Evolution in a nutshell

EMBL PhD student symposium on evolution

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The EMBL PhD Student Symposium on Evolution, supported by the European Commission High-Level Scientific Conferences, was held at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, November 9–10, 2001. This event was organised entirely by PhD students from EMBL and was the second in a continuing series of symposia initiated in 2000 with 'From Genes to Thoughts'. The next event of this kind, 'Life within Boundaries: Membranes & Compartments in Biology', is scheduled for November 14–16, 2002.

Introduction

The European Molecular Biology Laboratory in Heidelberg hosted an exciting 2-day symposium last November covering various topics of evolution. The symposium was unusual in two respects: first, it had been organised entirely by PhD students; secondly, the invited speakers had been encouraged not only to talk about their most recent work, but also to present a general overview of their field of study. This made the symposium useful for both those specialised in the study of evolution and those committed to other research areas. The central importance of the evolutionary theory in most, if not all, areas of biology cannot be debated. Yet, as researchers often intellectually confined within the boundaries of our experimental paradigms and model organisms, we often forget to look at things from this perspective. The symposium provided an opportunity to experience the thrills and excitement of some of the hot topics in evolutionary theory, including the origin of life and human evolution, and to marvel at some fascinating novel experimental and technological applications, such as *in vitro* enzyme evolution and evolutionary robotics.

The early history of life

E. Szathmáry (Budapest, Hungary) presented some of his views on the origin of life and early molecular evolution (Maynard Smith and Szathmáry, 1999). He emphasised that, in this field of research, elegant experiments and intelligent theories could easily prove to be irrelevant. When researchers attempt to make meaningful statements about the early phases of life some billions of years ago, they rely on 'scenarios', the plausibility of which is judged by 'constrained speculations'. Some of these speculations now seem to overturn the most famous theory of the origin of life, that of the primordial soup. Furthermore, the relevance of the type of experiments first carried out by Stanley Miller, in which organic molecules were produced by simulating the ancient atmosphere exposed to lightning, is also diminishing. The problems arise partly from the incompatibility of the chemical reactions under such conditions and partly from uncertainties about whether the ancient atmosphere was reducing in nature, as would have been necessary for the proposed reactions to occur.

The emerging new paradigm relies on autocatalytic reaction cycles able to build up various biomolecules from simple starting materials (e.g. the 'formose reaction', a complex network of reactions producing sugars from pre-existing sugars and formaldehyde), with minerals (e.g. clay or pyrite) providing a binding surface for some of those reactions. Mineral surfaces could have played multiple roles in such a scenario. For example, their absorption of organic molecules would have increased the local concentrations of the molecules on the surface and generally improved the thermodynamics of chemical reactions. Although these and similar arguments point, quite compellingly, to abandoning the idea of the primordial soup, much more needs to be done to complement the surface theory. For example, no one has yet been able to create an autocatalytic reaction cycle that works on a mineral surface and provides a constant supply of organic molecules.

Researchers interested in understanding how the first replicating genetic molecules (most likely RNA) arose face problems in

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addition to that of explaining autocatalysis. As pointed out by Szathmáry, the length of genetic polymers is maximised by Eigen's 'error threshold'. Beyond a length of ~100 nucleotides, no sequence can be maintained simply by natural selection. This is because of the high mutation rate in the absence of replicase molecules, which cannot be encoded by a short genetic polymer. One way of circumventing this problem is to have a segmented genome composed of individual RNA molecules. However, in this type of scenario, one is faced with the problem of competition, with some molecules replicating faster than others and overrunning the system. Using a new theoretical formulation, Szathmáry showed that metabolic coupling between individual genes on a mineral surface could have solved this problem. Selfish replicators cannot undermine the system, as they do in a liquid environment, because if they overgrow they will no longer be supplemented metabolically by the other replicators. Indeed, these theoretical models of the RNA world, where different RNA molecules act as enzymes (ribozymes) able to catalyse either a variety of chemical reactions or the replication of other RNAs, are becoming more realistic as new experimental data on ribozymes come to light.

The origin of eukaryotes

T. Cavalier-Smith (Oxford, UK) discussed key events in the origin and diversification of eukaryotes. Some of these events involved radical changes in the biology of the cell. First, the bacterial cell with a hard exoskeleton, the cell wall, had to be transformed into one with a soft surface and an actin-tubulin endoskeleton. This could have led to the origin of mitosis, of an endomembrane system with vesicle budding and targeting, and of the nucleus, i.e. the eukaryotic cell, ~800–850 million years ago. A cell with a soft surface could later have engulfed the bacterial ancestor of mitochondria (Figure 1). Subsequently, eukaryotes split into two major branches that are clearly separated from one another according to phylogenetic trees based on ribosomal RNA sequences. One branch comprises the so-called opisthokonts (cells with a single flagellum in the back). By diversification, this group gave rise to the ancestors of fungi, animals and choanozoa (a group of flagellated protozoans).

In the other branch, which includes amoebozoia and bikonts (cells with two cilia), further major symbiogenetic events occurred. Roughly 580 million years ago, the cyanobacterial ancestor of chloroplasts was engulfed to form the plant lineage. Another group, the chromalveolates, arose by enslavement of a red alga by a biciliate protozoan host (Figure 1). This group diversified to give rise to brown algae and diatoms, among other taxa. This secondary symbiogenetic event, i.e. the permanent evolutionary merger of two eukaryotic cells, resulted in very interesting cellular architecture. For example, protein products that are encoded by the host genome and destined for the red algal chloroplast need to cross four membranes. In some lineages, the chimaeric chromalveolates have lost the chloroplasts and thus the ability to photosynthesise. Even when the chloroplasts are retained, most of the genes of the red alga have also been lost or transferred into the host nucleus. A miniature nucleus, the nucleomorph, remains only in the group termed cryptomonads (Douglas *et al.*, 2001). Based on the variation of the size of this nucleomorph in different cryptomonads and on the universal

Fig. 1. Secondary symbiogenetic origin and membrane topology of cryptomonads. Reprinted with permission from *Nature* (Douglas *et al.*, 2001).

correlation between nuclear genome size and cell volume, Cavalier-Smith presented compelling arguments for rejecting the selfish and junk DNA interpretation of the evolution of non-coding DNA, which posits that DNA can easily accumulate in genomes, even if it is non-functional. The fact that the size of the nucleomorph in cryptomonads is highly reduced and gene-dense shows that non-functional nuclear DNA can be eliminated very efficiently by selection. This means that the large amounts of non-coding DNA in the host nuclei of these chimeras, and in nuclei in general, must be maintained by positive selection. Based on these arguments, Cavalier-Smith suggested that non-coding DNA can have a skeletal function. According to this hypothesis, nuclear DNA functions as the basic framework for the assembly of the nucleus, and the total genomic DNA content functions in determining nuclear volume and thereby cell volume.

The Cambrian explosion and predictability in evolution

The Cambrian explosion ~550 million years ago, in which metazoan phyla rapidly diversified, continues to attract wide attention. S. Conway Morris (Cambridge, UK) presented new fossil findings from the Lower Cambrian that provide insights into the origin of metazoan body plans. Brachiopods (two-shelled marine invertebrates), for example, seem to have evolved from slug-like ancestors with a shell at each end of the body. Similarly, the origin of the deuterostome clade can be traced back to fossil animals with primitive gill slits (Shu *et al.*, 2001). Conway Morris also discussed the process of convergence and how it relates to the question of predictability of evolutionary trends. One of his major goals is to establish some degree of structural predictability for the way that complex organisms are likely to evolve in biospheres. He argued that, since evolutionary processes are

strongly constrained, the emergence of certain features, including the origin of intelligent bipeds (two-footed organisms), is inevitable. This raises interesting questions about what could happen elsewhere in the Galaxy.

S.J. Gould (Cambridge, MA) was not convinced by these arguments. He pointed out that the fact that intelligent bipeds appeared only once on the evolutionary tree is equally consistent with two extreme suppositions, namely that such organisms evolve every now and then in the universe or, alternatively, that the chances that they arise twice are vanishingly small. Gould additionally discussed the historical setting in which Darwin published his work and the radical nature of Darwin's new ideas in the intellectual atmosphere of his days. He also claimed, based on the punctuated patterns of speciation (short periods of speciation followed by long periods of stasis) observable in the fossil record, that, at least on a geological timescale, species are individual entities with definite birth and death points and a characteristic lifespan. Gould proposed that, if this is indeed the case, natural selection could also act at the level of species, not only at that of genes or organisms.

Genes and the evolution of development

When we try to understand the genetic changes underlying past evolutionary events, a comparison of the expression patterns of developmental genes in extant organisms can prove to be very fruitful. M. Averof (Heraklion, Greece) talked about the generation of segment diversity during arthropod evolution, relating differences in expression patterns to differences in morphology. Segment and appendage diversification was prevalent during the evolution of the arthropods, and Hox genes (a group of transcription factors central to segment specification) are likely to underlie many of these changes. Since all members of the Hox family are found throughout arthropod classes, the mechanism of change cannot have been extensive gene duplications, and it is possible that changes in expression patterns contributed to segment diversity. To test this, Averof, together with N. Patel, looked at the expression patterns of the Hox genes *Ubx* and *AbdA* in various crustacean species (Figure 2; Averof and Patel, 1997). They found that *Ubx* and *AbdA* were expressed in all thoracic segments in species with uniform segment morphology, but *Ubx* and *AbdA* expression was specifically lacking in those segments in species where some of the thoracic segments were transformed. As emphasised by Averof, other morphological changes may have resulted from alterations in the expression of downstream genes. This complication, combined with the long evolutionary histories of the species involved, make these analyses particularly challenging.

Many of the problems discussed above do not exist if one is studying relatively recent evolutionary transformations. D. Stern (Princeton, NJ) discussed small phenotypic variation between closely related species and the genetic causes of such variation. When Stern, together with E. Sucena, looked at specimens of the fruit fly *Drosophila sechellia* and its sibling species, they found a striking difference between the patterns of fine cuticular hairs on the larvae of these animals (Sucena and Stern, 2000). In contrast to the hairy larval cuticle of its close relatives, *D. sechellia* larvae were almost completely naked. When looking for the genetic causes of this variation, they were surprised to find that this difference is caused entirely by evolution at a single locus,

Fig. 2. Phylogenetic distribution of different patterns of segmental specialisation and *Ubx-AbdA* expression among crustaceans. Reprinted with permission from *Nature* (Averof and Patel, 1997).

namely *ovo/shaven-baby*. This gene is required to make the larval hairs; when it is mutated in *D. melanogaster*, larvae with a naked cuticle develop. Whereas the *shaven-baby* transcript is present in the cells that differentiate the hairy larval cuticle in the embryos of *D. melanogaster*, it is absent from the corresponding ones in *D. sechellia*; therefore, these cells only differentiate naked cuticle. Although the *shaven-baby* locus in *D. sechellia* was clearly defective in this respect, other functions were unaffected. This indicates that the changes leading to the *D. sechellia* phenotype occurred in the *cis*-regulatory region of the gene. This example represents a mechanism of evolutionary transition where distinct functions of a pleiotropic gene can evolve independently by subtle changes in the *cis*-regulatory region.

Evolution in the laboratory

The study of evolution is no longer solely reliant on theory, fossils and the comparison of extant organisms: a fascinating new science of experimental evolution has recently started to emerge. This area was well represented at the symposium.

A. Griffiths (Cambridge, UK) presented methods for the evolution of enzymes in the laboratory. The future technological benefits of these tailor-made enzymes is hard to overestimate, and these endeavours could also provide invaluable information for those who would like to understand the origin and evolution of natural enzymes.

To produce tailor-made enzymes by *in vitro* evolution, one must start with a sufficiently large gene library. Proteins encoded by these genes are then synthesised and tested for their activity, and those that have the best properties are selected out. Subsequently, the genes encoding the selected proteins are mutagenised to produce additional variation. One can hope that, as multiple mutation-selection cycles are carried out, a good catalyst will evolve. The phenotype-genotype linking required to keep the protein and the encoding gene together is one of the most challenging aspects of such applications. Griffiths presented an ingenious new way of achieving this (Griffiths and Tawfik, 2000). The method relies on the *in vitro* compartmentalisation of individual members of a gene library

into tiny lipid vesicles. The library, with each gene bound to a bead, is emulsified such that each lipid compartment contains only one gene in a buffer containing all of the transcription and translation machinery. The individual genes are then transcribed and translated in the compartments, and the proteins that are produced are immediately coupled to the bead (e.g. by synthesising polypeptides fused to streptavidin that are attached to biotin beads). The beads are then released from the compartments, washed and, in a next round of compartmentalisation, substrates are added. Since translation is separated from the enzyme reaction step, the conditions for the latter can be varied extensively. The extension of this method opens up new opportunities for the evolution of better enzymes with diverse activities, the understanding of protein function and evolution and the sampling of the prodigious protein diversity of the biosphere.

When studying naturally evolved organisms, researchers face the problems of complex and undefined selection histories and uncertainties about phylogenies. These limitations can be circumvented if one is studying evolution in the laboratory. As explained by G.J. Velicer (Tübingen, Germany), microbes are particularly well suited for such studies because of their short generation times, the easy adjustment of selection conditions and the straightforward way of generating a fossil record in a laboratory freezer. Velicer has addressed experimentally the problems of the evolution of social behaviour in the microbial world using a species belonging to a group of social microbes, the myxobacteria. *Myxococcus xanthus* is a bacterium that forms multicellular fruiting bodies upon starvation. These structures are composed of a number of differentiated cell types, only one of which is able to form spores. The ability to develop the fruiting body partly relies on the presence of two distinct motility systems and also on the production of specialised pili, both of which probably impose extra costs ('costs of socialisation') upon their carrier. To examine the fate of social behaviour under asocial growth conditions, Velicer established replicate populations from a common ancestor and grew them for many hundred generations in nutrient-rich media. He found that the bacteria could rapidly adapt to this new situation. The strains that evolved grew faster, and most had lost both their social motility and the ability to develop a fruiting body. Losing these traits increased the fitness of the bacteria, whereas the artificial introduction of functional social motility genes from the ancestor both restored motility and reduced competitive fitness in evolved clones. Socialisation is thus cumbersome when it is unnecessary. Moreover, some of the asocial laboratory-evolved genotypes showed 'cheating behaviour' during fruiting-body formation when mixed with their socially proficient ancestor. In other words, although they were defective in pure-culture spore production, these genotypes were even more efficient at spore production than their ancestor when mixed with them at a low frequency (Velicer *et al.*, 2000).

Experimental evolutionary science does not exclusively deal with the organic world. Machines can also be made to evolve. As presented by D. Floreano (Lausanne, Switzerland), evolutionary robotics is a powerful alternative to less flexible robot design. Floreano's robots, small machines with two wheels for locomotion and eight infrared sensors for light perception, are connected to a computer and operate following the instructions of an artificial neuronal network. In a series of experiments, these robots had to accomplish simple tasks in different environments (e.g. navigation

through a maze). The parameters of the neural networks were made to change by allowing mutation and recombination, and the most successful robots were selected according to various selection criteria. After many generations of selection, neural networks of remarkable competence evolved. When Floreano analysed the behaviour of the evolved networks in the computer, he found some artificial neurons detecting the distance from the light source, as well as others changing activity according to the position of the robot (analogous to the place cells in mammalian brains). Quite amazingly, when the ability to change the parameters of synaptic transmission was also introduced into the evolving artificial robot brains, the performance of these learning networks was superior to those lacking this plasticity (Urzalai and Floreano, 2001). The finding that intelligent and flexible behaviours can emerge out of very simple networks challenges some of the current tenets of robotics, artificial intelligence and cognitive sciences.

Human evolution

Whatever the truth about the inevitability of the evolution of intelligent bipeds might be, human evolution is certainly an issue of general interest. S. Pääbo (Leipzig, Germany) explained how the analysis of DNA from humans and great apes could clarify our evolutionary history and elucidate some of the genetic traits that define humans as a species. An analysis of intraspecific DNA sequence diversity in humans and great apes carried out in Pääbo's laboratory indicated that the extent of DNA sequence variation in the great apes is several-fold greater than that in humans (Figure 3). Humans are thus unique among the apes in having a low level of genetic variation, which is a sign of recent population expansion (Kaessmann *et al.*, 2001). In another approach, the relative levels of expression of 20 000 genes in humans and chimps were analysed. Striking differences in the rate of evolutionary change, i.e. to what extent expression levels change during a given period of time, were found between different organs. For example, whereas the rates of change in the liver and the blood have been similar between chimps and humans, in the case of the human brain, the rate has accelerated ~3-fold, indicating that evolution has been shaping this organ strongly in the human lineage. Many individual genes with significantly altered expression levels have been found, including one that may be involved in the development of complex cognitive and language skills in humans. This gene

Fig. 3. Phylogenetic tree of human, chimpanzee, bonobo, gorilla and orang-utan Xq13.3 sequences. Reprinted with permission from *Nature Genetics* (Kaessmann *et al.*, 2001).

encodes the FOXP2 transcription factor, the mutation of which leads to language problems (Lai *et al.*, 2001). Pääbo found strong indications of selection for changes in this gene in humans in the recent past. Since FOXP2 is thought to be required for the fine motor control of the mouth and the larynx, it is easily conceivable that selection on this locus could have contributed to the refinement of a primitive form of vocal communication in early humans.

Some unresolved issues

The symposium ended with a panel discussion about questions of microevolution (evolution within the species) and macroevolution (evolution after speciation). The issue at stake was whether extrapolation from the selection theory operating on organisms is sufficient to explain all patterns of macroevolution. In other words, do we need an independent body of theory to explain the changes occurring above, as opposed to at, the species level? There was no general agreement among the panel members. It seems that the jury is still out on this important question. Similarly, although the question of species level selection was debated, the arguments seemed not to lead to a consensus. However, there was agreement on other questions. Many speakers emphasised the role of internal constraints, which had not been considered in conventional Darwinian thinking. Constraints set, for example, by developmental gene networks, and probably many other unforeseen rules of complexity, define the boundaries of what is possible. As phrased by Szathmáry, 'If we want to know what can happen in evolution, we must have an idea about the possible'. That is why we must develop theories of gene networks, development, neural networks and, more generally, emerging complexity.

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References

- Averof, M. and Patel, N.H. (1997) Crustacean appendage evolution associated with changes in Hox gene expression. *Nature*, **388**, 682–686.
- Douglas, S. *et al.* (2001) The highly reduced genome of an enslaved algal nucleus. *Nature*, **410**, 1091–1096.
- Griffiths, A.D. and Tawfik, D.S. (2000) Man-made enzymes—from design to *in vitro* compartmentalisation. *Curr. Opin. Biotechnol.*, **11**, 338–353.
- Kaessmann, H., Wiebe, V., Weiss, G. and Pääbo, S. (2001) Great ape DNA sequences reveal a reduced diversity and an expansion in humans. *Nature Genet.*, **27**, 155–156.
- Lai, C.S., Fisher, S.E., Hurst, J.A., Vargha-Khadem, F. and Monaco, A.P. (2001) A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature*, **413**, 519–523.
- Maynard Smith, J. and Szathmáry, E. (1999) *The Origins of Life: From the Birth of Life to the Origin of Language*. Oxford University Press, Oxford, UK.
- Shu, D.G., Morris, S.C., Han, J., Chen, L., Zhang, X.L., Zhang, Z.F., Liu, H.Q., Li, Y. and Liu, J.N. (2001) Primitive deuterostomes from the Chengjiang Lagerstätte (Lower Cambrian, China). *Nature*, **414**, 419–424.
- Sucena, E. and Stern, D.L. (2000) Divergence of larval morphology between *Drosophila sechellia* and its sibling species caused by *cis*-regulatory evolution of *ovolshaven-baby*. *Proc. Natl Acad. Sci. USA*, **97**, 4530–4534.
- Urzalai, J. and Floreano, D. (2001) Evolution of adaptive synapses: robots with fast adaptive behavior in new environments. *Evol. Comput.*, **9**, 495–524.
- Velicer, G.J., Kroos, L. and Lenski, R.E. (2000) Developmental cheating in the social bacterium *Myxococcus xanthus*. *Nature*, **404**, 598–601.

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