Gene and genome duplications in vertebrates: the one-to-four (-to-eight in fish) rule and the evolution of novel gene functions

Axel Meyer* and Manfred Schartl†

One important mechanism for functional innovation during evolution is the duplication of genes and entire genomes. Evidence is accumulating that during the evolution of vertebrates from early deuterostome ancestors entire genomes were duplicated through two rounds of duplications (the 'one-to-two-to-four' rule). The first genome duplication in chordate evolution might predate the Cambrian explosion. The second genome duplication possibly dates back to the early Devonian. Recent data suggest that later in the Devonian, the fish genome was duplicated for a third time to produce up to eight copies of the original deuterostome genome. This last duplication took place after the two major radiations of jawed vertebrate life, the ray-finned fish (Actinopterygia) and the sarcopterygian lineage, diverged. Therefore the sarcopterygian fish, which includes the coelacanth, lungfish and all land vertebrates such as amphibians, reptiles, birds and mammals, tend to have only half the number of genes compared with actinopterygian fish. Although many duplicated genes turned into pseudogenes, or even 'junk' DNA, many others evolved new functions particularly during development. The increased genetic complexity of fish might reflect their evolutionary success and diversity.

Addresses
*Department of Biology, University of Konstanz, 78457 Konstanz, Germany; e-mail: axel.meyer@uni-konstanz.de
†Physiologische Chemie I, Biozentrum der Julius-Maximilians Universität, Würzburg, 97074 Würzburg, Germany; e-mail: phchl@biozentrum.uni-wuerzburg.de

Current Opinion in Cell Biology 1999, **11**:699–704

0955-0674/99/$ – see front matter

Abbreviations

Dhh	Desert hedgehog
hh	hedgehog
Ihh	Indian hedgehog
Shh	Sonic hedgehog
Twhh	Tiggy-winkle hedgehog

Introduction

Duplication of genes and entire genomes are two of the major mechanisms that facilitated the increasing complexity of organisms in the evolution of life. Gene duplications might be responsible for the functional diversification of genes, the creation of gene families and the generally increased genomic, and possibly also phenotypic, complexity [1,2]. Protostomes, such as *Drosophila*, and deuterostome ancestors of vertebrates tend to have single copies of genes whereas chordate genomes typically have more genes, often four; the copies belong to the same gene family [3–7].

The most commonly used model to explain the evolution of the vertebrate genome is the 'one-two-four' (or 1-2-4) rule. It assumes that the genome underwent two rounds of duplication leading from a single ancestral deuterostome genome to two after the first duplication, and then to four genomes after the second genome duplication. Evidence in favor of the 1-2-4 hypothesis is the observation that genes from the same gene family are often arranged in linked clusters that maintain the same gene order on different chromosomes (e.g. see [8]). This synteny of gene clusters (the location of two genes within a linkage group but on different chromosomes) is often retained across large evolutionary distances, such as between fish, mice and humans. Clearly, this synteny could have also arisen by duplications of only portions of the entire genome (e.g. the chromosomes or parts of them) but it cannot be explained easily by many independent individual gene-duplication events.

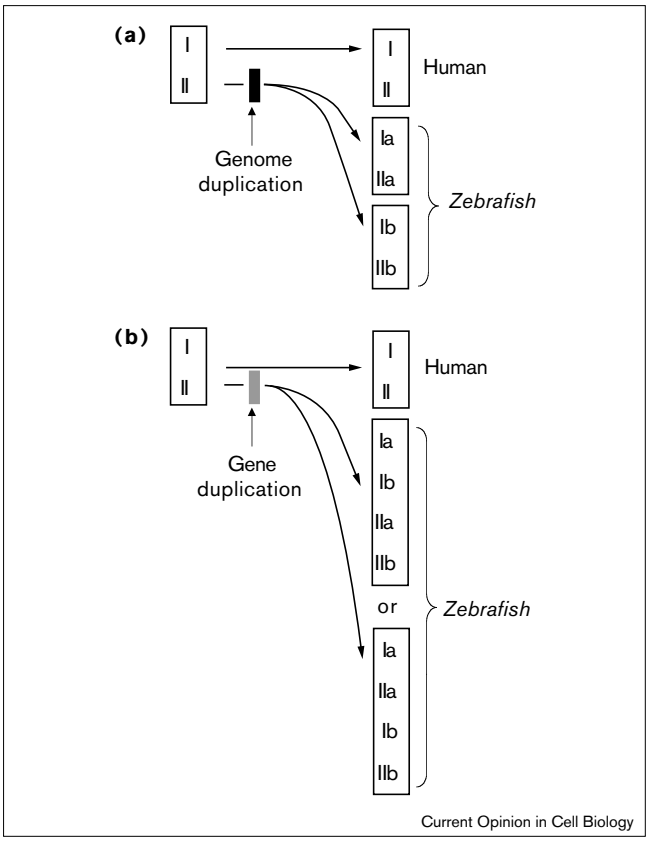
The evidence for the 1-2-4 model it not unequivocal, however, because not all gene families in vertebrates are made up of four genes [9–11]. Obviously, genes can also be lost during evolution. The simplest explanation for only three copies of genes in tetrapod gene families, rather than the expected number of four under the 1-2-4 model, is that one copy was lost after the second round of genome duplication. If only two genes in a gene family are found it might be explained by a second copy being lost after the first round of genome duplication, and the remaining one being duplicated in the second round of genome duplication. The arrangement of genes in clusters might add a particular selective advantage and hence, these gene orders could have arisen repeatedly and independently [10] rather than through simple duplication [11]. Moreover, phylogenetic analyses of the four genes do not always confirm the predicted topology of genes within gene families that would arise if the 1-2-4 hypothesis were correct [10,12,13].

Recent data [14,15••] suggest an additional entire genome duplication in the fish lineage, extending the 1-2-4 to a 1-2-4-8 rule. Here, we review some of the evidence in favor of the 1-2-4-8 hypothesis and suggest ways in which this model can be tested against alternatives such as the hypothesis involving several independent duplications.

Even more genes in fish?

All tetrapods, as well as the lungfish (lungfish also belong to the sarcopterygian lineage) [16], have only four *Hox*-gene clusters. Evidence is now accumulating that ray-finned fish (actinopterygia) have more genes than sarcopterygians [14,15••,17,18], although, there are examples (e.g. from odorant receptor genes [19,20]) of fishes with fewer copies of genes in some gene families. The most convincing support for the hypothesis that fish have more genes than tetrapods comes from work on the *Hox* gene clusters. Zebrafish (and data is forthcoming for other

Figure 1



The left box shows the gene order of two genes (I and II) on a chromosome in a common chordate ancestor of human and zebrafish. **(a)** The black bar represents the duplication of an entire genome, leading to an initial doubling of the number of chromosomes after duplication of the entire genome. Synteny of the genes on the duplicated chromosomes would be maintained. Later, the number of chromosomes would decrease to a modal number around the original number of chromosomes. In this reduced number of chromosomes, synteny relationships would tend to be maintained. **(b)** Depending on the mechanisms of gene duplication (gray bar) the relative positions of genes would change. If both genes I and II duplicate independently, their copies Ib and IIb would be arranged in a tandem array next to their progenitor genes Ia and IIa. If a portion of the chromosome that included both genes I and II were to duplicate, then gene Ia would continue to be located next to gene IIa and the duplicated gene Ib next to the duplicated gene IIb. In either case synteny relationships would have changed.

teleost fishes as well) have more (typically five to seven) than the expected number of four *Hox* gene clusters [14,15••]. But, in terms of numbers of chromosomes, most fishes have about the same number of chromosomes as mammals (e.g. zebrafish has 50, medaka 48, and human and mouse have 46).

Two mechanisms could account for the observation that fish have more genes than tetrapods: first, an additional (third) entire genome duplication taking place during the evolution of actinopterygian fish that other vertebrates did not experience — the 1-2-4-8 rule [15••,17,18]; second, several rounds of independent gene duplications along the lineage leading to fishes, but not along the sarcopterygian lineage, leading to an increase in the number of genes in the fish genome. The prediction of the first hypothesis would be that we would, on average, expect to find that gene families are twice as large in fishes than in tetrapods. The second mechanism would predict that some genes, gene families or chromosomes were duplicated, and hence that only some genes would be present in larger numbers in fishes than in mammals. If genome duplications occurred a long time ago their traces might be obliterated by the loss of genes, fusion of chromosomes and lineage-specific gene amplifications, rendering the distinction between the two competing explanations for the observation of more genes in fishes much more difficult.

One strong line of evidence for distinguishing between the mechanisms of gene duplication comes from comparative genomic analyses of gene order. During genome duplications, genes should retain their positions relative to each other — thus conserving their linkage and relative positions on the chromosome, that is their synteny (Figure 1a), but on duplicated chromosomes. However, if individual genes were duplicated one would expect to find the duplicated copies near, or next, to the original copy of the gene and at least initially on the same chromosome (Figure 1b). Single gene duplications would create tandem arrays of genes on the same chromosomes, or in the case of single chromosome duplications, a chromosome complement would result.

There are clear examples in fish of individual duplications that are, as expected, mapped onto chromosomes in tandem arrays and in closely linked gene clusters [21,22]. Although humans and zebrafish last shared a common ancestor about 380 million years ago, there are a growing number of studies [15••,23] that show extensive conserved synteny between zebrafish and human chromosomes. This evidence favors the 1-2-4-8 rule of additional genome duplication in fish because in the three gene pairs studied so far, where the mapping information is complete [23] the paralogous copies are linked on two pairs of chromosomes indicating synteny of each of the paralogous copies, as predicted by the scenario of complete genome duplications. The question is, once more orthologous genes have been mapped in both the zebrafish and mammals, will they still be most syntenic? The possibility that synteny arose independently three times over seems highly remote. We therefore suspect that a fish-specific genome duplication took place during the Devonian.

Not surprisingly, the number of genes in fish gene families is not always an even multiple of that in mammals (as would be expected from the third genome duplication hypothesis), because genes can be lost. Again, evidence for this comes from work on the *Hox* gene clusters in fish. A reconstruction of the phylogenetic timing and genomic architecture of the *Hox* gene clusters in fish would predict that a common ancestor of all gnathostome vertebrates

probably possessed more than 42 *Hox* genes arranged in four clusters [15••,18]. After the fish-specific genome duplication event the number of genes is likely to have increased to 84, in 8 *Hox* gene clusters. The observation that the zebrafish only has 47 *Hox* genes left in its 7-8 *Hox* clusters implies that it retained only 5 of these 42 additional genes. This phylogenetic reconstruction, coupled with additional results, show that most gene copies of the zebrafish (as well as the medaka) are quite divergent in function, which supporting the notion that the duplication event occurred in the ancient past. Given the antiquity of the actinopterygian group, and the different selective regimes that they might have been exposed to, we would expect to find that the numbers of genes and the genomic architecture (e.g. the morphology of the *Hox* gene clusters) among fish are highly diverse ([18]; Malaga-Trillo E, Meyer A, unpublished data).

To lose or not to lose?

Since the functions of genes are redundant after gene and genome duplications and a single copy of a gene is usually sufficient to perform its function, daughter copies of genes might accumulate detrimental mutations due to relaxed selection on one of the duplicates. It had long been assumed that one copy of the duplicated genes might almost always become a pseudogene, unrecognizable 'junk' DNA or nonfunctional ('silenced') relatively quickly, in the order of only a few million years at best, because of the potentially rapid accumulation of deleterious mutations [24]. Among other forces, several population-wide genetic factors might determine how quickly duplicated genes decay into non-functional genes [2,10,25,26,27•]. However, the rate of gene silencing is often much slower than predicted by most population genetic models and the functions of duplicated genes, despite the fact they are doubly covered, are often maintained for an unexpectedly long time.

Evidence for this retention of function comes from polyploid organisms. Genome duplications are initially increases in ploidy. Tetraploidy is widespread in salmonid fish, hence they often have four alleles per locus and twice the number of chromosomes compared with most other groups of fish. Increases in ploidy are also known in some, but not all, cypriniform fish — a group that also includes the (diploid) zebrafish. Comparative studies in the salmonid fish and the tetraploid frog *Xenopus laevis* showed that duplicated genes on duplicated chromosomes retain their function for a surprisingly long time — for 50 million years, and possibly even 100 million years. For example, in salmonids only about 50% of genes lost their function after 50 million years [28–31], instead of being lost they took on new functions and are retained rather than being turned into 'junk', which might be the more typical fate of duplicated genes after all. Evidence for this also comes from the first two rounds of genome duplications. The first round may have occurred shortly before the Cambrian explosion (about 590 million years ago) and the second genome duplication probably took place a surprisingly long time afterwards — up to 150 million years later [32]. If these estimates are correct, then the majority of genes persisted without being lost for the 150 million years in between these two genome duplication events. Even more interesting is that most genes appear to have survived since the second genome-duplication in the Devonian more than 440 million years ago.

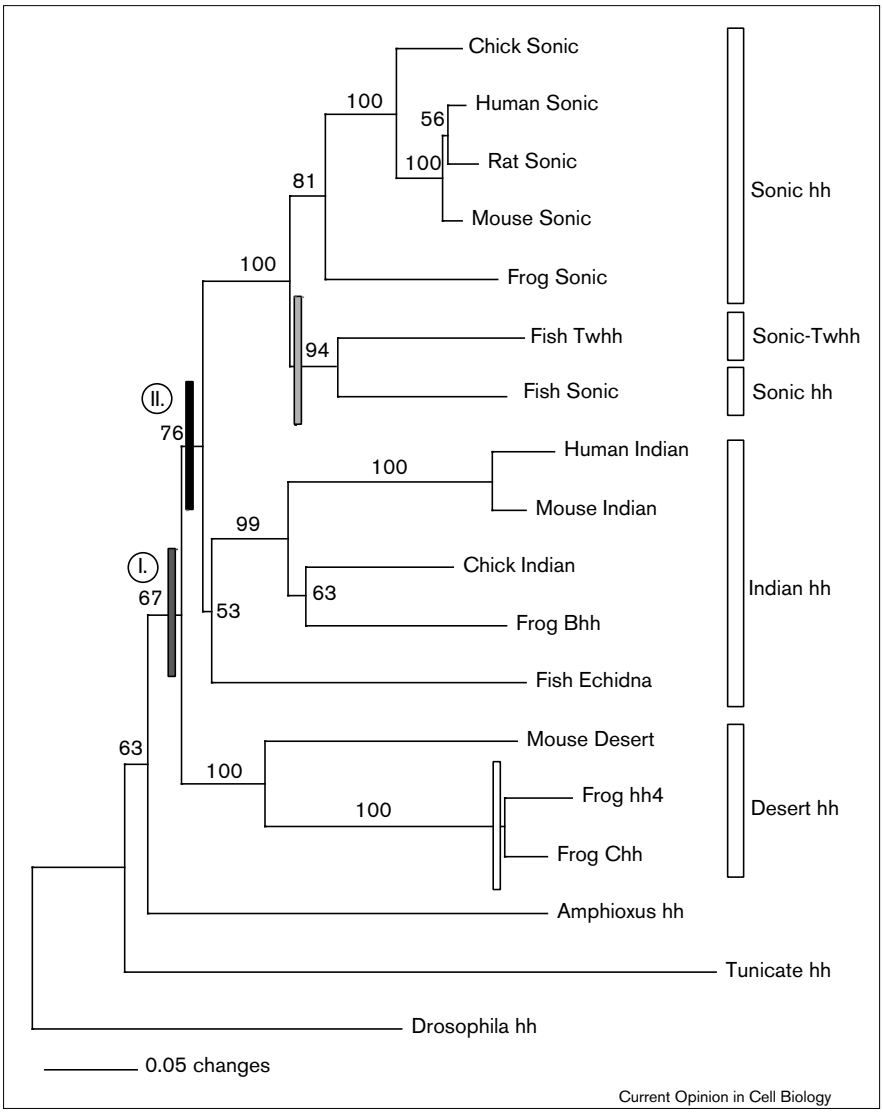
Other, indirect, evidence for the long evolutionary retention of genes, even if they are not continuously expressed or are present in duplicate, comes from phylogenetic comparison of phenotypes. For example, *Pax-6* is one of the major genes involved in the formation of the eye throughout metazoans (reviewed in [33]), but it retains its ability to produce eyes even in animals that do not have eyes. This might be due to the fact that many genes (at least developmental-control genes, which are often transcription factors) are multifunctional and participate in several genetic programs and signaling cascades simultaneously and therefore *Pax-6* may have been retained to carry out an unrelated function [26,34]. The retention of genetic programs may be a common evolutionary mechanism through which evolution can tinker with programs without having to reinvent them [35], by reusing 'tools' even if they have not been applied in a particular developmental context for a long time [34]. This multifunctionality of genes might also create a 'backup' copy and might be causally related to developmental stability [36,37].

Taking on new functions: the case of *hedgehog* genes

Duplicate genes might diversify and take on new functions rather quickly. This can occur through various forms of regulatory evolution such as divergence in expression patterns. If the new function confers a selective advantage then this might prevent the loss of genes through the accumulation of deleterious mutations (e.g. see [31]). Many evolutionary models, too numerous to be covered in detail here, for new functions of genes have been proposed. An interesting new one includes the 'duplication-degeneration-complementation model' [27•]. Other models of regulatory evolution often invoke changes in *cis* acting elements or genes [38–40].

Phylogeny reconstruction is a necessary tool for deciding if genes can be considered to be orthologous (and therefore be most informative in terms of studying the evolution and diversification of function of genes across different species) or merely paralogous copies, arising from some sort of duplication. Phylogenetic analyses of gene families provide insights into the evolutionary history not only of genes, but also of genomes and the organisms that contain them. But there are a number of caveats that need to be considered in this approach. In general, prior knowledge of the phylogenetic relationships among the organisms that are being compared is required in order to be able to correctly interpret the evolution of the function of the genes [12]. Phylogenetic

Figure 2



A phylogenetic tree of the family of hedgehog genes based on an amino acid alignment that included all members of the *hh* gene family in chordates. The phylogenetic analysis was conducted with 1000 bootstrap replications of the neighbor-joining method. *Drosophila* was used as outgroup. The circles denote the postulated phylogenetic timing of entire genome duplications during the evolution of chordates.

analyses of gene families are, of course, limited in their ability to explain gene evolution by the availability of sequences from all members of the gene family and the thoroughness of these genomic searches. Also, the completeness of the species sampling from model systems might influence the interpretation of that particular gene family's evolution. If gene/genome duplication predates speciation events then each of the particular portions of gene trees should reflect the evolutionary history of the organisms that contain those two gene copies (Figure 2).

Some of the proposed genomic events during the evolution of vertebrates can be illustrated by the imperfect example of the evolution of *hedgehog* (*hh*) genes. Three members of the *hh* gene family, which all perform different functions during development, are currently known in most vertebrates [41]. The topology (Figure 2) of the three major portions of the *hh* gene family tree, *Sonic* (*Shh*), *Indian* (*Ihh*) and *Desert* (*Dhh*), reflects three times over the phylogenetic relationships of the vertebrates from which those genes were sequenced: birds are more closely related to mammals and amphibians share a more recent common ancestor with birds or mammals than fish.

In the family of *hh* genes, the ancestral chordate condition (as exemplified in the lancelet, *Amphioxus*) was likely a single, possibly *Amphioxus-hh* like, copy of the *hh* gene [42•]. Then, before the origin of vertebrates (as far as is known, since the condition in hagfish and lamprey is not currently known) two rounds of genome duplications (dark gray and black bars, I and II, in Figure 2) occurred in relatively quick succession. This is also indicated by the internal branches being short (Figure 2). Since there are only three rather than the expected four copies of genes in this gene family, one *hh* copy (probably on the *Dhh* branch) may have been lost early on after the second genome duplication

[43,44]. Alternatively, only one complete genome duplication might have occurred, followed by a gene or chromosome duplication (dark gray bar, II, Figure 2) that led from ancestral gene to both *Shh* and *Ihh*. Note that the branches of the zebrafish *Twhh* and *Shh* genes are long, suggesting that the duplication (indicated by the light gray bar) that gave rise to the extra copy of a *Shh*-like gene, the *Twhh* gene, was probably ancient. These two genes also perform slightly different functions during development. This duplication (light gray bar in Figure 2) is likely to have been a fish-specific gene duplication rather than a genome duplication since *Twhh* genes have not been found yet in other vertebrates.

The *hh* example does not fully support the '1-2-4-8 hypothesis' since we would expect to find six *hh* gene family members in fish, but it might be able to serve as an example of what increases in ploidy might look like on a gene phylogeny tree. The remnants of this recent (notice the relative shortness of branches) increase in ploidy in frogs (typified by the tetraploid *Xenopus*) might be the presence of two frog *Dhh* genes, called *Banded* and *Cephalic hh*. However, one would have expected to have two copies of all *hh* genes in *Xenopus*. These other copies of *Shh* and *Ihh* in *Xenopus* have either not been found yet, or they were quickly lost after the polyploidization event. The zebrafish *Echidna* gene was shown to be identical to the zebrafish copy of the *Ihh* gene [41,42]. The *Echidna* gene (should better be called *Ihh*) of the zebrafish would be expected to be more like the *Ihh* genes of other vertebrates in function and sequence since they belong to that orthologous portion of the gene family.

Conclusions

It is too early to conclude with certainty what the role of genomewide duplications was in producing the architectures of vertebrate genomes. The answers will lie partly in the data collected in the current genome projects and particularly from the data from future genome projects on early deuterostomes. Because of financial and time constraints only a small number of model systems can be studied, but the evolutionary insights would be much clearer if genomic information was available for evolutionarily important chordates such as tunicates, cephalochordates and cyclostome fishes [45]. However, since all these lineages are more than 400 million years old a lot of changes have accumulated that make the reconstruction of genomic evolution difficult. Knowledge of the history and the mechanisms that shaped the evolution of the vertebrate genome might also help to better understand how new genes arise and how existing genes change their function. These data might also help to explain the relative importance of these genetic processes in canalizing, restricting or even permitting and shaping the phenotypic evolution of organisms. The genome duplication and the diversification of the fish lineage possibly coincided in time; fish, with 25,000 species or so, are by far the most evolutionarily successful group of vertebrates and this might suggest a cause-effect relationship between gene copy number and species diversity.

Acknowledgements

We thank Xun Gu for permitting us to cite unpublished data and Rick Grosberg and Yves Van de Peer for helpful comments on the manuscript. Support from grants of the U.S. National Science Foundation, the University of Konstanz to AM, the Deutsche Forschungsgemeinschaft, and the Fond der Chemischen Industrie to AM and MS, and the commission of the EU to MS, are gratefully acknowledged.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ohno S: *Evolution by Gene Duplication*. New York: Springer Verlag; 1970.

2. Sidow A: **Gen(om)e duplications in the evolution of early vertebrates.** *Curr Opin Genet Dev* 1996, **6**:715-722.

3. Holland PWH, Garcia-Fernandez JD: **Hox genes and chordate evolution.** *Dev Biol* 1996, **173**:382-395.

4. Sharman AC, Holland PWH: **Conservation, duplication, and divergence of developmental genes during chordate evolution.** *Neth J Zool* 1996, **46**:47-67.

5. Spring J: **Vertebrate evolution by interspecific hybridization - are we polyploid?** *FEBS Lett* 1997, **400**:2-8.

6. Ohno S: **The one-to-four rule and paralogues of sex-determing gene.** *Cell Mol Life Sc*i 1999, **55**:824-830.

7. Holland PWH: **Vertebrate evolution: something fishy about Hox genes.** *Curr Biol* 1997, **7**:570-572.

8. Pebusque MJ, Coulier F, Birnbaurm D, Pontarotti P: **Ancient large-scale genome duplications: phylogenetic linkage analyses shed light on chordate genome evolution.** *Mol Biol Evol* 1998, **15**:1145-1159.

9. Skrabanek L, Wolfe KH: **Eukaryote genome duplication - where's the evidence?** *Curr Opin Genet Dev* 1998, **8**:694-700.

10. Hughes AL: **Phylogenetic tests of the hypothesis of block duplication of homologous genes on human chromosomes 6, 9, and 1.** *Mol Biol Evol* 1998, **15**: 854-870.

11. Smith NGC, Knight R, Hurst LD: **Vertebrate genome evolution: a slow shuffle or a big bang?** *BioEssays* 1999, **21**:697-703.

12. Meyer A: **The evolution of body plans: HOM/*Hox* cluster evolution, model systems and the importance of phylogeny.** In *New Uses for New Phylogenies.* Edited by Harvey PH, Leigh Brown AJ, Maynard Smith J, Nee S. Oxford University Press: 1996:322-340.

13. Bailey WJ, Kim J, Wagner GP, Ruddle FH: **Phylogenetic reconstruction of vertebrate Hox cluster duplications.** *Mol Biol Evol* 1997, **14**:843-853.

14. Prince VE, Joly L, Ekker M, Ho RK: **Zebrafish Hox genes: genomic organisation and modified colinear expression patterns in the trunk.** *Development* 1998, **125**:407-420.

15. •• Amores A, Force A, Yan Y-L, Joly L, Amemiya C, Fritz A, Ho RK, Langeland J, Prince V, Wang Y-L, Westerfield M *et al.*: **Zebrafish Hox clusters and vertebrate genome evolution.** *Science* 1998, **282**:711-714.

This study demonstrates the existences of at least seven *Hox* gene clusters in the zebrafish and reconstructs an evolutionary scenario for the evolution of *Hox* gene clusters in vertebrates.

16. Longhurst TJ, Joss JMP: **Homeobox genes in the Australian lungfish, Neocerodus forsteri.** *Mol Dev Evol* 1999, **285**:140-145.

17. Wittbrodt J, Meyer A, Schartl M: **More genes in fish?** *Bioessays* 1998, **20**:511-515.

18. Meyer A, Málaga-Trillo E: **Vertebrate genomics: more fishy tales about *Hox* genes.** *Curr Biol* 1999, **9**:R210-R213.

19. Barth AL, Justice NJ, Ngai J: **Asynchromous onset of odorant receptor expression in the developing zebrafish olfactory system.** *Neuron* 1996, **16**:23-34.

20. Weth F, Nadler W, Korsching S: **Nested expression for odorant receptors in zebrafish olfactory epithelium.** *Proc Natl Acad Sci USA* **93**:13321-13326.

21. Frönzler D, Altschmied J, Nanda I, Kollo R, Baudler M, Schmid M, Schartl M: **The *Xmrk* oncogene promoter is derived from a novel amplified locus of unusual organiszation.** *Genome Res* 1996, **6**:102-113.

22. Adam D, Dimitrijevic, Schartl M: **Tumor suppression in *Xiphophorus* by an accidentall acquired promoter.** *Science* 1993, **259**:816-819.

23. Gates MA, Kim L, Egan ES, Cardozo T, Sirotkin HI, Dougan ST, Lashkari D, Abagyan R, Schier AF, Talbot WS: **A genetic linkage map for zebrafish: Comparative analysis and localization of genes and expressed sequences.** *Genome Res* 1999, **9**:334-347.

24. Nei M: **Gene duplication and nucleotide substitution in evolution.** *Nature* 1969, **221**:40-42.

25. Li W-H: **Rate of gene silencing at duplicate loci: a theoretical study and interpretation of data from tetraploid fishes.** *Genetics* 1980, **95**:722-731.

26. Wagner A: **The fate of duplicated genes: loss or new function?** *Bioessays* 1998, **20**:785-788.

27. • Force Am Lynsch M, Picketrt FB, Amores A, Yan Y-L, Postleweith J: **Preservation of duplicate genes by complementary, degenerative muations.** *Genetics* 1999, **151**:1531-1545.

This paper presents an innovative model of diversification of gene function after duplication.

28. Allendorf FW, Utter FM, May BP: **Gene duplication within the family Salmonidae. II. Detection and determination of the genetic control of duplicate loci through inheritance studies and the examination of populations.** In *Isozymes Genetics and Evolution*. Edited by Market CL. New York: Academic Press: 1975:415-432.

29. Bailey GS, Poulter RTM, Stockwell PA: **Gene duplication in tetraploid fish: a model for silencing at unlinked duplicated loci.** *Proc Natl Acad Sci USA* 1978, **75**:5575-5579.

30. Ferris, SD, Whitt GS: **Evolution of differential regulation of duplicate genes after polyploidization.** *J Mol Evol* 1979, **12**:267-317.

31. Hughes MK, Hughes AL: **Evolution of duplicate genes in a tetraploid animal, *Xenopus laevis*.** *Mol Biol Evol* 1993, **10**:1360-1369.

32. Wang Y, Gu X: **Dating the two-round genome duplications in early vertebrates**. *Trends Genet* 1999, 'in press.'

33. Treisman JE: **A conserved blueprint for the eye?** *Bioessays* 1999, **21**:843-850.

34. Meyer A: **Homology and homoplasy: the retention of genetic programmes.** In *Homology*. Edited by G Bock. Chichester: Wiley & Son; 1999:141-157.

35. Jacob F: **Evolution and tinkering.** *Science* 1977, **196**:1161-1166.

36. Wilkens AS: **Canalization: a molecular genetic perspective.** *Bioessays* 1997, **19**:257-262.

37. Cooke J, Nowak MA, Boerlijst M, Maynard-Smith J: **Evolutionary origins and maintenance of redundant gene expression during metazoan development.** *Trends Genet* 1997, **13**:360-364.

38. Davidson EH: In *Gene Activity in Early Development*. edn 3. Orlando Florida: Academic Press; 1986.

39. Davidson EH, Ruckun G: **Themes from a NASA workshop on gene regulatory processes in development and evolution.** *J Exp Zool* 1999, **285**:104-115.

40. Palopoli MF, Patel NH: **Neo-Darwinian developmental evolution: can we bridge the gap between pattern and process?** *Curr Opin Genet Dev* 1996, **6**:502-508.

41. Hammerschmid M: **The world according to hedgehog.** *Trends Genetics* 1997, **13**:14-21.

42. • Shimeld SM: **The evolution of the hedgehog gene family in chordates: insights from amphioxus hedgehog.** *Dev Genes Evol* 1999, **209**:40-47.

In this paper the author reconstructs the evolution of *hh* genes in chordates. The diversification of functions in paralogous copies of hedgehog genes that arose through gene and genome duplication during the evolution of chordates is described.

43. Zardoya R, Abeiheif E, Meyer A: **Evolutionary analyses of *hedgehog* and *Hoxd*-10 genes in fish species closely related to the zebrafish.** *Proc Natl Acad Sci USA* 1996, **93**:13036-13041.

44. Zardoya R, Abeiheif E, Meyer A: **Evolution and orthology of *hedgehog* genes.** *Trends in Genet* 1996, **12**:496-497.

45. Ruddle FH: **Vertebrate genome evolution - a decade ahead.** *Genomics* 1997, **46**:171-173.