary independently of one another. The change in population size is shown here as a contraction (bottleneck), but the independence of parameters equally allows for a population expansion to be modeled.

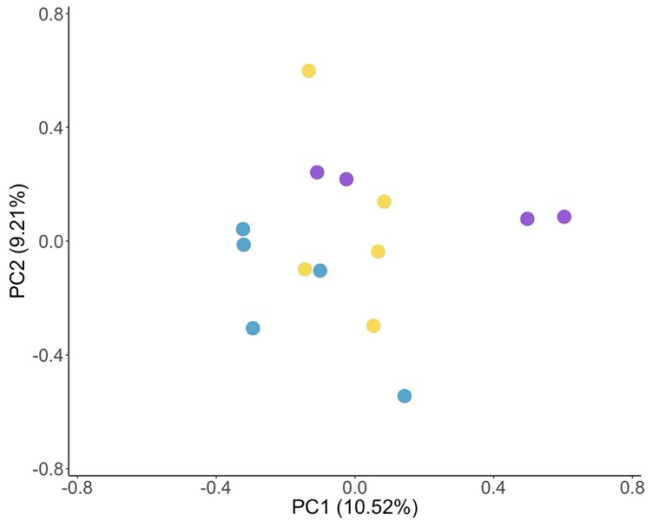


Figure 2a. PCA of Hawaiian monk seal synonymous sites. Points are colored by geographic location (purple: Main Hawaiian Islands (Kauai, Oahu, Hawai'i); yellow: mid-archipelago islands (French Frigate Shoals, Laysan, Lisianski; blue: far Northwest Hawaiian Islands (Pearl and Hermes Atoll, Midway Atoll, Kure Atoll)).

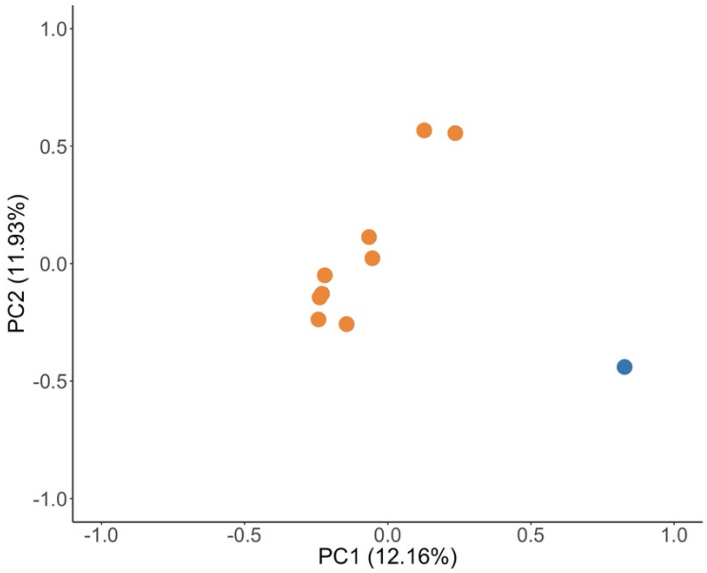


Figure 2b. PCA of Weddell seal synonymous sites. Samples in orange are from Erebus Bay. Sample in blue is from publicly available data with sampling location unknown.

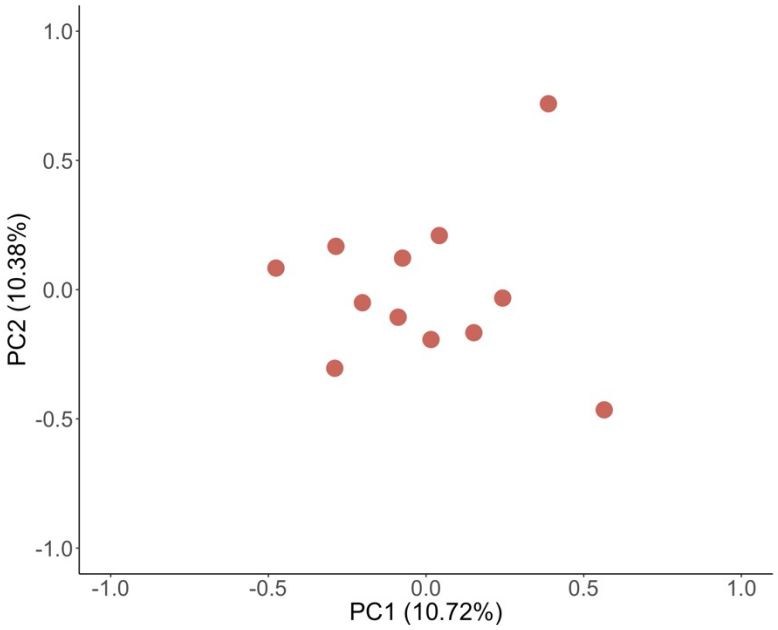


Figure 2c. PCA of northern elephant seal synonymous sites. All samples are from the same geographic location.

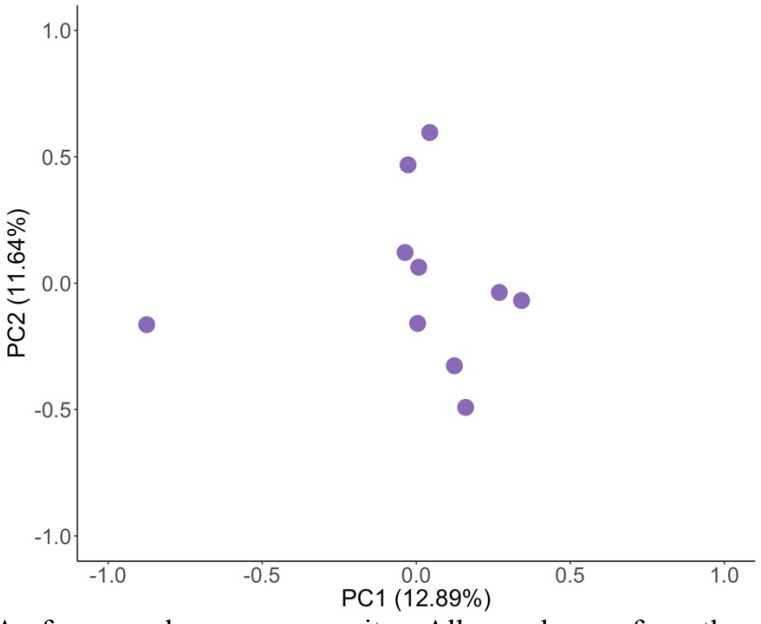


Figure 2d. PCA of grey seal synonymous sites. All samples are from the same geographic region (Baltic Sea).

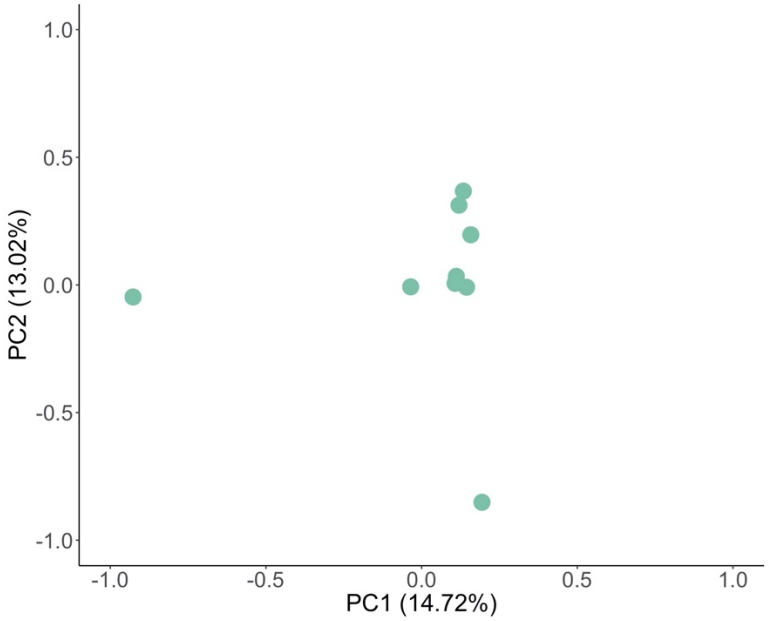


Figure 2e. PCA of Baltic ringed seal synonymous sites. All samples are from the same geographic location (Baltic Sea).

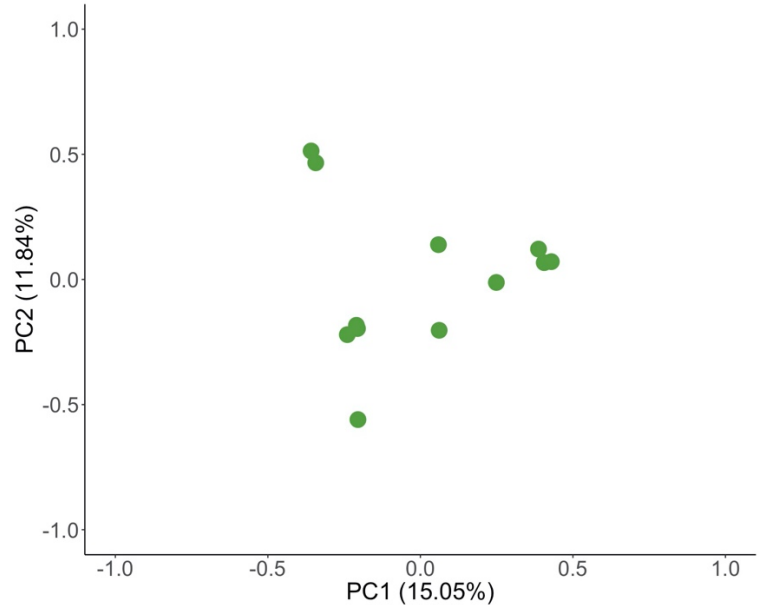


Figure 2f. PCA of Saimaa ringed seal synonymous sites. All samples are from the same geographic location (Lake Saimaa).

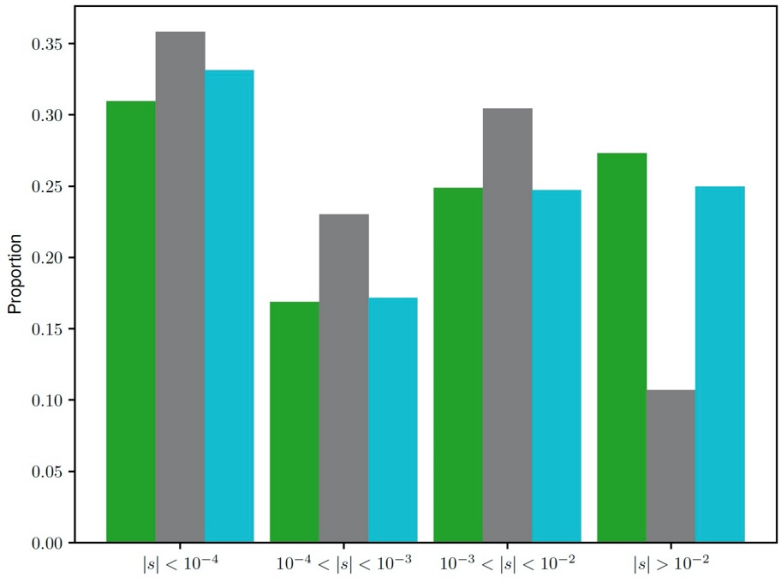


Figure 3a. The proportion of alleles with a selection coefficient in each interval. Bars correspond to human (green), mouse (grey), and Hawaiian monk seal (light blue).

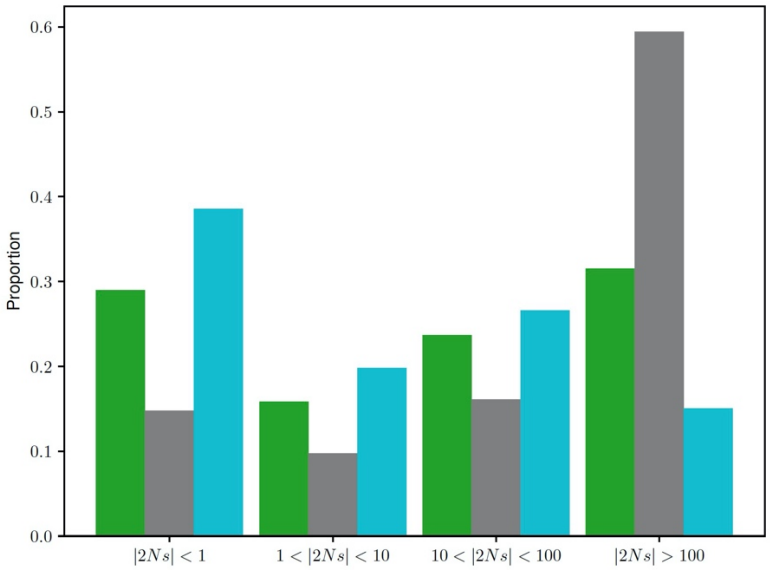


Figure 3b. The proportion of alleles with a scaled selection coefficient in each interval. Bars correspond to human (green), mouse (grey), and Hawaiian monk seal (light blue).

Table 1. Parameter estimates from demographic models for each taxon. The model for the Saimaa ringed seal consistently pushed the lower bounds of *nu* and *T* to unrealistic values.

Species	***Nu***	***T***	$\theta_{S\text{-}Ancestral}$	**Time change (years ago)**	$N_{e\text{-}Ancestral}$	$N_{e\text{-}Current}$	**likelihood**
Grey seal	0.192	0.0314	4814.249	17,814	23,676	4541	-53.554
Hawaiian monk seal	0.047	0.0145	883.105	1512	4343	202	-60.393
Northern elephant seal	0.014	0.0307	11351.158	41,131	55,824	798	-75.575
Baltic ringed seal	2.013	2.8693	7447.307	2,522,180	36,625	73,716	-600.681
Saimaa ringed seal	NA	NA	NA	NA	NA	NA	NA
Weddell seal	2.362	0.6094	6623.880	476,452	32,576	76,957	-60.958
Hawaiian monk seal (down-sampled)	*0.100*	*0.0395*	*934.532*	*5432*	*5745*	*576*	*-52.702*

Table 2. Parameter estimates from selection models for each taxon. The models for the northern elephant seal and Baltic ringed seal consistently pushed the upper bound of β estimates to unrealistic values ($|s| > 0.5$). The selection model for the Saimaa ringed seal could not be run because the demographic model did not converge.

Species	**Shape (α)**	**Scale (β)**	**De-scaled β**	**likelihood**
Grey seal	0.1611	39016	0.6592	-51.71338
Hawaiian monk seal	0.1823	576	0.0663	-69.83949
Northern elephant seal	NA	NA	NA	NA
Baltic ringed seal	NA	NA	NA	NA
Saimaa ringed seal	NA	NA	NA	NA
Weddell seal	0.1725	16680	0.2048	-77.96850
Hawaiian monk seal (down-sampled)	*0.1123*	*14804*	*1.2885*	*-56.02394*

Table 3a. The expected (mean) selection coefficient (E[|*s*|]) and variance in selection coefficient (Var[|*s*|]) for each species, as well as the proportion of alleles with a selection coefficient in each interval.

	E[|*s*|]	**Var[|*s*|]**	**$|s| < 10^{-4}$**	**$10^{-4} < |s| < 10^{-3}$**	**$10^{-3} < |s| < 10^{-2}$**	**$10^{-2} < |s|$**
Human	0.01406	0.001040	0.309419	0.16889	0.24865	0.273043
Mouse	0.00352	0.000056	0.358165	0.230317	0.304459	0.107059
Grey seal	0.10617	0.069986	0.26098	0.117116	0.168743	0.434025
Hawaiian monk seal	0.01209	0.000801	0.331314	0.171786	0.247089	0.249811
Weddell seal	0.03533	0.007237	0.289824	0.141065	0.206052	0.362727
Hawaiian monk seal (down-sampled)	0.1447	0.1865	0.364883	0.107684	0.139075	0.348679

Table 3b. The expected (mean) selection coefficient (E[|2*Ns*|]) and variance in selection coefficient (Var[|2*Ns*|]) for each species, as well as the proportion of alleles with a selection coefficient in each interval.

	E[|2*Ns*|]	**Var[|2*Ns* |]**	**|2*Ns*	< 1**	**1 < |2*Ns*	< 10**	**10 < |2*Ns*	< 100**	**100 < |2*Ns* |**
Human	198.8	208025	0.289727	0.158392	0.236656	0.315225
Mouse	1990.912	18016957	0.147575	0.097294	0.160786	0.594345
Grey seal	2513.811	39231226	0.227154	0.101972	0.147406	0.523467
Hawaiian monk seal	52.5053	15121	0.385609	0.19836	0.265706	0.150325
Weddell seal	1151.037	7679970	0.236415	0.115227	0.170458	0.4779
Hawaiian monk seal (down-sampled)	831.576	6155592	0.388322	0.114575	0.147663	0.34944

Supplemental Material

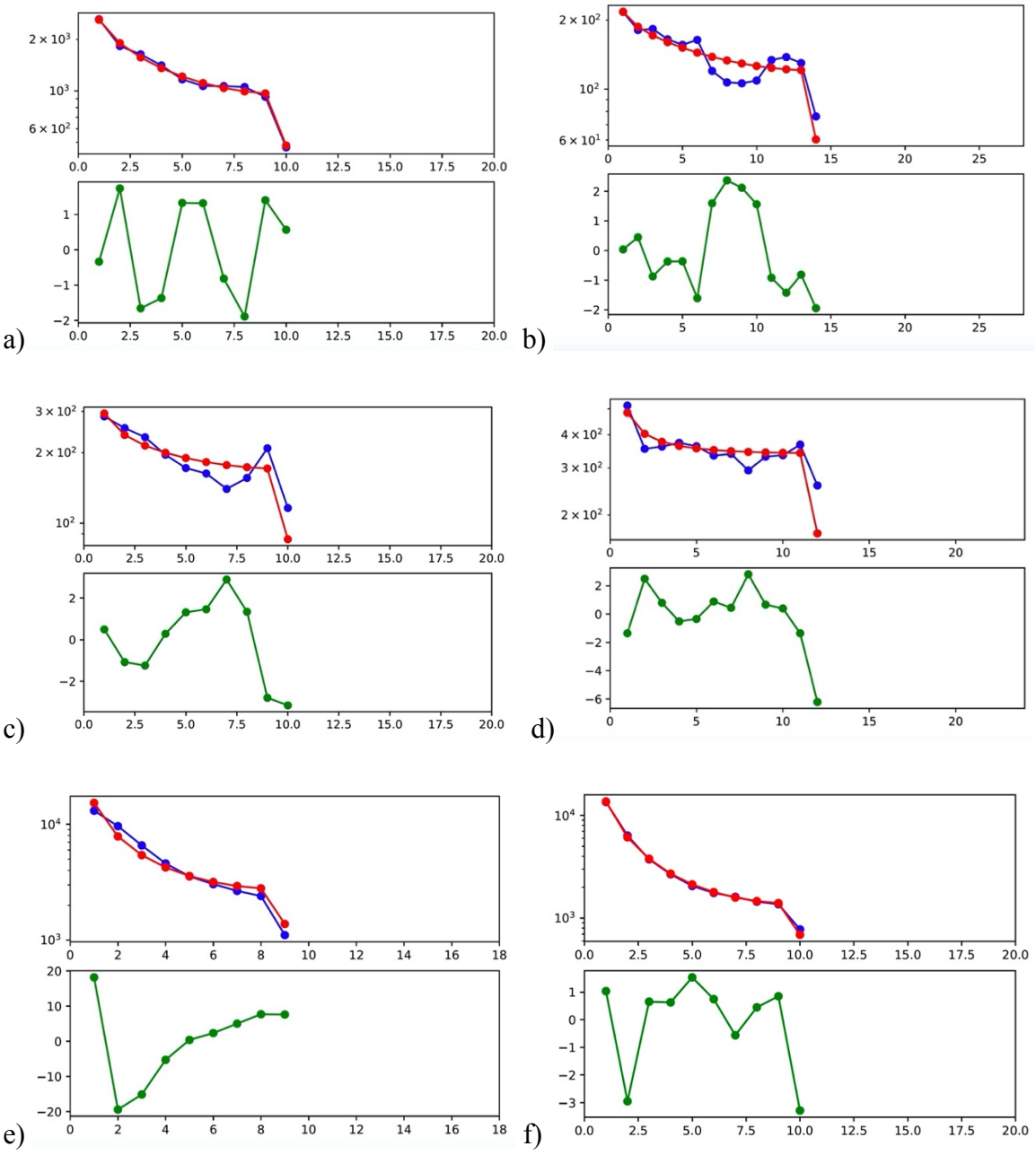


Figure S1. *Above*: Synonymous site frequency spectra comparisons between model (red) and data (blue). *Below*: Poisson residuals between model and data. **a)** grey seal **b)** Hawaiian monk seal (full data set) **c)** Hawaiian monk seal (down-sampled data set) **d)** northern elephant seal **e)** Baltic ringed seal **f)** Weddell seal.

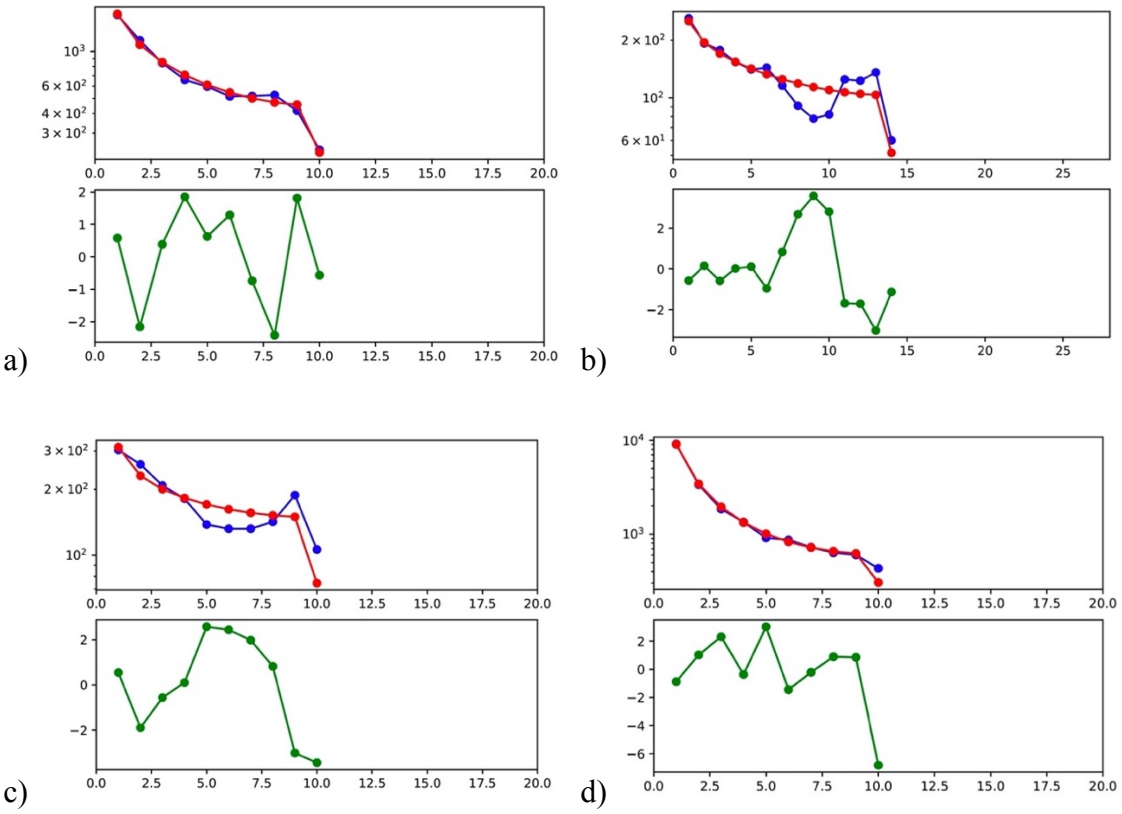


Figure S2. *Above*: Nonsynonymous site frequency spectra comparisons between model (red) and data (blue). *Below*: Poisson residuals between model and data. **a)** grey seal **b)** Hawaiian monk seal (full data set) **c)** Hawaiian monk seal (down-sampled data set) **d)** Weddell seal.

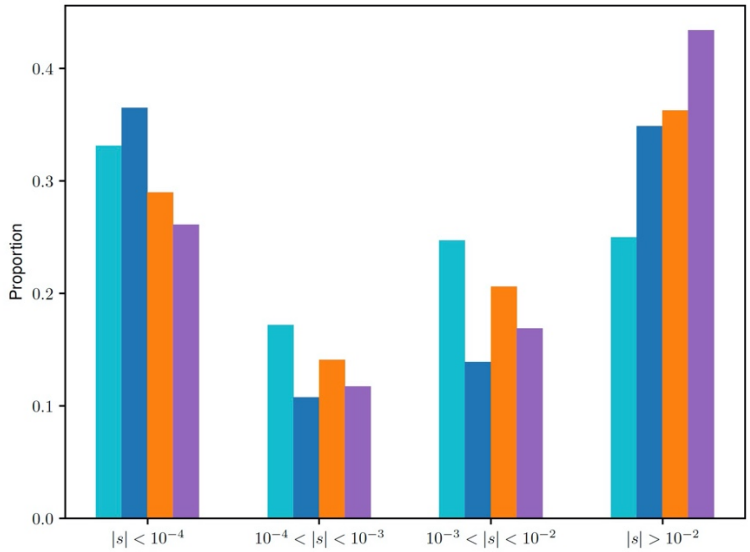
Low-confidence comparisons of taxa with smaller sample sizes

The down-sampling analysis I performed with Hawaiian monk seals showed that smaller sample size (10 individuals) and lower sequencing depth (average 10X) may be insufficient to accurately estimate the parameters of the DFE. Specifically, this smaller sample size tends to overestimate large-effect alleles in the population. In light of the Hawaiian monk seal results, the results from the Weddell seal (n=10) and grey seal (n=10)

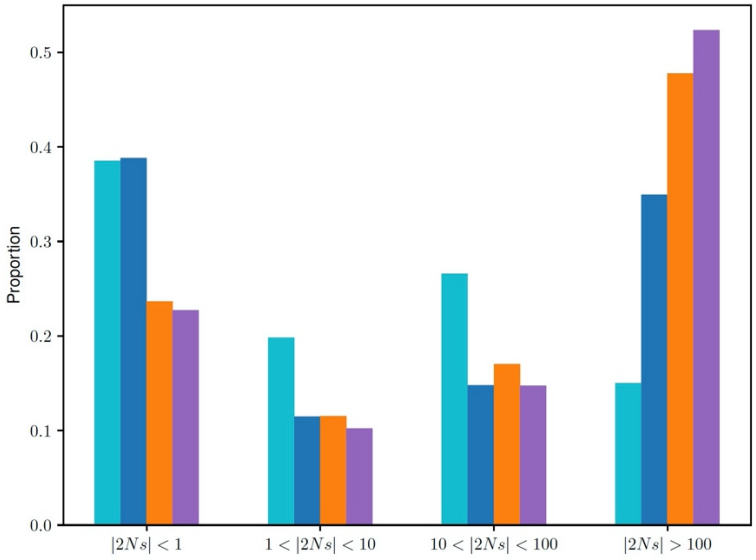
should be viewed cautiously. This cautious view is especially needed in grey seals, which have an average sequencing depth of 10X or below and therefore could have additional error in the SFS.

The shape parameters for both grey seal (α = 0.161077) and Weddell seal (α = 0.172512) were similar to those estimated in the full Hawaiian monk seal data set, as well as those in human and mouse (Table 2). The scale parameters, however, were much higher than the parameter estimates for the other mammalian species (Table 2). This inflation of the scale parameters in a smaller sample size was also observed in the down-sampled Hawaiian monk seal data set, and is almost certainly an artefact of sample size.

However, in the Hawaiian monk seal the full data set and down-sampled data set both estimated nearly identical proportions of nearly neutral alleles (Figure S1b). This one statistic may therefore be robust to the error that comes from differences in sample size. This makes statistical sense, given that nearly neutral alleles are expected to be at higher frequencies than strongly deleterious alleles, and therefore easier to describe with fewer individuals. If this estimate of nearly neutral alleles is accurate across species, the pattern shows that the Hawaiian monk seals has a higher proportion of nearly neutral alleles (0.386), while the proportion of nearly neutral alleles is lower in the grey seal (0.227) and Weddell seal (0.236; Table 3b and Figure S1b).



Supplemental table S1a. The proportion of alleles with a selection coefficient in each interval. Bars correspond to Hawaiian monk seal (light blue), down-sampled Hawaiian monk seal (dark blue), Weddell seal (orange) and grey seal (purple).



Supplemental table S1b. The proportion of alleles with a scaled selection coefficient in each interval. Bars correspond to Hawaiian monk seal (light blue), down-sampled Hawaiian monk seal (dark blue), Weddell seal (orange) and grey seal (purple).

GENERAL DISCUSSION

Population genetics and molecular evolution originated as mathematical disciplines, but transformed into an empirical disciplines with the advent of experimental and molecular genetics (Chakravarti 1999). However, only in the past few years has genomic sequencing made it possible to truly bridge the gap between evolutionary theory and the patterns of molecular evolution in natural populations. In this dissertation, I took advantage of these new methods to test fundamental ideas of molecular evolution in a unique evolutionary system: phocid seals. This charismatic clade is one of the few mammal lineages to return to the sea, forcing them to evolve a suite of adaptations to thrive in a marine environment. Importantly for this study, the colonization of different oceanic habitats has led not just to distinct adaptations across species but also differences in carrying capacity and therefore population size. Finally, species in tropical and temperate regions with smaller populations have also suffered disproportionately from human exploitation, making an understanding of their evolutionary history an important piece in developing an effective plan for their survival.

In both my first and third chapters, I used population genetic modelling to estimate the historical population sizes and trends for these phocid species. In Chapter 1, I showed that tropical and temperate species, including the Hawaiian monk seal, Mediterranean monk seal, northern elephant seal, and grey seal, have historically smaller population sizes. Furthermore, these species all apparently experienced declines during the last glaciation starting around 120,000 years ago. Cooler ocean temperatures, changing sea levels, or disrupted nutrient cycling all may have affected these species during the glaciation. On the other hand, the Arctic (ringed seal) and Antarctic (Weddell seal and southern elephant seal)

species all showed larger historical population sizes but varying responses to the glaciation, likely dependent on their individual environmental contexts. In my third chapter, I was only able to confidently estimate the demographic history of the Hawaiian monk seal, but the estimates matched with the results from Chapter 1. Because the site frequency spectrum-based modelling allowed me to examine more recent time periods, I was further able to show that the Hawaiian monk seal population experienced a reduction in size of about 95% after the arrival of humans in the Hawaiian archipelago. In combination, this history has resulted in the Hawaiian monk seal having the lowest genome-wide heterozygosity of any mammal studied so far. This detailed history matches with archeological, anthropological, and historical records from Hawaii (Kittinger *et al.* 2011). It also paints a clear picture of the particular vulnerability of the Hawaiian monk seal.

Despite the small population sizes of Hawaiian monk seals, Mediterranean monk seals, and grey seals, however, all of these species showed lowered rates of mutation accumulation compared to closely-related species with larger populations. This unexpected finding suggests that the underlying mechanisms of molecular evolution may evolve quickly in response to effective population size, perhaps reaching new equilibria that are not accounted for in many simple models of molecular evolution (Cherry 1998).

In particular, species with smaller populations may fix deleterious alleles that in turn change the distribution of fitness effects (DFE) of new alleles through epistatic interactions. I attempted to test this idea directly in Chapter 3. Due to sampling limitations, however, I could only confidently estimate the DFE for the Hawaiian monk seal. By comparing the DFE from Hawaiian monk seal to that from mouse and human, I showed that DFE clearly corresponds to long-term effective population size. This pattern is in

agreement with other theoretical and empirical work supporting the geometric model as an appropriate theoretical framework for understanding molecular evolution (Tenaillon 2014, Huber *et al.* 2017). Without comparisons from other seal species, it is difficult to conclude if the DFE differs among seals enough to account for the mutation accumulation pattern observed in Chapter 1. I showed in Chapter 3 that the smaller sample sizes for Weddell seal and grey seal may still be sufficient to describe the nearly neutral portion of the DFE. If that is true, the Hawaiian monk seal has a much higher proportion of nearly neutral mutations than the Weddell seal or grey seal, which would conflict with the results from Chapter 1 as the Hawaiian monk seal would be expected to have a higher substitution rate due to the fixation of nearly neutral mutations. While I cannot resolve this in my dissertation, a more expansive study of the DFE in seals still offers an exciting opportunity to test for the rapid evolution of DFE.

In addition to patterns of purifying selection across seal species, I also find abundant evidence of positive selection in the genomes of phocid seals. In Chapter 2, I show how genes underlying blubber composition, and especially collagen genes, have been under strong, ongoing positive selection across seal lineages. Given how important this thermos-insulating blubber is to the survival of marine mammals, and the fact that it is a derived trait in this lineage, this strong signal is unsurprising. Other functional categories—such as genes related to immune function and to sperm motility—are also under consistent positive selection across lineages.

Hundreds of other genes are under positive selection in seals. Among the most interesting genes are those that are putatively related to hypoxia tolerance in the deep-diving elephant seals and Weddell seal. The extreme physiological stress of hypoxia on the

tissues and organs, but especially on the heart and brain (Blix 2018, Hindle 2020), have led to multiple molecular adaptations in the genomes of elephant seals and the Weddell seal. In particular, genes relating to cardiac development and cardiac signal transduction may help explain how these seals have evolved inducible bradycardia, with heart rates as low as 2–3 beats per minute (Andrews *et al.* 1997). Modern tissue culture techniques would all for an important experimental look at role of these genetic changes in cardiac cell development and function.

Besides providing insight into the molecular adaptations of physiological traits in seals, the results from Chapter 2 also reveal important patterns of adaptive molecular evolution more general. In particular, by finding examples of parallel molecular evolution across seal lineages I show how, in closely related species, the evolution of complex adaptive phenotypes can occur in parallel sets of genes. Interestingly, though, I showed that genes that are evolving in parallel need not evolve in the same way, with positive selection acting different regions of the same gene in different seal lineages.

Studies of molecular evolution in natural populations offer opportunities to test hypotheses from theoretical models and parameterize models for further theoretical work. In this dissertation, I showed that phocid seals represent an exciting model system for studying the impact of long-term population size on molecular evolution. The results from this work support the use of the geometric model as a framework for understanding molecular evolution, and future studies should explore the application of this model to natural populations. In addition, these results provide important insight into the biology, evolutionary history, and future survival of these unique marine mammals.

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