Convergent Evolution of Mitochondrial Genes in Deep-Sea Fishes

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Deep seas have extremely harsh conditions including high hydrostatic pressure, total darkness, cold, and little food and oxygen. The adaptations of fishes to deep-sea environment apparently have occurred independently many times. The genetic basis of adaptation for obtaining their energy remains unknown. Mitochondria play a central role in aerobic respiration. Analyses of the available 2,161 complete mitochondrial genomes of 1,042 fishes, including 115 deep-sea species, detect signals of positive selection in mitochondrial genes in nine branches of deep-sea fishes. Aerobic metabolism yields much more energy per unit of source material than anaerobic metabolism. The adaptive evolution of the mtDNA may reflect that aerobic metabolism plays a more important role than anaerobic metabolism in deep-sea fishes, whose energy sources (food) are extremely limited. This strategy maximizes the usage of energy sources. Eleven mitochondrial genes have convergent/parallel amino acid changes between branches of deep-sea fishes. Thus, these amino acid sites may be functionally important in the acquisition of energy, and reflect convergent evolution during their independent invasion of the harsh deep-sea ecological niche.

Keywords: deep-sea adaptation, mtDNA, adaptive evolution, positive selection, convergent evolution

INTRODUCTION

Oceans constitute the largest habitat on Earth. Most marine organisms live in the shallower, illuminated depths, and only a few live in the vast darkness of the deep seas (Randall and Farrell, 1997). Deep-sea creatures live below the photic zone and experience hundreds of atmospheres of hydrostatic pressure and constant extreme cold (Robison, 2004). Photosynthesis occurs only down to about 100–200 m, and sunlight disappears altogether at 1,000 m or less. At this depth, there is no light for photosynthesis and animals depend on very limited food floating down from the photic zone. Oxygen is also a limited resource in the deep sea (Childress and Seibel, 1998). Thus, deep-sea organisms must survive in extremely harsh conditions.

Fishes are the charismatic megafauna of the deep sea. Evolutionary adaptations to deep-sea life apparently have occurred independently in at least 22 orders of fishes (Randall and Farrell, 1997). Genetic adaptations for vision to the dark environment of the deep sea is studied well (Hunt et al., 2001; Davies et al., 2009), yet other adaptations remain largely unknown. Biomass available as energy at depths exceeding 1,000 m drops to less than 5% of that available in surface waters (Marshall, 1980). Aerobic metabolism yields much more energy per unit of source material than anaerobic metabolism. Thus, aerobic metabolism should play a greater role than anaerobic metabolism to maximize the use of limited energy sources. However, paradoxically, the level oxygen is low in the deep sea (Childress and Seibel, 1998).
Furthermore, high hydrostatic pressure affects the functioning of lipid membranes, enzymes, and other macromolecules (Macdonald, 1997; Robison, 2004). Therefore, proteins involved in the aerobic metabolism of deep-sea fishes must function efficiently to obtain energy in the absence of abundant food and oxygen.

Mitochondria play the most prominent role in aerobic metabolism of the cell by producing ATP through the electron transport chain. All 13 of the mitochondrial protein-coding genes are involved in this. Functional constraints on mitochondrial DNA (mtDNA) genes have been suggested to influence the evolution of locomotion (Shen et al., 2009; Shen et al., 2010), climatic adaptation (Sun et al., 2011), high elevation adaptation (low-oxygen and cold climate) (Luo et al., 2008; Gering et al., 2009; Scott et al., 2011; Wang et al., 2011; Gu et al., 2012; Zhou et al., 2014), adaptive evolution in mammals (da Fonseca et al., 2008), and high hydrostatic pressure adaption of deep-sea animals (Siebenaller and Garrett, 2002). Considering the important role played by mtDNA in aerobic respiration, the environment of deep-sea fishes, and the independent occupation of the deep sea by lineages of fishes, herein we test the hypothesis that adaptive evolution of mitochondrial genes plays a role in deep-sea adaptation.

**MATERIALS AND METHODS**

**Source of Data and Primary Treatments**

A total of 2,161 complete mitochondrial genomes of 1,042 fish species were downloaded from NCBI. Considering that human mtDNA is widely studied, we used Cambridge reference sequence for human mtDNA (NC_012920) as the reference to standardize our data. All sequences were aligned by MAFFT with option (FFT-NS-2) (Katoh and Toh, 2010). Information of the depth ranges of these fishes was collected from FishBase (http://www.fishbase.org). Deep-sea fishes often have been considered as those maximum living depth below 1,000 m (Angel, 1997; Pradillon and Gaill, 2007). The depth ranges of 115 species of 1,042 bony fishes fell below 1,000 m (Supplementary Table 1). For some orders of fishes, such as Ateleopodiformes, Lophiiformes, Myctophiformes, and Notacanthiformes, either few data was available, or all of the fishes in the orders were deep-sea dwelling without shallow water-dwelling sister taxa; herein, these fishes were not considered further. Finally, the remaining 77 deep-sea fishes were used in this study. They were classed into nine groups according their phylogenetic positions (Betancur et al., 2013): 1) Anguilliformes; 2) Beryciformes and Stephanocybiformes; 3) Ophidiiformes; 4) Osmeriformes and Stomiiformes; 5) Perciformes; 6) Carangimorpha; 7) Scorpaeniformes; 8) Pleuronectiformes; and 9) Scorpaeniformes.

**Phylogenetic Analysis**

Phylogenetic analyses were conducted from 13 concatenated protein-coding genes (ATP6, ATP8, COXI, COX2, COX3, CytB, ND1, ND2, ND3, ND4, ND4L, ND5, and ND6) for nine groups separately by ML and Bayesian inference (BI) analysis as implemented in MrBayes v3.0 (Ronquist et al., 2010). The best-fit models for nucleotide substitutions were selected by jModelTest v0.1.1 (Posada, 2008; Posada, 2009) (Table 1).

| Groups                          | Best-fit model         | Among-site rate variation |
|--------------------------------|------------------------|---------------------------|
| 1 Anguilliformes               | GTR+I+G                | 0.3317 (0.5836)           |
| 2 Beryciformes and Stephanocybiformes | GTR+I+G            | 0.3941 (0.8536)           |
| 3 Ophidiiformes                | GTR+I+G                | 0.2293 (0.5730)           |
| 4 Osmeriformes and Stomiiformes| GTR+I+G                | 0.3826 (0.7774)           |
| 5 Perciformes (Thunnus maccoyi) | TVM+I+G                | 0.7784 (0.7134)           |
| 6 Perciformes (Anarhichas denticulatus) | GTR+I+G        | 0.4948 (1.0584)           |
| 7 Perciformes (Tetrapturus angustirostris) | TVM+I+G            | 0.3400 (0.9908)           |
| 8 Carangimorpha                | TVM+I+G                | 0.5949 (1.6423)           |
| 9 Scorpaeniformes              | GTR+I+G                | 0.4500 (1.0906)           |

1, proportion of invariant sites; and α, gamma distribution shape parameter.

**Selection Analyses**

Alignments and consensus trees were used for subsequent molecular evolutionary analyses. Positive selection analyses were restricted to those branches leading to the deep-sea fishes. We used a gene-level approach based on the ratio (ω) of nonsynonymous (Ku) to synonymous (Ks) substitutions rates (ω = Ku/Ks) to identify potential positive signals of selection. This analysis employed likelihood ratio tests in the CODEML algorithm of the PAML package (Yang, 1997). Topologies based on single mitochondrial protein-coding genes did not stabilize, while phylogenetic trees that based on combined 13 mitochondrial protein-coding genes were robust. Therefore, we used the combined trees (13 mitochondrial protein-coding genes) for nine groups as guide trees for PAML analyses. The following tests were conducted for 13 genes for nine groups respectively: 1) one-ratio model, which assumes an identical ω value for all branches, was used to detect the overall ω of a gene; and 2) branch-site model was used to determine if these genes had undergone positive selection on a foreground branch. In order to detect whether the positive selection signals were significant, LRT statistics were calculated between branch-site model vs. branch-site model with fixed ω Burlington = 1 (null model).

**Analyses of Convergent and Parallel Evolution**

The sequence reconstruction of the ancestor was carried out using the CODEML program implemented in PAML (Yang, 1997). Convergent and parallel amino acid substitutions along deep-sea branches were detected. The statistical significance of the convergent/parallel evolution between two branches was tested by using the method of Zhang and Kumar (1997). Intracellular domains, TM domains, and the extracellular domains of 11 mitochondrial genes were delineated by TMHMM (Sonnhammer et al., 1998; Krogh et al., 2001). Convergent/parallel amino acid sites were mapped to the three-dimensional (3D) protein structures of ND (PDB:5LIX) (Zhu et al., 2016), CytB (PDB:1PP6) (Huang et al., 2005), and COX (PDB:1V54) (Tsukihara et al., 2003) by using VMD v1.9.3 (Humphrey et al., 1996).
RESULTS

Positive Selection on Nine of 13 Mitochondrial Genes in Deep-Sea Fishes

In this study, 77 of 115 deep-sea fishes, which divided into nine taxonomic groups, were selected for further analysis (Figure 1). The topologies of these nine groups were further constructed and used as the guide tree for positive selection analyses. Analyses using PAML (Yang, 1997) found significant signals of positive selection in the following nine deep-sea branches (Figure 2).

Group 1, the true eels (Anguilliformes). Four independent branches of the phylogeny were suggested to have experienced deep-sea adaptation (Supplementary Figure 1). Significant signals of positive selection occurred in ND1 and ND2 in a cluster of deep-sea eels (Supplementary Table 2).

Group 2, Beryciformes and Stephanoberyciformes. At least three independent deep-sea branches were identified (Supplementary Figure 2). ATP6 on branch g (Anoplogaster cormuta) and ATP8, COX2, COX3, ND5, and ND6 on branch f (Diretmoides veriginae and Diretmus argenteus) showed significant signals of positive selection (Supplementary Table 3).

FIGURE 1 | Maximum-likelihood (ML) phylogenetic tree of 1,042 bony fishes. The tree displays 47 orders. The nine groups studied in this study were marked in red. Numbers in the brackets denote available mitochondrial genomes.
Group 3 (Ophidiiformes), which included a cluster of deep-sea fishes (Supplementary Figure 3). Selection analyses showed that ATP6 and ND6 had significant signals of positive selection on this branch (Supplementary Table 4).

Group 4 (Osmeriformes and Stomiiformes) had three branches of deep-sea fishes (Supplementary Figure 4). Branch i (Lipolagus ochotensis) had significant positive selection signals in COX3, CytB, and ND6, and branch j had significant signals in ND2 and ND5 genes (Supplementary Table 5).

Group 5 (Perciformes). This group had at least three independent events of deep-sea adaptation (Supplementary Figure 5). However, no deep-sea branch had significant signals of positive selection (Supplementary Table 6).

Group 6 (Carangimorpha). The group had one deep-sea branch (Supplementary Figure 6), but no significant signal of positive selection (Supplementary Table 7).

Group 7 (Scombriformes). As Group 6, it had one deep-sea branch (Supplementary Figure 7), but no significant signal of positive selection (Supplementary Table 8).

Group 8 (Pleuronectiformes) had a deep-sea branch p (Hippoglossus and Reinhardtius; Supplementary Figure 8). ND2 showed significant signals of positive selection in this branch (Supplementary Table 9).

Group 9 (Scorpaeniformes). No any signal of positive selection was detected on its deep-sea branch (Supplementary Figure 9, and Supplementary Table 10).

Convergent/Parallel Evolution on 11 of 13 Mitochondrial Genes in Deep-Sea Fishes

Among the 13 mitochondrial protein-genes, 11 genes had convergent/parallel amino acid sites between deep-sea fishes,
including 5 convergent amino acid changes and 29 parallel amino acid changes (Figure 2; Table 2). ND1 had one statistically significant ($P < 0.01$) parallel amino acid change (L251F) and one convergent change (S/T250A, $P < 0.001$). ND2 had four significant sites of parallel evolution (L154I, T310I, T229S, and F21L), and two sites of significant convergent evolution (A/M261I and T/P322I). ND3 had one statistically significant ($P < 0.01$) parallel amino acid change (L251F) and one convergent change (S/T250A, $P < 0.001$). ND2 had four significant sites of parallel evolution (L154I, T310I, T229S, and F21L), and two sites of significant convergent evolution (A/M261I and T/P322I). ND3 had one statistically significant ($P < 0.01$) parallel-evolved site (V94I) on branches f and g. ND4 had four parallel amino acid substitutions on deep-sea branches: A20T and I442V on branches f and m ($P < 0.001$); G111S on branches c and o ($P < 0.01$); and I96L on branches g and i ($P < 0.05$). For ND6, branches c and o shared statistically significant ($P < 0.01$) convergent mutation L/I94M.

Table 2 | Convergent/parallel sites in 11 of 13 mitochondrial protein-coding genes in deep-sea fishes.

| Gene | Amino acid change | Branches | Significance | Convergent evolution |
|------|------------------|----------|--------------|----------------------|
| ND1  | L251F            | g, i     | $P < 0.01$   |                      |
|      | S/T250A          | i, o     | $P < 0.001$  |                      |
| ND2  | L154I            | e, f     | $P < 0.05$   |                      |
|      | A/M261I          | f, m     | $P < 0.01$   |                      |
| ND3  | V94I             | f, g     | $P < 0.05$   |                      |
|      | M92I             | a, b     | $P < 0.01$   |                      |
|      | S/120I           | g, i     | $P < 0.01$   |                      |
| ND4  | V460I            | a, b     | $P < 0.001$  |                      |
|      | S155T            | f, i     | $P < 0.05$   |                      |
|      | I94L             | c, o     | $P < 0.05$   |                      |
|      | L120F            | g, i     | $P < 0.001$  |                      |
|      | V153A            | c, f     | $P < 0.01$   |                      |
|      | T193A            | g, o     | $P < 0.01$   |                      |
|      | S296F            | d, f     | $P < 0.05$   |                      |
| COX1 | V155I            | a, b     | $P < 0.001$  |                      |
|      | M92I             | a, g     | $P < 0.01$   |                      |
|      | I97M             | c, i     | $P < 0.01$   |                      |
|      | V416I            | a, b     | $P < 0.01$   |                      |
|      | S47T             | b, i     | $P < 0.01$   |                      |
|      | M100I            | g, i     | $P < 0.01$   |                      |
|      | V460I            | a, b     | $P < 0.001$  |                      |
|      | S47T             | b, i     | $P < 0.01$   |                      |
|      | I97V             | a, c     | $P < 0.01$   |                      |
|      | S155T            | f, i     | $P < 0.01$   |                      |
|      | T193A            | f, j     | $P < 0.05$   |                      |
|      | S296F            | d, f     | $P < 0.05$   |                      |
| Cyb  | I94L             | g, i     | $P < 0.01$   |                      |
|      | L120F            | g, i     | $P < 0.01$   |                      |
|      | V153A            | c, f     | $P < 0.05$   |                      |
|      | T193A            | g, o     | $P < 0.01$   |                      |
|      | S296F            | d, f     | $P < 0.05$   |                      |

*P < 0.05; **P < 0.01; ***P < 0.001.
CytB had five parallel amino acid changes: I94L on branches g, I, and o; S296F on branches d, f, and g; L120F on branches g and i (P < 0.001); V153A on branches c and f (P < 0.05); and T193A on branches g and o (P < 0.001).

Convergent/parallel amino acid sites in deep-sea fishes were mapped to the available 3D structure of CytB (Figure 3), ND (Supplementary Figure 10), and COX (Supplementary Figure 11) to facilitate interpretations of their positions. Eighteen convergent/parallel amino acid changes located in the transmembrane (TM) domain and the remaining 16 changes were located in other domains. However, all five parallel amino acid changes in CytB protein occurred in TM subunits; two of them (T193A and S296F) changed from neutral polarity to nonpolar amino acids, while the other three did not change their polarity.

**DISCUSSION**

Due to the limited sources of food, deep-sea fishes appear to have maximized their usage of energy sources. Aerobic metabolism yields much more energy per unit of source material than anaerobic metabolism. Our study reveals signals of adaptive selection (positive selection and convergent/parallel evolution) in mitochondrial genes of deep-sea fishes (Figure 2). This result corresponds with our hypothesis that aerobic metabolism plays an important role in deep-sea fishes to maximize the usage of limited energy sources.

Positive selection drives the accumulation of advantageous mutations, and thus is associated with the adaptation of new environment and the evolution of new function (Nielsen, 2005; Xiang et al., 2018). During the deep-sea invasions, nine genes (ND1, ND2, ND5, ND6, COX2, COX3, ATP6, ATP8, and CytB) appear to have experienced positive selection. A previous study suggested that ND4, CytB, and ATP8 played a role in the origin of flight in bats to fit for the huge change in energy demand (Shen et al., 2010). Further, ATP6, ND2, and ND4 genes were suggested to associate with high-elevation adaptation in galliform birds (Zhou et al., 2014). All of the 13 mitochondrial genes play important roles in aerobic metabolism, and this may explain why different studies discover different adaptive genes.

Deep-sea fishes have independently invaded deep-sea habitats several times. Due to the similar environmental pressures, convergent/parallel evolution in key genes likely occurs, when similar morphological or physiological changes occur in multiple lineages (Zhang and Kumar, 1997; Shen et al., 2012). To identify genes and amino acid sites that are important for the multiple deep-sea adaptations, our analyses of convergent/parallel evolution detect 5 convergent amino acid changes and 29 parallel amino acid changes in 11 mitochondrial genes (Figure 2, Table 2). Some parallel amino acid changes occur on several branches. For example, I97V in COX2 occurs on four deep-sea branches, and V416I and M921f of COX1 and I94L and S296F of CytB occur on three. Some deep-sea branches have many parallel amino acid changes in one gene. For example, in COX1, branch a has four parallel amino acid changes and branch b has three. Previous assays revealed that convergent/parallel amino acid changes were responsible for convergent/parallel functional changes (Yokoyama and Radlwimmer, 1998; Zhang, 2006). The multiple occurrences of convergent/parallel evolution in mitochondrial proteins in deep-sea fishes suggest that mitochondria play important roles in adaptation of fishes to deep sea. This may also reflect that only a few amino acid sites are critical for mitochondrial adaptation to deep seas.

For the complex of electron transport chains, TM and other domains have approximately the same numbers of convergent/parallel amino acid changes (18 vs. 16, respectively). For the former changes, two of the five parallel amino acid changes (T193A and S296F) in CytB occur in TM subunits of the protein and they change polarity of the amino acids from polar to nonpolar (Figure 3). CytB catalyzes reversible electron transfer from ubiquinol to cytochrome c coupled to proton translocation (Trumpower, 1990). Nonpolar amino acids are mainly hydrophobic. Thus, the parallel hydrophobic changes in the TM subunits likely make the protein more stable. For deep-sea animals, the high hydrostatic pressure orders phospholipid bilayers, causing the fatty acyl chains to pack together more tightly. This property can have extremely detrimental effects on the functioning of the lipid membrane of cells, influencing membrane enzymatic processes (Siebenaller and Garrett, 2002). Proteins of the oxidative phosphorylation (OXPHOS) system are all membrane proteins. The parallel hydrophobic changes in the TM subunits are likely a better fit of the protein to the more viscous membrane fluidity due to high hydrostatic pressure.
Except for ND4L and ND5, the other 11 mitochondrial genes exhibit either positive selection or convergent/parallel signals. Six sites appear to have undergone both positive selection and convergent/parallel evolution (Figure 2). However, further investigation is necessary to determine the roles these genes and sites play in deep-sea adaptation.

Some deep-sea branches do not exhibit signals of positive selection or convergent/parallel evolution. However, adaptive evolution has other mechanisms such as increased densities of mitochondria (Mahalingam et al., 2017), larger mitochondria, changes of gene expression levels, and phenotypic plasticity.

Deep-sea creatures are among the most amazing forms of life. They survive in extremely harsh conditions, such as hundreds of atmospheres of hydrostatic pressure, small amounts of oxygen, very little food, no sunlight, and constant extreme cold. The genetic basis of their adaptation to the deep-sea ecological niche remains a mystery. Our study reveals multiple signals of adaptive evolution (positive selection and convergent/parallel evolution) on mitochondrial genes in deep-sea fishes. Positive selection on mitochondrial genes may help deep-sea fishes to maximize the usage of limited energy sources, and thus drive energetic survival in harsh deep-sea environment. In addition, multiple convergent/parallel changes in mitochondrial genes may reflect that these amino acid sites are functional importance for the mitochondria to acquire energy, and reflect convergent evolution during their independently invaded harsh deep-sea habitats.

**DATA AVAILABILITY STATEMENT**

Publicly available datasets were analyzed in this study. These data can be found here: All the accession numbers were listed in Supplementary Table 1.

**AUTHOR CONTRIBUTIONS**

YS conceived and designed the research. XS, ZP, and XC collected and analyzed the data. YS and XS wrote the manuscript. RM revised the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2019.00925/full#supplementary-material
