

C. elegans germline-specific transcripts which was proposed to be a consequence of X-chromosome condensation in the male germline and/or X-chromosome dosage compensation in hermaphrodites¹⁶.

Another striking observation, made by Gönczy *et al.*, is the similarity of RNAi phenotype for genes with a particular cell biological function². For example, RNAi for all tested genes that encode components of the translation machinery disrupted the female meiotic divisions, and RNAi for genes encoding proteins involved in DNA replication caused a delay in pronuclear envelope breakdown in the one-cell embryo. With hindsight, such similarity of phenotype is perhaps not so surprising, but it could be of great value, particularly in suggesting specific functions for novel genes.

With these four studies^{1–4}, a third of the genes in the *C. elegans* genome have been subjected to functional analysis by RNAi. In the future, the rest of the genome will be similarly analysed, and selected RNAi phenotypes will be more carefully characterized. The RNAi results should make it possible to link half of the genes, identified by forward genetics, rapidly to the molecular data. The bacteria expressing specific dsRNAs (Ref. 1), effectively an RNAi library, generated by the Ahringer group, will be a particularly valuable resource because of the ease with which they allow specific gene activity to

be disrupted. Worms grown on these bacteria will allow screens for more subtle or conditional phenotypes, and, although mixed feeding reduces RNAi potency¹⁵, might allow identification of synergistic and epistatic interactions.

For the first time, functional genomics provides a global biological image of a multicellular organism, even if noise and resolution initially limit the quality of the picture. Fine-scale tuning will eventually improve the image, but the original picture, for the metazoa, will have been generated with broad-cast, dsRNA-mediated interference.

Acknowledgements

I.A.H. is currently supported with a Royal Society Leverhulme Trust Senior Research Fellowship. I thank Pierre Gönczy and Julie Ahringer for comments on the manuscript and Tony Hyman for permission to assemble the figure.

References

- 1 Fraser, A.G. *et al.* (2000) Functional genomic analysis of *C. elegans* chromosome I by systematic RNA interference. *Nature* 408, 325–330
- 2 Gönczy, P. *et al.* (2000) Functional genomic analysis of cell division in *C. elegans* using RNAi of genes on chromosome III. *Nature* 408, 331–335
- 3 Piano, F. *et al.* (2000) RNAi analysis of genes expressed in the ovary of *Caenorhabditis elegans*. *Curr. Biol.* 10, 1619–1622
- 4 Maeda, I. *et al.* (2001) Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. *Curr. Biol.* 11, 171–176

- 5 The *C. elegans* sequencing consortium (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282, 2012–2018
- 6 Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71–94
- 7 Kenyon, C. (1988) The nematode *C. elegans*. *Science* 240, 1448–1452
- 8 Hodgkin, J. *et al.* (1995) The nematode *Caenorhabditis elegans* and its genome. *Science* 270, 410–414
- 9 Zwaal, R.R. *et al.* (1993) Target-selected gene inactivation in *Caenorhabditis elegans* by using a frozen transposon insertion mutant bank. *Proc. Natl. Acad. Sci. U. S. A.* 90, 7431–7435
- 10 Jansen, G. *et al.* (1997) Reverse genetics by chemical mutagenesis in *Caenorhabditis elegans*. *Nat. Genet.* 17, 119–121
- 11 Fire, A. *et al.* (1998) Potent and specific genetic interference by double stranded RNA in *C. elegans*. *Nature* 391, 806–811
- 12 Domeier, M.E. *et al.* (2000) A link between RNA interference and nonsense-mediated decay in *Caenorhabditis elegans*. *Science* 289, 1928–1930
- 13 Tabara, H. *et al.* (1998) RNAi in *C. elegans* – soaking in the genome sequence. *Science* 282, 430–431
- 14 Timmons, L. and Fire, A. (1998) Specific interference by ingested dsRNA. *Nature* 395, 854
- 15 Kamath, R.S. *et al.* (2000) Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *Genome Biol.* 2, 1–10
- 16 Reinke, V. *et al.* (2000) A global profile of germline gene expression in *C. elegans*. *Mol. Cell* 6, 605–616

I.A. Hope

School of Biology, University of Leeds, Leeds, UK LS2 9JT.
e-mail: i.a.hope@leeds.ac.uk

Genome duplication, divergent resolution and speciation

John S. Taylor, Yves Van de Peer and Axel Meyer

What are the evolutionary consequences of gene duplication? One answer is speciation, according to a model initially called Reciprocal Silencing and recently expanded and renamed Divergent Resolution. This model shows how the loss of different copies of a duplicated gene in allopatric populations (divergent resolution) can promote speciation by genetically isolating these populations should they become reunited. Genome duplication events produce thousands of duplicated genes. Therefore, lineages with a history of genome duplication might have been especially prone to speciation via divergent resolution.

Genome duplications have been credited, albeit controversially, with providing the

genetic raw material necessary for major transitions in evolution, including the evolution of multicellularity, bilateral symmetry and the evolution of vertebrates^{1–5}. The controversy stems largely from the results of phylogenetic studies that raise doubts about the timing of certain genome duplication events relative to major transitions and show that many gene trees do not have the topology predicted by genome duplication hypotheses^{6–10}. Despite doubts over the origins of duplicated genes, the mechanistic link between gene duplication and evolutionary transitions has been less contentious. Although there are only a few examples of duplicated genes evolving completely new functions^{11–13}, Ohno's proposition that redundant genes

produced during large-scale gene duplication events can evolve previously nonexistent functions important for the evolution of phenotypic 'complexity' has become well entrenched in the literature.

Recently, several studies have reported evidence for a complete genome duplication in the ancestor of actinopterygian fishes (ray-finned fishes)^{14–22}. Two distantly related actinopterygian species, zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*), possess seven unlinked Hox gene clusters^{14,18}. In Sarcopterygii (the sister group to Actinopterygia that includes lobe-finned fish, amphibians, reptiles and mammals) there appear to be only four unlinked Hox gene clusters. Thus, a genome duplication event in the ancestor of ray-finned fishes

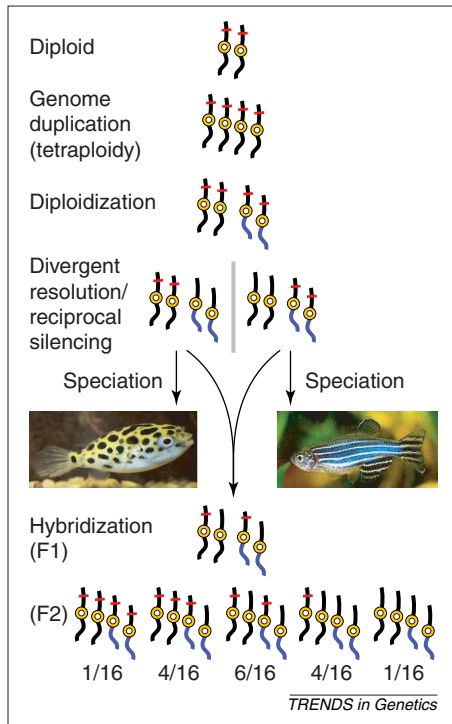


Fig. 1. Speciation through genome duplication and divergent resolution. The hypothesis that natural selection will favour speciation over hybridization in populations fixed for different unlinked copies of a duplicated locus. Red bars represent a locus duplicated (along with all other loci) during a tetraploidy event. In this hypothetical example, diploidization is driven by a reciprocal translocation depicted by a change in chromatid shape and colour. A pufferfish (left) and a zebrafish (right) are shown as examples of the descendants of the two populations. It will be possible to test the hypothesis that divergent resolution has occurred in ray-finned fish when the genome sequencing projects for these two species are completed. Hybridization of populations fixed for different copies of a duplicated locus produces heterozygous individuals possessing a functional allele and a pseudogene at each locus of the duplicated gene. Crosses between these F1 individuals produce F2 individuals with between zero and four alleles in the proportions shown.

could explain the observation that zebrafish and medaka have almost twice as many Hox clusters as, for example, humans. Additional evidence favouring the fish-specific genome duplication hypothesis comes from comparative mapping studies, which have identified a large number of mammalian genes with two zebrafish 'co-orthologs'^{19–21}. Furthermore, most of these zebrafish co-orthologs are related in the way predicted by the fish-specific genome duplication hypothesis²². Amores *et al.*¹⁴ proposed that this fish-specific genome duplication event provided gene copies that helped spur the teleost radiation, and Meyer and Schartl¹⁷ suggested that there might be a cause–effect relationship between gene copy number and species diversity. This hypothesis, that new genes produced during a genome duplication facilitated the teleost radiation, mirrors

Ohno's proposal that 'big leaps' in evolution required the creation of new gene loci with previously nonexistent functions¹. However, instead of promoting speciation, the fate awaiting most duplicated genes appears to be loss or silencing due to degenerative mutations in coding and/or regulatory regions^{23–26}.

Lynch and Conery²⁶ and Lynch and Force²⁷ have recently presented a model that suggests that 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 Conery²⁶ described how the loss of different copies of a duplicated gene in geographically separated populations could genetically isolate these populations, should they become reunited (Fig. 1). Their model is as follows. Consider individuals from populations that are fixed for different unlinked copies of a duplicated gene. If these individuals mate, their 'hybrid' progeny would be heterozygous, possessing a functional allele and a pseudogene at each locus of the duplicated gene. Crosses between the F1 individuals produce some F2 individuals with only pseudogenes at both loci in question (~6%). The remaining F2 progeny would receive between one and four functional alleles at these loci. The loss of different duplicates in different populations is called 'divergent resolution', and Lynch and Conery stated that with 'tens to hundreds' of gene duplicates present in most eukaryotic genomes, divergent resolution would result in the passive build-up of reproductive isolation.

Lynch and Force²⁷ expanded this model of speciation by divergent resolution by pointing out that for 'haploinsufficient' loci, the consequence of inheriting one functional allele can be as severe as inheriting only pseudogenes (haploinsufficiency occurs when the gene product from one functional allele is inadequate to support normal function). Lynch and Force also brought together 'subfunctionalization' and divergent resolution. Subfunctionalization occurs when duplicated genes divide the functions of their single ancestral gene²⁸. Unless the subdivision of expression by duplicate genes follows an identical pattern in different populations, hybrids will lack one or more subfunctions even in the absence of gene loss²⁷. Lastly, Lynch and Force make the connection between divergent resolution and genome duplication events. Divergent resolution of the thousands to

tens of thousands of genes and regulatory regions produced by a genome duplication event could be a much more effective isolating mechanism than the one involving independently duplicated genes originally described by Lynch and Conery.

'Lynch and Conery²⁶ and Lynch and Force²⁷ ... [suggest] that 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.'

Data from the family Salmonidae are consistent with this hypothesis that reproductive isolation via divergent resolution following genome duplication can lead to speciation. Salmonid fishes (e.g. salmon, trout, whitefish and grayling) evolved from a tetraploid ancestor and are in various stages of diploidization^{29,30}. This is important because divergent resolution following genome duplication genetically isolates populations only when the genes involved occur on chromosomes that have re-established disomic inheritance. As predicted by the hypothesis that tetraploidy promotes speciation, the Salmonidae includes many more species (c. 70) than its diploid sister taxon, Osmeridae (smelt and their relatives), which contains only about ten species³¹. The hypothesis that divergent resolution has played a role in salmonid speciation is consistent with the results of isozyme studies. These demonstrate that in this family the number of duplicated loci retained varies among populations and among species^{32,33}.

Ferris *et al.*³⁴ provided different but equally intriguing data consistent with the hypothesis that divergent resolution leads to speciation in the fish family Catostomidae. Like salmonids, catostomids (suckers) evolved from a tetraploid ancestor³⁴. Within this family the loss of expression of duplicated genes appears to be correlated with speciation – the clades within Catostomidae containing the most species are those clades with the most gene silencing³⁴. Ferris *et al.* suggested that gene loss might have been enhanced by reductions in population size accompanying speciation events; that is, the speciation process might have caused duplicate gene silencing. However, an alternative interpretation of the data is that gene loss (i.e. divergent resolution) was at least partially responsible for speciation in these fishes.

Many examples of variation in locus copy number among tetraploid populations can also be found in the plant literature^{35–37}. *Asplenium bradleyi* is a tetraploid fern formed by the hybridization of *A. platyneuron* and *A. montanum*, which are both diploid³⁶. Comparisons of isozyme expression patterns in these species showed that one of the *A. bradleyi* populations had lost the isocitrate dehydrogenase (IDH) locus that came from *A. montanum*³⁶. Bryan and Soltis³⁷ surveyed diploid, triploid and tetraploid populations of another fern species, *Polypodium virginianum*. Diploid populations all possessed a single leucine aminopeptidase (LAP) locus. Thirteen triploid or tetraploid populations possessed two LAP loci. However, in one tetraploid population, only the Lap-1 locus was observed and in a second allopatric tetraploid population, only Lap-2 was observed. From these isozyme studies in ferns, Werth and Windham³⁸ developed a model that is very similar to the divergent resolution model, in which 'reciprocal silencing' (the loss of different copies of duplicated genes in allopatric populations) would promote speciation.

Comparisons between tetraploid taxa and their diploid sister groups can determine if the association between genome duplication and speciation that we see in Paracanthopterygii (the group that includes salmonids and osmerids) is widespread. Some 50–70% of angiosperms appear to have experienced genome doubling³⁹, therefore it might be especially prudent to survey this group for a phylogenetic link between polyploidy and speciation. Genome sequencing projects will allow more extensive testing of the hypothesis that divergent resolution follows genome duplication. Ray-finned fish and flowering plants (Magnoliophyta) are both examples of rapid radiations, and polyploidy occurs at the base of both lineages^{14–21,39–41}. To find examples of divergent resolution in these groups it will be necessary to identify paralogous regions within genomes and orthologous regions between genomes for distantly related species within each group. The pufferfish (*Fugu rubripes*), zebrafish (*Danio rerio*), *Arabidopsis* and rice (*Oryza sativa*) sequencing projects will facilitate this task.

Acknowledgements

J.S.T. is supported by a postdoctoral fellowship from NERC (Canada). The

authors acknowledge support from the Deutsche Forschungsgemeinschaft and the University of Konstanz. A.M. thanks the Joint Genome Institute, Walnut Creek, USA, for their hospitality during his sabbatical stay there, while this manuscript was prepared. We thank Gerrit Begemann, Felix Breden and Henner Brinkmann; also Tancred Frickey, Joel Shore and Charles Werth for discussion and comments. This manuscript also benefited from the comments of three anonymous referees.

References

- Ohno, S. (1970) *Evolution by Gene Duplication*, Springer-Verlag
- Lundin, L.-G. (1999) Gene duplications in early metazoan evolution. *Cell Dev. Biol.* 10, 523–530
- Sidow, A. (1996) Gen(om)e duplications in the evolution of early vertebrates. *Curr. Opin. Genet. Dev.* 6, 715–722
- Holland, P.W.H. (1999) The effect of gene duplication on homology. In *Homology* (Bock, G.R. and Cardew, G., eds), pp. 226–242, Wiley
- Patel, N.H. and Prince, V.E. (2000) Beyond the Hox complex. *Genome Biol.* 1, 1027.1–1027.4
- Hughes, A.L. (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
- Wang, Y. and Gu, X. (2000) Evolution patterns of gene families generated in the early stages of vertebrates. *J. Mol. Evol.* 51, 88–96
- Martin, A.P. (1999) Increasing genomic complexity by gene duplication and the origin of vertebrates. *Am. Nat.* 154, 111–128
- Rivinsky, I. et al. (2001) Phylogenetic analysis of T-Box genes demonstrates the importance of *Amphioxus* for understanding evolution of the vertebrate genome. *Genetics* 156, 1249–1257
- Friedman, R. and Hughes, A. (2001) Gene duplication and the structure of eukaryotic genomes. *Genome Res.* 11, 373–381
- Cheng, C.-H.C. and Chen, L. (1999) Evolution of an antifreeze glycoprotein. *Nature* 401, 443–444
- Dulai, K.S. et al. (1999) The evolution of trichromatic colour vision by opsin gene duplication in New World and Old World primates. *Genome Res.* 9, 629–638
- Manzanares, M. et al. (2000) Conservation and elaboration of Hox gene regulation during evolution of the vertebrate head. *Nature* 408, 854–857
- Amores, A. et al. (1998) Zebrafish hox clusters and vertebrate genome evolution. *Science* 282, 1711–1714
- Wittbrodt, J. et al. (1998) More genes in fish? *BioEssays* 20, 511–512
- Meyer, A. and Málaga-Trillo, E. (1999) Vertebrate genomics: More fishy tales about Hox genes. *Curr. Biol.* 9, R210–R213
- Meyer, A. and Schartl, M. (1999) Gene and genome duplications in vertebrates: the one-to-four (to-eight in fish) rule and the evolution of novel gene functions. *Curr. Opin. Cell Biol.* 11, 699–704
- Naruse, K. et al. (2000) A detailed linkage map of Medaka, *Oryzias latipes*: Comparative genomics and genome evolution. *Genetics* 154, 1773–1784
- Gates, M.A. et al. (1999) A genetic linkage map for zebrafish: comparative analysis and localization of genes and expressed sequences. *Genome Res.* 9, 334–347
- Postlethwait, J.H. et al. (2000) Zebrafish comparative genomics and the origins of vertebrate chromosomes. *Genome Res.* 10, 1890–1902
- Woods, I.G. et al. (2000) A comparative map of the zebrafish genome. *Genome Res.* 10, 1903–1914
- Taylor, J.S. et al. Comparative genomics provides evidence for an ancient genome duplication event in fish. *Philos. Trans. R. Soc. London Ser. B* (in press).
- Nei, M. (1969) Gene duplication and nucleotide substitution in evolution. *Nature* 221, 40–42
- Allendorf, F.W. (1978) Protein polymorphism and the rate of loss of duplicate gene expression. *Nature* 272, 76–78
- Li, W.-H. (1980) Rate of gene silencing at duplicated loci: a theoretical study and interpretation of data from tetraploid fishes. *Genetics* 95, 237–258
- Lynch, M. and Conery, J.S. (2000) The evolutionary fate and consequences of duplicate genes. *Science* 290, 1151–1155
- Lynch, M. and Force, A.G. (2000) The origin of interspecific genomic incompatibility via gene duplication. *Am. Nat.* 156, 590–605
- Force, A. et al. (1999) The preservation of duplicate genes by complementary, degenerative mutations. *Genetics* 151, 1531–1545
- Hartley, S.E. (1987) The chromosomes of salmonid fishes. *Biol. Rev.* 62, 197–214
- Bailey, G.S. et al. (1978) Gene duplication in tetraploid fish: Model for gene silencing at unlinked duplicated loci. *Proc. Natl. Acad. Sci. U. S. A.* 75, 5575–5579
- Nelson, J.S. (1984) *Fishes of the World* (2nd edn), Wiley
- Allendorf, F.W. et al. (1984) Silencing of duplicate genes: A null allele polymorphism of lactate dehydrogenase in brown trout (*Salmo trutta*). *Mol. Biol. Evol.* 1, 238–248
- May, B. et al. (1975) Biochemical genetic variation in pink and chum salmon. *J. Hered.* 66, 227–232
- Ferris, S.D. et al. (1979) The roles of speciation and divergence time in the loss of duplicate gene expression. *Theor. Popul. Biol.* 15, 114–139
- Wilson, H.D. et al. (1983) Loss of duplicate gene expression in tetraploid *Chenopodium*. *Biochem. Syst. Ecol.* 11, 7–13
- Werth, C.R. et al. (1985) Recurring origins of allopolyploid species in *Asplenium*. *Science* 228, 731–733
- Bryan, F.A. and Soltis, D.E. (1987) Electrophoretic evidence for allopolyploidy in the fern *Polypodium virginianum*. *Syst. Bot.* 12, 553–561
- Werth, C.R. and Windham, M.D. (1991) A model for divergent, allopatric speciation of polyploid pteridophytes resulting from silencing of duplicate-gene expression. *Am. Nat.* 137, 515–526
- Wendel, J. (2000) Genome evolution in polyploids. *Plant Mol. Biol.* 42, 225–249
- Ku, H.-M. et al. (2000) Comparing sequenced segments of the tomato and *Arabidopsis* genomes: Large-scale duplication followed by selective gene loss creates a network of synteny. *Proc. Natl. Acad. Sci. U. S. A.* 97, 9121–9126
- Poethig, R.S. (2001) Life with 25,000 genes. *Genome Res.* 11, 313–316

J.S. Taylor*

Y. Van de Peer

A. Meyer

Dept of Biology, University of Konstanz,
78457 Konstanz, Germany.

*e-mail: john.taylor@uni-konstanz.de