

Paper III

Vertebrate Vitellogenin Gene Duplication in Relation to the “3R Hypothesis”: Correlation to the Pelagic Egg and the Oceanic Radiation of Teleosts

Roderick Nigel Finn*, Børge A. Kristoffersen

Department of Biology, University of Bergen, Bergen, Norway

The spiny ray-finned teleost fishes (Acanthomorpha) are the most successful group of vertebrates in terms of species diversity. Their meteoric radiation and speciation in the oceans during the late Cretaceous and Eocene epoch is unprecedented in vertebrate history, occurring in one third of the time for similar diversity to appear in the birds and mammals. The success of marine teleosts is even more remarkable considering their long freshwater ancestry, since it implies solving major physiological challenges when freely broadcasting their eggs in the hyper-osmotic conditions of seawater. Most extant marine teleosts spawn highly hydrated pelagic eggs, due to differential proteolysis of vitellogenin (Vtg)-derived yolk proteins. The maturational degradation of Vtg involves depolymerization of mainly the lipovitellin heavy chain (LvH) of one form of Vtg to generate a large pool of free amino acids (FAA 150–200 mM). This organic osmolyte pool drives hydration of the oocyte while still protected within the maternal ovary. In the present contribution, we have used Bayesian analysis to examine the evolution of vertebrate Vtg genes in relation to the “3R hypothesis” of whole genome duplication (WGD) and the functional end points of LvH degradation during oocyte maturation. We find that teleost Vtgs have experienced a post-R3 lineage-specific gene duplication to form paralogous clusters that correlate to the pelagic and benthic character of the eggs. Neo-functionalization allowed one paralogue to be proteolyzed to FAA driving hydration of the maturing oocytes, which pre-adapts them to the marine environment and causes them to float. The timing of these events matches the appearance of the Acanthomorpha in the fossil record. We discuss the significance of these adaptations in relation to ancestral physiological features, and propose that the neo-functionalization of duplicated Vtg genes was a key event in the evolution and success of the teleosts in the oceanic environment.

Citation: Finn RN, Kristoffersen BA (2007) Vertebrate Vitellogenin Gene Duplication in Relation to the “3R Hypothesis”: Correlation to the Pelagic Egg and the Oceanic Radiation of Teleosts. PLoS ONE 2(1): e169. doi:10.1371/journal.pone.0000169

INTRODUCTION

Ancestral Gene Landscapes

Gene duplication has long been recognized as a powerful mechanism in evolution [1–3]. As noted by Haldane [1], and Ohno [2] the products of such events may acquire new functions (neo-functionalization), survive due to dosage effects (sub-functionalization), become dysfunctional due to mutational insertions, deletions and/or substitutions (pseudogenes), or be lost, without obligate deleterious effects to the host. Whole genome duplications (WGD) have been argued to be the bases of major events in the evolution of vertebrates [2,4–15]. However, the massive gene loss that may occur in the aftermath of WGD, can mask the ancestral ploidy of an organism [2,16–18]. Similarly the propensity for certain lineages to acquire transposable elements; to independently duplicate genes through cis- or trans- events; to retain or lose extra chromosomes (aneuploidy) or a complete set of chromosomes (tetraploidy, haploidy, diploidization) during meiosis or early germline mitosis [17] with subsequent deletion or translocation of genes or chromosomes, further complicates the deciphering of ancestral gene landscapes [4,16,19,20]. Indeed, while teleosts are considered to have doubled their genes in relation to humans (see below), chromosomal loss, or diploidization [16,17] seems to have been rampant in this group, since similar numbers exist between teleosts (~20–25) and rodents and primates (19–22 autosomes). As a result, the number of WGD, and whether such events have occurred at all, has remained controversial [9,12,15,18,21–25].

Recent comparative genomic analyses of paralogous chromosomal regions (paralogons) between humans and other vertebrate and invertebrate model organisms have provided convincing evidence that two rounds of WGD occurred during early chordate

evolution [8,12,20,26]. Similar analyses between humans and the gene maps of actinopterygian fishes revealed a double conserved synteny between the chromosomes of the teleosts compared to those of a human [11,27–30], and thus demonstrated that a fish-specific WGD first postulated by Ohno [2] and later proposed by Amores *et al.* [5] had indeed occurred. Most recently, Crow *et al.* [31] provided strong evidence that the fish-specific WGD took place after the separation of the crown group of teleosts from bowfin, a neopterygian fish. Hence three rounds (R1, R2, R3) of WGD have occurred during the evolution of teleost fishes from early chordate roots, and traces of these ancient events should be detectable in the many thousands of extant species [13,32–34]. Any phylogenetic reconstruction of genes should thus reconcile the chronology of these events with the proposed tree model and the fossil record.

Academic Editor: Jean-Nicolas Volff, University of Würzburg, Germany

Received November 1, 2006; Accepted December 22, 2006; Published January 24, 2007

Copyright: © 2007 Finn, Kristoffersen. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors claim no financial interests in the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* To whom correspondence should be addressed. E-mail: nigel.finn@bio.uib.no

A Brief History of Teleosts

The origin of the Actinopterygii dates back to paleonisciform fishes during the late Silurian (410 mya) some 40 million years after separating from the Sarcopterygii [35–39]. One of the most primitive actinopterygian fishes was *Cheirolepis*, a freshwater fish that did not enter seawater [37,40]. While some descendants, such as *Mimia* and *Bigeria* did apparently enter seawater, their lines became extinct long ago and thus did not contribute to the teleost lineage. Only those groups that remained in freshwater exist today. These are the Chondrostei including the Polypteriformes (bichirs) and Acipenseriformes (sturgeons and paddlefishes), and the neopterygian Semionotiformes (gars) and Amiiformes (bowfin). Despite anadromic behaviour in sturgeons, all of these fishes are obligate freshwater spawners, as indeed are the more ancestral Hyperoartia (lampreys). The monophyletic primitive teleosts [41,42] did not appear in the fossil record until 235 mya [37], but are suggested to have arisen 250–290 mya [32,39,43–46]. Despite some teleost fossils being recorded from marine deposits, it is not known whether this is the result of anadromous behaviour, or represents true marine species spawning in the ocean. The oldest modern teleosts, however, are the Osteoglossomorpha (bony tongues), all of which, both extinct and extant, were and are freshwater species [44,47]. The first extant modern marine teleosts appeared as Elopomorpha (e.g. eels and tarpons) during the Jurassic while subsequent Clupeomorpha (e.g. herrings and shads) had both freshwater and anadromous forms. The most successful group of freshwater teleosts, the Ostariophysi (carps and catfishes), were the next to appear (~245 mya, [39]), and today represent more than 75% of all freshwater fishes of the world with ~8000 species (30% of all teleosts) [47–49]. However, they only achieved their great diversity in freshwater long after the appearance of the Acanthomorpha [37]. It was not until the appearance of Acanthomorpha ~55–125 mya that the unprecedented radiation and speciation of the teleosts occurred. Extant Acanthomorpha comprise ~16,000 species, equivalent to 86% of non-ostariophysan teleosts [47], (and see supplementary material Fig. S1). According to Maissey [37]

“Charts showing the range of fishes through geological time suddenly sprout hundreds of new families. Many of the fossils come from the marine strata of Monte Bolca in Northern Italy. These and other fossils from around the world leave no doubt that modern family-level acanthomorph diversity is rooted in the early Eocene (about 55 million years ago), and that this teleost explosion was the most dramatic evolutionary radiation ever seen in vertebrate history, eclipsing the evolution of mammals and birds in numbers of families and species.”

The basis of this teleost explosion has never been satisfactorily explained. Although the long freshwater ancestry of the teleosts is documented in the fossil record, it can also be inferred from their physiology. All teleosts are hypo-osmotic to seawater, and hyper-osmotic to freshwater. The hypo-osmotic condition of marine teleosts as a group is unique among animal taxa, and is generally assumed to reflect their freshwater past [50,51]. A related argument for a freshwater origin concerns the presence of a glomerular kidney and a distal tubule segment, the main functions of which are to produce copious volumes of hypo-osmotic urine to counteract the osmotic influx of water in the freshwater teleost [52,53]. Glomerular kidneys are also present in the more ancient Chondrichthyes, with glomerular filtration rates (GFR: 1–4 mL • kg⁻¹ • h⁻¹) equivalent to the GFRs of freshwater teleosts (2–10 mL • kg⁻¹ • h⁻¹) [53–55]. Indeed the presence of glomerular kidneys led Homer Smith [52] to suggest that the Chondrichthyes,

and even the extinct placoderms, had a freshwater origin. He [52] argued that the glomerulus, the sole function of which is to filter extracellular fluids, could only have arisen in the presence of excess water – i.e. freshwater. In contrast, the marine teleosts have considerably reduced glomeruli, and fractional GFRs (0.1–0.5 mL • kg⁻¹ • h⁻¹) compared to their freshwater relatives and the Chondrichthyes. Some marine teleosts are even aglomerular (a unique condition among vertebrata), relying entirely on secretion rather than filtration for ion regulation [51–53]. Reduction or loss of glomeruli may be considered an adaptation facilitating the conservation of water in the functionally arid marine environment.

Spawning in the Sea – the Water Problem

The long freshwater ancestry of teleosts implies that also their eggs, which are freely broadcast into the environment by their oviparous parents, had become adapted to the freshwater conditions in the rivers and lakes. When, however, the teleosts eventually started to spawn in the sea, the eggs met new, opposite osmotic problems. The yolk osmolarity is similar to the parental body fluids, ~350 mOsm [56,57], and thus hypo-osmotic to seawater. Hence, instead of an osmotic influx, the problem in seawater is a continuous osmotic water efflux. Thus, since the osmoregulatory systems (mitochondrial rich cells, intestine, kidneys, and gills) are not yet developed, the egg must be endowed with a water reservoir before it can be spawned in the hyper-osmotic seawater. A water reservoir in the egg is a prerequisite and a necessary pre-adaptation before the teleosts could complete their life cycles in the oceans and truly establish themselves as marine organisms. Without this egg feature, the adult fish had to remain anadromous and return to their freshwater habitats to spawn their eggs. Indeed anadromy is typical of the more primitive fishes including lampreys, sturgeons, shads and salmonids.

Already Fulton [58,59] and Milroy [60] noted the remarkable volume increase during oocyte maturation in the eggs of marine teleosts, and intuitively proposed that an osmotic mechanism caused a watery fluid to be secreted into the oocytes. This unique oocyte hydration in marine teleosts has been confirmed for a wide range of species with the degree of hydration being much greater in species that spawn pelagic eggs (pelagophils) compared to those that spawn benthic eggs (benthophils). In benthophils the mechanism mostly involves the differential movement of inorganic ions, and a high concentration of the amino acid analogue taurine, while in pelagophils, free amino acids (FAA) due to the maturational proteolysis of vitellogenin (Vtg)-derived yolk proteins, are the main osmolytes driving the hydration [56,57,61–74]. This growing body of literature shows that up to three forms of Vtg are expressed and differentially processed in pelagophil and benthophil teleosts.

Vitellogenins and the Organic Osmolyte Pool of Pelagic Eggs

In teleosts Vtg genes are linearly organized as large monomeric structures with multiple sub-domains consisting of a lipovitellin heavy chain (LvH), phosphovitin (Pv), lipovitellin light chain (LvL), and a von Willebrand factor type D domain (vWFD) that is split into a beta-component (β') and a C-terminal coding region (CT). Once assembled and secreted by the liver, Vtgs are taken up via clathrin-mediated endocytosis by the growing oocytes and cleaved by cathepsin D (CatD) in the early endosomes to form the primary yolk proteins [75–78]. The CatD processing represents the primary cleavage event in the degradation of yolk proteins. Recent studies have shown that developmentally regulated V-class ATPases (proton pumps) acidify the yolk platelets during oocyte maturation

[66,79,80]. This disassembles the crystalline, or in some species non-crystalline yolk and activates other cathepsins (CatL or CatB, dependent upon species) that hydrolytically attack the yolk proteins [75,76] [66,77,80,81]. This latter processing is known as the secondary cleavage event and is unique to marine teleosts. In some freshwater species, such as zebrafish, electrophoretic band-shifts of the LvH occurs during oocyte maturation due to nicking [82], but no proteolysis, and no buildup of FAA occurs (Finn, unpublished data), which would be osmotically disadvantageous to the freshwater embryo. During oocyte maturation in marine benthophils the yolk proteins are either not processed, or are partially cleaved and hydrolyzed with the release of a small pool of FAA [69,73], while in marine pelagophils, the yolk proteins are not only cleaved but undergo extensive differential proteolysis of particularly one of the LvH domains resulting in the buildup of the large pool of FAA. The transient increase in osmolarity during oocyte maturation [57] causes water influx via specialized aquaporins that are temporally inserted in the plasma membrane during this period [83,84]. This hydration provides the early embryo of marine teleosts with a vital water reservoir before a drinking mechanism is developed [57].

Fulton [59] also noted that the greater hydration of the pelagic eggs compared to the benthic eggs caused them to float and hence acquire their pelagic nature. More recently, in a comprehensive review of the early life history stages of fishes and their characters, Kendall *et al.* [85] revealed that most extant marine fishes, regardless of systematic affinities, demersal or pelagic habits, coastal or oceanic distribution, tropical or boreal ranges, spawn pelagic eggs (see also supplementary material, Fig. S1). These findings suggest that the rise of the pelagic egg was an important event in the evolution of the teleosts.

Vitellogenin Genealogy

Since no evidence of the ancestral spawning habits of teleost fishes has been recorded in the fossil record, we can only infer it. The best means of achieving this is through Bayesian phylogenetic inference [86–88] for genes involved in the reproductive physiology of the parent and the survival of the embryo. The hill-climbing, and proportional hill-hopping Markov Chain Monte Carlo algorithm of Bayesian methods is superior to traditional methods of phylogenetic inference [87]. Since Vtg-derived yolk proteins are the major components of the egg that sustain the embryo during early development, and are implicated in the pre-adaptation to the marine environment, we analyzed all available vertebrate Vtg genes (sequenced and genomic) and the related homologue apolipoprotein B (apoB) in relation to WGD. Previous studies have shown that apoB, which is related to Vtgs [89–91], is also incorporated in the yolk of birds [92], and may represent a neo-functional product of gene duplication. We therefore included the first ~1000 amino acids of apoB proteins, which represent the large lipid transfer module (LLTM) that is homologous the LvH of Vtgs. Other studies have claimed or continue to cite that the phosvitinless class of Vtg, which lacks the polyserine domain, is more closely related to insect Vtgs [93–95]. We therefore included cnidarian and molluscan Vtgs, which predate the arthropods, as outgroups in order to reconcile the topology of the tree with the fossil record, currently accepted phylogenies, and the “3R hypothesis”.

The notion that R3, which is estimated to have occurred prior to the appearance of the crown group of teleosts >290 mya [11,15,32,39,43–45], is the primary cause of the diversity of teleosts seems improbable due to the ~200 million year gap between the WGD and the rapid diversification. Based upon the present analyses, we propose that the radiation and speciation of

the acanthomorph teleosts is rooted in the adaptation of their eggs to the marine environment. We argue that the origin of the LLTM, which predates the bilateria [96], is the molecular harbinger of key adaptations that facilitated the dramatic radiation, while retention of 3R gene products latently contributed to speciation of the Acanthomorpha during the late Cretaceous and Eocene epochs.

MATERIALS AND METHODS

Sequences

Based on our recent sequencing of full length Vtg genes from haddock [67] and Atlantic halibut (Finn, unpublished data), related members of this family of low density lipoproteins were identified using the NCBI BLAST interfaces (www.ncbi.nlm.nih.gov/BLAST). Homologies were confirmed using the blast 2 sequence tool [97]. Using this approach we identified 38 full length vertebrate Vtg homologues, and 56 partial sequences of which only 10 salmonid sequences were included due to their sufficient length and position at the N-terminal domain. We further accessed all available Vtg constructs for vertebrates from the Ensembl genome database (www.ensembl.org). This resulted in the inclusion of 8 more constructs from chicken, zebrafish, 3-spined stickleback, medaka and torafugu. In addition we included the first ~1000 amino acids of apoB proteins from 5 model organisms. In total ~83,000 aa were aligned in 73 sequences.

Since there is some debate in the literature as to the antiquity of the phosvitinless Vtg genes [93,95,98], two invertebrates (galaxy coral and Pacific oyster) were used as outgroups. All sequences studied are summarized in Table S1.

In order to corroborate these analyses and confirm the number of gene forms within teleosts, we retrieved all available Vtg genes from currently sequenced teleost genomes (www.ensembl.org). This resulted in separate analyses of 2 genes from torafugu, 11 genes from 3-spined stickleback, 6 genes from medaka, and more than 16 genes from zebrafish. Due to recent updates of the zebrafish genome, we included both the new fragments from Ensembl release 41 (October 2006), and full genes from release 38 (April, 2006) to match the published data of Wang *et al.* [98].

Multiple Sequence Alignments

Multiple amino acid (aa) alignments of the Vtg homologues were achieved using several programs. Initial alignments were performed on the full length Vtg protein data set with default settings using t-coffee, Clustal W, Muscle and Probcons [99–102]. Extensive modelling was performed using Blossum and PAM matrices and by varying gap-open and gap-extension penalties. This strategy allowed us to make a high quality alignment for the first ~1075 aa, and the last ~500 aa. The remaining polyserine segment was separately aligned using t-coffee and re-inserted into the full alignment. In addition, we further modelled the alignment in Clustal X profile mode using the lamprey structure mask for Vtg [103,104]. This latter approach allowed us to minimize gaps in regions associated with secondary structures. Based on these outputs we manually adjusted the sequences to give a final full length multiple alignment. A second data set included the 10 partial salmonid sequences that were added to the full data set using Clustal X in profile mode. All data sets were then converted to codon alignments using the University of Bergen computational biology unit alignment to coding tool (www.bioinfo.no).

To determine the most likely tree topology, the full aa and codon alignments were analyzed using phylogenetic programs, and then re-examined after removal of the signal peptides, and polyserine domain which showed the least consensus. The

alignments were further examined after removal of regions that contained gaps in more than 70% of the taxa, and again after removal the C-terminal vWFD domain that only occurs in most VtgA type homologues. Two separate sets of the first 270 aa and 810 nucleic acids, which included the partial salmonid sequences, with and without gaps were also analysed. A summary of the domains analysed is shown below the multiple sequence alignment (see supplementary information, Fig. S2).

Phylogenetic Analyses

Bayesian analyses (MrBayes 3.1.2; [105]) was used for each of the aligned aa and codon data sets. The following settings were used for codon alignments: nucmodel = 4by4, nst = 2, rates = gamma; and aa alignments: aamodel = mixed, with 1,000,000 generations, sampled every 100 generations using 4 chains and a burnin of 3,500. For each run, a Majority rule consensus tree together with posterior probabilities from the last 6,500 trees, representing 650,000 generations, was rendered with ATV [106]. The codon alignments were examined for clock-like behaviour using the MrBayes strict-clock model. Speciation or duplication events were inferred using the method of Zmasek and Eddy [107].

To corroborate the Bayesian results [108], maximum likelihood analyses of the codon alignments and maximum parsimony and neighbour-joining analyses of the aa alignments with 1000 bootstrap replicates were conducted using PAUP 4.0b10 [109]. In order to understand the evolution of the sub-domain structure of Vtgs, the ratio of non-synonymous (Ka) to synonymous (Ks) nucleotide substitution rates were estimated using the Ka/Ks web service at the computational biology unit, University of Bergen [110,111].

Vitellogenin Gene Nomenclature

In previous reports cloned teleost cDNAs encoding Vtgs with >1600 aa have been considered complete and classified as either (Vg/Vit), Vtg1, VtgI or VtgA and Vtg2, VtgII or VtgB, while cloned teleost cDNAs encoding Vtgs with <1400 aa and lacking a polyserine domain have been considered incomplete and classified as either Vtg3, VtgIII or (Vg)VtgC [65,67,72,74,93,95, 112–116]. This nomenclature sometimes mixed between the types of Vtg, and sometimes placed the Vtgs of more basal teleosts with higher teleosts without establishing whether the genes were orthologous. Based on the Bayesian phylogenies that were supported by 100% posterior probability at 82% of the nodes and by >95% posterior probability at 95% of the nodes, we reclassified the gene nomenclature according to WGD, lineage-specific gene duplications and the functional properties of the LvH domains (see Fig. 1, Fig. 2 and supplementary information, Table S1).

RESULTS AND DISCUSSION

Evolution of Vertebrate Vitellogenin Genes

As in previous studies [90,91,117] we found good homology between the LLTMs of vertebrate LvHs and apoBs, and for the separate vWFD domains (see supplementary material, Fig. S2 for alignment). Computed Ka/Ks ratios for each of these domains over the whole tree were not significantly different (0.411 ± 0.181 and 0.375 ± 0.177 between the LvH and vWFD domains, respectively), but were significantly lower ($p < 0.05$) than for the LvL domains (0.492 ± 0.163). Excluding the Pv domain, these Ka/Ks ratios indicate that the LvL domain is the least conserved, and that vertebrate Vtgs are not under strong purifying selection [110,118,119], but are evolving. This notion is corroborated by the Bayesian strict-clock model analyses, which showed that the evolution of the Vtg genes is not clock-like.

Although apoB is known to be related to Vtgs [89–91], the N-terminal LLTM has yet to be examined in the context of WGD. To investigate whether apoB could have arisen as a consequence of R1, we accessed the NCBI and Ensembl databases for related vertebrate and invertebrate lipoproteins. 128 of these sequences were used in an alignment consisting of ~100 000 aa for Bayesian analysis. We followed the methodology as described above. These studies revealed that apoB is not a directly duplicated product of Vtg, but is secondarily derived from microsomal triglyceride transfer protein. To illustrate this, we included a kink in the descending branch of the tree that leads to the apoB cluster (Fig. 1).

Consensus Bayesian reconstruction of the evolution of vertebrate Vtg proteins (Fig. 1) was well supported by codon analyses (data not shown), and was congruent with both the fossil record [35–37] and current theory of vertebrate phylogeny [38,39,42–44,46,47,120]. The great majority of nodes were supported by 100% posterior probability providing a solid statistical basis for interpretation of the tree topology. Moreover, switching between the cnidarian and molluscan outgroups did not alter the topology of the tree, and firmly established silver lamprey at the root of the vertebrate Vtgs. This finding reveals that the phosvitinless class of Vtgs, which to date has only been reported in teleosts, is more closely related to the Vtgs of vertebrates rather than insects. We propose that the phosvitinless class of Vtg is a neo-functional product of R2 (Fig. 2, and see later). The other methods of phylogenetic inference, maximum parsimony of the proteins, and maximum likelihood of the codons, also corroborated these findings (data not shown).

To reconcile the multiplicity of extant Vtg genes with the “3R hypothesis” of WGD we used a discontinuous evolutionary model of the Hox gene clusters in accordance with the literature [5–7,31,121–126]. Apparently, an ancient proto-Hox gene diverged to a para-Hox system and the Hox system [121,123,125], and the latter underwent several cis-duplications (intrachromosomal) yielding up to 13–14 genes before undergoing trans-duplications (interchromosomal) during R1, R2 and R3 to give rise to two, four and eight Hox clusters, respectively. Subsequent loss of genes has confused this pattern. For example until very recently zebrafish was thought to have lost the HoxDb cluster [124], but this has now been found in a degenerate form [127]. The HoxDb cluster is present in the medaka and torafugu, but these species have each apparently lost the HoxCb cluster [6]. Thus eight Hox clusters are known for the teleosts, while four are known in tetrapods, and only one Hox cluster is known in the early chordate ancestors (cephalochordates and urochordates). The presence of two Hox clusters for lamprey is congruent with scenario B proposed by Irvine *et al.* [128], and the observation that even though up to four clusters may exist in the Hypochoarta, they are not orthologous to the gnathostomes and hence duplicated independently [129]. We have therefore adopted an (AB)(CD) system for effects of WGD on the Vtg gene in accordance with Hox gene nomenclature. We recognize that the results of autotetraploidy followed by variable rates of diploidization may not generate symmetrical trees [17], and that Hox gene nomenclature was established through the chronology of discovery, rather than by phylogenetic analyses as is presently proposed for the Vtg genes. We further recognize that the ancestral vertebrate containing two Hox-clusters remains a logical hypothesis [6,122].

Since the Vtg genes have the same longevity and metazoan heritage as the Hox clusters, there should be four Vtg genes in tetrapods, and eight Vtg genes present in teleosts. To date, up to four Vtg genes are known in amphibians (African clawed frog), of which two are sequenced [130,131] and included in the tree. Currently three Vtg gene fragments are annotated in the genome

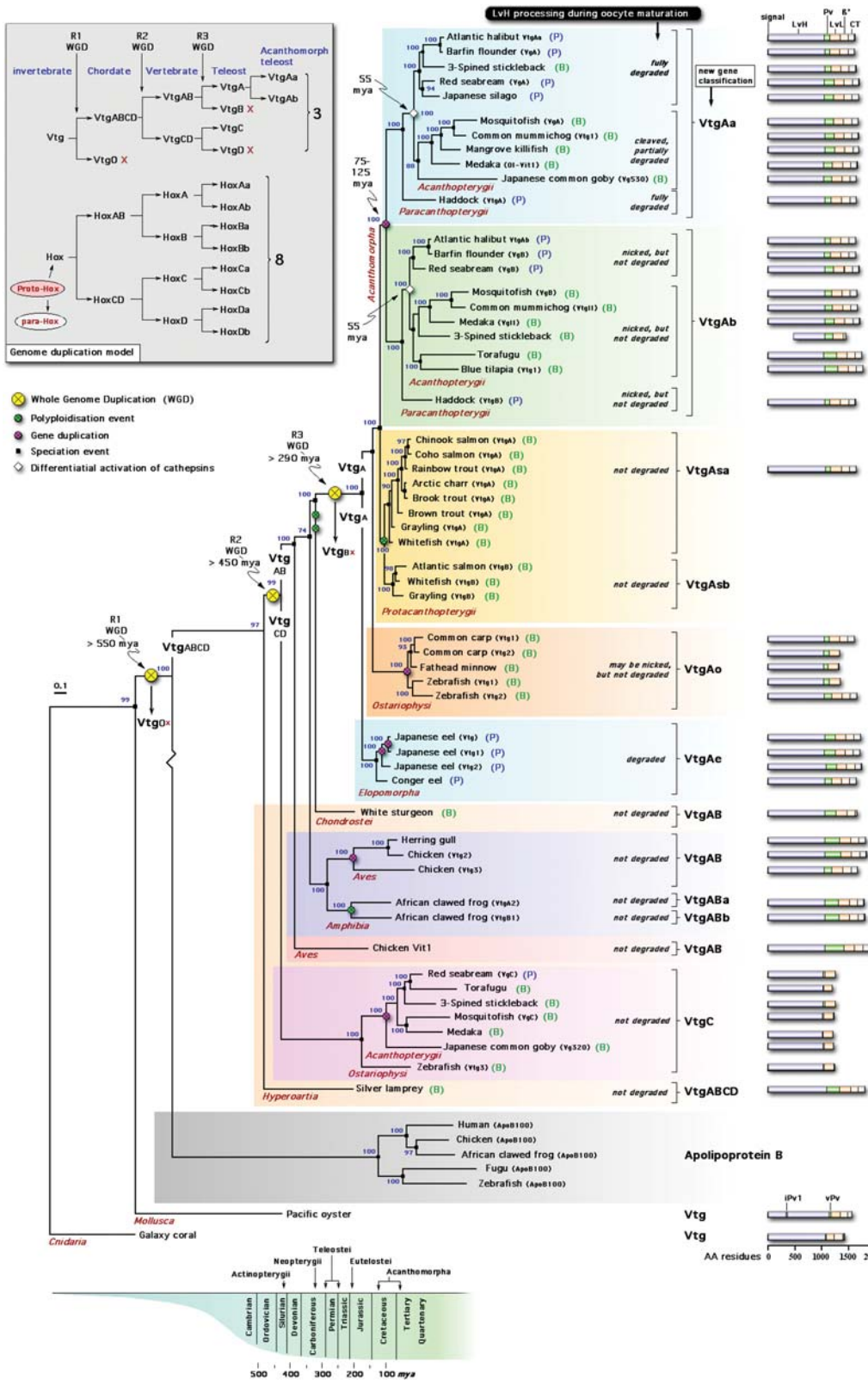


Figure 1. Bayesian majority rule consensus phylogenetic tree for the amino acid alignment of vertebrate large lipid transfer modules rooted with galaxy coral. Numbers beside nodes indicate posterior probabilities (consensus of 6,500 trees from 650,000 generations). See methods for further details of phylogenetic analyses and supplementary material (Table S1) for accession numbers. Schematic linear scale representations of the structure of the vitellogenin genes are drawn for each taxa with complete sequences in the databanks. Sub-domain structures were identified from conserved cleavage sites. The iPv1 of Pacific oyster is proposed to be the origin of the first polyserine domain in the vitellogenins of insects. The geological scale provides an estimate of the divergence of the genes. Since the Vtg genes were not clock-like, this scale is approximate. See text and Fig. 2 for an explanation of the gene duplication model. X = gene lost; (P) = pelagic egg; (B) = benthic egg. doi:10.1371/journal.pone.0000169.g001

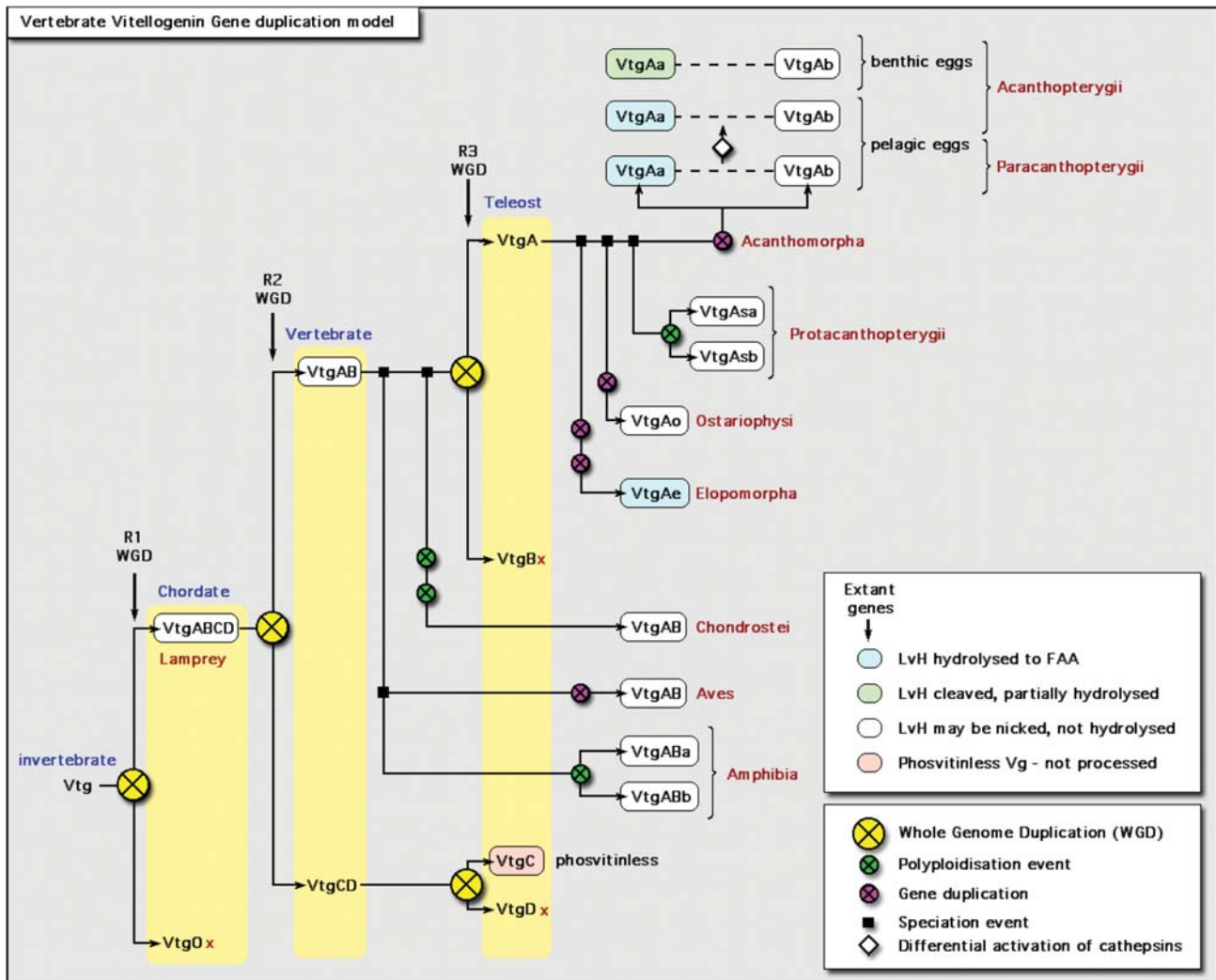


Figure 2. Proposed scheme for the classification of vertebrate vitellogenin genes based on three rounds (R1, R2, R3) of whole genome duplications (WGD), and lineage-specific gene duplications. The genes are classified according to their cluster pattern in Fig. 1 and Fig. 3 and the differential degradation of the lipovitellin heavy chain (LvH) during oocyte maturation. The loss of VtgD as is currently indicated in the scheme remains a logical conclusion since there is currently no evidence for its existence in the sequence or genome databases. Due to the low number of species examined to date, however, we do not preclude its existence. The white diamond within the acanthomorph lineage indicates differential regulation of cathepsins among pelagophils and benthophils.
doi:10.1371/journal.pone.0000169.g002

of Western clawed frog (www.ensembl.org). The multiplicity of the Vtg system in amphibians, however, is the consequence of their tetraploidy [92] rather than the heritage of a WGD. In birds, three Vtg genes are known for the chicken, but all three are linked on chromosome 8 (www.ensembl.org). The major and minor Vtg genes, VtgAB2 and VtgAB3, respectively (see supplementary material Table S1), are known to have cis-duplicated and formed pseudogenes [132], and are co-located ~2 Mbp upstream of VtgAB1. Interestingly, VtgAB1 does not cluster with the other avian Vtg genes, indicating that it has diverged functionally.

In teleosts, only three forms of Vtg have been found, which indicates that possibly up to five have been lost. Using the heuristic maxim of Occam's razor, we believe a more parsimonious explanation would be loss of a single Vtg gene paralogue (Vtg0) after R1 (see Fig. 1). This suggestion is semi-compatible with the arguments of von Shantz *et al.* [20], who, based on paralogon analyses of the circadian clock *Period* genes, concluded that one paralogue (*Per4*) was lost during the early rounds of WGD. Since

there is greater similarity between *Per1* and *Per2* compared to *Per3* [133], the loss of *Per4* may have occurred after R2 rather than R1. This remains open to interpretation, however, since von Shantz *et al.* [20] concluded that the data did not conclusively support one pairing above others.

In the present context, the proposed loss of Vtg0 would explain the single gene in silver lamprey and the cluster of phosvitinless genes as the neo-functional product of R2. In support of this latter argument, it is noteworthy that phosvitinless Vtgs have not only lost the Pv domain, but also the β and CT domains comprising the vWFD (see Vtg bar structures, Fig. 1, Fig. 3). This is true for all currently sequenced VtgC forms, and those present in available teleost genomes. The propensity to lose domains is not restricted to the phosvitinless class of Vtg. Current evidence for genomic Vtg genes (3-spined stickleback, medaka and zebrafish) indicates that several fragments or truncated genes are present (see Fig. 3). Among the Ostariophysi, the major Vtg genes of zebrafish, common carp, and fathead minnow (VtgAo1) have also lost the

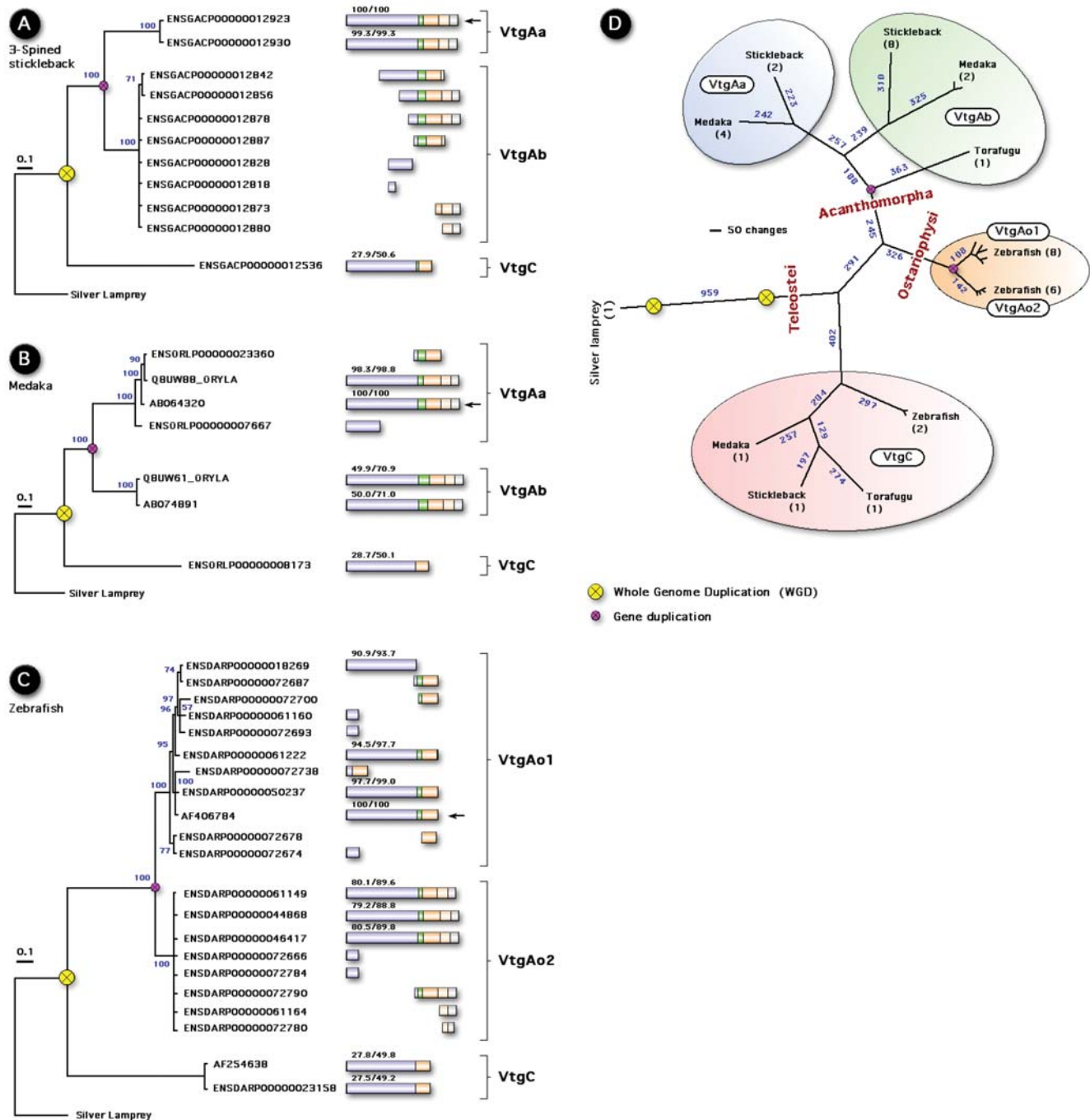


Figure 3. Bayesian majority rule consensus phylogenetic trees for the amino acid alignment of known and novel genomic vitellogenins. Panel A: 3-spined stickleback (*Gasterosteus aculeatus*); Panel B: Medaka (*Oryzias latipes*); Panel C: Zebrafish (*Danio rerio*) includes sequenced genes, and full genes ensembl release 38 and fragments from ensembl release 41; Panel D: Maximum parsimony phylogram of all known teleost genomic vitellogenin genes rooted with silver lamprey. The Vtg bars were constructed from conserved cleavage sites as described in Fig. 1. Homology identity and similarities for the full LvH domains are shown above each bar based on comparison to the reference sequence (arrow). See text and methods for further details of phylogenetic analyses. doi:10.1371/journal.pone.0000169.g003

vWFD domain. In zebrafish up to seven genes, including one pseudogene have been reported [93,98], however, many more Vtg genes appear to exist in the genome (www.ensembl.org). Fourteen of these genes are tightly linked on chromosome 22, while the phosvitinless gene is located on chromosome 11. The last gene is not yet localized. We initially subjected predicted full genes (ensembl release 38, April 2006) to Bayesian analysis to find two

closely related clusters, VtgAo1 and VtgAo2 and a third branch containing the more distantly related phosvitinless gene. With release 41 (October 2006) many of the earlier predicted genes are only present as fragments. We analyzed these fragments and compared them to the previously predicted genes to find that the three clusters VgAo1 (truncated), VgAo2 (complete), and VgC (phosvitinless) was supported by 100% posterior probabilities

(Fig. 3c). Interestingly, the mRNA of the major gene, VtgAo1, is expressed 100–1000× higher than those of either the complete VtgAo2 or the phosphatidylcholine form [98], suggesting that deleterious mutations have occurred in the cis-regulatory elements of the VtgAo2 genes. These analyses illuminate three points. The first is that Vtgs other than the phosphatidylcholine class can lose domains. The second is that Vtg gene forms may be present in the genome, but are not significantly expressed, while the third strengthens the probability that the phosphatidylcholine class is indeed a product of R2 since it is located on a separate chromosome. A caveat to the third point is that the VtgC genes of 3-spined stickleback and medaka are currently located on the same group (stickleback: group VIII) or chromosome (medaka: chromosome 4) ~0.5–0.7 Mb from their VtgAa and VtgAb forms. However, since the chromosomal loci of the zebrafish VtgC genes have been very recently reallocated from chromosome 15 to chromosome 11 in the latest Ensembl release (41), and the genomes of 3-spined stickleback and medaka are newly released, further analyses will be necessary to establish the definitive gene maps. Despite this caveat, maximum parsimony analysis of all genomic Vtg genes supports the topology of the tree in Fig. 1, and the division of the major forms of Vtgs in teleosts (Fig. 3D).

Placing the R3 at the base of the crown group of teleosts as recently proposed by Crow *et al.* [31] illustrates why the timing of this WGD, or whether it in fact occurred, has remained controversial [9,12,15,18,22–25]. The gene products of a WGD should show paralogous gene clustering. However, below the level of the Protacanthopterygii (salmonids), the Vtg genes do not show paralogous clustering. One interpretation for this could be the differential retention of paralogues among the different groups yielding an asymmetric tree in which the Elopomorpha, Ostariophysi and Protacanthopterygii have retained one paralogue (VtgB), and the Acanthomorpha both paralogues (VtgA and VtgB). However, with the exception of the top cluster encompassing Atlantic halibut to Japanese silago (Fig. 1), the identities and similarities for the LvH of each of these groups (50–65%, and 70–80%, respectively; see supplementary information Fig. S3) suggest otherwise. Further, since no phylogenetic method yielded orthologous relationships between the Vtgs of either the Elopomorpha, Ostariophysi, Protacanthopterygii and the Acanthomorpha or paralogous clustering of the Vtg genes immediately following R3, we suggest VtgB has been lost, and that all post-R3 teleost Vtg genes are derived from an ancestral VtgA type.

Both the Elopomorpha and Ostariophysi have duplicated their Vtg genes, but our analyses show that this has occurred in a lineage-specific manner after the WGD (Fig. 1, Fig. 3C&D). Similarly, although the Vtg genes of the salmonids do show paralogous clustering, this is argued to be the consequence of an independent polyploidy event that occurred between 25–50 mya [134]. Our current data match the first tree for salmonid Vtgs proposed by Buisine *et al.* [113]. Interestingly, with the exception of catfish, which are also tetraploid, the eggs of salmonids are among the largest shed by any broadcast spawning teleost. The loss of a paralogue in the *Oncorhynchus* genera after separation from *Salmo* genera ~25 mya [39] resulted in local duplications of up to 31 copies of the other paralogue [113], suggesting a possible compensation for the synthesis of the large amounts of yolk. Notably, the WGD at the base of the crown group of teleosts coincides with appearance of telolecithal eggs and meroblastic cleavage. With the exception of Coelacanthomorpha, all other osteichthyan fishes below the level of teleosts, have mesolecithal eggs and holoblastic cleavage.

Above the level of the Protacanthopterygii, all Vtg genes show paralogous clustering giving rise to the proposed VtgAa and VtgAb paralogues among the Paracanthopterygii and Acanthop-

terygii (Fig. 1). This matches the appearance of the Acanthomorpha in the fossil record 75–100 mya [35,37] and their estimated origin 125 mya [39]. Our analyses also indicate that a second putative lineage-specific duplication of the VtgA genes could have occurred with the appearance of the Acanthopterygii 55 mya. This suggests that four genes should exist in these species, but to date only dual Vtgs are known. Since we cannot preclude the existence of silent, or near silent genes as found for zebrafish (VtgAo2), this latter event may somehow represent a linked duplication or reflects a diversification of the Aa and Ab paralogues. No data are currently available for the chromosomal loci of these genes in the Acanthomorpha, and further studies will be necessary to clarify this issue. Presently we have indicated that this clustering is the result of differential cathepsin activation, since benthophils also express the VtgAa gene, but either do not, or only partially degrade the LvH during oocyte maturation [73,73,81].

Remarkably, the gene clustering was highly correlated to the pelagic or benthic character of the egg. Since most marine teleosts spawn pelagic eggs [85], and only three Vtg forms from marine pelagophils have been fully sequenced to date, we suggest that the present tree does not reflect the bias toward VtgAa type genes. The observation that only the VtgAa forms are predominantly degraded during oocyte maturation, implies that this form of the gene has evolved novel sensitivity to the developmentally regulated activation of acid hydrolases (see later).

The proposed scheme of WGD and lineage-specific duplication of the Vtg genes precisely matches the observed number of vertebrate Vtg genes in accordance with the discontinuous Hox model (Fig. 1 inset). For lineage-specific VtgA duplications that resulted in paralogues, we have adopted the a, b convention. To avoid confusion with the independent tetraploidization amongst the Protacanthopterygii, we used VtgAa and VtgAb to denote paralogous genes that were previously classified as Vtg-A and Vtg-B, respectively [113]. The homologous Vtg genes of the Elopomorpha and Ostariophysi are preliminary classified as VtgAe and VtgAo, respectively. Further sequencing will be necessary to better establish their homology associations.

Neo-Functionalization of the Vtg Genes

The mechanism of hydration differs between benthophils and pelagophils. In benthophils, the degree of oocyte hydration is less dramatic [69,135] [73] and mostly dominated by an increase in inorganic ions and the presence of taurine [62,64,79], while in pelagophils, oocyte hydration is driven by an increase in the FAA content followed by inorganic ions [56,57,61,67,69,84,136]. Recent studies of the structure and disassembly of the yolk proteins during oocyte hydration in Atlantic halibut (Finn, unpublished data) and other marine pelagophils have demonstrated that the origin of the FAA pool stems mostly from the LvH of the VtgAa paralogue [65,67,74]. As in the more ancestral VtgABCD of lamprey [103] and VtgAo1 of zebrafish [82], the VtgAb LvH may be nicked, but is essentially not degraded.

Following fertilization and formation of the yolk syncytial layer, the surviving VtgAa and VtgAb gene products (yolk proteins) are also degraded by cathepsins as substrate for the developing embryo during yolk resorption [137–139], while the FAA generated from the maturational degradation of VtgAa become the dominant catabolic substrate for energy metabolism during embryogenesis (reviewed by [68]). In this respect the VtgAb paralogues maintain their original yolk function, but the present and previously published data demonstrate a post-duplication neo-functionalization of the VtgAa paralogues. This notion is supported by Ka/Ks ratios of 0.415 versus 0.294 for the branches that lead to the VtgAa and VtgAb clusters, respectively.

Intriguingly, the VtgAa paralogue expressed in benthophils may also be partially cleaved and hydrolyzed during oocyte maturation, while the VtgAb paralogue remains intact for the developing embryo [73]. Confirmation that the VtgAa genes have undergone neo-functionalization arises from observations that all of the yolk proteins, including those that are derived from the VtgAb clusters become exposed to the acid hydrolases during the hydration event. However, it is primarily the LvH of the VtgAa paralogues that is degraded.

Many investigations have observed the maturational disappearance of high molecular weight proteins or the appearance of the FAA pool in other species that spawn pelagic eggs, including the Elopomorpha [140–142]. Indeed the FAA content of a newly fertilized teleost egg may be regarded as the signature of the pelagic egg (reviewed by [68]). The observation that Elopomorpha also have pelagic eggs, that arise with the disappearance of a high molecular weight Lv (110 kD) and a concomitant increase in FAA [140–142] indicates that the same mechanism has evolved in this group. Up to three forms of Vtg are known in Elopomorpha (Fig. 1 and supplementary material, Table S1), but these genes are not orthologous to the Vtgs of Acanthomorpha or indeed any other teleost group. For the Japanese eel, the LvH of VtgAe1 and VtgAe2 are 98% identical, while the LvH of VtgAe3 is only 86% identical indicating that at least two forms of Vtg exist in this group. Similar to the dual Vtgs of the Acanthomorpha, only the 110 kD Lv disappears during oocyte maturation of the Japanese eel [140]. However, due to the non-orthologous nature of eel Vtgs, we suggest that the pelagic eggs in this group have arisen by convergent evolution.

Solving the Water Problem

We argue that the solution realized by marine teleosts that spawn pelagic eggs is the generation of a large pool of organic osmolytes (FAA) that drive hydration of the oocyte while still protected within the maternal ovary. Interestingly, this adaptation is analogous to the oviparous Chondrichthyes that store the organic end-products of protein metabolism (urea and trimethylamine oxides) in their eggs [143–145]. The functional significance of these mechanisms conforms to the compatible osmolyte hypothesis [146]. Unlike charged ions, neutral amino acids, which dominate the FAA pool [57,67,69,136,141,142,147–151] do not compromise enzyme function. We suggest that in teleosts, the retention of the organic end products of protein degradation was made possible by a post-R3 lineage-specific duplication of their Vtg genes, and the differential activation of acid hydrolases during oocyte maturation. Instead of converting their depolymerized yolk protein products to urea and trimethylamine oxides, they retain them in a free form as FAA, which, due to their transiently increased concentration in the yolk relative to the maternal body fluids, drive hydration of the oocyte. Once the eggs are broadcast into the sea, the formation of the virtually impermeable vitelline membrane during the cortical reaction [152–156] prevents the loss of this water of life until osmoregulatory mechanisms develop during embryogenesis. The greater degradation of the VtgAa forms in pelagophils led to the highly hydrated egg and caused them to float, while the FAA comprise the major substrate that fuels embryonic development. This mechanism appears to have independently evolved in the Elopomorpha.

REFERENCES

1. Haldane JBS (1932) *The Causes of Evolution*. New York: Cornell University Press. 235 p.

Oceanic Radiation and Speciation

The greater hydration associated with the spawning of pelagic eggs (>90% water) in vast numbers would have severely loaded the maternal ovary with water, and probably led to batch spawning, which is the prevalent means of reproduction in the extant Acanthomorpha. We thus argue that the pelagic nature of the egg, which arose due to neo-functionalization of the Vtg paralogues, provided the allopatric means of radiation in the oceans, while the retention of 3R gene products, latently yielded the genetic means for adjustments in pattern formation and speciation. The rapid acanthomorph colonization of the oceans occurred when competition and predation was low [157,158] following the Cretaceous-Tertiary boundary extinction. Such a lack of competition and reduced predation pressure may have provided the opportunity for a flotilla of teleost invaders in the oceans.

SUPPORTING INFORMATION

Figure S1 Phylogenetic organisation of the fishes illustrating the fraction of species spawning benthic (B) or pelagic (P) eggs, or having viviparous/ovoviviparous (V) reproduction in seawater or freshwater. A plus indicates that the mode of reproduction occurs in the given order. Estimates of minimum paleontological dates or calculated divergence times (millions of years ago; mya) according to the fossil record or mitogenomic data (Inoue et al., 2005) are given for the appearance of the major groups. Model species that are currently undergoing complete genome sequencing belong to orders highlighted in grey.

Found at: doi:10.1371/journal.pone.0000169.s001 (0.10 MB PDF)

Figure S2 Multiple sequence alignment of vertebrate vitellogenins and apolipoprotein B100. Reclassification of the genes as shown in Figs 1–3 in manuscript are indicated. Sub-domains of the vitellogenin monomers are indicated by labeled bars beneath the alignment. Conserved cleavage sites are annotated above the sequences. Data sets for phylogenetic analyses are illustrated by grey bars under the relevant domains.

Found at: doi:10.1371/journal.pone.0000169.s002 (8.47 MB JPG)

Figure S3 Similarity and identity scores for the lipovitellin heavy chains in the multiple sequence alignment shown in Fig. S2. Cells are colored according to score.

Found at: doi:10.1371/journal.pone.0000169.s003 (0.04 MB PDF)

Table S1 Accession numbers of sequences and taxa used in the analyses.

Found at: doi:10.1371/journal.pone.0000169.s004 (0.06 MB PDF)

ACKNOWLEDGMENTS

We thank Hans Jørgen Fyhn, Mathew Betts, David Liberles, Tim Hughes and Cedric Notredame for their discussions and assistance

Author Contributions

Conceived and designed the experiments: RF. Performed the experiments: RF. Analyzed the data: RF BK. Contributed reagents/materials/analysis tools: RF. Wrote the paper: RF.

3. Taylor JS, Raes J (2004) Duplication and divergence: the evolution of new genes and old ideas. *Annu Rev Genet* 38: 615–643.
4. Ohno S (1999) The one-to-four rule and paralogues of sex-determining genes. *Cell Mol Life Sci* 55: 824–830.
5. Amores A, Force A, Yan YL, Joly L, Amemiya C, et al. (1998) Zebrafish hox clusters and vertebrate genome evolution. *Science* 282: 1711–1714.
6. Amores A, Suzuki T, Yan YL, Pomeroy J, Singer A, Amemiya C, Postlethwait JH (2004) Developmental roles of pufferfish Hox clusters and genome evolution in ray-finned fish. *Genome Res* 14: 1–10.
7. Meyer A, Schartle M (1999) Gene and genome duplications in vertebrates: the one-to-four (-to-eight in fish) rule and the evolution of novel gene functions. *Current Opin Cell Biol* 11: 699–704.
8. McLysaght A, Hokamp K, Wolfe KH (2002) Extensive genomic duplication during early chordate evolution. *Nat Genet* 31: 200–204.
9. Van de Peer Y, Taylor JS, Meyer A (2003) Are all fishes ancient polyploids? *J Struct Funct Genomics* 3: 65–73.
10. Hoegg S, Brinkmann H, Taylor JS, Meyer A (2004) Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. *J Mol Evol* 59: 190–203.
11. Vandepoele K, De Vos W, Taylor JS, Meyer A, Van de Peer Y (2004) Major events in the genome evolution of vertebrates: paranome age and size differ considerably between ray-finned fishes and land vertebrates. *Proc Natl Acad Sci U S A* 101: 1638–1643.
12. Dehal P, Boore JL (2005) Two rounds of whole genome duplication in the ancestral vertebrate. *PLoS Biol* 3: e314.
13. Donoghue PC, Purnell MA (2005) Genome duplication, extinction and vertebrate evolution. *Trends Ecol Evol* 20: 312–319.
14. Steinke D, Hoegg S, Brinkmann H, Meyer A (2006) Three rounds (1R/2R/3R) of genome duplications and the evolution of the glycolytic pathway in vertebrates. *BMC Biol* 4: 16.
15. Steinke D, Salzburger W, Braasch I, Meyer A (2006) Many genes in fish have species-specific asymmetric rates of molecular evolution. *BMC Genomics* 7: 20.
16. Wolfe KH (2001) Yesterday's polyploids and the mystery of diploidization. *Nat Rev Genet* 2: 333–341.
17. Furlong RF, Holland PWH (2002) Were vertebrates octoploid? *Philos Trans R Soc Lond B Biol Sci* 357: 531–544.
18. Furlong RF, Holland PWH (2004) Polyploidy in vertebrate ancestry: Ohno and beyond. *Biol J Linn Soc* 82: 425–430.
19. Lynch M, O'Hely M, Walsh B, Force A (2001) The probability of preservation of a newly arisen gene duplicate. *Genetics* 159: 1789–1804.
20. von Schantz M, Jenkins A, Archer SN (2006) Evolutionary history of the vertebrate *Period* genes. *J Mol Evol* 62: 701–707.
21. Schmidtke J, Weiler C, Kunz B, Engel W (1977) Isozymes of a tunicate and a cephalochordate as a test of polyploidisation in chordate evolution. *Nature* 266: 532–533.
22. Skrabanek L, Wolfe KH (1998) Eukaryote genome duplication - where's the evidence? *Curr Opin Genet Dev* 8: 694–700.
23. Hughes AL (1999) Phylogenies of developmentally important proteins do not support the hypothesis of two rounds of genome duplication early in vertebrate history. *J Mol Evol* 48: 565–576.
24. Robinson-Rechavi M, Marchand O, Escriva H, Laudet V (2001) An ancestral whole-genome duplication may not have been responsible for the abundance of duplicated fish genes. *Curr Biol* 11(12): R458–9.
25. Venkatesh B (2003) Evolution and diversity of fish genomes. *Curr Opin Genet Dev* 13: 588–592.
26. Abi-Rached L, Gilles A, Shiina T, Pontarotti P, Inoko H (2002) Evidence of en bloc duplication in vertebrate genomes. *Nat Genet* 31: 100–105.
27. Postlethwait JH, Yan YL, Gates MA, Horne S, Amores A, et al. (1998) Vertebrate genome evolution and the zebrafish gene map. *Nat Genet* 18: 345–349.
28. Christoffels A, Koh EG, Chia JM, Brenner S, Aparicio S, Venkatesh B (2004) Fugu genome analysis provides evidence for a whole-genome duplication early during the evolution of ray-finned fishes. *Mol Biol Evol* 21: 1146–1151.
29. Jaillon O, Aury JM, Brunet F, Petit JL, Stange-Thomann N, et al. (2004) Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. *Nature* 431: 946–957.
30. Woods IG, Wilson C, Friedlander B, Chang P, Reyes DK, Nix R, Kelly PD, Chu F, Postlethwait JH, Talbot WS (2005) The zebrafish gene map defines ancestral vertebrate chromosomes. *Genome Res* 15: 1307–1314.
31. Crow KD, Stadler PF, Lynch VJ, Amemiya C, Wagner GP (2006) The “fish-specific” Hox cluster duplication is coincident with the origin of teleosts. *Mol Biol Evol* 23: 121–136.
32. Taylor JS, Van de Peer Y, Braasch I, Meyer A (2001) Comparative genomics provides evidence for an ancient genome duplication event in fish. *Philos Trans R Soc Lond B Biol Sci* 356: 1661–1679.
33. Taylor JS, Braasch I, Frickey T, Meyer A, Van de Peer Y (2003) Genome duplication, a trait shared by 22000 species of ray-finned fish. *Genome Res* 13: 382–390.
34. Mulley J, Holland P (2004) Comparative genomics: small genome, big insights. *Nature* 431: 916–917.
35. Romer AS (1966) *Vertebrate Paleontology*. Chicago: University of Chicago Press.
36. Long JA (1995) *The rise of fishes. 500 million years of evolution*. Baltimore: The John Hopkins University Press.
37. Maissey JG (1996) *Discovering fossil fishes*. New York: Westview Press.
38. Gardiner BG, Schaeffer B, Masserie JA (2005) A review of the lower actinopterygian phylogeny. *Zool J Linnean Soc* 144: 511–525.
39. Inoue JG, Miya M, Venkatesh B, Nishida M (2005) The mitochondrial genome of Indonesian coelacanth *Latimeria menadoensis* (Sarcopterygii: Coelacanthiformes) and divergence time estimation between the two coelacanths. *Gene* 349: 227–235.
40. Bemis WE, Findeis EK, Grande L (1997) An overview of Acipenseriformes. In: Birstein V, Waldman JR, Bemis WE, editors. *Sturgeon Biodiversity and Conservation*. Dordrecht: Kluwer Academic Publishers. pp. 25–71.
41. de Pinna MCC (1996) Teleostean monophyly. In: Stiassny MLJ, Parenti LR, Johnson GD, editors. *Interrelationships of Fishes*. New York: Academic Press. pp. 147–162.
42. Inoue JG, Miya M, Tsukamoto K, Nishida M (2003) Basal actinopterygian relationships: a mitogenomic perspective on the phylogeny of the “ancient fish”. *Mol Phylogenet Evol* 26: pp. 110–120.
43. Kumazawa Y, Yamaguchi M, Nishida M (1999) Mitochondrial molecular clocks and the origin of euteleostean biodiversity: familial radiation of Perciformes may have predated the Cretaceous/Tertiary boundary. In: Kato M, editor. *The biology of biodiversity*. Hong Kong: Springer-Verlag. pp. 35–52.
44. Kumazawa Y, Nishida M (2000) Molecular phylogeny of osteoglossoids: a new model for Gondwanian origin and plate tectonic transportation of the Asian arowana. *Mol Biol Evol* 17: 1869–1878.
45. Hurley I, Hale ME, Prince VE (2005) Duplication events and the evolution of segmental identity. *Evol Dev* 7: 556–567.
46. Steinke D, Salzburger W, Meyer A (2006) Novel relationships among ten fish model species revealed based on a phylogenomic analysis using ESTs. *J Mol Evol* 62: 772–784.
47. Nelson JS (2006) *Fishes of the World*. Hoboken New Jersey: John Wiley & Sons, Inc. 601 p.
48. Fink SV, Fink WL (1996) Interrelationships of Ostariophysan Fishes (Teleostei). In: Stiassny MLJ, Parenti LR, GD J, editors. *Interrelationships of Fishes*. New York: Academic Press. pp. 251–332.
49. Saitoh K, Miya M, Inoue JG, Ishiguro NB, Nishida M (2003) Mitochondrial genomes of ostariophysan fishes: perspectives on phylogeny and biogeography. *J Mol Evol* 56: 464–472.
50. Griffith RW (1987) Freshwater or marine origin of the vertebrates? *Comp Biochem Physiol* 87A: 523–531.
51. Evans DH (1993) Osmotic and ionic regulation. In: Evans DH, editor. *The physiology of fishes*. Boca Raton/Florida: CRC Press. pp. 315–341.
52. Smith HW (1953) *From fish to philosopher. The story of our internal environment*. USA: CIBA Pharmaceutical Products Ltd.
53. Marshall WS, Grosell M (2006) Ion transport, osmoregulation, and acid-base balance. In: Evans DH, Claiborne JB, editors. *The physiology of fishes*. Boca Raton/Florida: Taylor & Francis. pp. 177–230.
54. Wilkie MP (2002) Ammonia excretion and urea handling by fish gills: present understanding and future research challenges. *J Exp Zool* 293: 284–301.
55. Evans DH, Piermarini PM, Choe KP (2004) Homeostasis: Osmoregulation, pH, regulation and nitrogen excretion. In: Carrier JC, Musick JA, Heithaus MR, editors. *Biology of sharks and their relatives*. Boca Raton: CRC Press. pp. 247–268.
56. Watanabe WO, Kuo CM (1986) Water and ion balance in hydrating oocytes of the grey mullet, *Mugil cephalus* (L.), during hormonal induced final maturation. *J Fish Biol* 28: 425–437.
57. Finn RN, Østby GC, Norberg B, Fyhn HJ (2002) *In vivo* oocyte hydration in Atlantic halibut (*Hippoglossus hippoglossus*); proteolytic liberation of free amino acids, and ion transport, are driving forces for osmotic water influx. *J Exp Biol* 205: 211–224.
58. Fulton TW (1891) *The comparative fecundity of sea fishes*. Fishery Board for Scotland 9th annual Report Part I: 243–268.
59. Fulton TW (1898) *On growth and maturation of the ovarian eggs of teleostean fishes*. 16th Annual Report of the Fishery Board for Scotland (1897) Part III: 83–134.
60. Milroy TH (1898) *The physical and chemical changes taking place in the ova of certain marine teleosteans during maturation*. Fishery Board for Scotland 16th Annual Report Part III: 135–152.
61. Craik JCA, Harvey SM (1987) The causes of buoyancy in eggs of marine teleosts. *J mar biol Ass UK* 67: 169–182.
62. LaFleur GJ Jr, Thomas P (1991) Evidence for a role of Na⁺,K⁺-ATPase in the hydration of Atlantic croaker and spotted seatrout oocytes during final maturation. *J Exp Zool* 258: 126–136.
63. Wallace RA, Greeley MS Jr, McPherson R (1992) Analytical and experimental studies on the relationship between Na⁺, K⁺, and water uptake during volume increases associated with *Fundulus* oocyte maturation *in vitro*. *J Comp Physiol* 162B: 241–248.
64. Thorsen A, Fyhn HJ, Wallace RA (1993) Free amino acids as osmotic effectors for oocyte hydration in marine fishes. In: Walther BT, Fyhn HJ, editors. *Physiological and Biochemical Aspects of Fish Development*. Bergen: University of Bergen. pp. 94–98.
65. Matsubara T, Ohkubo N, Andoh T, Sullivan CV, Hara A (1999) Two forms of vitellogenin, yielding two distinct lipovitellins, play different roles during oocyte

- maturation and early development of barfin flounder, *Verasper moseri*, a marine teleost that spawns pelagic eggs. *Dev Biol* 213: 18–32.
66. Matsubara T, Nagae M, Ohkubo N, Andoh T, Sawaguchi S, Hiramatsu N, Sullivan CV, Hara A (2003) Multiple vitellogenins and their unique roles in marine teleosts. *Fish Physiol Biochem* 28: 295–299.
 67. Reith M, Munholland J, Kelly J, Finn RN, Fyhn HJ (2001) Lipovitellins derived from two forms of vitellogenin are differentially processed during oocyte maturation in haddock (*Melanogrammus aeglefinus*). *J Exp Zool* 291: 58–67.
 68. Wright PA, Fyhn HJ (2001) Ontogeny of nitrogen metabolism and excretion. In: Wright P, Anderson PM, editors. *Fish Physiology Volume 20, Nitrogen Excretion*. New York: Academic Press. pp. 149–200.
 69. Finn RN, Wamboldt M, Fyhn HJ (2002) Differential processing of yolk proteins during oocyte hydration in fishes (*Labridae*) that spawn benthic and pelagic eggs. *Mar Ecol Prog Ser* 237: 217–226.
 70. Hiramatsu N, Matsubara T, Weber GM, Sullivan CV, Hara A (2002) Vitellogenesis in aquatic animals. *Fish Sci* 68: 694–699.
 71. Ohkubo N, Mochida K, Adachi S, Hara A, Hotta K, Nakamura Y, Matsubara T (2003) Development of enzyme-linked immunosorbent assays for two forms of vitellogenin in Japanese common goby (*Acanthogobius flavimanus*). *Gen Comp Endocrinol* 131: 353–364.
 72. Ohkubo N, Andoh T, Mochida K, Adachi S, Hara A, Matsubara T (2004) Deduced primary structure of two forms of vitellogenin in Japanese common goby (*Acanthogobius flavimanus*). *Gen Comp Endocrinol* 137: 19–28.
 73. LaFleur GJ Jr, Raldúa D, Fabra M, Carnevali O, Denslow N, Wallace RA, Cerdà J (2005) Derivation of major yolk proteins from parental vitellogenins and alternative processing during oocyte maturation in *Fundulus heteroclitus*. *Biol Reprod* 73: 815–824.
 74. Sawaguchi S, Kagawa H, Ohkubo N, Hiramatsu N, Sullivan CV, Matsubara T (2006) Molecular characterization of three forms of vitellogenin and their yolk protein products during oocyte growth and maturation in red seabream (*Pagrus major*), a marine teleost spawning pelagic eggs. *Mol Reprod Dev* 73: 719–736.
 75. Carnevali O, Carletta R, Cambi A, Vita A, Bromage N (1999) Yolk formation and degradation during oocyte maturation in seabream *Sparus aurata*: involvement of two lysosomal proteinases. *Biol Reprod* 60: 140–146.
 76. Carnevali O, Cionna C, Tosti L, Lubzens E, Maradonna F (2006) Role of cathepsins in ovarian follicle growth and maturation. *Gen Comp Endocrinol* 146: 195–203.
 77. Mosconi G, Carnevali O, Habibi HR, Sanyal R, Polzonetti-Magni AM (2002) Hormonal mechanisms regulating hepatic vitellogenin synthesis in the gilthead seabream, *Sparus aurata*. *Am J Physiol Cell Physiol* 283: 673–678.
 78. Romano M, Rosanova P, Anteo C, Limatola E (2004) Vertebrate yolk proteins: A review. *Mol Reprod Dev* 69: 109–116.
 79. Selman K, Wallace RA, Cerdà J (2001) Bafilomycin A1 inhibits proteolytic cleavage and hydration but not yolk crystal disassembly or meiosis during maturation of sea bass oocytes. *J Exp Zool* 290: 265–278.
 80. Raldúa D, Fabra M, Bozzo MG, Weber E, Cerdà J (2006) Cathepsin B-mediated yolk protein degradation during killifish oocyte maturation is blocked by an H⁺-ATPase inhibitor: effects on the hydration mechanism. *Am J Physiol Regul Integr Comp Physiol* 290: R456–66.
 81. Fabra M, Cerdà J (2004) Ovarian cysteine proteinases in the teleost *Fundulus heteroclitus*: molecular cloning and gene expression during vitellogenesis and oocyte maturation. *Mol Reprod Dev* 67: 282–294.
 82. Dosch R, Wagner DS, Mintzer KA, Runke G, Wiemelt AP, Mullins MC (2004) Maternal control of vertebrate development before the midblastula transition: mutants from the zebrafish I. *Dev Cell* 6: 771–780.
 83. Fabra M, Raldúa D, Power DM, Deen PM, Cerdà J (2005) Marine fish egg hydration is aquaporin-mediated. *Science* 307: 545.
 84. Fabra M, Raldúa D, Bozzo MG, Deen PM, Lubzens E, Cerdà J (2006) Yolk proteolysis and aquaporin-10 play essential roles to regulate fish oocyte hydration during meiosis resumption. *Dev Biol* 295: 250–262.
 85. Kendall AW, Ahlstron EH, HG M (1984) Early life history stages of fishes and their characters. In: Moser HG, editor. *Ontogeny and systematics of fishes*. *Am Soc Ichthyol Herpetol Special publ* 1. pp. 11–22.
 86. Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310–2314.
 87. Holder M, Lewis PO (2003) Phylogeny estimation: traditional and Bayesian approaches. *Nat Rev Genet* 4: 275–284.
 88. Glenner H, Hansen AJ, Sorensen MV, Ronquist F, Huelsenbeck JP, Willerslev E (2004) Bayesian inference of the metazoan phylogeny; a combined molecular and morphological approach. *Curr Biol* 14: 1644–1649.
 89. Baker ME (1988) Is vitellogenin an ancestor of apolipoprotein B-100 of human low-density lipoprotein and human lipoprotein lipase? *Biochem J* 255: 1057–1060.
 90. Babin PJ, Bogerd J, Kooiman FP, Van Marrewijk WJ, Van der Horst DJ (1999) Apolipoprotein II/I, apolipoprotein B, vitellogenin, and microsomal triglyceride transfer protein genes are derived from a common ancestor. *J Mol Evol* 49: 150–160.
 91. Perez LE, Fenton MJ, Callard IP (1991) Vitellogenin-homologs of mammalian apolipoproteins? *Comp Biochem Physiol B* 100: 821–826.
 92. Byrne BM, Gruber M, Ab G (1989) The evolution of egg yolk proteins. *Prog Biophys Mol Biol* 53: 33–69.
 93. Wang H, Yan T, Tan JT, Gong Z (2000) A zebrafish vitellogenin gene (*vg3*) encodes a novel vitellogenin without a phosphotyrosine domain and may represent a primitive vertebrate vitellogenin gene. *Gene* 256: 303–310.
 94. Hiramatsu N, Cheek AO, Sullivan CV, Matsubara T, Hara A (2006) Vitellogenesis and endocrine disruption. In: Mommens TP, Moon TW, editors. *Biochemistry and molecular biology of fishes*. Vol 6. Amsterdam: Elsevier Science BV. pp. 431–471.
 95. Mikawa N, Utoh T, Horie N, Okamura A, Yamada Y, Akazawa A, Tanaka S, Tsukamoto K, Hirono I, Aoki T (2006) Cloning and characterization of vitellogenin cDNA from the common Japanese conger (*Conger myriaster*) and vitellogenin gene expression during ovarian development. *Comp Biochem Physiol B Biochem Mol Biol* 143: 404–414.
 96. Hayakawa H, Andoh T, Watanabe T (2006) Precursor structure of egg proteins in the coral *Galaxea fascicularis*. *Biochem Biophys Res Comm* 344: 173–180.
 97. Tatusova GA, Madden TL (1999) BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol Lett* 174: 247–250.
 98. Wang H, Tan JT, Emelyanov A, Korzh V, Gong Z (2005) Hepatic and extrahepatic expression of vitellogenin genes in the zebrafish, *Danio rerio*. *Gene* 356: 91–100.
 99. Notredame C, Higgins DG, Heringa J (2000) T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* 302: 205–217.
 100. Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD (2003) Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res* 31: 3497–3500.
 101. O'Sullivan O, Suhre K, Abergel C, Higgins DG, Notredame C (2004) 3DCoffee: combining protein sequences and structures within multiple sequence alignments. *J Mol Biol* 340: 385–395.
 102. Do CB, Mahabhashyam MS, Brudno M, Batzoglou S (2005) ProbCons: Probabilistic consistency-based multiple sequence alignment. *Genome Res* 15: 330–340.
 103. Anderson TA, Levitt DG, Banaszak LJ (1998) The structural basis of lipid interactions in lipovitellin, a soluble lipoprotein. *Structure* 6: 895–909.
 104. Thompson JR, Banaszak LJ (2002) Lipid-protein interactions in lipovitellin. *Biochemistry* 41: 9398–9409.
 105. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
 106. Zmasek CM, Eddy SR (2001) ATV: display and manipulation of annotated phylogenetic trees. *Bioinformatics* 17: 383–384.
 107. Zmasek CM, Eddy SR (2001) A simple algorithm to infer gene duplication and speciation events on a gene tree. *Bioinformatics* 17: 821–828.
 108. Mossel E, Vigoda E (2005) Phylogenetic MCMC algorithms are misleading on mixtures of trees. *Science* 309: 2207–2209.
 109. Swafford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other models). Version 4.0b10 for macintosh. SunderlandMass: Sinauer Associates Inc.
 110. Liberles DA (2001) Evaluation of methods for determination of a reconstructed history of gene sequence evolution. *Mol Biol Evol* 18: 2040–2047.
 111. Silberg J, Liberles DA (2002) A simple covarion-based approach to analyse nucleotide substitution rates. *J Mol Biol* 15: 588–594.
 112. LaFleur GJ Jr, Byrne BM, Kanungo J, Nelson LD, Greenberg RM, Wallace RA (1995) *Fundulus heteroclitus* vitellogenin: the deduced primary structure of a piscine precursor to noncrystalline, liquid-phase yolk protein. *J Mol Evol* 41: 505–521.
 113. Buisine N, Trichet V, Wolff J (2002) Complex evolution of vitellogenin genes in salmonid fishes. *Mol Genet Genomics* 268: 535–542.
 114. Fujiwara Y, Fukada H, Shimizu M, Hara A (2005) Purification of two lipovitellins and development of immunoassays for two forms of their precursors (vitellogenins) in medaka (*Oryzias latipes*). *Gen Comp Endocrinol* 143: 267–277.
 115. Sawaguchi S, Koya Y, Yoshizaki N, Ohkubo N, Andoh T, Hiramatsu N, Sullivan CV, Hara A, Matsubara T (2005) Multiple vitellogenins (Vgs) in mosquitofish (*Gambusia affinis*): identification and characterization of three functional Vg genes and their circulating and yolk protein products. *Biol Reprod* 72: 1045–1060.
 116. Miracle A, Ankley G, Lattier D (2006) Expression of two vitellogenin genes (*vg1* and *vg3*) in fathead minnow (*Pimephales promelas*) liver in response to exposure to steroidal estrogens and androgens. *Ecotoxicol Environ Safety* 63: 337–342.
 117. Chen JS, Sappington TW, Raikhel AS (1997) Extensive sequence conservation among insect, nematode, and vertebrate vitellogenins reveals ancient common ancestry. *J Mol Evol* 44: 440–451.
 118. Skibinski DO, Ward RD (2004) Average allozyme heterozygosity in vertebrates correlates with Ka/Ks measured in the human-mouse lineage. *Mol Biol Evol* 21: 1753–1759.
 119. McInerney JO (2006) The causes of protein evolutionary rate variation. *Trends Ecol Evol* 21: 230–232.
 120. Inoue JG, Miya M, Tsukamoto K, Nishida M (2004) Mitogenomic evidence for the monophyly of elopomorph fishes (Teleostei) and the evolutionary origin of the leptocephalus larva. *Mol Phylogenet Evol* 32: 274–286.
 121. Brooke NM, Garcia-Fernández J, Holland PW (1998) The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. *Nature* 392: 920–922.
 122. Meyer A, Malaga-Trillo E (1999) Vertebrate genomics: More fishy tales about Hox genes. *Curr Biol* 9: R210–3.

123. Ferrier DE, Holland PW (2001) Ancient origin of the Hox gene cluster. *Nat Rev Genet* 2: 33–38.
124. Prince VE, Pickett FB (2002) Splitting pairs: the diverging fates of duplicated genes. *Nat Rev Genet* 3: 827–837.
125. Ferrier DE, Dewar K, Cook A, Chang JL, Hill-Force A, Amemiya C (2005) The chordate ParaHox cluster. *Curr Biol* 15(20): R820–2.
126. Kamm K, Schierwater B, Jakob W, Dellaporta SL, Miller DJ (2006) Axial patterning and diversification in the cnidaria predate the Hox system. *Curr Biol* 16: 920–926.
127. Woltering JM, Durston AJ (2006) The zebrafish hoxDb cluster has been reduced to a single microRNA. *Nat Genet* 38: 601–602.
128. Irvine SQ, Carr JL, Bailey WJ, Kawasaki K, Shimizu N, Amemiya CT, Ruddle FH (2002) Genomic analysis of Hox clusters in the sea lamprey *Petromyzon marinus*. *J Exp Zool* 294: 47–62.
129. Force A, Amores A, Postlethwait JH (2002) Hox cluster organization in the jawless vertebrate *Petromyzon marinus*. *J Exp Zool* 294: 30–46.
130. Gerber-Huber S, Nardelli D, Haefliger JA, Cooper DN, Givel F, Germond JE, Engel J, Green NM, Wahli W (1987) Precursor-product relationship between vitellogenin and the yolk proteins as derived from the complete sequence of a *Xenopus* vitellogenin gene. *Nucleic Acids Res* 15: 4737–4760.
131. Yoshitome S, Nakamura H, Nakajo N, Okamoto K, Sugimoto I, et al. (2003) *M*₂₅ 000 protein, a substrate for protein serine/threonine kinases, is identified as a part of *Xenopus laevis* vitellogenin B1. *Dev Growth Differ* 45: 283–294.
132. Silva R, Fischer AH, Burch JB (1989) The major and minor chicken vitellogenin genes are each adjacent to partially deleted pseudogene copies of the other. *Mol Cell Biol* 9: 3557–3562.
133. Tauber E, Last KS, Olive PJ, Kyriacou CP (2004) Clock gene evolution and functional divergence. *J Biol Rhythms* 19: 445–458.
134. Allendorf FW, Thorgaard G (1984) Tetraploidy and the evolution of salmonid fishes. In: Turner BJ, editor. *Evolutionary Genetics of Fishes*. New York: Plenum Press. pp. 1–45.
135. Greeley MSJ, Hols H, Wallace RA (1991) Changes in size, hydration, and low molecular weight osmotic effectors during meiotic maturation of *Fundulus* oocytes *in vivo*. *Comp Biochem Physiol* 100A: 639–647.
136. Matsubara T, Koya Y (1997) Course of proteolytic cleavage in three classes of yolk proteins during oocyte maturation in barfin flounder *Verasper moseri*, a marine teleost spawning pelagic eggs. *J Exp Zool* 278: 189–200.
137. Ohkubo N, Matsubara T (2002) Sequential utilization of free amino acids, yolk proteins and lipids in developing eggs and yolk-sac larvae of barfin flounder *Verasper moseri*. *Mar Biol* 140: 187–196.
138. Ohkubo N, Sawaguchi S, Hamatsu T, Matsubara T (2006) Utilization of free amino acids, yolk proteins and lipids in developing eggs and yolk-sac larvae of walleye pollock *Theragra chalcogramma*. *Fish Sci* 76: 620–630.
139. Tingaud-Sequeira A, Cerdà J (2006) Phylogenetic relationships and gene expression pattern of three different cathepsin L (Ctsl) isoforms in zebrafish: Ctsla is the putative yolk processing enzyme. *Gene* 386: 98–106.
140. Okumura HK, T, Kazeto Y, Hara A, Adachi S, Yamauchi K (1995) Changes in the electrophoretic patterns of lipovitellin during oocyte development in the Japanese eel *Anguilla japonica*. *Fish Sci* 61: 529–530.
141. Seoka M, Yamada S, Iwata Y, Yanagisawa T, Nakagawa T, Kumai H (2003) Differences in the biochemical content of buoyant and non-buoyant eggs of the Japanese eel, *Anguilla japonica*. *Aquaculture* 216: 355–362.
142. Seoka M, Yamada S, Kumai H (2004) Free amino acids in Japanese eel eggs obtained by hormonal inducement. *J Fish Biol* 65: 595–596.
143. Needham J, Needham DM (1930) Nitrogen excretion in selachian ontogeny. *J Exp Biol* 7: 7–18.
144. Kormanik GA (1993) Ionic and osmotic environment of developing clasmobranch embryos. *Environ Biol Fish* 38: 233–240.
145. Steele SL, Yancey PH, Wright PA (2004) Dogmas and controversies in the handling of nitrogenous wastes: osmoregulation during early embryonic development in the marine little skate *Raja erinacea*; response to changes in external salinity. *J Exp Biol* 207: 2021–2031.
146. Hochachka PWH, Somero GN (2002) *Biochemical adaptation. Mechanism and process in physiological evolution*. Oxford: Oxford University Press.
147. Rønnestad I, Finn RN, Groot EP, Fyhn HJ (1992) Utilization of free amino acids related to energy metabolism of developing eggs and larvae of lemon sole, *Microstomus kitt*, reared in the laboratory. *Mar Ecol Prog Ser* 88: 195–205.
148. Rønnestad I, Robertson R, Fyhn HJ (1996) Free amino acids and protein content in pelagic and demersal eggs of tropical marine fishes. In: MacKinlay DD, Eldridge M, editors. *The Fish Egg American Fisheries Society*. pp. 81–84.
149. Finn RN, Fyhn HJ, Evjen MS (1995) Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. *Mar Biol* 124: 355–369.
150. Finn RN, Fyhn HJ, Henderson RJ, Evjen MS (1996) The sequence of catabolic substrate oxidation and enthalpy balance of developing embryos and yolk-sac larvae of turbot (*Scophthalmus maximus* L.). *Comp Biochem Physiol* 115A: 133–151.
151. Thorsen A, Fyhn HJ (1996) Final oocyte maturation *in vivo* and *in vitro* in marine fishes with pelagic eggs; Yolk protein hydrolysis and free amino acid content. *J Fish Biol* 48: 1195–1209.
152. Gray J (1932) The osmotic properties of the eggs of trout (*Salmo fario*). *J Exp Biol* 9: 277–299.
153. Krogh A, Ussing HH (1937) A note on the permeability of trout eggs to D₂O and H₂O. *J Exp Biol* 14: 35–37.
154. Potts WTW, Rudy PP (1969) Water balance in the eggs of Atlantic salmon. *J Exp Biol* 50: 223–237.
155. Loeffler CA, Lovtrup S (1970) Water balance in the salmon egg. *J Exp Biol* 52: 291–298.
156. Jørgensen NC, Scmalbach H (1984) The eggs of the freshwater fish *Epiplatys dageti* have tight plasma membranes without intermembrane particles. *Cell Tissue Res* 235: 643–646.
157. Everhart MJ (2000) Last of the great marine reptiles. *Prehistoric Times* 44: 29–31.
158. Keller G (2001) The end-cretaceous mass extinction in the marine realm: year 2000 assessment. *Planetary and Space Science* 49: 817–830.

Supporting Information

Figure S1.

<http://www.plosone.org/article/firstRepresentation.action?uri=info:doi/10.1371/journal.pone.000169.s001>

Figure S2. Very large image, quality not retained in print so download to view:

<http://www.plosone.org/article/firstRepresentation.action?uri=info:doi/10.1371/journal.pone.000169.s002>

Figure S3.

<http://www.plosone.org/article/firstRepresentation.action?uri=info:doi/10.1371/journal.pone.000169.s003>

Table S1.

<http://www.plosone.org/article/firstRepresentation.action?uri=info:doi/10.1371/journal.pone.000169.s004>

Figure S1: Finn and Kristoffersen

Phylogenetic organisation of the fishes illustrating the fraction of species spawning benthic (B) or pelagic (P) eggs, or having viviparous/ovoviviparous (V) reproduction in seawater or freshwater. A plus indicates that the mode of reproduction occurs in the given order. Estimates of minimum paleontological dates or calculated divergence times (millions of years ago; mya) according to the fossil record or mitogenomic data are given for the appearance of the major groups. Model species that are currently undergoing complete genome sequencing belong to orders highlighted in grey.

Sources

- Inoue JG, Miya M, Tsukamoto K, Nishida M (2004) Mitogenomic evidence for the monophyly of elopomorph fishes (Teleostei) and the evolutionary origin of the leptocephalus larva. *Mol Phylogen Evol* 32: 274-284
 Inoue JG, Miya M, Venkatesh B, Nishida M (2005) The mitochondrial genome of Indoseian coelacanth *Latimeria menadoensis* (Sarcopterygii: Coelacanthiformes) and divergence time estimation between the two coelacanths. *Gene* 349: 227-235
 Kumazawa Y, Nishida M (2000) Molecular phylogeny of Osteoglossoids: A new model for Gondwanian origin and plate tectonic transportation of the asian aruwana. *Mol Biol Evol* 17: 1869-1878
 Long JA (1995) The rise of fishes. 500 million years of evolution. The John Hopkins University Press, Baltimore
 Maissey JG (1996) Discovering fossil fishes. Westview Press, New York
 Moser HG (1983) Ontogeny and systematics of fishes. American Society Ichthyologists Herpetologists Special publication Number 1. Symposium 15-18 Aug, La Jolla, California
 Nelson JS (2006) Fishes of the world. John Wiley & Sons Inc, New York
 Russell FS (1976) The eggs and planktonic stages of British marine fishes. Academic Press, New York
 Stiassny MLJ, Parenti LR, Johnson GD (1996) Interrelationships of fishes. Academic Press, New York

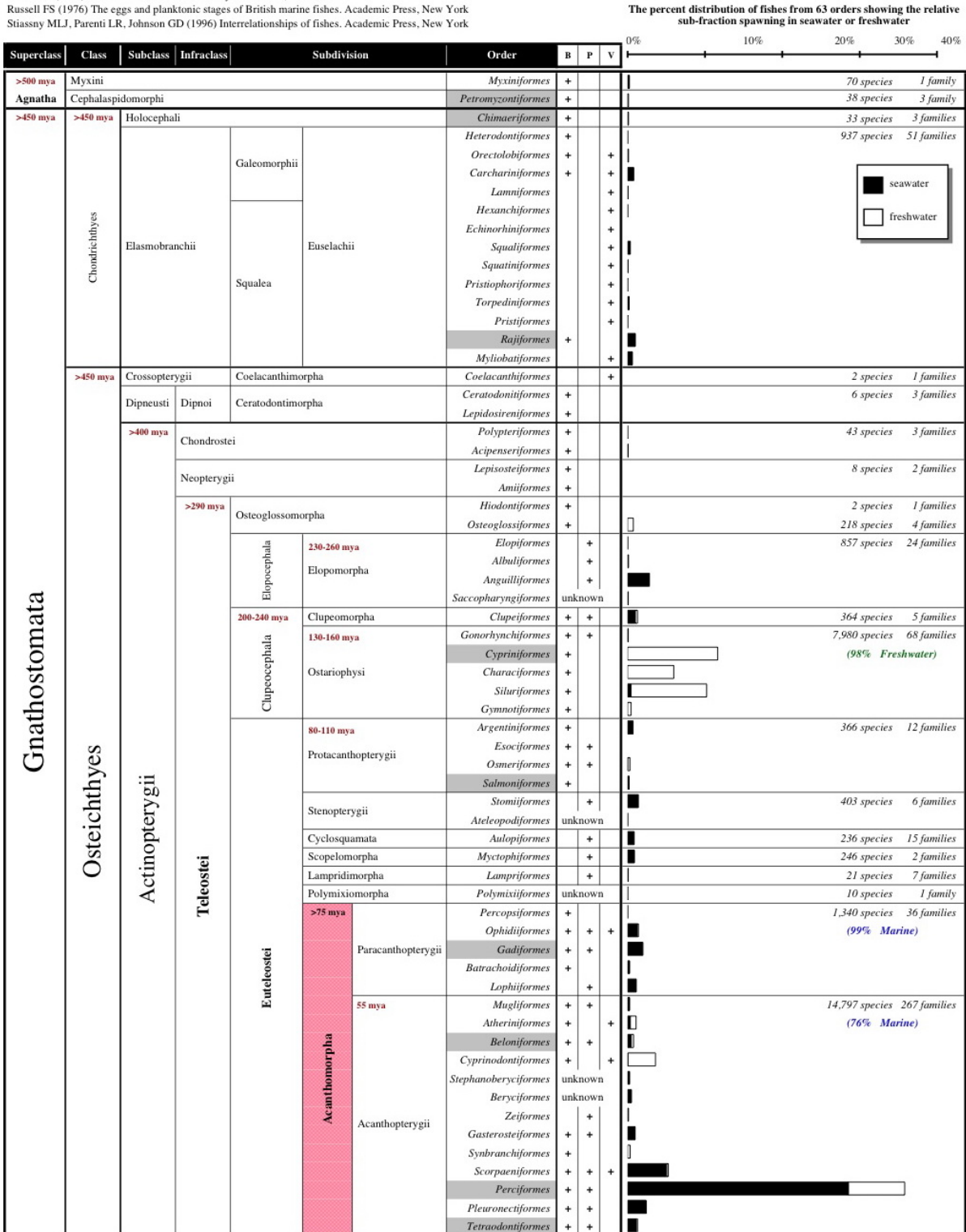
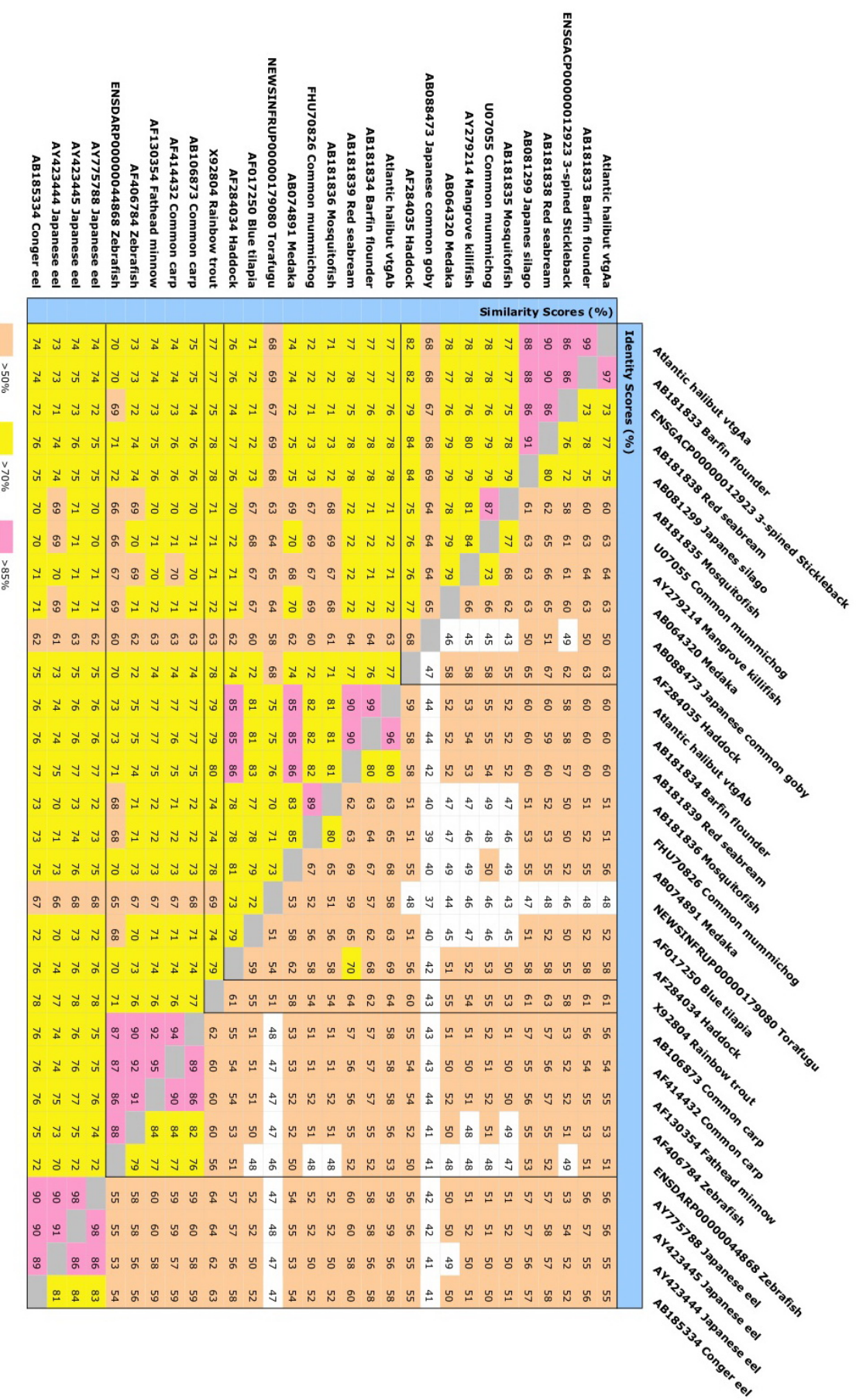


Fig S3: Finn and Kristoffersen
 Similarity and identity scores for complete teleost vitellogenin lipovitellin heavy chains. Cells are coloured according to score



>50% >70% >85%

Table S1: Supplementary material: Finn and Kristoffersen

Accession numbers of sequences and taxa used in the analyses

Accession # (NCBI / Ensembl)	Database		Classified Rank/Class	Order	Animal	Species	Length		
	ID	homologue					bp	aa	Source
1 HUMAPOBB	apo-B100	apoB100	Eutheria	Primates	Human	<i>Homo sapiens</i>	13,689	4,563	Law et al. (1986)
2 ENSGALP0000026550	apo-B100	apoB100	Aves	Galliformes	Chicken	<i>Gallus gallus</i>	12,267	4,089	Ensembl (2006)
3 D89547	VitI	vtgAB1			Chicken	<i>Gallus gallus</i>	5,736	1,912	Mabuchi et al. (1999)
4 NM_001031276	vtgII	vtgAB2			Chicken	<i>Gallus gallus</i>	5,550	1,850	van het Schip et al. (1999)
5 ENSGALT0000002890	novel	vtgAB3			Chicken	<i>Gallus gallus</i>	5,058	1,686	Ensembl (2006)
6 AY045719	vitellogenin	vtgAB		Charadriiformes	Herring gull	<i>Larus argentatus</i>	5,496	1,832	Lorenzen et al. (2001)
7 AAHS4229	apo-B100	apoB100	Amphibia	Anura	African clawed frog	<i>Xenopus laevis</i>	1,311	437	Klein et al. (2002)
8 M18061	vtgA2	vtgABa			African clawed frog	<i>Xenopus laevis</i>	5,421	1,807	Gerber-Huber et al. (1987); Nardelli et al. (1987)
9 AB092605	vtgB1	vtgABb			African clawed frog	<i>Xenopus laevis</i>	5,451	1,817	Yoshitome et al. (2003)
10 NEWSINFRUP00000127842	apo-B100	apoB100	Acanthopterygii	Tetraodontiformes	Torafugu	<i>Takifugu rubripes</i>	11,145	3,715	Ensembl (2006)
11 NEWSINFRUP00000179080	novel	vtgAb			Torafugu	<i>Takifugu rubripes</i>	5,274	1,758	Ensembl (2006)
12 NEWSINFRUP00000131263	novel	vtgC			Torafugu	<i>Takifugu rubripes</i>	3,594	1,198	Ensembl (2006)
13 hhvtgAa1	vtgAa1	vtgAa		Pleuronectiformes	Atlantic halibut	<i>Hippoglossus hippoglossus</i>	4,899	1,633	Finn et al. (2006)
14 hhvtgAb1	vtgAb1	vtgAb			Atlantic halibut	<i>Hippoglossus hippoglossus</i>	4,941	1,647	Finn et al. (2006)
15 AB181833	VgA	vtgAa			Barfin flounder	<i>Verasper moseri</i>	4,884	1,628	Sawaguchi et al. (2005)
16 AB181834	VgB	vtgAb			Barfin flounder	<i>Verasper moseri</i>	4,953	1,651	Sawaguchi et al. (2005)
17 AF017250	Vtg1	vtgAb		Perciformes	Blue Tilapia	<i>Oreochromis aureus</i>	5,364	1,788	Lim et al. (1997)
18 AB088473	Vg-530	vtgAa			Japanese common goby	<i>Acanthogobius flavimanus</i>	4,992	1,664	Ohkubo et al. (2004)
19 AB088474	Vg-320	vtgC			Japanese common goby	<i>Acanthogobius flavimanus</i>	3,714	1,238	Ohkubo et al. (2004)
20 AB081299	VgA	vtgAa			Japanese silago	<i>Sillago japonica</i>	5,103	1,701	Yoon et al. (2002)
21 AB181838	VgA	vtgAa			Red seabream	<i>Pagrus major</i>	5,145	1,715	Sawaguchi et al. (2006)
22 AB181839	VgB	vtgAb			Red seabream	<i>Pagrus major</i>	5,148	1,716	Sawaguchi et al. (2006)
23 AB181840	VgC	vtgC			Red seabream	<i>Pagrus major</i>	3,816	1,272	Sawaguchi et al. (2006)
24 ENSGACP00000012923	novel	vtgAa		Gasterosteiformes	3-spined stickleback	<i>Gasterosteus aculeatus</i>	4,962	1,654	Ensembl (2006)
25 ENSGACP00000012842	novel	vtgAb			3-spined stickleback	<i>Gasterosteus aculeatus</i>	2,952	984	Ensembl (2006)
26 ENSGACP00000012536	novel	vtgC			3-spined stickleback	<i>Gasterosteus aculeatus</i>	3,813	1,271	Ensembl (2006)
27 AB181835	VgA	vtgAa		Atheriniformes	Mosquitofish	<i>Gambusia affinis</i>	5,085	1,695	Sawaguchi et al. (2005)
28 AB181836	VgB	vtgAb			Mosquitofish	<i>Gambusia affinis</i>	5,025	1,675	Sawaguchi et al. (2005)
29 AB181837	VgC	vtgC			Mosquitofish	<i>Gambusia affinis</i>	3,726	1,242	Sawaguchi et al. (2005)
30 AB064320	OI-vit1	vtgAa		Belontiiformes	Medaka	<i>Oryzias latipes</i>	5,034	1,678	Arai et al. (2001); Fujiwara et al. (2002)
31 AB074891	vtgII	vtgAb			Medaka	<i>Oryzias latipes</i>	5,175	1,725	Murakami and Nakai (2003)
32 ENSORLP00000008173	novel	vtgC			Medaka	<i>Oryzias latipes</i>	3,663	1,221	Ensembl (2006)
33 AY279214	vtgI	vtgAa		Cyprinodontiformes	Mangrove killifish	<i>Kryptolebias marmoratus</i>	5,133	1,711	Kim et al. (2004)
34 U07055	vtgI	vtgAa			Common nummichog	<i>Fundulus heteroclitus</i>	5,112	1,704	LaFleur et al. (1995)
35 FHU70826	vtgII	vtgAb			Common nummichog	<i>Fundulus heteroclitus</i>	5,061	1,687	LaFleur et al. (1995)
36 AF284035	VtgA	vtgAa	Paracanthopterygii	Gadiformes	Haddock	<i>Melanogrammus aeglefinus</i>	4,995	1,665	Reith et al. (2001)
37 AF284034	VtgB	vtgAb			Haddock	<i>Melanogrammus aeglefinus</i>	4,929	1,643	Reith et al. (2001)
38 AF454748	VtgA	vtgAa	Protacanthopterygii	Salmoniformes	Chinook salmon	<i>Oncorhynchus tshawytscha</i>	804	268	Buisine et al. (2002)
39 AF454747	VtgA	vtgAa			Coho Salmon	<i>Oncorhynchus kisutch</i>	807	269	Buisine et al. (2002)
40 X92804	vtgI	vtgAa			Rainbow trout	<i>Oncorhynchus mykiss</i>	4,977	1,659	Mouchel et al. (1996)
41 AF454751	VtgA	vtgAa			Arctic charr	<i>Salvelinus alpinus</i>	810	270	Buisine et al. (2002)
42 AF454752	VtgA	vtgAa			Brook trout	<i>Salvelinus fontinalis</i>	807	269	Buisine et al. (2002)
43 AF454749	VtgB	vtgAsb			Atlantic salmon	<i>Salmo salar</i>	849	283	Buisine et al. (2002)
44 AF454750	VtgA	vtgAa			Brown trout	<i>Salmo trutta</i>	807	269	Buisine et al. (2002)
45 AF454745	VtgA	vtgAa			Whitefish	<i>Coregonus lavaretus</i>	807	269	Buisine et al. (2002)
46 AF454746	VtgB	vtgAsb			Whitefish	<i>Coregonus lavaretus</i>	807	269	Buisine et al. (2002)
47 AF454753	VtgA	vtgAa			Grayling	<i>Thymallus thymallus</i>	807	269	Buisine et al. (2002)
48 AF454754	VtgB	vtgAsb			Grayling	<i>Thymallus thymallus</i>	807	269	Buisine et al. (2002)
49 AF414432	vitellogenin	vtgAa1	Ostariophysi	Cypriniformes	Common carp	<i>Cyprinus carpio</i>	4,059	1,353	Chang et al. (2001); Lai et al. (2001)
50 AB106873	vitellogenin	vtgAa2			Common carp	<i>Cyprinus carpio</i>	4,872	1,624	Han et al. (2004)
51 AF130354	vtg	vtgAa1			Fathead Minnow	<i>Pimephales promelas</i>	4,017	1,339	Korte et al. (2000)
52 ENSDARP000000062792	apo-B100	apoB100			Zebrafish	<i>Danio rerio</i>	11,346	3,782	Ensembl (2006)
53 AF406784	vtg1	vtgAa1			Zebrafish	<i>Danio rerio</i>	4,086	1,362	Islinger et al. (2003)
54 ENSDARP00000018269	novel	vtgAa1			Zebrafish	<i>Danio rerio</i>	3,147	1,049	Ensembl (2006)
55 ENSDARP000000061222	novel	vtgAa1			Zebrafish	<i>Danio rerio</i>	4,977	1,659	Ensembl (2006)
56 ENSDARP000000044868	novel	vtgAa2			Zebrafish	<i>Danio rerio</i>	5,010	1,670	Ensembl (2006)
57 ENSDARP000000046417	novel	vtgAa2			Zebrafish	<i>Danio rerio</i>	5,022	1,674	Ensembl (2006)
58 ENSDARP000000061149	novel	vtgAa2			Zebrafish	<i>Danio rerio</i>	4,890	1,630	Ensembl (2006)
59 ENSDARP000000061160	novel	vtgAa2			Zebrafish	<i>Danio rerio</i>	5,061	1,687	Ensembl (2006)
60 ENSDARP000000061164	novel	vtgAa2			Zebrafish	<i>Danio rerio</i>	5,064	1,688	Ensembl (2006)
61 AF254638	vg3	vtgC			Zebrafish	<i>Danio rerio</i>	3,753	1,251	Wang et al. (2000)
62 AY775788	Vtg	vtgAe1	Elopomorpha	Anguilliformes	Japanese eel	<i>Anguilla japonica</i>	5,226	1,742	Wang and Lou (2006)
63 AY423445	VTG1	vtgAe2			Japanese eel	<i>Anguilla japonica</i>	5,202	1,734	Okumura et al. (2003); Mikawa et al. (2006)
64 AY423444	VTG2	vtgAe3			Japanese eel	<i>Anguilla japonica</i>	5,277	1,759	Okumura et al. (2003); Mikawa et al. (2006)
65 AB185334	vtg	vtgAe1			Conger eel	<i>Conger myriaster</i>	4,989	1,663	Mikawa et al. (2006)
66 U00455	vitellogenin	vtgAB	Chondrostei	Acipenseriformes	White Sturgeon	<i>Acipenser transmontanus</i>	5,031	1,677	Bidwell and Carlson (1995)
67 M88749	vitellogenin	vtgABCD	Hyperoartia	Petromyzontiformes	Silver lamprey	<i>Ichthyomyzon unicuspis</i>	5,469	1,823	Sharrock et al. (1992)
68 AB084783	vg	vtg	Mollusca	Ostreoida	Pacific oyster	<i>Crassostrea gigas</i>	4,749	1,583	Matsumoto et al. (2002)
69 AB179781	Vg	vtg	Anthozoa	Scleractinia	Galaxy Coral	<i>Galaxea fascicularis</i>	4,317	1,439	Hayakawa et al. (2004; 2006)

