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Origins and evolution of extreme lifespan in Pacific Ocean rockfishes

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Abstract

Pacific Ocean rockfishes (genus *Sebastes*) exhibit extreme variation in lifespan with some species amongst the most long-lived extant vertebrates. We *de-novo* assembled the genomes of 88 rockfish species and from this identified repeated signatures of positive selection in DNA repair pathways in long-lived taxa and 137 longevity-associated genes with effects on lifespan through insulin signaling, and pleiotropic effects through size and environmental adaptations. A genome-wide screen of structural variation reveals copy number expansions in the immune modulatory butyrophilin gene family in long-lived species. The evolution of different rockfish life-histories is coupled to genetic diversity and reshapes the mutational spectrum driving segregating CpG->TpG variants in long-lived species. These analyses highlight the genetic innovations that underlie life-history trait adaptations and, in turn, how they shape genomic diversity.

One Sentence Summary:

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Author contributions: P.H.S. conceptualized and designed the experiment. A.S. C.J., K.S. J.A.V, K.M., M.M., M.W.S, J.W.R. and M.L. coordinated sample collection efforts. S.R.R.K., G.L.O., J.M.V. and P.H.S. performed all analyses. K.C. and D.B. performed HiC experiments for genome assembly. P.H.S., G.L.O., S.R.R.K. and M.L. wrote the manuscript.

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Supplementary Materials:
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Genomes of 88 rockfish species identify the genetic drivers of longevity and how life-history adaptations can reshape patterns of genetic diversity.

Decades of theoretical work on the evolution of aging have demonstrated that senescence is a *"theoretically inevitable"* consequence of the increased selective impact of genes that influence early life survival and fecundity compared to genes that act late in life (1). Nonetheless, across vertebrates alone lifespan varies by more than 3 orders of magnitude ranging from the 5-week lifecycle of the pygmy goby (2) to the ~400-year lifespan of the Greenland shark (3) highlighting exceptional diversity of this trait across species. Understanding evolutionary transitions in lifespan can highlight diverse genetic mechanisms of lifespan control and the subsequent impacts of life-history shifts on patterns of genetic diversity.

Across organisms aging is accompanied by several molecular hallmarks including genome instability, loss of protein homeostasis, and mitochondrial dysfunction among others (4). Vertebrates in particular also exhibit a number of distinct hallmarks of aging which are directly linked to human disease and healthspan such as immuno-senescence, inflammation, and stem-cell exhaustion (5). Thus, understanding the underpinnings of lifespan-variation across vertebrate taxa can provide critical insights into the maintenance of human health and vigor in old age.

Rockfishes (genus *Sebastes*) of the Pacific Ocean exhibit lifespans ranging from 11 years (*Sebastes minor*) to more than 200 years for the Rougheye Rockfish (*Sebastes aleutianus*) (6) (Fig. 1A). This phenotype exhibits a relatively uniform distribution across the more than 75 different species of this clade for which detailed longevity information is available (Fig. S1). Rockfishes are thus unique in that while some species are among the longest-lived vertebrates known to exist, lifespan can widely range even among closely related taxa. More than 120 different species of rockfish are found throughout the Northeast and Northwest Pacific Ocean (7) (Fig. 1B). This abundance of species with vastly differing life histories represents a unique example of repeated, recent adaptations that have reshaped longevity phenotypes.

Results

Sequencing and assembly of 102 rockfish genomes

To dissect the genetic underpinnings of lifespan variation and adaptation we sequenced and *de novo* assembled the genomes of 102 rockfish individuals encompassing 88 different species, including 79 members of the *Sebastes* clade and 9 closely related outgroup taxa (Fig. S1, Fig. S2). Long-read Pacific Biosciences genome assemblies were generated for 6 *Sebastes* species and the outgroup *Sebastolobus alascanus* (6.1Mbp mean contig N50, Fig. 1C). Five of these *Sebastes* genomes were scaffolded with long-range HiC data resulting in chromosome-scale assemblies (34.1Mbp mean scaffold N50, Fig. 1C, Table S1, Fig. S3). High-coverage Illumina based assemblies were generated for 71 additional *Sebastes* species (73.8Kbp mean contig N50). Genome completeness (assessed by BUSCO scores (8)) ranged from an average of 97.9% for long-read based genomes to 91.2% for Illumina

based assemblies and the majority (5 of 7) of the long-read based genomes reached QV40 base quality (average QV39 for all 7) (Fig. S4). Transcriptomes were generated from brain and eye tissue for 8 species including 6 individuals for which we generated long-read based genome assemblies to assist in gene annotation. In total, we identified ~25,000 protein-coding genes on average across the seven long-read genome assemblies (Table S3). Together, these genome assemblies encompass the majority of species represented in the *Sebastes* genus and almost all representatives for which detailed lifespan information has been cataloged, including a chromosome level assembly of the ultra-long-lived *S. aleutianus*.

We established the phylogenetic relationships and speciation times among rockfish species by constructing an ultrametric species phylogeny (See supplementary methods (9)) (Fig. 1A). The topology of this tree is consistent with previous phylogenetic analyses of rockfishes (7, 10). The varied placement of long-lived taxa in the tree suggests repeated life-span related evolution (Fig. 1A), which is supported by character mapping analysis which shows multiple independent gains of long lifespan (Fig. S7).

Repeated signatures of positive selection in DNA repair pathways in long-lived taxa

We next sought to determine the genetic underpinnings of lifespan in Pacific rockfish. We used a branch-site model test of codon evolution to identify candidates of positive selection in the longest-lived (≥105 years) and shortest-lived (≤20 years) species (lifespan decile tails, n=7) identifying 772 and 873 genes, respectively. Most of these positively selected genes (PSGs) were lineage-specific, although ~12–15% of genes (127 and 118 respectively) were present in two or more species representing either selection on the ancestral branch or convergent evolution. Additionally, 180 PSGs were found in both long- and short-lived individuals, highlighting that many of these genes may be under selection for other traits. While no pathways were enriched in PSGs in short-lived species after multiple testing correction, PSGs in long-lived species were enriched for DNA double stranded break repair pathways (Fig. 2A, Table S5).

The enrichment in DNA replication, repair, and maintenance is driven by 16 genes (Fig. 2B), five of which exhibit selective signatures in more than one species. These include genes in pathways associated with lifespan across organisms, such as telomere maintenance (e.g. *WRAP53*, *DCLRE1B*) and base excision repair (e.g. *FEN1*). Intriguingly adaptive signatures in *DCLRE1B* and *FEN1* have been identified in the Bowhead whale and giant tortoise respectively (11, 12). MCM6, a core member of the DNA replication helicase machinery, exhibits signatures of positive selection in *S. ruberrimus* and *S. nigrocinctus*. These signatures of selection suggest that repeated transitions in lifespan in rockfish are likely enabled by parallel evolution of different genes in shared critical DNA maintenance pathways.

Genes associated with lifespan adaptations through direct and pleiotropic effects

We next examined the role of evolution in convergent genes and pathways on rockfish lifespan by comparing the relative evolutionary rates of genes across the phylogeny (13). This approach allows us to identify genes with evolutionary rates that are correlated, either positively or negatively, with lifespan (Fig. 2C, Table S14). While these correlations do

not prove causation, they can guide insights in experimentally intractable systems, such as rockfish. At a q-value cutoff of 0.05 we discovered 91 genes significantly associated with lifespan including candidates with roles in cell growth and proliferation (e.g. *NRG1*), DNA repair (e.g. *BRIP1*) and suppression of apoptosis (e.g. *TNFRSF6B*) (Fig. 2D, Fig. S9). However, lifespan in rockfish is correlated with body size and environmental factors, such as depth (14). Thus, some of these genes associated with lifespan may act by influencing growth and size or may facilitate adaptations to environments that promote longevity.

To identify genes associated with rockfish longevity, independent of other factors, we constructed a linear model using the two most predictive variables for lifespan, size at maturity and maximum depth (Fig. 2E). This model described 59% of the variation in lifespan (Fig. 2F), comparable to simple models of lifespan and body size in mammals (15). Using the residuals of this model as phenotypes we identified 56 genes associated with lifespan, independent of size at maturity or depth (Fig. 2D, Table S14). These were overrepresented for insulin and glucose signaling (Hypergeometric test, P=1.8e-6, Fig. 2G, Fig. S10) including genes with roles in lifespan extension across many organisms (*19, 20*). The rate of evolution for most of these genes was negatively correlated with lifespan emphasizing the importance of nutrient sensing maintenance in long-lived species. We also identified genes associated with reproductive aging in mice (16), the antiviral innate immunity factor *TRAF3IP3* (17), and the tumor suppressor *DYRK2* (18).

Of the 91 genes associated with lifespan, prior to correcting for size and depth, only 10 overlapped with those identified as associated with the lifespan residual suggesting that the majority (n=81) of the genes we identified in *Sebastes* act indirectly by influencing size or facilitating adaptations to depth. To parse apart the axes along which these lifespan-associated genes act, we correlated their relative evolutionary rates with either the growth-associated component of lifespan in our linear model (S, size); the depth associated component of lifespan (D); or the residual of the model (R) (Fig. 2H). The size axis was associated with the most genes (n=33, S>0.5) in comparison to depth or the residual (n=18, D>0.5; n=17, R>0.5) in agreement with the importance of growth and size related pathways underlying differences in lifespan among different organisms (19). Pathways enriched along this axis included mTOR signaling, DNA and telomere maintenance, and cancer (Fig. 2I). Genes and pathways along the depth axis reflect environmental adaptations including lipid metabolism and synthesis. The R-axis was enriched for insulin signaling, and protein homeostasis with ribosome assembly pathways along the DR axis. Apoptosis genes were more closely clustered with the R axis intermediate to S and D and antigen processing and presentation genes were also clustered along the SD axis. Together, these results suggest that the genetic basis of longevity in rockfish species may be due to the combined effect of genes acting directly on lifespan alongside genes impacting ecological and growth phenotypes which pleiotropically influence lifespan.

Expansion of the immune modulatory butyrophilin gene family in long-lived species

Gene duplications and structural rearrangements can drive evolutionary innovations (20, 21). To identify copy number changes associated with variation in lifespan, we performed a genome-wide screen using windowed read-depth based copy estimates across chromosomes.

Controlling for phylogenetic signal, we found an enrichment for positive associations between lifespan and copy number, whether we used a short- or long-lived species reference (χ-squared, P < 1e-15). The greatest cluster of positive associations was on chromosome 1 (Fig. 3A); it extended for almost 250kb and overlapped 9 genes (Fig. 3B) ranging from 0–7 copies among different species (Fig. 3C). These 9 genes included 6 members of the butyrophilin gene family (*BTN* and *BTNL* genes, Fig. 3D), a family of immune regulators associated with human inflammatory diseases (22). Using our linear model we find that this signature is primarily driven by the depth-associated component of lifespan (Fig. 2H, Fig. S15). Mutations in *BTNL2* cause sarcoidosis in humans (23) and variants in *BTN3A2* have been associated with human lifespan (24).

Butyrophilins are members of the B7 immunoglobulin superfamily and in response to inflammatory stimuli inhibit cytokine secretion and production in T-cells, thus serving an immuno-suppressive function. We resolved the underlying segmental duplication (SD) architecture of the lifespan-associated butyrophilin duplication in the *S. aleutianus* genome (Fig. 3E) identifying 22 large (>1kb, >90% identity) SD blocks across the locus. The majority of the locus is contained in three pairs of large adjacent SDs in direct orientation which together encompass 163kb. Two large, high-identity (>95%) inverted duplications add additional complexity to the locus. While the copy number of *BTN/BTNL* genes at this locus correlates significantly (Fig. 3C) with lifespan in rockfish, we identified non-lifespan associated *BTN/BTNL* copies as well, largely localized to two clusters on chromosomes 1 and 22 (Fig. 3F). Among our 6 *Sebastes* reference genome assemblies the total number of *BTN/BNTL* genes ranges from 18–36 highlighting expansion of this gene family across taxa. Teleost fishes exhibit diverse immune defense strategies and gene expansions of the major histocompatibility complex (MHC) I locus have occurred numerous times (25). Here a distinct class of immuno-regulatory genes in rockfish may have facilitated adaptations to extreme lifespan.

Life history transitions are tightly coupled to patterns of genetic diversity

To assess genetic diversity across the *Sebastes* clade we performed variant calling on samples mapped to each of their respective genome assemblies. We identified a more than 13-fold range in heterozygosity among *Sebastes* species ($\pi = 2.9\times10^{-4}$–3.8×10^{-3} per bp, Fig. 4A) and a 20-fold range when the outgroup taxa were also considered. These levels of diversity correspond to a range in effective population size (N_e) of 7.2×10^4–9.6×10^5 in the *Sebastes* clade assuming a mutation rate of 1e-9.

We applied multiple sequentially Markovian coalescent (26) (MSMC) analysis to dissect the demographic histories underlying these differences in diversity. We grouped MSMC trajectories by lifespan quartile and compared the mean N_e over the last 10^6 generations (Fig. 4B). While we observed a wide range of different demographic trajectories, short-lived species exhibited increases in N_e over the past ~10^6 generations. Note that the short-lived African killifish exhibits reduced lifespan linked to severe population bottlenecks (27). However, rockfish lifespan was significantly negatively correlated with N_e as a function of maximum lifespan (PGLS P=0.005, Fig. 4C).

Body mass is strongly positively correlated with both census population sizes and lifespans across terrestrial vertebrates (28, 29). In rockfish too, size is correlated with lifespan (Fig. 2E) and indeed size is a better predictor of N_e than lifespan (Fig. 4D, PGLS P=6.6e-5). These results suggest that life-history transitions to shortened life and smaller body size are potentially adaptive, in contrast to the drift-driven transitions observed in killifish. These results also underscore the inherent genetic vulnerability of larger long-lived marine fish species which are often the targets of commercial fisheries (30).

Shifts in lifespan reshape the mutational spectrum of segregating genetic variation

Long-lived, larger, terrestrial vertebrates with smaller N_e often exhibit low rates of fecundity, increased maturation times, and higher survival of offspring (31). Long-lived rockfish species also exhibit extremely long maturation times ranging to over a decade (3). We modelled survival, maturation, and fecundity in 34 different rockfish species for which detailed information could be ascertained to understand the evolutionary tradeoffs driving rockfish life histories (Fig. 5A–D, Tables S15–17). Similar to other marine fishes, rockfish exhibit a “type-III” survivorship curve with high mortality at young ages and very few individuals surviving to old age (32) (Fig. 5A). However, fecundity increases rapidly as a function of age, proportional to size (Fig. 5B) and thus aging imparts minimal declines in their reproductive values (Fig. 5C). In extreme cases such as in yelloweye rockfish (*Sebastes ruberrimus)*, 150 year-old individuals can produce more than 1 million offspring per season. Even compared to other marine fishes, which exhibit non-linear scaling in reproductive output with size (33), older, larger rockfish have significantly higher reproductive output per unit weight (Fig. S20, Wilcoxon P=6.3e-6).

The disproportionate reproductive output of older fish results in generation times that span from 5–45 years across different species (Fig. 5D, Table S17). We find that these differences in generation time and lifespan are associated with reduced nucleotide substitution rates (resulting in shortened phylogenetic branch lengths) in longer-lived species, as observed in terrestrial mammals (Fig. S21) (34). Generation time can also have an influence on the mutational spectrum with certain classes of mutations more likely to occur in older parents (35, 36). We thus classified species-specific segregating single nucleotide variants (SNVs) by mutation type and tri-nucleotide context (Fig. S22). Correlations between mutation types and lifespan (Fig. 5E) were seen with enrichments of CpG->TpG mutations; longer-lived species exhibited a significantly increased proportion of CpG transitions in all *CG->*TG contexts (Fig. 5F, PGLS $P=1\times10^{-4}$). CpG->TpG transitions are characteristic of spontaneous methylated cytosine deamination, a mutational signature that occurs independent of DNA-replication (37) and is the dominant age-associated mutational profile of human tumors (38).

In humans a reduced proportion of CpG variants in European populations has been hypothesized to have been driven by shortened generation times (39), which, if due to similar processes in fish, may be consistent with our findings. A number of mutational signatures were also at reduced frequency across long-lived Rockfish species, including A->C and C->G transversions (Fig. 5E), however it is unclear what the underlying mutational mechanism of this signature might be. Together, these results indicate that shifts in life histories can in turn reshape patterns of segregating genetic diversity.

Discussion

The vast diversity of life histories in Pacific Ocean rockfishes presents an opportunity to dissect the genetic adaptations that shape life-history transitions in vertebrates. Our results highlight selective signatures in pathways underlying “hallmarks of aging” (4) that are conserved across all eukaryotes (e.g. DNA damage and nutrient sensing pathways) as well as in vertebrate-specific hallmarks such as immunity and inflammation (5). Chronic inflammation (“inflammaging”) in particular has emerged as a key therapeutic target in humans and our results highlight a novel gene family, the butyrophilins, which may play a role in modulating lifespan in rockfish. We also highlight that the genetic adaptations that enable extreme longevity in rockfish do so both directly, by influencing insulin signaling and other key pathways, as well as indirectly, by influencing size and adaptations to depth. Our results further highlight that such life-history transitions themselves reshape patterns of genetic diversity. Long-lived rockfish species exhibit reduced genetic diversity in contrast to short-lived species and the mutational spectrum of segregating genetic variation is also altered by lifespan. Indeed, the disproportionately higher reproductive output in older, larger rockfish, is likely directly linked to their extreme longevity. Our work further highlights the utility of genus-wide genome assembly efforts to answer questions that have thus far been limited to representative species from broad taxonomic groups (e.g. vertebrates). The advent of additional complete genome assemblies from vertebrates will further our understanding of the generality of our findings across evolutionary and physical scales.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data and materials availability:

All sequencing data have been depositing in the European Nucleotide Archive under accession PRJEB42258. Complete methods are described in the supplementary materials (9) and all code required to recapitulate analyses, including BUSCO gene alignments and gene trees, has been posted at https://zenodo.org/record/5535007#.YVOk9y1h3wo (40).

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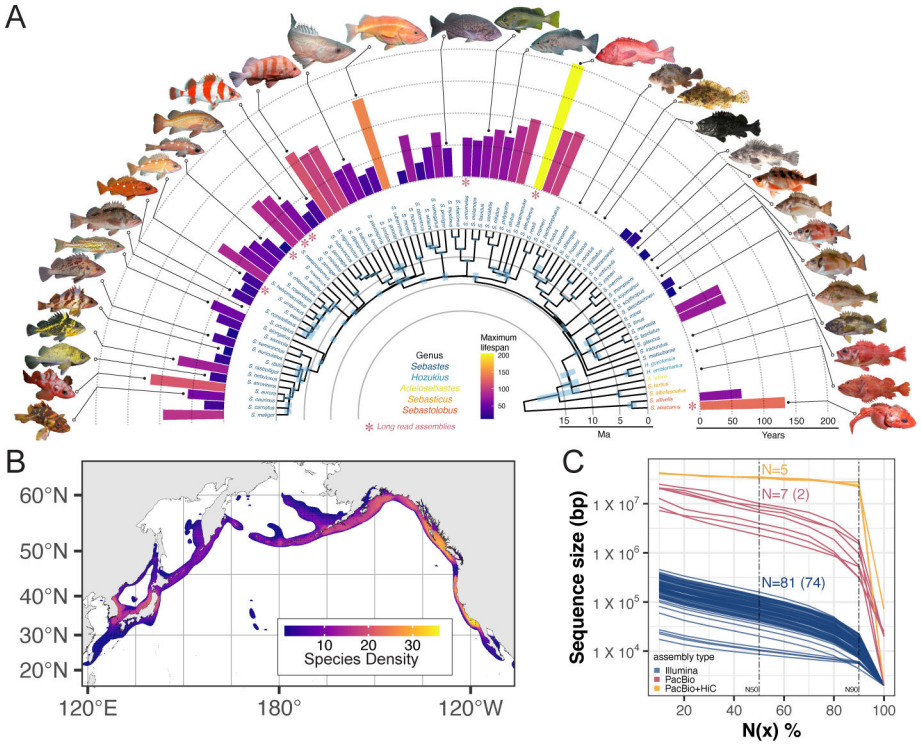

Figure 1 - Genome assemblies and relationships among rockfish species:

A) Ultrametric tree of the rockfish species sequenced in this study and their associated maximum lifespans along with representative images (node timing confidence intervals in light blue) created using IQ-tree, ASTRAL and BPPR. Asterisks indicate individuals for which long-read sequencing-based genomes were assembled. **B**) The density of rockfish species (heatmap colors) throughout the Pacific Ocean. **C**) Genome assembly statistics for 81 species, blue and pink represent N(x)-contig lengths while orange indicate N(x) scaffold lengths.

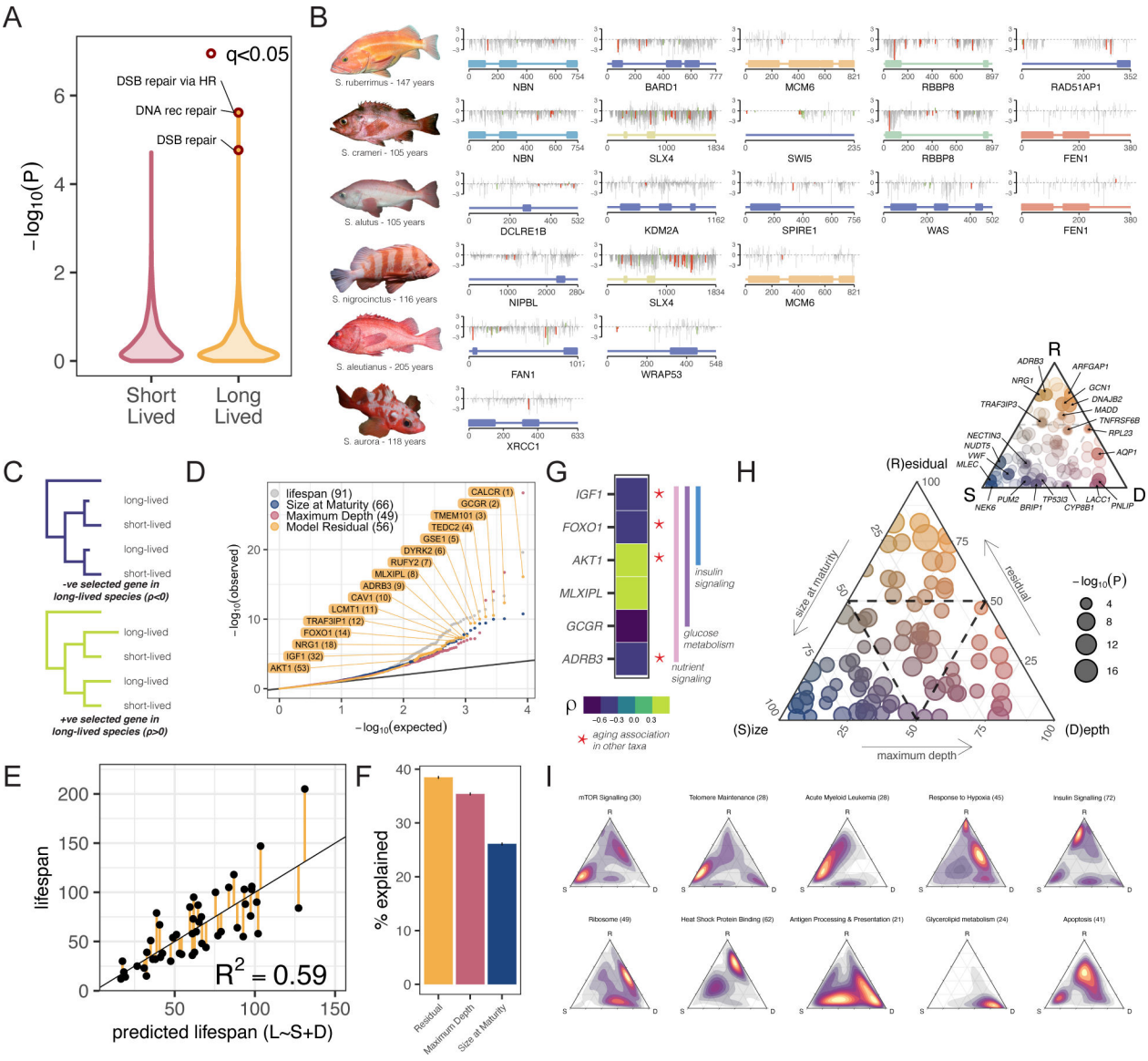

Figure 2 –. Genetic underpinnings of lifespan adaptations:

A) Distribution of pathway enrichment P-values for genes under positive selection in short- and long-lived species. **B)** Schematics of the 16 DNA replication/repair/maintenance genes exhibiting positive selection in long-lived rockfish. Domain structure (bottom), grey lines indicate putative functional consequence of rockfish amino acid compared to human (PROVEAN score). Red and green bars indicate mutations distinct from all non-long-lived taxa colored by SIFT score - Tolerated (green) / Damaging (red). **C)** Schematic representation of relative evolutionary rate test and **D**) P-value distribution of genes from relative evolutionary rate test (RERconverge) of correlation between evolutionary rate and individual traits. Lifespan and the individual components of a predictive linear model of lifespan (E) are used as traits. Gene names are highlighted for the top 12 genes associated with the linear model residual as a trait in addition to four other significant gene candidates previously associated with aging (full gene list in Table S14). **E)** Linear regression model

between maximum lifespan and predicted lifespan based on body size and depth for different species. **F)** Proportion of variation in lifespan explained by size, depth, and the residual from the linear model. **G)** Relative evolutionary rates of six genes involved in glucose, insulin, and nutrient signaling four of which have been associated with aging in other taxa (Table S14). **H)** Ternary plot of 91 lifespan associated genes plotted as a function of the relative importance along linear model axes with dotted line at 50% (inset with gene labels) and heatmaps of pathway enrichment along axes (**I**).

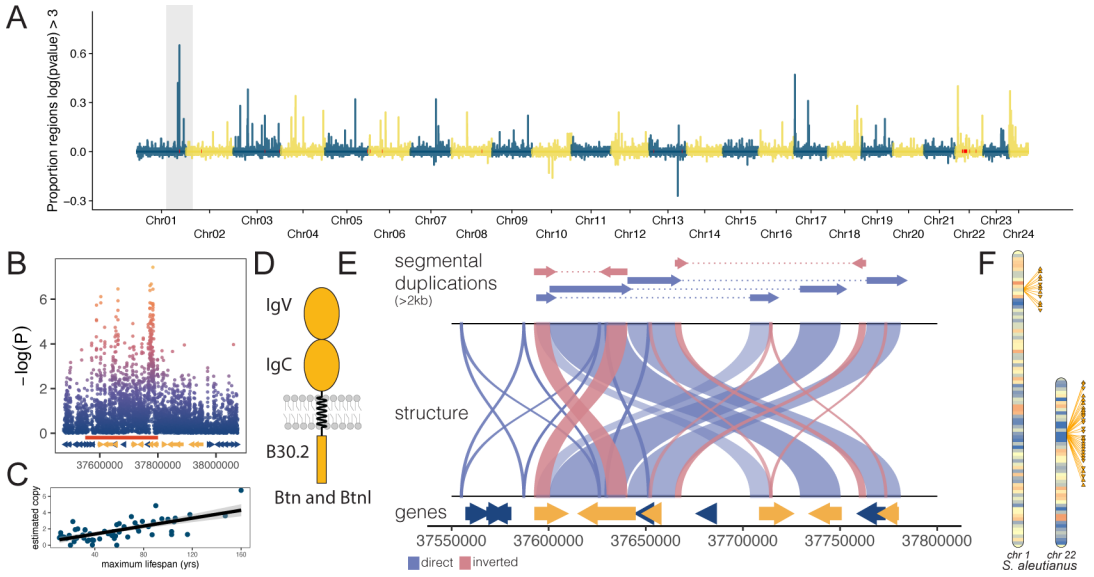

Figure 3 –. Lifespan associated butyrophilin gene duplications:

A) Miami plot of proportion of 100bp regions with significant phylogenetic least squares linear model (PGLS) correlations between read-depth inferred copy number and lifespan (10kb windows) across the *S. aleutianus* reference genome. **B**) Manhattan plot of ~250kb chromosome 1 butyrophilin locus and copy number association in **C**. Butyrophilin genes shown in orange. **D**) Protein structural organization of BTN and BTNL gene family members. **E**) Genome structure of the butyrophilin locus in the *S. aleutianus* genome assembly. Segmental duplications are highlighted above a self-alignment of the locus to show the underlying duplication architecture with genes below. **F**) Karyotypes of chromosomes 1 and 22 in *S. aleutianus* (color indicates gene-density) highlighting the two butyrophilin gene family clusters in *Sebastes* species.

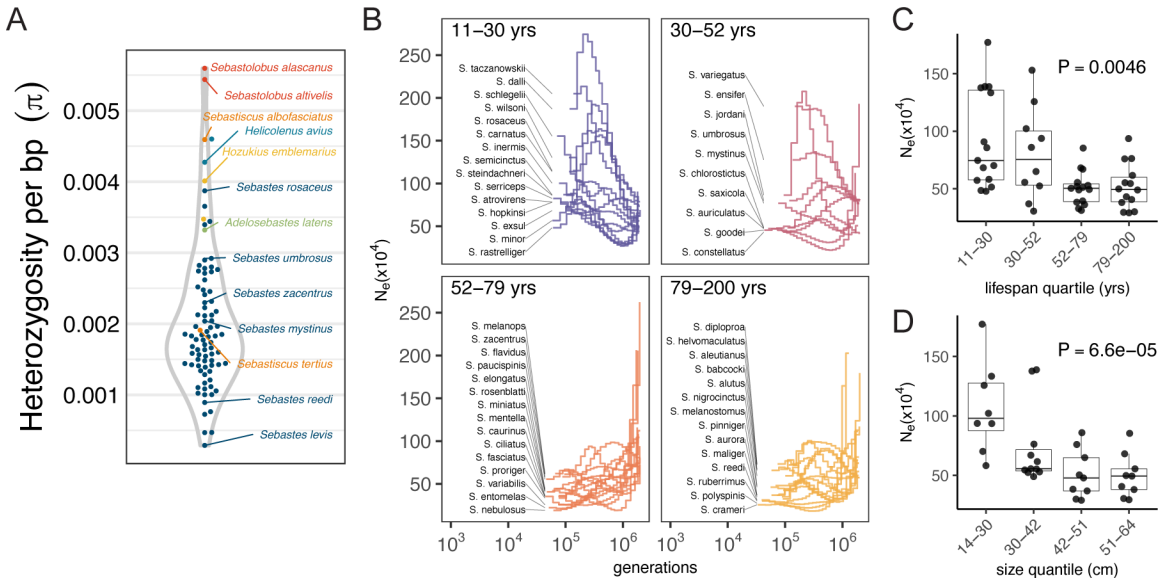

Figure 4 - Life history transitions are associated with patterns of diversity:
A) Nucleotide diversity across 88 different species. **B)** MSMC based estimates of effective population sizes grouped by lifespan quartile over the last ~10^6 generations and averaged N_e grouped by lifespan quartile in **C** or grouped by size quartile in **D**. Box and whisker plots indicate the median and interquartile range (IQR) with tails at the min and maximum values within 1.5*IQR. P-values from PGLS linear model.

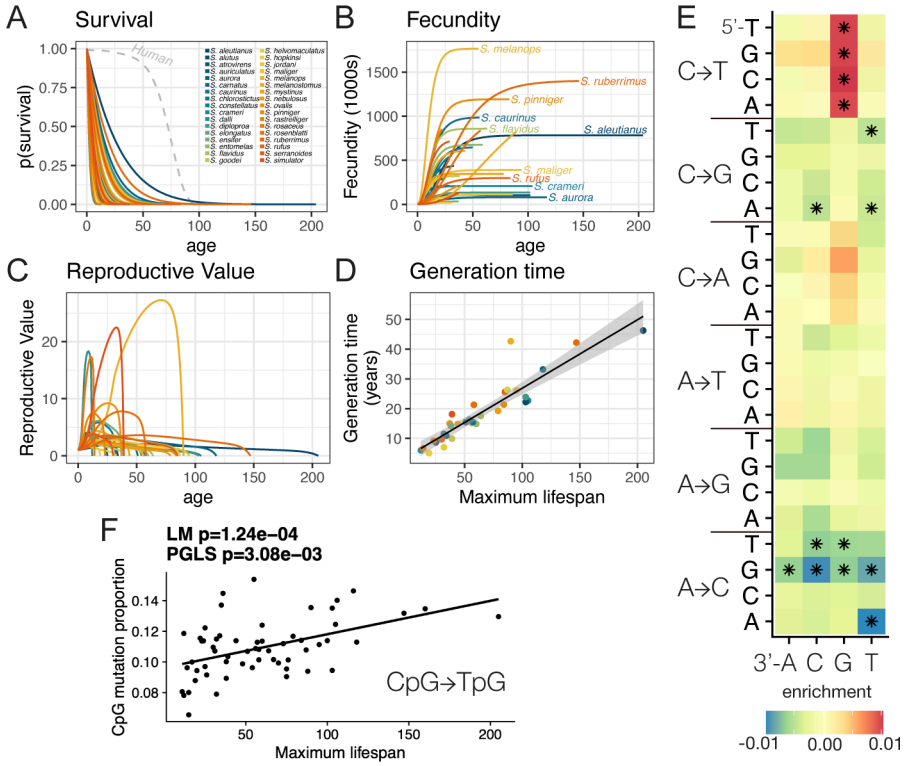

Figure 5 –. Shifts in life history traits reshape the mutational spectrum of segregating genetic variation:

A) Survival curves for 34 rockfish species. Humans, shown for comparison, exhibit similar maximum lifespans to many rockfish species but different survival curves (Type I versus Type III survival). **B**) Rockfish fecundity (births per season) plotted as a function of age. **C**) Reproductive value plotted as a function of age. **D**) Generation times estimated from survival and fecundity plotted as a function of lifespan. **E**) Association of segregating single nucleotide variant mutation types with lifespan across all trinucleotide contexts. Asterisks indicate age-associated mutational profiles significant after multiple testing correction ($q<0.05$). **F**) The proportion of segregating CpG->TpG mutations as a function of maximum lifespan.