

EVOLVING CONCEPTS: THE ROLE OF GENE LOSS FROM AN ANCESTRAL GENETIC TOOL-KIT IN ANIMAL EVOLUTION

EDWARD M. DE ROBERTIS

Introduction

In this meeting dedicated to evolving concepts of nature I would like to discuss some aspects of animal evolution. It is now clear from genome sequencing studies that bilateral animals evolved through the differential use of an ancestral “genomic tool-kit” shared by all bilateral animals. We will examine three main points:

- 1) Whole-genome duplications followed by massive gene loss were very important evolutionary events.
- 2) Tandem duplications are accompanied by reciprocal gene deletions. Gene deletions are common and copious, but limit the potential for future evolutionary change.
- 3) Although a conserved set of genes is used by all animals, similar functions can be fulfilled by genes of different structure. In particular, we will examine the case of self-avoidance in neurons, which is mediated by different proteins in insects and humans.

1. Genomes contain the record of our evolutionary history

1.1. *Developmental genes determine body shapes in animals*

As explained by François Jacob (1982), “It is during embryonic development that the instructions contained in the genetic program of an organism are expressed, that the genotype is converted into phenotype. It is mainly the requirements of embryonic development that, among all possible changes in phenotype, screen the actual phenotypes”. Animals can be grouped into about 34 phyla, which have their cells arranged in such a way as to generate distinct body plans. Of these, 30 constitute the bilateral animals which are most of the animals we see around us. These derive from a hypothetical common ancestor designated *Urbilateria* (Ur=primeval, bilateria=bilateral animal) that existed 560 million years ago (De Robertis and Sasai, 1996). One of the central problems in the field of evolution and development (Evo-Devo) is how morphologically complex this ancestral animal was.

Genomes contain the record of our evolutionary history and we know that the vast majority of the genes used by animals today were present in *Urbilateria* and in its non-bilateral ancestors such as Cnidarians (e.g., sea anemones and Hydra). These genes are used to construct the protostome (mouth-first) animals which include most invertebrates, and the deuterostomes (mouth-second) which include chordates (such as the vertebrates, amphioxus and ascidians), hemichordates and echinoderms. *Urbilateria* patterned its body using conserved systems of developmental control genes such as the Chordin/BMP network that patterns Dorsal-Ventral (D-V) differentiation and the Hox genes that pattern the Antero-Posterior (A-P) axis.

1.2. *D-V gene losses in mammals*

The Chordin/BMP network patterns D-V differentiation in vertebrates, the fruit fly *Drosophila*, and many other animals. We have described a self-regulating network of secreted proteins that regulates gastrulation and is conserved in fishes, amphibians, reptiles and birds (De Robertis, 2008a). The platypus (*Ornithorhynchus*) is a mammalian ancestor that, surprisingly, has lost one of these genes, called ADMP, from its genome. Although platypus is a mammal, it still lays eggs in a nest. Although its reptile ancestors had two or three *vitellogenin* (yolk protein) genes, the platypus has retained only one (Warren et al. al., 2008). In higher mammals, two additional genes of this regulatory network, *Crescent* and *Sizzled*, were also deleted from the genome. Interestingly, in placental mammals (and also in marsupials) the *vitellogenin* gene was also completely lost. The Chordin/BMP network probably evolved to self-organize a morphogen gradient in cells actively migrating in a yolk egg. As soon as yolk disappeared, so did some genes in the network. Another possibility is that the lost genes were important in adjusting to differences in temperature during early development, which became unimportant when embryogenesis took place inside the mother. In a systematic search for genes lost in mammals but present in the chick genome, Kuraku and Kuratani (2011) found 147 losses of protein-coding genes. These losses of early patterning genes prompted our interest on the role of gene loss in evolution.

1.3. *Gene losses in the Hox system*

In *Drosophila*, homeotic genes specify A-P segment identity. Edward B. Lewis found that there was a colinearity between their order in the DNA and the regions of the body they specify. Walter J. Gehring and others showed that all homeotic genes contained a conserved region called the homeodo-

main of 60 amino acids, which encodes a DNA-binding domain (Gehring, 1998). A *Drosophila* homeobox gene was used to isolate what constituted “the first development-controlling gene identified in vertebrates” thirty years ago (Carrasco et al., 1984). Hox gene complexes have been isolated through the work of many groups and found to conserve the colinearity in *Drosophila* and chordates. From these studies, one can conclude that a Hox complex consisting of at least six genes was present in *Urbilateria* (De Robertis, 2008a; Holland, 2013). Mice and men have four Hox complexes that represent duplications from an ancestral gene set consisting of 13 paralogues (duplicated genes). Within these complexes 13 individual genes have been lost, illustrating that genes are not only gained but also readily lost.

As discussed in section 2.3, sequencing of the genome of the chordate ancestor amphioxus (*Branchiostoma*) has proven particularly informative. It contains a single Hox complex, which indicates that the vertebrates underwent two whole-genome duplications during the early evolution of a fish ancestor.

2. Evolution by whole genome duplication followed by gene loss

2.1 Susumu Ohno and evolution by gene duplication

In 1970 Susumu Ohno published a prescient book entitled “Evolution by gene duplication”. After looking at chromosomes and quantifying DNA contents in nuclei after staining DNA with Feuglen reagent, he predicted that two whole-genome duplications events (4X) had occurred early during vertebrate evolution. These duplications probably were key to the evolutionary success of the vertebrates.

Ohno also predicted that bony fishes (teleosts) had undergone an additional round of genome duplications (8X) when compared to their cartilaginous ancestors. In fact, fishes are the most successful of all vertebrate animals in terms of having the most species and number of individuals. He also noted that the surf smelt (8X) had half the genome size of the trout (16X), and the salmon (32X) had double that of the trout. Therefore, these familiar fish constitute a polyploid series of nutritious animals. Polyploidization events have occurred multiple times in fishes and amphibians, but ceased in reptiles, birds and mammals, when sex chromosomes became distinct. The role of gene duplication – both by whole genome and by tandem DNA duplication – in providing a substratum for evolutionary innovation is now well recognized. When two genes are duplicated one of the copies can adopt different functions or a different regulation. The role of gene deletion is less generally recognized.

2.2. Tandem duplications are accompanied by deletions

When two chromosomes undergo crossing over in meiosis (or sister chromatid exchange in germ line mitoses) they usually align exactly. However, if the two DNA strands are not aligned correctly (unequal crossing over) this will result in a tandem duplication of one DNA strand. What is less frequently realized is that for each duplication there is an accompanying reciprocal deletion in the other DNA molecule.

Deletions by unequal crossing over must be very frequent, because they provide the molecular mechanism by which deleterious sequence changes are eliminated in repeated genes. For example, the *Xenopus laevis* nucleolar organizer has 450 tandemly repeated large ribosomal RNA (rDNA) genes (Brown and Dawid, 1968). Mutations must occur at random in rDNA, just as in any other DNA sequence. To keep the 450 rDNA genes homogeneous in sequence, constant deletions accompanied by new tandem duplications are required. Through these continuous gene deletions, the nucleolar organizer can cleanse itself of genes that have become inactivated by mutation. In *Drosophila*, there are normally about 100 rDNA copies. The *bobbed* mutant has an insufficient number of rDNA copies for vigorous growth, resulting in small flies. However, the *bobbed* mutation can easily revert by unequal crossing over, resulting in an increase in rDNA genes and normal growth. In this way, the rDNA gene sequences are maintained invariant by continuous deletion and duplication events mediated by unequal crossing over (Ritossa et al., 1966; Ohno, 1970).

These examples argue that gene loss must be copious and easily achieved though unequal crossing over events that generate deletions and duplications. Natural selection (Darwin, 1858) provides the constant policing required to ensure that all genes required for propagation of the species with maximal reproductive fitness are preserved. All animals produce many more progeny than the numbers needed to maintain their population. An extreme example is provided by the *Echinus esculentus* sea urchin female, which releases 20 million eggs, even though only two surviving adult progeny are required to maintain the same number of individuals in the population. The price of gene loss is that many offspring will not be viable, but that is a small price to pay for variation when reproduction is so exuberant.

Genomes are probably constantly being streamlined by removal of unwanted DNA. As computer programmers know, as a large program grows over time the software becomes unwieldy because many layers of no longer useful code remain. Some programmers specialize in locating these unnecessary instructions and deleting them. In the case of the genome, DNA

deletions are constantly purifying genomes of unwanted DNA code. Gene loss can serve the useful function of increasing fitness.

2.3. *The amphioxus and yeast genomes have been very informative*

Sequencing of the amphioxus genome confirmed Ohno's proposal that vertebrates had undergone two whole-genome duplication events. Amphioxus is an early chordate that did not undergo genome duplication. In principle, humans should have four copies of every chordate gene. In the case of the Hox gene complexes, all four copies have been preserved. Comparison of the amphioxus and human genomes showed that 25% of human genes have two or more copies resulting from the two genome duplications (Putnam et al., 2008). Massive gene losses, through the process known as rediploidization, have left humans with 75% of their protein-coding genes as single copies. Developmental control genes appear to have been conserved in duplicated form more frequently than housekeeping genes.

Comparison of the amphioxus and human genomes has shown that the tunicate class of chordates has undergone massive gene losses. These animals, also known as ascidians or sea squirts, have a larval swimming form with a notochord but then adopt a sessile life form in which they attach to rocks and become essentially a large water-filtering pharynx. Ascidians have not undergone whole genome-duplications and would be expected to have a similar number of genes as amphioxus. However, the ascidian genome has lost a total of 2,250 – out of a total of about 8,000 – gene families that are present both in amphioxus and humans (Holland et al., 2008). Adaptation to this particular life form came at the cost of future evolution for these animals.

The baker's yeast *Saccharomyces cerevisiae* evolved from a single whole-genome duplication followed by massive gene losses (Wolfe and Shields, 1997). Sequencing of a diploid ancestral yeast (*Kluyveromyces waltii*) species revealed that a tetraploidization was followed by massive gene losses through gene deletions (Kellis et al., 2004). In fact, one copy of each duplicated gene has been deleted for 88% of all *S. cerevisiae* gene loci through a process involving deletions comprising multiple adjacent genes (Kellis et al., 2004). In present-day *S. cerevisiae* only 12% of the genes have been maintained in duplicated form. Frequently, the protein encoded by one of the duplicated genes has diverged significantly in sequence from the ancestral gene pair. The yeast genome has provided very strong support for the hypothesis that gene duplication allows one of the duplicated copies to become different in function (Ohno, 1970). Genome duplication is thus followed by rapid loss of many, but not all, genes.

3. Loss of genes and anatomical structures in evolution

3.1. Losses by disuse

The old adage that “what is not used atrophies” was recognized from the time of French naturalist Buffon. When a change in habits or in the environment occurs, it seems likely that all variations that are compatible with the new behavior will be allowed to escape the strictures of Darwinian natural selection. There are many examples of this.

The case of the gastrointestinal tract of the vampire bat provides a striking illustration of adaptations that follow a change in feeding habits. The external morphology of the vampires is very similar to that of the insectivorous bats from which they evolved. However, the esophagus has become very thin, with a lumen so narrow that only liquids can pass (Kent, 1973). In addition, the stomach has been modified to provide a lateral sac for the accumulation and dehydration of blood. These gastrointestinal adaptations have rendered vampires unable to feed on insects as the other bats do. These internal adaptations are related to the change in feeding habit and likely occurred in a relatively short evolutionary period because vampires are very similar in external morphology to their close insectivorous relatives. These variations would also occur spontaneously in insectivorous bat species, but the guiding hand of natural selection would have prevented them from appearing in the population for they would not have been able to swallow insects.

Laboratory mice have been bred in captivity for centuries, starting by the breeding of “fancy” mice pets in China and then Japan. Unlike field mice which reproduce seasonally, under domestication the artificial selection imposed by human breeders has favored reproduction throughout the year. The change was caused by loss-of-function mutations in two genes which encode enzymes required for the conversion of serotonin into melatonin in the pineal gland (Olson, 1999). Melatonin secretion normally monitors the length of daylight hours, adjusting reproduction to the seasons. In addition, most laboratory strains of mice are deaf, which indicates another loss-of-function.

Darwin himself dealt with the effects of disuse in his *Origin of Species*. He noted that beetles in windswept islands become flightless (200 out of the 550 beetle species in the island of Madeira). Darwin also discussed other examples of adaptive organ losses such as flightless birds and moles with rudimentary eyes (Darwin, 1859).

When a change in habits or in the environment occurs, it seems likely that all variations that are compatible with the new behavior will be al-

lowed to escape natural selection, with adaptations secondary to a change in life habits following. These adaptations will become fixed when new speciation events occur.

3.2. *DNA deletions and the adaptation of fish to new environments*

An important question is which genes were actually mutated during evolution in nature. Three-spined sticklebacks are marine fish that spawn in fresh water. They became entrapped independently in many lakes after the last ice age. Only 10,000 to 20,000 years ago Canada and the great lakes were covered by the Laurentide ice sheet. As the glaciers receded by global warming, lakes were formed and the local stickleback populations diversified rapidly and dramatically. These events were recent enough that genetic crosses can still be carried out. Isolation can cause variations that constrain reproduction (such as different mating dances), but fertile progeny will result if sperm and eggs are squeezed into a Petri dish. This allows genes responsible for morphological adaptations to be mapped genetically (Jones et al., 2012).

Sticklebacks have spines that prevent predators from swallowing them. They have two prominent pelvic spines that have been lost repeatedly in different lakes. Why would sticklebacks ever lose the pelvis? Shallow lakes often contain dragonfly nymphs that grasp small fish and eat them. The pelvic spines greatly facilitate the grasping predators. Genetic mapping and sequencing has shown that the mutation is due to the deletion of a DNA enhancer element that is required for the expression of a gene called *Pitx1* specifically in the pelvis (Chan et al., 2010). Enhancer elements direct the expression of genes to specific tissues. Deletion of enhancer DNA has the advantage that the *Pitx1* protein gene, which is also expressed in other regions, is left in place. Loss of the *Pitx1* protein coding region would otherwise be lethal. The extraordinary finding that three different deletions occurred independently of each other, always removing the same gene enhancer. Adaptation by gene loss must be frequent as it occurred repeatedly in a short time from an evolutionary perspective.

Sticklebacks also protect themselves with armored plates, which have been lost in many populations (Jones et al., 2012). This mutation is caused by deletion of the enhancer for a growth factor gene called *ectodysplasin* that induces the formation of bony plates in the skin. Unlike the previous case, the deletion of the enhancer was present in the marine population before sticklebacks became isolated at the end of the ice age. Thus, all populations carry the same mutation. It does not manifest itself in the large marine

population because it is a rare recessive trait. As the armor is probably energetically expensive, if not needed in the absence of predators, this mutation was naturally selected for in some of the isolated lake populations.

Loss-of-function mutations can also cause deletions in the protein coding genes themselves in addition to their enhancer elements. Many cave animals, such as shrimp, salamanders and fish lose pigmentation and eyes when they adapt to permanent darkness. Mexican Tetra river fish became entrapped in subterranean caves multiple independent times. Genetic crosses have mapped their albinism to *Oca2*. The ocular and cutaneous albinism-2 protein encodes a Tyrosine transporter; Tyrosine is the amino acid building block for melanin. *Oca2* encodes a very large gene which appears not to have many pleiotropic effects and its mutation is the most frequent cause of albinism in humans. Interestingly, independent deletions in the *Oca2* genes occurred repeatedly in various Tetra cave populations (Protas et al., 2006; Rohner et al., 2013). Variation in evolution seems to follow the path of least resistance. The ancestral genetic tool-kit has a profound influence, channeling the type of variations that result in adaptations.

Humans carry enormous numbers of recessive mutations that could serve as a reservoir of variation in case of a drastic change in the environment. Sequencing of human genomes indicates that each of us carries about 100 mutations that would inactivate protein-coding genes, usually in heterozygote form, with about 20 protein genes having been deleted (MacArthur et al., 2012).

4. Urbilateria: a complex or simple animal

4.1. *Urbilateria* had a complex life cycle

A central question in Evo-Devo is how complex the urbilaterian ancestor was from a morphological point of view. From a genetic point of view it must have contained the vast majority of the genes present in bilateral animals today. *Urbilateria* probably had a complex life cycle with an initial ciliated free-swimming (pelagic) larval phase in oceanic plankton. Many phyla of the two main branches of bilateral animals (e.g., annelids, molluscs, hemichordates and echinoderms) have planktonic larvae with two ciliary bands that collect food into the mouth by beating in opposite directions, an apical eye, and an apical ciliary tuft. These larvae then settle on the ocean bottom for their adult life (benthic) phase. The ancestral planktonic phase of the life cycle has been lost repeatedly in many marine animal lineages.

Much debate has centered on whether *Urbilateria* was segmented (De Robertis, 2008b). The segment formation mechanism in *Drosophila* is very

different from that of the vertebrates. In vertebrates, segmentation is caused by an oscillating network of genes of the Notch pathway (Dequéant et al., 2006). Studies on the segmentation of the cockroach embryo have also shown oscillatory expression of Notch pathway genes, indicating that this might be the ancestral mode of segmentation (Pueyo et al., 2008). The ability to form segments may have been lost secondarily in non-segmented bilateral animals, such as planarians and nematodes. The alternative view is that the ancestor was a simple organism such as a planarian and metamerism arose multiple times in evolution.

4.2. *The axochord as a homolog of the notochord*

The chordate phylum is characterized by a dorsal flexible rod that is used for undulatory swimming and as a signaling center in the embryo. In the vertebrates the notochord serves as an induction center for the formation of the vertebrae, and later degenerates remaining only as the nucleus pulposus of the intervertebral disc. In a recent study Detlev Arendt and colleagues discovered that the ventral midline of annelid embryos has a medial muscle called the axochord. They found that the axochord muscle expresses many genes similar to those found in the vertebrate notochord. These include homologs of *Brachyury*, *Twist*, *FoxD*, *Fox1*, *Collagen A*, *Netrin*, *Slit*, *Noggin* and *Hedgehog* (Lauri et al., 2014). The combination of such genes in one anatomical structure is found only in the axochord and notochord. In annelids, mollusks and chaetognaths, a contractile muscular axochord-like organ serves for the attachment of oblique muscles to its collagenous sheath. In *Drosophila*, the muscular nature was lost, but the signaling activity was retained by the mesodermal midline glia cells.

In amphioxus, the notochord became more rigid through a process of intracellular vacuolization that turned the notochord into a rod of fixed length. Interestingly, amphioxus notochord cells still have a muscular nature. Contraction of the amphioxus notochord serves to regulate the stiffness of this rod essential for undulatory swimming (which in amphioxus can take place both in forward and reverse directions; Webb, 1973). These notochord muscle fibers were lost in the vertebrates. In mammals, the vacuolization of the notochord was lost and it remained as a very thin structure serving as a signaling center in development but which is no longer used in locomotion. Thus, it appears that the urbilaterian ancestor had a muscular mesodermal midline that then became vacuolized, increased its collagenous nature, and finally served as an inducing center for the formation of the vertebral column.

5. Using a common tool-kit in different ways can generate diversity

5.1. Conserved and novel functions in the central nervous system (CNS)

There are many examples of genes that have highly conserved functions in the CNS of vertebrates and invertebrates (De Robertis, 2008a). The most celebrated case of gene conservation is *Pax6*, the master control gene used for eye development in all animals (Gehring, 1998).

While a conserved gene tool-kit is shared by animals, genes are not always used in the same way. As Pontifical Academy President Werner Arber has said: “Nature is very inventive and able to find different ways to reach a specific goal”. One of the best examples of this incredible versatility is provided by the molecular solution to the problem of neuronal self-avoidance.

Self-avoidance is an essential property of neurons by which dendrites and axons from the same neuron never contact each other. Projections are repelled if they contact a membrane from the same cell. There are 100 billion neurons in the human CNS and each one has its own identification tag. This may be considered similar to the license plates of cars, which gives them uniqueness. Self-avoidance is essential for neurons to form proper circuits. For example, it ensures that a neuron will cover an entire area without making contact with its sister branches. In *Drosophila* this problem has been resolved by the elaboration of a single, remarkable gene.

Work by the laboratory of Larry Zipursky at UCLA has shown that mutation of *Dscam1* (Down syndrome cell adhesion molecule-1) eliminates repulsion between branches of the same neuron. *Dscam1* evolved multiple exon sequences for three of its nine immunoglobulin domains. These segments are then spliced together at the mRNA level to encode a transmembrane protein. The three variable exons have 12, 48 and 33 different possibilities respectively, as well as 2 different transmembrane domains that target Dscam1 to axons and dendrites (Zipursky and Grueber, 2013). Alternative splicing from this one gene can generate a staggering number of different proteins: $12 \times 48 \times 33 \times 2 = 38,016$, all from one gene. This is an amazing number of proteins, considering that the entire *Drosophila* genome encodes a total of 15,000 (and the human one about 20,000) different protein-coding genes.

Self-avoidance occurs only when the three variable domains of Dscam1 match each other exactly. When two Dscam1 proteins bind homotypically in different membranes, this triggers repulsion via the intracellular domain and the cytoskeleton machinery, and the two sister cell projections separate. Each neuron expresses about 10–20 different Dscam1 isoforms in a stochastic fashion, and this provides an enormous amount of combinatorial possibilities for each individual neuron (Miura et al., 2013).

Remarkably, humans use a different mechanism for neuronal self-avoidance. We do have two *Dscam* genes, but they lack all the alternative exons found in insects and therefore encode only one protein each. In addition, *Dscam* is also not variable in the annelids. This suggests that *Urbilateria* had an ancestral *Dscam* gene that was adapted specifically in the insect lineage to generate the self-avoidance system.

Recent work by Tom Maniatis and Josh Sanes has shown that in mammals the self-avoidance function is fulfilled by an entirely different gene family called the clustered protocadherins. Rather than using splicing to generate variability, protocadherins utilize alternative promoters that result in the splicing of different extracellular domains to a common intracellular region (Lefebvre et al., 2012). Several protocadherins are expressed in a stochastic fashion in neurons. They also have highly specific homophilic binding. Although there are only 50 different protocadherin genes, these proteins form hetero-tetramers which greatly increases the combined variability. As in the case of *Dscam*1, homophilic binding triggers repulsion and self-avoidance (Thu et al., 2014). Protocadherins are very different trans-membrane proteins from *Dscam*. Homotypic recognition occurs through cadherin domains instead of immunoglobulin domains. However, the intracellular region also has the capacity of transmitting to the cytoskeleton a message of repulsion.

Surprisingly, insect genomes do not contain a single protocadherin gene. However, spiders do have one protocadherin. This indicates that *Urbilateria*, and perhaps its ancestors, already had a protocadherin gene containing six cadherin domains. Different solutions to the physiological problem of self-avoidance have been achieved through the gain of variability in different proteins. The resourcefulness of evolution is enormous.

6. Chance and Necessity in evolution

Darwinian evolution has been used many times as an argument against Judeo-Christian faith. In a very interesting book entitled “In the Beginning...” Cardinal Joseph Ratzinger of Munich (later member of our Academy and Pope Benedict XVI), wrote: “There has been a conflict between natural sciences and theology that has been a burden to the Church. This did not have to be”. This book contains the four Lent homilies Ratzinger gave in the Frauenkirche Cathedral in response to the highly influential book “Chance and Necessity” written in 1970 by the famous French geneticist Jacques Monod. The Cardinal’s audience must have been very faithful and patient to sit through this thoughtful argument on Evolution

and Faith in the course of four weeks (in fact, he gratefully dedicated his book to them).

Monod's essay on the natural philosophy of modern biology proposed a new "ethics of knowledge". The main guiding principle was the principle of objectivity (in essence similar to an earlier proposal by physiologist Claude Bernard) by which hypotheses must be tested by experiment and the results analyzed by reason, and not influenced by any other values or ideologies. His essay, however, was influenced by atheism and his book ended in a dark philosophical message: "The ancient covenant is in pieces; man knows at last that he is alone in the universe's unfeeling immensity, out of which he emerged only by chance".

François Jacob was the close collaborator of Monod in their Nobel prize-winning work on gene regulation. He took a more optimistic view in his own book on evolution: "Western science is founded on the monastic doctrine of an orderly universe founded by God who stands outside of nature and controls it through laws accessible to human reason" (Jacob, 1982). The response from Cardinal Ratzinger was related, making the argument that God is also Reason and set up universal laws by the act of Creation. A similar reflection was made by Pope Francis in the *Domus Sanctae Marthae* homily cited in the introduction to this meeting on *Evolving Concepts of Nature*: "God made things – each one – and he let them go with the interior, inward laws which he gave to each one, so that they would develop, so they would reach fullness".

Cardinal Ratzinger explains at the end of "In the Beginning..." why this is an important issue to the Catholic Church: "Only if the Redeemer is also Creator can he really be Redeemer. That is why the question of what we do is decided by the ground of what we are. We can win the future only if we do not lose creation". For Ratzinger a Creator that set the rational natural world is essential; otherwise, he could not have sent his Son to save us.

The fields of religion and science are separate. The scientific mind would argue that invoking a Creator because we need salvation is a circular argument. It indeed is, but for Christians it is clear how much good Faith can do in the daily lives of so many people. Conversely, in the field of science we know that a natural world from which universal laws can be uncovered by experiment must clearly exist, because of the beneficial effects that scientific development has brought in the improvement of human life.

As pointed by Monod, the principle of science is that we must deduce the laws of the natural world with complete objectivity, not influenced

by biases or ideologies. It was in the Church that the separation between the pagan (e.g., Greek philosophy) and the sacred was first made. In the natural sciences hypotheses are tested by experiments interpreted by our own human reason. It is therefore immaterial from the point of view of the advancement of everyday science whether the first principle is Chance as in Monod's view, or through a Creator as in the Judeo-Christian view. All that is needed is a rational universe governed by natural laws.

7. Conclusions

7.1. *Evolving concepts of evolution*

Work in developmental genetics has revealed that animal evolution is generated through variations in ancestral gene networks that were present in *Urbilateria*, the common ancestor of all bilateral animals and in its metazoan ancestors. Animal morphologies depend on the arrangement of cells with respect to each other and this is controlled by ancient developmental gene networks. The discovery of conserved Hox genes thirty years ago, revealed that the mechanisms of development were common to all animals. The use of developmental control networks placed evolutionary constraints on the types of animal anatomies that can evolve by natural selection. The "Necessity" aspect of animal evolution resides on the conserved use of genes of the machinery of embryonic development. Not all possible body plans can evolve; only those that are compatible with the developmental gene networks, which are in turn determined by our previous history.

An important source of variation in evolution is through duplications and deletions of this ancient set of genes. The urbilaterian genome must have had a surprisingly complete set of the genes used in all animals. Tandem gene duplications provided a way of generating new genes that can then adopt new functions or be expressed in specialized regions. Each tandem duplication is accompanied by a reciprocal deletion, which plays a fundamental role in policing the genome. Natural selection provides the constant vigilance required to ensure that all genes required for propagation of the species with maximal reproductive fitness are preserved.

Genome-wide duplications provided a very effective way of providing new genes that can then diverge in function. Whole-genome duplications occurred many times in plants, yeasts, and early vertebrates. Two successive genome-wide duplications were key to the evolutionary success of vertebrates. These events were followed by massive gene loss of genes that did not acquire a specialized function. If there were no gene losses all human

genes should be present in four copies, but in fact 75% of them are now single-copy.

The adaptive role of gene loss in evolution has not been fully appreciated. When the environment changes, losses of DNA enhancer elements or of protein-coding regions have been documented in stickleback and cave fishes. In lakes without predators, sticklebacks rapidly lose their armor, using a deletion mutation that exists at low frequency in the large marine population. Extra features such as body armor come at a fitness cost. Once there are no predators, it becomes adaptive to lose the unneeded feature. Cave fish lose their eyes because the energetic cost of vision is high; vision consumes more energy in the dark. Gene losses can result in better adaptation, but at the same time limit the potential for future evolution. The bilateral animal common ancestor started this lineage with an enormous reservoir of genetic diversity which was trimmed and elaborated by natural selection of the fittest, in some lineages more than in others. Life on earth evolved through a series of unique events such as the establishment of single genetic code, of the cell membrane, of photosynthesis, of multicellular animals and of a common tool-kit for bilateral animals.

On other side of the coin, very different genes can be used to achieve the same physiological needs. We have reviewed this in the case of self-avoidance in neurons, which is mediated by alternatively spliced Dscam1 insects and by tandemly repeated protocadherins in humans. Although different, both proteins used the principle of homotypic recognition to trigger repulsion at the level of the cell cytoskeleton.

In sum, bilateral animals evolved through variations in ancestral developmental gene networks in their DNA. It seems a miracle that we humans got to where we are today through evolutionary gains and losses of an ancient set of genes.

Bibliography

- Brown, D.D. and Dawid, I.B. (1968), specific gene amplification in oocytes. *Science* 160, 272-280.
- Carrasco, A.E., McGinnis, W., Gehring, W.J. and De Robertis, E.M. (1984), Cloning of a *Xenopus laevis* gene expressed during early embryogenesis that codes for a peptide region homologous to *Drosophila* homeotic genes: implications for vertebrate development. *Cell* 37, 409-414.
- Chan, Y.F. et al. (2010). Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. *Science* 327, 302-305.
- Darwin, C. (1859), *On the Origin of Species by Means of Natural Selection, or Preservation of Favored Races in the Struggle for Life*,

- Murray, London
- Deqéant, M.L. et al. (2006), A complex oscillating network of signaling genes underlies the mouse segmentation clock. *Science* 314, 1595–1598.
- De Robertis, E.M. (2008a), Evo-Devo: Variations on ancestral themes. *Cell* 132, 185–195.
- De Robertis, E.M. (2008b), The molecular ancestry of segmentation mechanisms. *Proc. Nat. Acad. Sci. USA* 105, 16411–16412.
- De Robertis, E.M. and Sasai, Y. (1996), A common plan for dorso-ventral patterning in Bilateria. *Nature* 380, 37–40.
- Gehring, W.J. (1998), *Master Control Genes in Development and Evolution: The Homeobox Story*, Yale Univ. Press, New Haven.
- Holland, P. (2013). Evolution of homeobox genes. *WIREs Dev. Biol.* 31, 31–45.
- Holland, L.Z. (2008), The amphioxus genome illuminates vertebrate origins and cephalochordate biology. *Genome Research* 18, 1100–1111.
- Jacob, F. (1982). *The Possible and the Actual*, University of Washington Press, Seattle.
- Jones, F.C. et al. (2012). The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* 484, 55–61.
- Kellis, M., Birren, B.W., and Lander, E.S. (2004). Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*. *Nature* 428, 617–624.
- Kent, G.C. (1973), *Comparative anatomy of the vertebrates*. The C.V. Mosby Company, Saint Louis.
- Kuraku, S., and Kuratani, S. (2011), Genome-wide detection of gene extinction in early mammalian evolution. *Genome Bio. Evol.* 3, 1449–1462.
- Lauri, A. et al. (2014), Development of the annelid axochord: Insights into notochord evolution. *Science* 345, 1365–1368.
- Lefebvre, J.L., Kostadinov, D., Chen, W.V., Maniatis, T., and Sanes, J.R. (2012), Protocadherins mediate dendritic self-avoidance in the mammalian nervous system. *Nature* 488, 517–521.
- MacArthur, D.G. et al. (2012) A systematic survey of loss-of-function variants in human protein-coding genes. *Science* 335, 823–828.
- Miura, S.K., Martins, A., Zhang, K.X., Graveley, B.R. and Zipursky, S.L. (2013). Probabilistic splicing of *Dscam1* establishes identity at the level of single neurons. *Cell* 155, 1166–1177.
- Monod, J. (1971), *Chance & Necessity*, Alfred A. Knopf, New York.
- Ohno, S. (1970), *Evolution by Gene Duplication*, Springer-Verlag, Heidelberg.
- Olson, M.V. (1999), Molecular Evolution '99. When less is more: gene loss as an engine of evolutionary change. *Am. J. Hum. Genet.* 64, 18–23.
- Protas, M.E., Hersey, C., Kochanek, D., Zhou, Y., Wilkens, H., Jeffery, W.R., Zon, L.I., Borowsky, R. and Tabin, C.J. (2006), Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism, *Nat. Gen.* 38, 107–111.
- Pueyo, J.I., Lanfear, R., Couseo, J.P. (2008), Ancestral notch-mediated segmentation revealed in the cockroach *Periplaneta Americana*. *Proc. Natl. Acad. Sci. USA* 105, 16614–16619.
- Putnam, N.H. et al. (2008), The amphioxus genome and the evolution of the chordate karyotype. *Nature* 453, 1064–1071.
- Ratzinger, J. (1995), *In the Beginning*, B. Eerdmans Publishing Co., Michigan.
- Ritossa, F.M., Atwood, K.C. and Speelman, S. (1966). A molecular explanation of the bobbed mutants of *Drosophila* as partial deficiencies of “ribosomal” DNA. *Genetics* 54, 819–834.
- Rohner, N., Jarosz, D.F., Kowalko, J.E., Yoshizawa, M., Jeffery, W.R., Borowsky, R.L., Linquist, S. and Tabin, C.J. (2013), Cryptic variation in morphological

- evolution: HSP90 as a capacitor for loss of eyes in cavefish. *Science* 342, 1372–1375.
- Thu, C.A. et al. (2014), Single-cell identity generated by combinatorial homophilic interactions between α , β , and γ protocadherins. *Cell* 158, 1045–1059.
- Warren, W.C. (2008), Genome analysis of the platypus reveals unique signatures of evolution. *Nature* 453, 175–183.
- Webb, J.E. (1973), The role of the notochord in forward and reverse swimming and burrowing in the amphioxus *Branchiostoma lanceolatum*. *J. Zool.* 170, 325–338.
- Wolfe, K.H., and Shields, D.C. (1997). Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* 387, 708–713.
- Zipursky, S.L. and Grueber, W.B. (2013), The molecular basis of self-avoidance. *Annu. Rev. Neurosci* 36, 547–568.