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# Differential genome duplication and fish diversity

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## Contents



**Key words:** genome evolution, genetic diversity, comparative genomics, chromosomes, gene families, fishes

## Abstract

The duplication of genes and entire genomes are believed to be important mechanisms underlying morphological variation and functional innovation in the evolution of life and especially for the broad diversity observed in the speciation of fishes. How did these fish species and their genetic diversity arise? The occurrence of three rounds of genome duplication during vertebrate evolution might explain why many gene families are typically about half the size in land vertebrates as they are in fishes. However, mechanisms of genetic diversity in fish lineages need to be further explained. Here we propose that differential genome duplication of from two to six rounds occurred in different fish lines, offering new opportunities during the radiation of fish lineages. This model provides a fundamental basis for the understanding of their speciation, diversity and evolution.

## Introduction

Their bony skeletons and broad diversity of forms characterize teleost fishes. This group, which includes most common fishes, is distinct from the cartilaginous fishes (such as sharks, rays and skates) and is composed of more than 24,000 species accounting for more than half of extant vertebrate species. This is the most successful and diverse group of vertebrates, and displays remarkable variation in morphological and physiological adaptations. How did these fish species and their genetic diversity arise? The variable occurrence of genome duplications (polyploidy) during fish lineages in evolution would contribute largely to the diversity.

## Polyploidization: the common events in fish evolution

Polyploidy has long been recognized in vertebrates, especially in fish species. Some species of primitive fish, such as the paddlefish (*Polyodon spathula*), shovelnose sturgeon (*Scaphirhynchus plato*

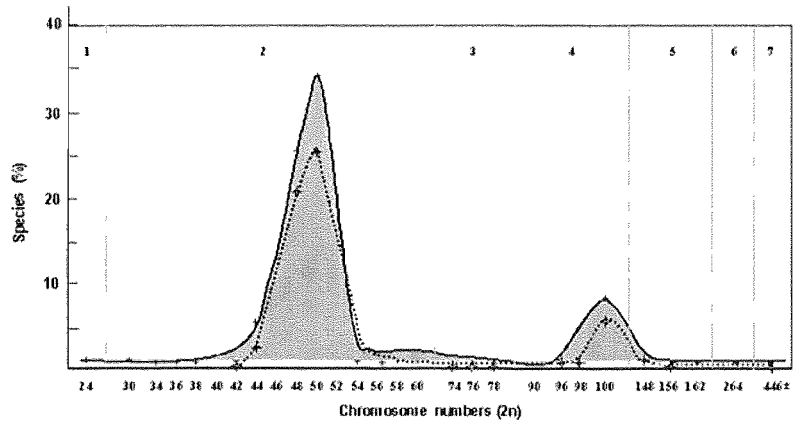
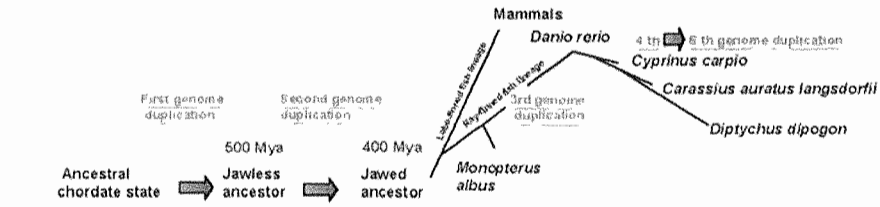



*Figure 1.* Distribution patterns of chromosome numbers of 235 species (Yu et al., 1989) of freshwater fishes (line) and of the order Cypriniformes (black dotted line). The first peak (Group 2) represents a diploid chromosome number of 50 and the second peak (Group 4) represents a chromosome number of 100. The cross symbol (+) indicates the percentage of the total number of species studied that have the corresponding chromosome number. Numbers across the top represent groupings based on potential genome duplications.

*rhynchus*), lungfish (*Protopterus dolloi*), and spotted gar (*Lepisosteus productus*) appear to be tetraploid in comparison to related groups (Dingerkus and Howell, 1976; Ohno et al., 1969; Vervoort, 1980). Recent tetraploids have been found in some species of cyprinds, live-bearing fishes, catostomids and salmonid fishes (Muramoto et al., 1968; Ohno et al., 1969). Many investigations of isozymes, such as lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and creatine kinase (CK), indicate duplicated genes in fishes (Markert et al., 1975; Ferris and Whitt, 1977a; 1979; Fisher et al., 1980; Allendorf and Thorgaard, 1984). Substantial homology between the duplicated genomes further supports the hypothesis that some tetraploid fishes arose when ancestral genomes were doubled through autopolyploidy (e.g., presence in extant salmonids of multivalents at meiosis), and while other tetraploid fishes such as catostomids (sucker species of North America) arose through allotetraploidy (Ferris and Whitt, 1980; Allendorf and Thorgaard, 1984). Furthermore, an extensive survey of chromosome patterns of the freshwater fishes in China (Yu et al., 1989) clearly shows that genome-doubling events gave rise to tetraploidy, and subsequent rounds of genome duplications appear to have occurred during the evolution of these lineages (Figure 1).

It is believed that duplication of genes and entire genomes are important mechanisms for enabling morphological and functional innovation in evolution (Ohno, 1970; Taylor et al., 2001; Wagner, 2001). Mammals generally have more (often four) copies of genes than do invertebrates which tend to have single copies of genes within given gene families. For example, there are generally four copies of the *Hox* gene clusters involved in patterning the anterior-posterior axis of vertebrates, whereas *Amphioxus* (an extant sister group to vertebrates) and invertebrates possess only a single copy. This supports the hypothesis that at least two rounds of genome duplication predated modern vertebrates during the evolution of vertebrates from early ancestors (the "one-to-four" model; Lundin, 1993; Garcia-Fernandez and Holland, 1994; Holland and Garcia-Fernandez, 1996; Holland, 1997; Aparicio et al., 1997; Skrabanek and Wolfe, 1998; Pebusque et al., 1998; Postlethwait et al., 1998; Prince et al., 1998; Ohno, 1999; Meyer and Schartl, 1999). The first round of genome duplication may have occurred shortly before the Cambrian explosion about 590 million years ago. The second genome duplication, dating to the Devonian more than about 440 million years ago, yielded a quadrupling of genome size in relation to ancestral chordates. After two rounds of genome duplication, the common ancestors of extant vertebrates, bony fishes, diverged to give rise to the lobe-finned fishes, from which mammals and other tetrapods evolved, and the ray-finned fishes, from which teleosts evolved (Figure 2).

Recent studies of *Hox* gene clusters of zebrafish (*Danio rerio*), pufferfish (*Takifugu rubripes*) and medaka (*Orizias latipes*) suggest that an additional genome duplication (the third genome duplication)




| | | | | | | |
|---|---|---|---|---|---|---|
| Chromosome numbers (2n) | | | 24 | 50 | 100 | >200 >400 |
| *Hox* cluster numbers | 2 | 4 | | 7 | | |
| Conserved blocks of the X chromosome | | | 2 | 4 | | |
| *Sox9* genes | | | 2 | 2 | | |
| *Dmrt* genes | | | 9 | | | |
| Haploid DNA content (pg) | | | 0.6-0.8 | 1.6-1.8 | >1.8 | |

*Figure 2.* A model of differential genome duplications for fish speciation and evolution is shown schematically. The diploid chromosome number (2N), numbers of *Hox* gene clusters, numbers of conserved blocks of the human X chromosome, numbers of *Sox9, Dmrt* genes, and the haploid DNA contents for each species or group are shown in the lower open block. Genome duplication events are shown underlined.

took place during the evolution of teleost fishes, around 360 million years ago (the "1-2-4-8" model) (Aparicio et al., 1997; Meyer and Schartl, 1999; Amores et al., 1998; Wittbrodt et al., 1998; Meyer and Malaga-Trillo, 1999; Kurosawa et al., 1999; Vogel, 1999; Aparicio, 2000; Naruse et al., 2000; Amores, A., Amemiya, C., and Postlethwait, J., personal communication). For example, at least 48 *Hox* genes have been observed in zebrafish, clustered in seven (with one lost) *Hox* gene complexes, while the number of *Hox* clusters in other vertebrates including mammals is four (Aparicio et al., 1997; Meyer and Schartl, 1999; Amores et al., 1998; Wittbrodt et al., 1998; Meyer and Malaga-Trillo, 1999; Kurosawa et al., 1999; Vogel, 1999; Aparicio, 2000; Naruse et al., 2000). Although it is believed that genome duplication occurred during vertebrate evolution and speciation, no substantial information exists on how many times the duplications occurred and which fish groups underwent additional genome duplications. Thus, questions surround the potential for a third round of genome duplication and the 1-2-4-8 model in fishes. Here we suggest that differential genome duplication of from two to six rounds in fish lineages contributed to the generation of fish diversity, and took place in fish lineages after the initial two rounds of duplication during vertebrate evolution. Differential genome duplication from before the third round of duplication to as much as a sixth round of duplication could have contributed to the genetic diversity of fishes and variable rates of speciation observed in different fish lineages.

## The differential genome duplication model (DGD)

Genome size has been of interest since species differences in DNA content were first studied in the late 1940s (Mirsky and Ris, 1951). Variation in genome size is considerable in fishes, ranging from less than 1 pg to more than 200 pg. The variation does not form a uniform distribution, nor is it random, but it can be evaluated at a number of levels (Wachtel and Tiersch, 1993) ranging across localized DNA (gene) duplications, chromosome polymorphisms (DNA aneuploidy such as heterochromatic blocks or extra arms), sex chromosome differences, chromosomal aneuploidy, and polyploidy (such as tetraploidy) produced through genome duplication. These mechanisms can work in combination and there can be losses as well gains in DNA content.

From the data available on comparative genome and gene analysis in fish (including the references in this review), we suggest that differential genome duplication (DGD) describes the hypothesis that there have been differing numbers of genome duplication events in different teleost lineages. These DGD events occurred after divergence from a common jawed ancestor more than 400 million years ago. Based on this model, some groups of fishes, such as those represented by the rice field eel, *Monopterus albus*, (diploid chromosome number of 24; Yu et al., 1989) retained a stable genome size (although individual gene duplications could occur), while other groups underwent a third genome duplication, such as in zebrafish and other species of the order Cypriniformes

(2N = 50). The fourth to sixth genome duplications took place for other groups some time afterwards (Figure 2). Certain groups, for example most species in the same Cyprininae (within the order Cypriniformes) have diploid chromosome numbers of 100 (Yu et al., 1989) indicating a fourth genome duplication, which was believed to constitute a tetraploid species. Moreover, a fifth duplication arose in other species of the Cyprininae (e.g., *Carassius auratus langsdorfii*; 2N = 206), and a sixth for other species within Cypriniformes (e.g., *Diptychus dipogon*, 2N = 446) took place afterwards. It is very rare for a vertebrate species to have more than 400 chromosomes. Genome doubling events would be a reasonable explanation for a genome with so many chromosomes. The duplicated genomes, yielding redundancy of genes, would initiate a process of functional diploidization and diversification of gene function followed by speciation. The duplicated genes could be lost, silenced, subfunctionalized or assume new functions. This loss or silencing of duplicated genes might be more important to the evolution of species diversity than the evolution of new functions in duplicated genes (Lynch and Conery, 2000; Lynch and Force, 2000). The duplicated genes and genomes could also supply genetic materials for fish evolution and speciation in diverse aquatic environments.

## Questions from the model

If the evidence from fishes supported an across-the-board third genome duplication (1-2-4-8), one would expect to find consistency in genome size (e.g., chromosome numbers, numbers of gene clusters) in modern teleost fishes. This is not so. For example, the diploid chromosome numbers of 235 species of freshwater fishes in China (Yu et al., 1989) ranged from 24 to 446 (Figure 1), and similar patterns of chromosome number were observed in all analyzed taxa (nine orders of fishes: Cypriniformes, Symbranchiformes, Perciformes, Scorpaeniformes, Atheriniformes, Siluriformes, Anguilliformes, Clupeiformes, Acipenseriformes; Yu et al., 1989). Overall, two peaks are evident from the distribution of chromosome numbers (Figure 1): one at 50 (Group 2) and another at 100 (Group 4). Many species of fish belong to Group 2, and if this peak resulted from the third genome duplication, the second peak (Group 4) would represent the fourth genome duplication. Moreover, for Group 7, represented by *Diptychus dipogon*, there would be at least six genome duplications predicted during their speciation. These results indicate that the timing of occurrence and number of genome duplications were variable among different fish lineages.

Fishes of Group 1, for example the rice field eel, have the fewest chromosomes, and may represent forms from before the third genome duplication. All chromosomes in this species are telocentric and the haploid genome size is among the smallest of vertebrates (0.6–0.8 pg). Variation in genome size is considerable in fishes, ranging from less than 1 pg to more than 200 pg (Wachtel and Tiersch, 1993; Yu et al., 1989; Li et al., 1983; Venkatesh et al., 2000). This suggests that chromosome fusion was not responsible for reducing the chromosome number, as fused chromosomes generally have two chromosome arms, and are not telocentric. The small genome size and low number of telocentric chromosomes suggest that the rice field eel would be a primitive species based on its genome. Additional evidence supporting the linkage of this group to early non-duplicated fish genomes comes from comparative chromosome painting (Yi et al., 2001). When the human X chromosome was used as a probe, two conserved X chromosome blocks were observed on the chromosomes of the rice field eel, which was two less than found in zebrafish (while the entire X chromosome was conserved in mammals). Based on these data, we favor the hypothesis that a third genome duplication did not occur during speciation of the rice field eel.

This raises the question of the origin of gene diversity in Group 1 fishes (such as the rice field eel) in the absence of genome duplication. Based on recent data, we infer that segmental (or individual) gene duplications were associated with speciation in Group 1 fishes. For example, *Sox9* (a member of the *SRY*-related family of genes) is required for cartilage formation. We identified two *Sox9* genes in rice field eel (GenBank access numbers: AF378150 and AF378151), and the same set is found in zebrafish (Chiang et al., 2001), while only one *Sox9* has been found in mammals and chicken (*Gallus gallus*). Furthermore, the two *Sox9* genes in rice field eel show similar expression patterns, and appear to be at an early stage of duplicate gene evolution, before their divergence. Moreover, we found nine members of the *Dmrt* (*D*oublesex and Mab-3 Related Transcription factor) family of genes belonging to five subfamilies in the rice field eel (GenBank access numbers: AF378149 and AF272954 to AF272961, Huang et al., 2002), in contrast to the five found in humans

(Ottolenghi et al., 2000; Marchand et al., 2000; Ottolenghi et al., 2002), which were grouped into four distinct subfamilies. The founding member of this gene family, *Dmrt1* is involved in sex determination and differentiation in vertebrates including humans. Moreover, we have just found multiple alternative splicing patterns that generate four different transcripts (*Dmrt1a* to *Dmrt1d*, GenBank accession numbers: AF421347 to AF421350) of the *Dmrt1* gene in the rice field eel. In all other vertebrates (e.g., mouse, human, and rainbow trout) there is only one transcript of the *Dmrt1* gene (Raymond et al., 1999; De Grandi et al., 2000; Marchand et al., 2000). This would enable diversification of gene function through segmental (or individual) gene duplications and alternative splicing in the absence of genome duplication. It is also possible that duplicated genes were an important force enabling successful speciation of Group 1 fishes by a mechanism called subfunctionalization of duplicated genes (Force et al., 1999).

Additional evidence to support the DGD hypothesis comes also from a recent study of 33 gene families of fishes (Robinson-Rechavi et al., 2001). The distribution of duplicated genes in different fish lineages was assessed as the number of gene families with duplication divided by the total number of gene families sampled for the lineage (Robinson-Rechavi et al. 2001). This yielded estimates of 41% within the Cypriniformes, 25% within Cyprinodontiformes, 11% within Siluriformes and 7% within Anguilliformes. Those groups with the largest genome sizes had the highest incidences of gene duplications. Interestingly, the duplication events of gene families were scarce in another kind of fish, the European eel (*Anguilla anguilla*).

It has been proposed that it is easier to create new genes by duplicating old ones than to create them *de novo*, and that genome duplication was a quick and easy way to produce vast numbers of duplicate genes (Ohno, 1970). Genome duplications in fish would provide genetic material to exploit different biochemical pathways. Following genome duplication, the chromosomal diploidization process would eventually transform the four homologs into two pairs of two, resulting in disomic inheritance and functional diploidization. The evolution and fates of the duplicated genes probably form a general pattern. At the early stages following gene duplication, gene structure and expression patterns are retained, for example, the *Sox9* genes of rice field eel. At this early stage, when the function of the duplicated gene is redundant, the silencing or loss of one of the gene copies can easily occur. Afterwards, there is a period of structural divergence brought about by the accumulation of mutations, which is characterized by variable expression of one or both of the duplicate copies. Eventually, propelled by the mutation process, one copy of the duplicated gene could lose its expression. Indeed, about only half of the potential duplicate gene expressions were observed in tetraploid fishes (Ferris and Whitt, 1977a, b; Fisher and Whitt, 1978). Final stage in the evolution of duplicate genes could appear when the original gene becomes independent in function to the newer additional locus. Accumulation of a number of these new genes may allow more rapid emergence of new species.

## Conclusions

Recent progress in understanding the evolution of vertebrate genomes has been rapid. The occurrence of three rounds of genome duplication in fish might explain why many gene families are typically about half the size in land vertebrates as they are in fishes. In addition, differential genome duplication offers another mechanism for addressing genetic diversity and variable rates of speciation and evolution in different fish lineages. This model is also useful to frame efficient schemes for conservation of fish breeds or species especially for endangered species of fishes, and to guide the development of new breeds for aquaculture by crossing among different species. This model can be tested as genetic information becomes available such as comparative gene mapping and chromosome painting to identify paralogous regions or chromosomes among distantly related species, and as additional DNA sequence information becomes available for more groups of fishes.

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