ZOONET: Perspectives on the Evolution of Animal Form. Meeting Report

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ABSTRACT   What drives evolution? This was one of the main questions raised at the final ZOONET meeting in Budapest, Hungary, in November 2008. The meeting marked the conclusion of ZOONET, an EU-funded Marie-Curie Research Training Network comprising nine research groups from all over Europe (Max Telford, University College London; Michael Akam, University of Cambridge; Detlev Arendt, EMBL Heidelberg; Maria Ina Arnone, Stazione Zoologica Anton Dohrn Napoli; Michalis Averof, IMBB Heraklion; Graham Budd, Uppsala University; Richard Copley, University of Oxford; Wim Damen, University of Cologne; Ernst Wimmer, University of Göttingen). ZOONET meetings and practical courses held during the past four years provided researchers from diverse backgrounds—bioinformatics, phylogenetics, embryology, palaeontology, and developmental and molecular biology—the opportunity to discuss their work under a common umbrella of evolutionary developmental biology (Evo Devo). The Budapest meeting emphasized in-depth discussions of the key concepts defining Evo Devo, and bringing together ZOONET researchers with external speakers who were invited to present their views on the evolution of animal form. The discussion sessions addressed four main topics: the driving forces of evolution, segmentation, fossils and phylogeny, and the future of Evo Devo. *J. Exp. Zool. (Mol. Dev. Evol.) 312B:679– 685, 2009.*   © 2009 Wiley-Liss, Inc.

DRIVING FORCES OF EVOLUTION

Evo Devo is constantly being redefined both conceptually and practically. Discussions during ZOONET's Budapest meeting highlighted the dualistic nature of Evo Devo. Macroevolution vs. microevolution, morphology vs. genotype, and

punctualism vs. gradualism illustrate how many areas of this science still await resolution. The meeting opened with a lecture by Wallace Arthur (National University of Ireland, Galway) who summarized the ongoing dispute between neo-Darwinists and developmental biologists. The discussion of whether evolution occurred gradually (neo-Darwinism) or by sudden leaps (punctuated equilibrium) naturally lends itself to a discussion of the mechanisms driving evolution. The processes underlying evolution are numerous and include (but are not exclusive to) the following: mutation, genetic drift, recombination, gene, and genome duplication and selection. At the core of all these processes are heritable differences in gene expression and function.

Cis-regulatory regions of genes, wherein information directing the temporal and spatial expression of genes is found, are the subject of many recent research programs in evolution and development (for review, see Wray, 2007; Prud'homme et al., 2007). Mutations in cis-regulatory sequences are considered to be very important for morphological evolution (Carroll, 2008). At the meeting, Benjamin Prud'homme (Institut de Biologie du Développement de Marseille-Luminy) presented examples emphasizing the importance of changes in cis-regulatory elements for the evolution of animal form (Prud'homme et al., 2006). He illustrated that, in *Drosophila*, a few mutations in a cis-regulatory element are sufficient to modify the expression of the *yellow* gene, leading to differences in pigmentation patterns. In contrast to mutations in coding regions, which can be highly pleiotropic, changes in cis-regulatory regions are considered to be less pleiotropic as a consequence of their modularity: many genes contain cis-regulatory enhancer modules that are responsible for specific spatial and temporal subsets of expression.

Despite the finding that a growing number of morphological differences between species are caused by cis-regulatory changes, this has also been the source of some controversy in the discipline (see Hoekstra and Coyne, 2007; Wray, 2007; Carroll, 2008; Wagner and Lynch, 2008). Arguments over the evolutionary impact of change in the coding sequences of genes, and thus change in the proteins produced, are at the heart of this discussion. Günter Wagner (Yale University) presented a study of the *Prolactin* gene during eutherian pregnancy to illustrate how mutations in coding regions as well as cis-regulatory regions may underlie phenotypic change (Lynch et al.,

2008). Wagner's example also highlighted that insertions of large DNA fragments like transposable elements is another important, albeit largely understudied, mechanism in evolution. Lynch et al. (2008) demonstrated that the transposable element MER20 became a novel enhancer for *Prolactin* after only a few mutations. This caused a fundamental change in *Prolactin*-expression during mammalian evolution. The consequence of this change was a new role for *Prolactin* during eutherian pregnancy through its involvement in the development of the invasive placenta. Furthermore, a key regulator of *Prolactin* is the transcription factor (TF) *HoxA11*. Mutations in the coding region of *HoxA11* caused a change in its protein structure, resulting in new protein–protein interactions and regulatory activities. Therefore, this study emphasized that TF protein structure is also often modular and can play an important role in evolutionary change.

Prud'homme speculated during the discussion following Wagner's talk that the whole genome duplication that occurred within the vertebrate lineage, and the accompanying redundancy in TF gene function, may have alleviated the negative pleiotropic affects that could accompany changes in coding sequences of TFs. It is also interesting here to note the work of Wittkopp et al. (2004, 2008a,b), who have studied the relative contributions of cis- vs. trans-variations within and between *Drosophila* species. These authors demonstrate a prevalence of trans-mutations within species and cis-mutations between species. They suggest that this may reflect the fact that trans-mutations with varying degrees of pleiotropic effects are maintained within populations by mutation/selection balance, whereas cis-mutations are more readily fixed within populations. Similar ideas have also been presented by Lemos et al. (2008), who add that additivity of allelic differences contributing to cis-effects is an important component contributing to preferential fixation between populations as compared with the fixation of trans-effects.

Observations such as those summarized above highlight that evolutionary change is the product of several processes operating across different taxonomic levels. It was clearly evident from all discussions that the perceived rift between those who choose to study cis-regulation as a driving force in evolution and those who investigate changes in proteins (for example, coding sequence changes or post-translational modifications) is not nearly as wide as has been recently suggested (Pennisi, 2008).

SEGMENTATION

In ZOONET and the wider Evo Devo community, there is an ongoing and controversial debate on the origin of segmentation, for example, its absence or presence in the last common ancestor of all bilaterians, the so-called urbilaterian. One prerequisite for fruitful discussion of this subject is to make clear what kind of segmentation is being referred to in comparisons of different animal groups. Comments on ''segmentation'' per se are too common and too vague, while questions such as: ''Did the last common ancestor of vertebrates and arthropods have a serially repeated mesoderm or serially repeated nephridia or serial repeated units in the nervous system?'' are sensible and tangible.

Approaches for determining the homology of segments between different phyla include the comparison of gene expression patterns during segmentation and functional analysis of the genes involved. However, it is apparent that ''similar'' expression of single genes is not sufficient to establish homology between distinct phyla. For example, the engrailed expression in the polyplacophoran mollusc *Lepidochitona caverna* indicates the development of shell glands rather than segment borders (Jacobs et al., 2000). A total evidence approach, involving a comprehensive examination of the mechanisms behind segment formation, is preferable when trying to identify homology of segmentation.

At least the reduction or loss of segmentation in connection with a small body size can be made plausible, e.g. in some archiannelids and in echiurans. The reduction of segmentation in echiurans can be inferred from the nervous system, which still displays segmented structures as found in other annelids (Hessling and Westheide, 2002). Also, segmentation can apparently be secondarily developed; basal rhabditophoran flatworms do not show any segmentation (apart from paratomy during asexual reproduction in some taxa), whereas the derived parasitic rhabditophorans, the Neodermata, are well-known for their body segmentation (e.g. tapeworms; Olson et al., 2008).

Which taxa in addition to the ''big three'' (annelids, arthropods, and vertebrates) should be studied for elucidating the phylogenetic signal of segmentation? Acoels, sometimes considered to be basally branching bilaterians, do not show morphological signs of segmentation (again, apart from paratomy during asexual reproduction).

Possible sister groups of the arthropods, such as kinorhynchs and priapulids, are potentially interesting, but can be difficult to obtain, maintain, and analyze in the laboratory. Nonetheless, the need for additional models to answer specific questions was stressed during this meeting.

Many researchers involved in ZOONET focus on segmentation and employ a comparative approach, a common strategy in Evo Devo research. During the Budapest meeting, segment formation in vertebrates was discussed by Olivier Pourquié (Stowers Institute for Medical Research, Kansas City), who summarized what is known about the vertebrate segmentation clock (the process of the formation of the somites) and the role of several genes, including members of the *Wnt* signalling pathway, *FGF*-signalling, *hairy*, *Notch*, and *Delta* (Gomez et al., 2008). Vertebrates display different numbers of somites from approximately 10 in frogs, and 55 in chickens to hundreds in snakes. The high number of segments in snakes is based on a higher frequency of segment formation during development (Gomez et al., 2008). The comparison with chicken embryos demonstrates that the segmentation clock in the posterior growth zone is relatively faster in snakes than in the chicken.

Some of the genes regulating segmentation in vertebrates are also involved in segmentation, or at least posterior development, in some arthropods such as spiders, beetles, and cockroaches (Damen, 2007; Bolognesi et al., 2008; Pueyo et al., 2008; McGregor et al., 2009). This suggests that aspects of the genetic regulatory network involved in posterior development were employed in the Urbilaterian, but further work is required to determine whether or not this conservation reflects an ancestral segmented condition of the urbilaterian ancestor.

FOSSILS AND PHYLOGENY

The use of comparative studies of recent phyla to reconstruct the body plan of a hypothetical ancestral species is one way to gain insights into evolution. A second source of information is the fossil record. Fossils provide a tangible record of how the evolution of morphology occurred through time, in particular, the order of acquisition of distinct characters.

Fossils can be placed within a phylogeny generated from morphological and/or molecular data by the application of the stem and crown group concept. Extant taxa are placed in a crown

group, which is a monophyletic grouping consisting of the last common ancestor of all extant members of the group and all of the descendents of that ancestor. Fossil organisms that are similar to a crown group but do not contain all of its morphological characteristics are placed in the stem group (Budd, 2001). This approach has been particularly successful when applied to the enigmatic fossils of the "Cambrian Explosion," which record the early evolution of most modern animal phyla approximately 510 million years ago. It may seem counter-intuitive to incorporate fossils, which are interpreted solely on morphological characteristics, into a molecular phylogeny of modern organisms, particularly because strictly morphology-based approaches to reconstructing the phylogeny of extant taxa have been less successful than some molecular approaches. Although missing information on soft part morphology, growth, and development can be substantial, the morphological characters of fossils that are preserved may be more plesiomorphic than those observed in modern taxa (Cloutier, '94), indicating that the morphology of fossils could be more reliable than the morphology of modern taxa when reconstructing phylogenies. Assuming that modern phyla diverged 550–560 million year ago, fossils from the Cambrian Explosion have only been subjected to evolutionary processes for 40–50 million years, whereas modern taxa have been accumulating reversals and convergences for nearly half a billion years. Thus, fossil taxa may, in some cases, give a clearer phylogenetic signal than living taxa.

Despite past tensions between molecular phylogeneticists and palaeontologists, it is becoming more common for molecular phylogenies to incorporate fossil data within the framework of the stem and crown group concept. Unfortunately, it is not always easy to identify stem group fossils, as Philip Donoghue (University of Bristol) described at the ZOONET meeting. It is often difficult to determine whether the lack of certain crown group characters in a fossil is because they are genuinely absent and that fossil is in the stem group, or because they are actually present but not preserved due to taphonomic (decay and fossilisation) processes (Donoghue and Purnell, 2009). Some taxa, in particular soft-bodied animals, are more susceptible to taphonomic effects than others, such as those with a hard skeleton, so their absence in the fossil record does not necessarily mean that those taxa did not exist. The interpretation of stem group fossils can also

vary widely, especially for those that branch off basally within the stem group. Problems of interpretation in palaeontology are sometimes aggravated by political situations within the field that can restrict the accessibility of specimens and result in the publication of insufficient descriptions and illustrations of new and critical fossils. In some disciplines, competition for funding and prestige between different institutional groups working on similar fossils can discourage cooperation within the field and create a pressure to rapidly publish new and important fossil material in high-ranking journals, often without including thorough, well-illustrated descriptions. Despite these problems, valuable information can be retrieved from fossils, provided that due diligence is paid to detailed and accurate descriptions of the material.

A relatively new approach in molecular phylogenetic reconstruction highlights another practical use of fossils. They can be used to date divergence times of different lineages in a molecular phylogeny using the principles of a "molecular clock." Davide Pisani (The National University of Ireland Maynooth) presented recent progress in this field, where previously static models are becoming more flexible by allowing substitution rates to vary, and by using minimum and soft maximum ages of fossils to provide a range within which divergence dates can vary (i.e. Pisani, 2004; Wilkinson et al., 2005; Smith et al., 2006). Also, the molecular phylogenies themselves are becoming more stable by using analyses in addition to parsimony, such as Bayesian inference and maximum likelihood methods. These methods have further been used to reconstruct ancestral character states for organisms or characters where fossil information is absent or scarce. Pisani suggested that ancestral character state reconstruction methods, particularly Bayesian inference, could also be employed by palaeontologists to compare reconstructed ancestral character states with those found in extinct organisms, as a more statistical evaluation of fossil material.

Recent work by Pisani incorporated various molecular phylogenetic techniques to shed light on the evolution of color vision within the animal kingdom (Pisani et al., 2006). Examination of different recent taxa led him to hypothesize that the evolution of color vision was coupled with the evolution of different types of opsins, since more than one opsin is required to distinguish light of different wavelength and therefore different color. But it is still unclear if merely the presence of a

second type of opsin is enough to infer that an organism had color vision. This question was also addressed by Gáspár Jékely (Max-Planck-Institut Tübingen) who presented his work on phototaxis. He showed that phototaxis is one of the simplest behaviors found in, for example, monocellular algae, but also in planktonic stages of marine animals such as the annelid *Platynereis dumerilii*. In *P. dumerilii*, only one neuron is required to transmit the light information to a locomotory organ, the ciliary belt, and create a response that varies the beat of the cilia around the ciliary belt to create movement. Using a computer model, Jékely could predict the swimming behavior of the larvae according to different light conditions and different beating behaviors of the cilia. Further investigations showed that older larvae turn from positive phototaxis to negative phototaxis. Jékely believes that this is triggered by the presence of a second type of rhabdomeric opsin in *P. dumerilii*, providing an example of how the presence of two rhabdomeric opsins did not result in color vision because they were used in different ways during development (Jékely et al., 2008). Thus, the evolution of color vision was not reliant on the simple presence or absence of a second opsin. It may often be the case that the evolution of functions and behaviors is more complex than can be inferred simply by the presence or absence of a gene. Elucidating the evolution of complexity may best be achieved by considering the genetic data as part of a large body of evidence that includes information from other fields including phylogenetic relationships, ecological data, and paleontology.

THE FUTURE OF EVO DEVO

While Evo Devo has its roots in the late 1800s with the important observations of Ernst Haeckel, Karl Ernst von Baer, and colleagues on the similarities in the development of different species, it is only in the last few decades that Evo Devo has emerged as a separate field of biological research. Today, Evo Devo has an important role in integrating comparative anatomy, embryology, molecular and developmental biology, and paleontology in an evolutionary context, as illustrated by Angelika Stollewerk's (Queen Mary University of London) work. Stollewerk employs a comparative approach in her research to investigate the evolution of the nervous system and presents an overview of neurogenesis in arthropods. Insects and malacostracan crustaceans show a relatively

similar mode of neuron formation based on the division of neuroectodermal cells that give rise to neuroblasts. These neuroblasts divide in a specific manner to form neurons and glial cells, which is different to myriapods and chelicerates where groups of neuron precursors invaginate from the neuroectodem to form glial and neuronal cells (Stollewerk and Simpson, 2005). So far, it is not clear how to interpret the distribution of characters described above. Following the Mandibulata-hypothesis, the mode of neurogenesis observed in chelicerates and myriapods is hypothesized to be the ancestral state present in the last common ancestor of all euarthropoda. If the Myriochelata-hypothesis is correct, it is unclear which mode of neuronal development is the ancestral one. Only the comparison with representatives of an outgroup such as onychoporans or tardigrads can help to resolve these questions.

The integration of information and methods from different fields within Biology gives Evo Devo a platform through which various research problems can be explored; however, the relevance of these findings in today's society may, at times, be unclear to the general public. Much of the research taking place within Evo Devo focuses on ''nonmodel'' organisms, as opposed to more traditional model organisms such as the fruit fly, the zebrafish, and, of course, *Homo sapiens*. It may not be immediately obvious to those outside the field how knowledge on the evolution and development of an obscure invertebrate taxon, or even nonhuman model organisms, can contribute to the general welfare of the modern world. Jason Scott Robert (Arizona State University) addressed this issue by focussing on the benefits Evo Devo provides to biomedical research. Experimentation on various nonmodel taxa can help identify the model organism that is best suited to study a disease or disease-related process. For example, not all human disease research needs to be conducted on nonhuman primates, as mice or other nonmodel organisms may serve the same purpose adequately and would allow research to happen at a faster pace because such animals are more abundant and easier to culture in a laboratory setting, have faster reproduction times, and are less costly to obtain (Jenner and Wills, 2007; Milinkovitch and Tzika, 2007). Unexpected but valuable results can come when researchers are forced to adapt standard research procedures to unusual model organisms. Evo Devo research provides information on biological processes, the application of which may not be immediately

obvious but which could over time prove to be invaluable.

With the celebration of Charles Darwin's 200th birthday this year, it seems appropriate to focus on the evolutionary aspect of Evo Devo. This unifying concept is the very foundation on which the diversity of animal form and function was built. All variations in the genotype, ontogeny, and morphology of an organism are the result of evolution, and the results of research conducted on these topics should always be interpreted in an evolutionary context. At times, workers within Evo Devo may focus too stringently on their specific field of research, without making enough attempt to fit their results into the broader picture of how and why life evolved the way it did—a pitfall that should be avoided. After all, an in situ hybridization is just a stain on an animal, and a fossil is just a piece of rock, until they are properly interpreted and their evolutionary implications elucidated.

Often, placing research results into the broader theme of evolution requires an integration of information from many different fields, which is why we consider collaboration among scientists as absolutely vital for the future of Evo Devo. As a research training network, ZOONET nurtured these efforts through scientific meetings and ZOONET/EMBO courses organized in Naples in 2006 and in Sweden in 2008, which not only provided valuable training and education, but also supplied a wealth of networking and collaborative opportunities. As a testament to the success of collaboration, approximately one-third of the papers published by ZOONET members in the last four years have been joint efforts between different laboratories. Research training networks also provide a venue for frequent informal meetings, which are a very welcome complement to larger events such as the biannual meetings of the European society for Evolution and Development (EED). A smaller meeting format helps especially younger scientists to feel comfortable contributing to discussion and to develop a strong personal network for their future as interdisciplinary researchers in Evo Devo.

Returning to our initial question: What drives evolution? The ZOONET meeting was not expected to produce definite answers, but the debate surrounding this question was a reflection of the current climate within Evo Devo. Although the nature of this field is often dualistic and prone to extensive debate, collaborative efforts pervade that bring together several different scientific fields to create a broader understanding of evolution. A collaborative spirit among Evo Devo workers combined with a critical approach to both experimental and theoretical evolutionary studies is particularly important in today's society where, for example, less than half of the American population believes in Evolution (e.g. Harris poll, November 2008, www.harrisinteractive.com), and there are movements proposing that Creationism and Intelligent Design should be taught in school as nonscientific ''alternatives'' to Darwinian evolution. Evo Devo is particularly well equipped to promote the study and teaching of evolution, because of its broad, interdisciplinary perspective on the topic. Research training networks, such as ZOONET, play a critical role in preparing young researchers for their future contributions to the field of Evo Devo. When the question ''What drives evolution?'' was asked at the Budapest meeting, the room of about 50 people could not come to a general consensus, but all present agreed that the field of Evo Devo was on the right track toward answering this fundamental question.

ACKNOWLEDGMENTS

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