annually degasses about 1 gigatonne (10^9 tonnes) of CO_2 (ref. 3), equivalent to 20 per cent of current anthropogenic output. The regression lines and intercepts of dissolved inorganic carbon, silicate and nitrate concentrations, measured in the upper 200 m of the EUZ, indicate that silicate availability regulates both carbon and nitrate uptake and fate. The slope of the highly significant regression between nitrate and silicate is 1, which coincides with the known requirement of these two elements by diatoms. The intercept represents the excess nitrate (about 4 mmol m^{-3}) that confers HNLC status on the EUZ.

Silicate is bound in mineral shells (biogenic silica) of no food value. In the EUZ model, the diatom shells are packaged in zooplankton faeces and exported wholesale from the surface layer. In contrast, nitrogen is selectively retained by the diatom grazers which, in the course of their metabolism, excrete ammonia; in turn the ammonia is assimilated by the photosynthesizing cyanobacteria (<2 μm) and small flagellates (2–10 μm) of the 'microbial loop'. This ubiquitous community (pico- and nanoplankton) is additionally composed of small protozoa that feed on the minute algae and bacteria, as well as on one another, which also release ammonia (see Fig. 2 of Dugdale and Wilkerson's paper, page 272). The ammonia-based production within this tightly geared community — termed regenerated production — contributed four-fifths of the total daily production (new plus regenerated) in the EUZ. Because of the low variability in nutrient and biomass concentrations observed over different cruises and seasons, the pelagic system of the EUZ seems to have settled into a steady state which can be likened to a silicate-limited chemostat.

The silicate-to-nitrate ratios in the other HNLC regions — the Southern Ocean and the subarctic Pacific — are much higher than in the EUZ, yet fairly similar community structures, total production rates and ratios of new-to-regenerated production are routinely measured there. Diatom growth is evidently not silicon-limited, so other factors, such as deep mixing, iron deficiency and heavy grazing pressure, have been invoked to explain the HNLC condition. An *in situ* iron fertilization experiment (IronEx II), carried out in the South Equatorial Current adjacent to the EUZ, yielded a spectacular diatom bloom[4] and showed that iron availability indeed limited new production in this region.

Dugdale and Wilkerson argue that, as the EUZ diatoms are already silicate-limited, adding iron should have no effect on them. They suggest that iron upwelling with the other nutrients in the EUZ is sufficient to meet the demands of the diatoms. In the iron-limited waters of the South Equatorial Current, iron-fertilized diatom growth would eventually be halted by silicate but not nitrate exhaustion.

Yet the paradox persists. Virtually all phytoplankton species, including the picoplankton, are able to use nitrate; indeed, phytoplankton other than diatoms routinely exhaust nitrate in the surface waters of the non-HNLC ocean. So why does this not happen in the EUZ and other low-silicate HNLC regions? Differences in grazing pressure have been proposed as an explanation[5]. One widely held view is that the small algae of the microbial loop are kept in check by heavy grazing pressure, whereas diatoms, because of their larger size (and possibly also the protection offered by the silica shell), are less prone to being grazed by the smaller protozoa[6]. So relaxation of a limiting factor (such as iron) results in accumulation of diatom but not picoplankton cells. Whether continued iron fertilization will eventually lead to nitrate exhaustion by non-diatom phytoplankton in low-silicate HNLC regions remains to be tested.

In Dugdale and Wilkerson's steady-state EUZ model, the biomass-to-production ratio of the diatoms indicates that they were growing at least as fast as, if not faster than, the microbial algae. To maintain steady state, grazing pressure on the diatoms, presumably by copepods (zooplanktonic crustacea, equipped with mandibles edged with silica the better to crush diatom shells with), must have been similar to or even higher than that on the microbial algae. The growth performance of the diatoms is all the more surprising as nitrate reduction requires energy and the mediating enzyme contains iron. Indeed, why diatoms can be so much more efficient than the other algae despite the nitrate handicap needs to be explained.

Balancing pelagic ecosystem budgets is still an art because we know so little about the abilities and predilections of the organisms and their interactions with one another[5]. Whatever the outcome of studies on the limiting factors in the various HNLC regions and their subsystems, the status of diatoms as key players will not be challenged. The workhorses running pelagic systems are recruited from this algal group: their new production not only fuels the food chains leading to fish but also provides the raw material driving the biological pump and ultimately the great biogeochemical cycles of the ocean. It is time we gained a better understanding of the properties that make diatoms so special. □

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Developmental biology

Hox gene variation and evolution

Axel Meyer

Both developmental and evolutionary biologists try to explain patterns in the diversity among organisms, and the Hox genes encode a class of transcription factors that have provided ample material for such discussions. Because they may be pivotal in specifying regional identity in body plans, differences in their expression could (at least partly) explain the evolution of animal phyla. The Hox genes are arranged in genomic clusters and, importantly, they are expressed in a spatially colinear fashion — anterior genes are expressed early in development and towards the front of the body, posterior genes later in development and in more distal portions of the body.

In invertebrates, only a single Hox gene cluster has been found (although it is split in *Drosophila*). The common ancestor of all chordates is surmised to have had a single cluster as well. This cluster is thought to have duplicated to four clusters (A–D) on different chromosomes, accompanying the increasing complexity of body plans during the evolution of vertebrates (Fig. 1). But a report by Prince *et al.*[1], shortly to appear in *Development,* is likely to cause some questioning of this commonly held hypothesis — that genomic and morphological complexity are causally linked[2–7].

Using an experimental approach based on the polymerase chain reaction, Prince *et al.* unambiguously identified 34 Hox genes and determined their linkage with somatic-cell hybrids. Surprisingly, they found that the zebrafish has three Hox genes (*Hox*C3, *Hox*A8 and *Hox*B10), with no direct mouse equivalents (Fig. 1). Moreover, the expression domains of the anterior Hox genes are partly overlapping, and restricted to a shorter anterior region. Possibly the most important finding is that the zebrafish has at least two additional Hox gene clusters for a total of six and not, as previously thought[8], the typical vertebrate number of four. All of the genes on these additional clusters have probably not yet been discovered, and three are reported so far[1].

These two additional clusters lead us to question a simple, 'more clusters, more complexity' model of evolutionary diversification — in terms of phenotypic complexity, however measured, a zebrafish is probably not 50 per cent more complex than a mouse

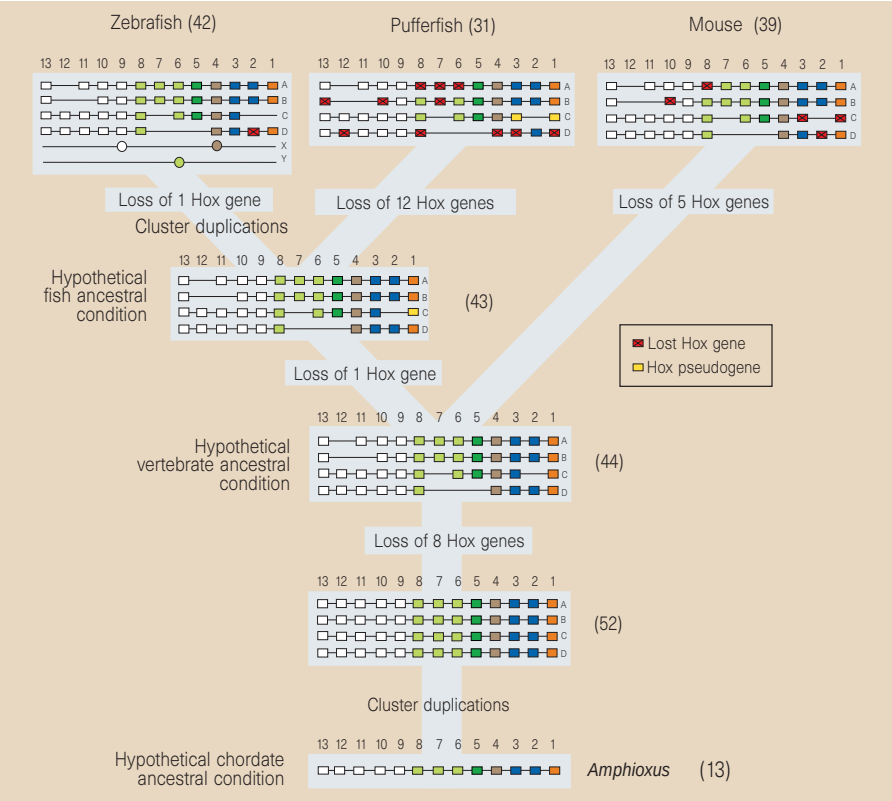

Figure 1 **Evolutionary conservation of the Hox gene clusters in zebrafish, pufferfish, mouse and *Amphioxus*. The zebrafish Hox gene clusters are shown with the 34 genes confirmed by Prince *et al.*[1], and an additional eight, for the total of 42 that the zebrafish is likely to have. Ten gene losses differentiate the mouse from the pufferfish, but there are only three losses between the mouse and zebrafish, even though fish and tetrapods shared their last common ancestor about 400 million years ago. The common ancestor of human and mouse probably lived around 60 million years ago, but the architectures of their Hox gene clusters are identical. The losses in the *AbdA (abdominal A)*-related genes (light green) in the D-cluster probably occurred early in the history of vertebrates. (Figure drawn by Heike Haunstetter.)**

or a human. The extra clusters cannot be explained by entire-genome duplications because, although polyploidy is known from other carp-like fish[9] and is common in salmonids, zebrafish are diploid. It also seems unlikely that the additional clusters are remnants of a polyploid ancestral condition. Instead, the Hox-cluster duplications in zebrafish might be a unique evolutionary event. But such events may turn out to be common, at least in fish.

Prince and colleagues' work on zebrafish[1], combined with studies on the pufferfish[10], now enables us to reconstruct the evolutionary history of the Hox gene clusters in vertebrates (Fig. 1). The initial chordate ancestral cluster of 13 Hox genes (the architecture that is still present in the cephalochordate *Amphioxus*[7]) probably duplicated in a three-step process, to form four complete clusters with a total of 52 genes. One phylogenetic study[11] indicates that the D-cluster is the most ancestral, and that the B- and C-clusters are the youngest. Hagfish and lamprey are phylogenetic intermediates between *Amphioxus* and more derived vertebrates such as zebrafish. So, if these fishes have only two or three clusters (which is not precisely known), they would be more likely to contain a D-like Hox gene cluster than a B- or C-cluster. The suggestion that the D- and A-clusters are the oldest also seems to fit the observation that the D-cluster is the most 'deteriorated' of all (Fig. 1).

Following the principle of Dollo parsimony — which assumes that losses of genes are much more common and likely than independent evolutionary origins[12] — we can speculate which Hox genes might have been present in the common ancestors of vertebrates, tetrapods and fish (Fig. 1). Based on the genomic organizations available so far, the rates of evolution of Hox clusters do not seem to be constant. For example, whereas the zebrafish is likely to have lost only one Hox gene since it shared a common ancestor with the pufferfish (probably more than 200 million years ago), the losses along the pufferfish lineage were possibly several times faster (12 Hox genes were lost, if the zebrafish really has 42 Hox genes) (Fig. 1). Ignoring the additional clusters of the zebrafish, and estimating that it has 42 Hox genes, we find that 13 differences separate the zebrafish from the pufferfish. In the pufferfish, the *Hox*C1 and C3 genes are still

100 YEARS AGO

Messrs. Swan Sonnenschein and Co. announce that they will shortly publish a work, entitled "The Wonderful Century: its Successes and its Failures," by Dr. Alfred R. Wallace, F.R.S. The object of the volume is to give a short descriptive sketch of all the more important mechanical inventions and scientific discoveries which are distinctive of the nineteenth century. ... The author maintains that our century is altogether unique; that it differs from the eighteenth or seventeenth centuries, not merely as those differed from the centuries which immediately preceded them, but that it has initiated a new era, and that it may be more properly compared with the whole preceding historical period.

The January number of the *National Review* has an admirable article by Mr. Gerald Arbuthnot, entitled "In Defence of the Muzzle." The temperate spirit in which it is written, and the conscientious manner in which the statistics referred to have been collected, ought to materially strengthen the hands of those who are upholding the muzzling order for dogs, in the face of the selfish and short-sighted opposition which it is receiving from a certain section of the public.

From *Nature* 13 January 1898.

50 YEARS AGO

A symposium arranged by the New York Academy of Sciences and held in December 1946 on "Nutrition in Relation to Cancer" covered a wide field and included a number of interesting articles which have now been published. ... Although it may seem disappointing that after so much study of cancer the fundamental cause or nature of it is unknown, the papers [in the symposium volume] show advances. The carcinogenic process can often be influenced by diet, which means that the process can be resolved into separate parts, and this must help in the understanding of the process. Many of the speakers at the symposium compared carcinogenesis to mutations. Both cancer and mutations can be induced in living organisms by similar agents. The hypothesis that cancer is a somatic mutation relates carcinogenesis to other biological changes and the stability of the nuclear and cytoplasmic genes.

From *Nature* 17 January 1948.

recognizable, but they are only pseudogenes (genes that are not transcribed). They might have lost their function at different points in the evolution of fish, because *Hox*C3, at least, is still present in the zebrafish.

The apparent acceleration of genomic evolution along the pufferfish lineage might be correlated with accelerated morphological evolution. Pufferfish belong to one of the most morphologically derived groups of fish, and they lack ribs, pelvic fins and the pelvic girdle. Are the missing genes those that are no longer necessary because these structures have been lost during evolution? If so, the missing Hox genes in the pufferfish might also be absent in the other groups of fish which have secondary loss of pelvic fins (such as eels) or even tail fins (for example, the ocean sunfish *Mola mola*). Moreover, the loss of Hox genes might also be accompanied by the secondary loss (or simplification) of appendages in land vertebrates, such as in limbless amphibians, reptiles and whales.

What selective forces maintain or modify genomic organizations? The observation that Hox genes are clustered, and that the architecture of these clusters is highly conserved in evolution, has led to the suggestion that the regulatory elements that control expression of the Hox genes cannot be separated from these genes without jeopardizing their proper functioning and, possibly, determination of morphology along the antero-posterior axis. For vertebrates[13,14] these ideas have been partially confirmed experimentally[15], and this tight functional linkage might be particularly strong along the lineage that leads to reptiles and mammals (Fig. 1).

The long-standing question of whether the evolution of genes or networks of interactions through regulatory elements drives most morphological diversification might, then, have different answers in different evolutionary lineages. In the most species-rich group of vertebrates — fish — organization of the Hox genes might not be completely constrained by interwoven regulatory networks, and differentiation might be driven by gene evolution. However, in the lineage that leads to reptiles and mammals, the driving force behind morphological diversification might have been newly evolving interactions in networks of regulatory elements[16].□

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Isotope astrophysics

Two cradles for the heavy elements

A. G. W. Cameron

Where do the Solar System's heavy elements come from? We know that many of the elements heavier than iron must have been formed in supernovae; but exactly how? Taken together, three new papers (one on page 261 of this issue[1], and two to appear in the *Astrophysical Journal*[2,3]) imply that two different types of supernova are responsible for elements in different mass regimes.

It is now some four decades since Suess and Urey[4], largely working from analyses of meteorites, assembled a table of abundances of the elements that enabled nuclear physicists to identify the principal processes in stellar interiors that had produced those elements. The actual nuclear physics of those processes became reasonably well understood within the following few years. However, it has taken considerably longer for us to understand the astrophysical environments within stellar interiors in which many of those processes take place. The process whose environment has proved most elusive has been the r-process, in which neutron capture takes place on a very rapid timescale.

In the evolution of a star considerably more massive than the Sun, nuclear fusion reactions build toward products in which the binding energy per nucleon becomes maximized. This produces an abundance peak at ^{56}Fe. Making nuclides much heavier than this involves neutron capture, which generally produces nuclides on the neutron-rich side of the region of stable elements, known as the valley of beta stability. Only in the violence of a supernova explosion can the nuclides on the neutron-deficient side be produced, primarily by losing nucleons in photodisintegration. It is possible to distinguish these processes by examining the abundances of nuclei in the Solar System, particularly of stable isobars, in which more than one stable nucleus exists at a given mass number.

There are three basic nuclear processes that produce heavy elements: the s-, r- and p-processes, standing for slow, rapid and proton-rich. The abundances produced by each of these[5] are shown in Fig. 1.

The s-process probably takes place in red giant stars that are burning helium, with a given nucleus capturing a neutron perhaps every few thousand years. But only fractions of a second separate successive photodisintegrations in the p-process or neutron captures in the r-process, implying that these processes must occur in supernova explosions. So some details of supernova explosions can be deduced from isotopic analysis of meteoritic material — in particular, interstellar grains that have undergone relatively little thermal processing during and since the formation of the Solar System.

One important recent discovery was that there are two different r-processes[6]: one responsible for the r-process nuclides up to about $A = 140$ (where A is the mass number), the other for nuclides above $A = 140$. The discovery stemmed from extinct radionuclides, which lived long enough to survive with measurable abundances from the time of their production to their injection into the forming Solar System, but not long enough to be measurably present now. They are detected through excesses of the daughter elements that result from their decay.

The abundance of ^{182}Hf in the early Solar System is consistent[6] with the continuous production of the actinides in the Galaxy, with mixing to maintain a roughly constant abundance level on a timescale consistent with the mean life of ^{182}Hf — about 10^7 years. But trouble arises from two lighter radionuclides, ^{107}Pd and ^{129}I. If these were made by the same r-process that produced the

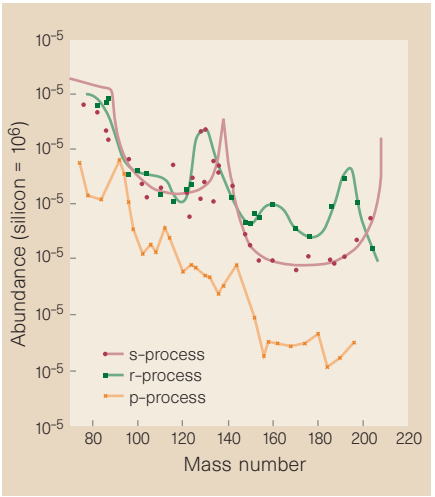

Figure 1 **The solar abundances of the nuclides, as a function of mass number, showing the p-, s- and r-process contributions. For the s- and r-processes, average smooth lines have been drawn through the characteristic zig-zag patterns of the odd and even mass numbers. (From ref. 5.)**