An increase in animal diversity was facilitated by ecologically-driven brain complexity throughout the Cambrian

Ariel D. Chipman

The Department of Ecology, Evolution & Behavior, The Alexander Silberman Institute of Life
Sciences, The Hebrew University of Jerusalem, Edmond J. Safra Campus, 9190401 Jerusalem,
Israel

* Author for correspondence: ariel.chipman@huji.ac.il

Abstract

The Cambrian Explosion is often seen as a singular event requiring an explanation. In fact, it is better represented as a cascade of linked events, each with numerous causes. The iconic middle Cambrian fauna, represented by sites such as the Burgess Shale, is a culmination of several phases of increases in taxonomic diversity and morphological complexity. I focus on an often-overlooked increase in complexity that took place in a limited number of phyla in parallel after the main "explosion". This increase in morphological complexity and in disparity was facilitated by an increase in the complexity of the central nervous system, which in itself was a selective response to the ecological complexity of the biosphere, which had been increasing from the late Ediacaran. Genetic regulatory components that contributed to an increasingly differentiated and regionalized central nervous system were developmentally co-opted to increase the differentiation and complexity of additional organ systems. This process took place convergently in arthropods, mollusks and annelids at different times throughout the Cambrian and, later in the Ordovician, also in vertebrates.

Introduction

The period between the late Ediacaran and the early Cambrian (roughly 550-520 million years ago) is the most dramatic period in the evolution of animal life on Earth [1-4]. This period represents a sequence of increases in animal complexity and diversity, during which the biosphere transitioned from including a low diversity of mostly sessile suspension or bottom feeders to a world with numerous animal body plans occupying a dynamic tiered ecosystem with diverse feeding modes, comprising a range of motile animals moving using different modes of locomotion in different spaces[5-7]. This transition is usually referred to as the Cambrian Explosion. However, it is becoming increasingly clear that this term is a misnomer, since it is neither exclusively Cambrian nor a true explosion[8-12]. The roots of the explosion are in the Ediacaran[13-19], and the increase in animal diversity continued into the Ordovician [9,20,21]

Over the years, there have been many attempts to explain the "cause" of the Cambrian Explosion[8,15,22-32]. These explanations have come from different disciplines and often disagree, because each explanation tries to pinpoint the driving force behind a specific event or process that took place within the period spanning the late Ediacaran and early Cambrian. While most of the attempted explanations are probably correct for the specific event or process they focus on, no one explanation can fully explain the entire transition.

Indeed, the events spanning the border between the Ediacaran and Cambrian are better represented as a cascade. Rather than a single explosive event, we should be thinking about a series of causally linked events or processes. Each of these is dependent on the ones before it and lays the ground for the ones after it. The sequence of events forming what can be called the Cambrian Cascade, include both abiotic changes and biotic changes[4]. In many cases, the abiotic changes are driven by biotic events, and vice versa (e.g. changes in the chemistry and sedimentation of the sea floor were driven by changes in the behavior of animals occupying the sea floor). Many of the events or processes making up the Cambrian Cascade were general to the entire biosphere, and thus were presumably drive by global changes. Some can be seen as "lineage-internal", meaning they occurred within a specific lineage of animals (e.g., the evolution of a specific innovation at the origin of a certain clade[33]), whereas others can be seen as "lineage-interactive", meaning they were driven by interaction between organisms of different lineages, ultimately affecting both (e.g., predator-prey evolutionary arms races or competition for resources).

When we look at the distribution of diversity in the animal kingdom, it is clear that it is not distributed evenly. Only a handful of phyla have more than a few thousand species. Furthermore, the phyla that are today the most speciose (Arthropoda, Annelida, Mollusca, and Chordata) are also the ones that display the most complex body plans (manifested by *e.g.* segmented and regionalized bodies, complex centralized nervous systems, highly differentiated digestive systems, complex excretory systems etc.) I will argue that the early stages of the Cambrian Cascade affected almost all lineages within Bilateria, consolidating the general bilaterian body plan, whereas later stages affected only specific lineages – those lineages that display higher complexity and higher diversity (See Text Box 1 for a discussion of defining and identifying complexity). Evolutionary events that drove an increase in complexity in a small number of lineages, ultimately led to an increase in taxonomic diversity of those lineages. The late evolution of the complexity of these lineages, strongly suggests an independent process in each one, while the similarity in process and outcome suggests a shared driving force, which may have included interactions among different lineages.

I will briefly review some of the central steps of the cascade, pointing out which were global, and which were lineage specific or interactive. I will then focus on the step that I suggest led to the convergent increase in complexity and diversity in three of these four lineages, and suggest a novel testable hypothesis for the cause of these increases.

The Cambrian Cascade

Wormworld

When did the Cambrian Cascade begin? As with any continuous event, choosing a starting point is somewhat arbitrary. Since the Cambrian Explosion is usually seen as an explosion in the evolution of Bilateria, I think it is reasonable to place the beginning of the Cascade at the late Ediacaran, in the so-called Wormworld of the Nama epoch [15]. While the earlier Ediacaran fauna includes within it organisms that are generally accepted to be the ancestors of bilaterians[14,16,34-38], the Wormworld fauna includes the first undisputed prevalence of motile Bilateria.

The Wormworld fauna includes small animals, with either light mineral or organic exoskeletons. They appeared about 550 million years ago (Ma), probably in the wake of a mass extinction event[15,39,40]. Most of them were probably motile, moving along the sea floor and grazing on the bacterial mat that covered it. The fossil record of this period also includes trace fossils supporting the existence of a plethora of worm-like animals moving along the ancient sea floor[13,14,16,41,42].

Bilaterian genetic toolkits and Urbilateria

These early bilaterians likely had a through gut[17,43]. Indeed, the through-gut has been suggested to be a specific adaptation to the type of life style that includes moving through a food source[30], as was probably the case for most Wormworld fauna. At the molecular level, it is highly likely that they already had a Hox cluster, with a division into (at least) anterior, central and posterior class Hox genes[30]. At the regulatory level, they already had distal enhancers, which allow a more complex and fine-tuned regulation of gene expression[44-46].

There is an important point (even if seemingly trivial) to be made about this view of the Wormworld fauna. If the crown group of Bilateria already appeared by this point – and there is good reason to believe that it did[16,17,47,48] – any increases in complexity beyond the basic bilaterian body plan must have occurred independently in each separate lineage. Somewhere within the Wormworld fauna was the actual *Urbilateria* – the common ancestor of all extant bilaterians[49]. Over the years there has been a debate how complex *Urbilateria* actually was[50-54]. There is no reason to assume it was significantly more complex than its contemporaneous worms. It thus follows that all bilaterian characters not found in Wormworld are lineage-specific autapomorphies.

Three-dimensional ecosystems

The next major step in the cascade occurred at the beginning of the Cambrian. The official base of the Cambrian at 538.8 Ma is defined as the first appearance of trace fossils that penetrate the surface[4]; the first animals that live in a three-dimensional world[28]. These fossils are indicative of a change in substrate utilization. Rather than only living on the microbial mat they fed on, animals started burrowing more deeply. This behavior disrupted the microbial mat, ultimately leading to the decline of microbial mats as a significant ecosystem, in what is often termed the “Cambrian substrate revolution”[3,55-57]. The disruption of microbial mats led to the extinction of the fauna that relied on it and to the disappearance of the Nama Wormworld[15,27,40].

The number and diversity of penetrating trace fossils increased significantly during the first stage of the Cambrian, known as the Fortunian, indicating that changes in behavior were taking place in several different bilaterian lineages[42,58]. This increasingly complex behavior paved the way for increasingly complex ecological interactions.

Predation and tiered ecosystems

Several other significant events took place at roughly the same time throughout the Fortunian. The sequence and interdependence of these events is not always clear and they appear to have occurred very rapidly[4]. The fossil record sees an increase in the diversity of small shelly fossils; fragments of exoskeletal structures, defensive spines, claws, feeding organs etc.[59]. The increased abundance of small shelly fossils is usually interpreted as indicating a rapid evolutionary arms race linked to the appearance of predation and of predator defense mechanisms[60]. There is evidence of some predation in the Nama[28], but it only became widespread and a significant evolutionary factor in the Cambrian[61].

There are very few complete body fossils of the animals of the Fortunian. Nonetheless, we can reasonably speculate that this stage saw an increase in cephalization and in body support structures, including both biomineralized skeletons[29,62] and coeloms[63]. Stem-group members of most extant phyla, as well as crown-group members of several phyla probably appeared by the end of the Fortunian[7,63]. These stem and crown-group members are recognizable by the existence of some or all of the defining synapomorphies of the phyla.

From a molecular regulatory point of view, this stage probably saw an increase in the complexity of chromatin structure and gene regulation[45,46,64]. However, most of the molecular machinery responsible for the events of the Fortunian is likely to have appeared in the Nama[8].

New body plans, new ecologies

The next stages of the early Cambrian are not formally defined and are referred to informally as Stage 2 (Beginning at 530 Ma) and Stage 3 (beginning at 521 Ma). These stages see an increase in the diversity of small shelly fossils[59], followed by an increase in diversity of body fossils, representing members of numerous different bilaterian lineages[4]. For example, trilobites first appear in the fossil record at the very beginning of Stage 3[65], indicating that the arthropod body plan had already been assembled by this point. The first putative crown annelid *Dannychaeta tucolus* also dates to this period[66]. Many of the famous sites of exceptional preservation, representing the iconic Cambrian fauna are from Stage 3 (e.g. *Kerygmachela* and *Halkieria* from the Sirius Passet and *Leanchoilia* and *Maotianchaeta* from Chengjiang)[67,68]. The fauna represented in these sites includes representatives of different lineages with varied body plans, occupying benthic, endobenthic and nektonic habitats. It includes a diversity of predators, suspension feeders, detritovores, herbivores and more[68], indicating a much more complex and tiered ecosystem than before.

The increase in diversity and ecological complexity that is evident in the fossil record of Cambrian Stage 3, is different from the increases seen in earlier periods. Unlike the first pulses of increase in diversity of the Nama and Fortunian, which were manifested throughout Bilateria, this increase in complexity and diversity only covered a few phyla[69]. This is best demonstrated by the dramatic and rapid increase that occurred in arthropods and their close relatives. Arthropods are completely absent from the body fossil record before the appearance of trilobites 521 Ma[65], although several earlier trace fossils have been interpreted as belonging to arthropods. Trilobites display the full complexity of the arthropod body plan. If their absence from the earlier fossil record is not an artifact, then the arthropod body plan was assembled very rapidly in the few million years before their first appearance in the fossil record[65].

The middle Cambrian, represented by the iconic Burgess Shale fauna[1,2], among others, sees a turnover in the dominant lineages, but there are no phylum-level body plans that appear later than the middle Cambrian. There are further increases in diversity, notably in the early Ordovician[20,21], but the main increases in morphological complexity are by and large complete by this point (but see the vertebrate exception below).

The Brain-First Hypothesis

An increase in complexity can drive an increase in diversity due to the ability to evolve specialized organs (see Box 1), and thus occupy an increased range of niches through taxon-specific adaptations of specialized organs. However, this is not a requirement, and diversity can increase without an increase in morphological complexity. A case in point are the nematodes, which are arguably the most species-rich phylum, while maintaining a morphologically simple and conserved body plan[70]. Similarly, priapulans probably reached their peak diversity in the middle Cambrian, but remained relatively simple animals, without strongly differentiated or specialized organs[71]. Another example is the brachiopods, which also increased in diversity over the Cambrian, but despite an increase in species number, did not evolve new or complex organ systems[72].

Conversely, the four phyla in question, Arthropoda, Mollusca, Annelida and Chordata, show a significant increase in complexity, followed shortly by an increase in diversity. In all four phyla, the increased complexity is manifested by most, if not all, of the following: a complex centralized nervous system composed of a ladder-like trunk nervous system and an expanded anterior brain, diverse sensory organs with a peripheral nervous system, a complete coelom often with distinct compartments, a circulatory system providing nutrition and oxygen to peripheral tissues, dedicated excretory organs linked to the circulatory system, a differentiated digestive system with a separation between digestion and absorption, and differentiated muscle bundles responsible for different types of movement and locomotion[73,74].

The increase in complexity and diversity is most notable in the arthropods, where a complex segmented body plan is evident already in the early stem group[75-77]. They rapidly became the most diverse animal phylum; a status they have held from the Cambrian to the present[76,78]. Annelids and mollusks attained their complex body plan somewhat later, probably by the mid-Cambrian, and increased in diversity more slowly. The annelid Cambrian fossil record is extremely sparse, and it is not clear whether this is an outcome of a preservation bias, or a true reflection of low diversity. Nonetheless, middle Cambrian annelids display the hallmarks of the typical annelid body plan[66,79-81]. The mollusk fossil record is significantly more extensive, mostly due to their mineralized shells. The main lineages within Mollusca must have split during the Cambrian or even earlier, since fossils of shells identified as belonging to individual lineages are found from the early Cambrian and onwards[82], although no body fossils are known from this period. Mollusks then underwent a gradual increase in diversity throughout the Cambrian and Ordovician[82-84]. The chordates, and specifically the vertebrates, did not increase in complexity or in diversity until the Ordovician[85,86]. For the sake of the following discussion, we will put the vertebrates aside, since their radiation cannot be disentangled from their hyper-mineralized skeletons, and we will focus only on the three “invertebrate” phyla.

Despite differences in the dynamics and timing of the evolution of these three phyla, their complexity is manifested in similar ways, such as the appearance of a ladder-like nervous system with an expanded anterior brain, a regionalized digestive system, and in two of the three, also segmented body plans[87-89]. This raises the questions whether a similar selective force drove the increase in complexity in all three, given the similarity in the outcome. If so, it is likely to have been a global selective force that acted on these three phyla convergently, but not on others (or at least, not as strongly). A selective force that works in parallel over time on several different lineages implies a long-lasting ecosystem-wide phenomenon. This could be an a-biotic factor, or a change in the biological interactions within the ecosystem. I suggest that the main selective force for the convergent increase in complexity was an increase in the need to respond to a more complex environment and process numerous complex environmental inputs. This was a direct outcome of the previous phases of the Cambrian Cascade, wherein the ecosystem became increasingly tiered and the number of trophic levels and the degree of interspecific interactions increased dramatically[4]. Organisms needed to receive, process and respond to an increased number of signals coming from different organisms and from different directions.

The outcome of this selective pressure was an improvement of sensory organs, and more importantly, an increase in the size and differentiation of the central nervous system. In order to process diverse sensory inputs, I suggest there was selection for the central nervous system to become increasingly specialized and regionalized. Processing inputs from different sensory organs was done in different parts of the central nervous system, and especially in the anterior regions of the central nervous system – the brain. Integrating data from different sensory systems required the evolution of additional regionalized sections of the brain, as did the execution of complex behaviors responding to a range of stimuli. The core of the hypothesis I present herein is that the brain was the first organ system to increase in complexity, and I refer to it hereafter as the “Brain-First Hypothesis” (Figure 1).

The more complex brain was composed of more types of neural cells and was divided into more individuated regions than in the ancestral condition. This regionalized and specialized brain was achieved by an increase in complexity of the developmental program responsible for forming the brain; developing a uniform structure requires much less regulation than developing a differentiated structure. The selection for an increasingly complex brain was manifested as a selection for more tightly regulated and nuanced gene expression patterns. I suggest that this resulted in more complex and combinatorial regulatory regions in the cis-regulatory modules of relevant genes, increased diversity of splice variants in these genes, more roles for non-coding RNAs, and duplication and sub-functionalization of both regulatory genes and structural genes involved in neurogenesis. It is these genes and the networks of interactions among them that drive the development of the morphological plan of the brain, and the more complex they are, the more complex the resulting brain will be[46,90-92].

While the main driving force for this increased regulatory complexity was the requirement for improved neural processing, it had additional far-reaching consequences. I suggest that once these expanded regulatory toolkits and the novel networks emerging from them were in place, they were rapidly and easily co-opted to regionalize and diversify additional organ systems. Co-option is a well-known and described phenomenon in the evolution of novelties[33,46,93-95]. Existing gene regulatory networks are re-activated or re-used in novel contexts to give rise to evolutionary novelties. With the increased regulatory complexity, evolution had an "open field" for numerous cases of co-option. I suggest that these co-option events allowed additional organ systems to become more highly differentiated, providing the raw material for the increase in general organismic complexity that occurred in the aforementioned three phyla throughout the Cambrian.

A co-option of neural developmental pathways into other organ systems has already been suggested for the evolution of the arthropod segmentation cascade[88], and indeed for segmentation in general. An increase in sensory functions as a driver of the Cambrian explosion is also an idea that has been suggested in the past[31,32], but has not gained traction. The Brain-First Hypothesis suggests that segmentation is only one example of a broader phenomenon, and suggests a mechanistic link between the increase in sensory functions and overall complexity.

More highly differentiated and regionalized structures and organs allowed an increase in morphological disparity (the degree to which organisms within a lineage are morphologically different from each other). Taxonomic diversity then increased through series of adaptive radiations making use of novelties that appeared as a result of new regulatory elements being recruited to new morphological structures[33]. Thus, the final – but indirect – outcome of the increased ecological complexity of the early Cambrian was an overall increase in taxonomic diversity, with most of this diversity stemming from three phyla with increased complexity.

This process of increasing sensory sensitivity, neural complexity, and morphological diversity probably did not occur at once, but in a gradual repeated feedback loop over an extended period during the Cambrian. It remains an open question why this process did not occur in other phyla, which were presumably experiencing the same ecological environment. Like many things in evolution, chance events probably had a significant role[1]. Perhaps these phyla already displayed some unknown exaptation to processing complex sensory inputs, allowing them to adapt and undergo an increase in neural complexity, with accelerating feedback driving the process more rapidly in these lineages. It also remains unknown what drove the early increase in diversity in morphologically less complex taxa (e.g. priapulans or brachiopods). Intriguingly, these phyla with early increases in diversity, but no parallel increase in complexity, declined with time (again, with the exception of nematodes).

Implications and predictions of the hypothesis

The hypothesis suggested above provides a simple explanation, based on first-principles, for the increase in complexity and diversity seen in a limited number of animal lineages after the main diversification of the early Cambrian. Furthermore, this explanation also allows us to formulate a number of predictions and ways to test them, using molecular data from extant organisms. These tests must allow us to differentiate between the Brain-First Hypothesis and alternate hypotheses: that increased overall complexity evolved earlier (for an unknown reason) and in turn repeatedly drove increased neural complexity (via an unknown mechanism), or that complexity in all organ systems evolved more or less in parallel (under unknown selective pressure).

First, we would need to test the link between increased morphological complexity and regulatory complexity in the three phyla: Mollusca, Annelida and Arthropoda. Intuitively, this link seems almost trivial, but it has never been empirically studied. We would expect to see the morphological complexity manifested in higher diversity of a range of regulatory elements. These should include not only regulatory genes such as transcription factors and signaling molecules, but also cis-regulatory modules, non-coding RNAs, alternative splice variants in structural genes and more. We should be able to distinguish between increases in regulatory elements that occurred before the diversification of Bilateria and those that occurred only in specific lineages by mapping the diversity of regulatory elements phylogenetically. This will allow us to elucidate which increases in regulatory complexity are shared across large phylogenetic distances and which have independently and convergently evolved in specific lineages. We would expect to see the most significant lineage-specific increases in regulatory diversity within the three focal phyla. The recent increased availability of high-quality genomes from representatives of numerous phyla, makes this type of analysis an achievable goal. Conversely, if there is no evidence for higher regulatory complexity in mollusks, annelids and arthropods, this would weaken the link between morphological and regulatory complexity and decrease support for the Brain-First Hypothesis.

Next, we would need to demonstrate that the increases in regulatory complexity evolved in the nervous system first and that the expanded regulatory toolkit was co-opted to other morphological structures. Under this assumption, we would expect the largest number and range of regulatory elements to be found in gene regulatory networks involved in the development of the brain in each of the three phyla. If complexity in other organs is a result of co-option of regulatory elements that originally evolved in the nervous system, only some elements of this neural-development toolkit should be found in the networks involved in developing other complex organ systems. Furthermore, if this co-option occurred independently in different organ systems, we would expect different regulatory elements to be found in the development of each system. Finally, this subset of the regulatory toolkit used in different systems, would also be different in each phylum. This would indicate that the co-option of regulatory elements occurred independently not only in each system but also in each lineage. Conversely, if complexity in different organ systems evolved independently of complexity of the nervous system (either before or after it), we would find system-specific regulatory elements, and would not expect the nervous system to display an unusual number or range of regulatory elements.

Finally, we would need to demonstrate that lineages that did not increase in complexity during the Cambrian, e.g. Priapula, Brachiopoda, Platyhelminthes etc., maintain a basic bilaterian regulatory toolkit representing the increase in complexity that occurred in the early phases of the Cambrian Cascade. Studying these lineages from a comparative genomic perspective should provide us with a baseline for what level of regulatory complexity was presumably found in the early Cambrian, before the increase in complexity seen in the three focal phyla. A phylogenetic comparison of the regulatory elements within these lineages should show that they date back to the last common ancestor of Bilateria, or possibly to the last common ancestor of one of the super-phyla (e.g. Ecdysozoa, Spiralia). We expect a much smaller number of lineage-specific regulatory innovations compared with the more complex phyla.

Concluding remarks

Reframing the Cambrian Explosion as a cascade removes the need to find an "explanation" for a singular event. Indeed, each phase in the Cambrian Cascade has its own cause (or several causes). The iconic fauna of the famous fossil sites of the middle Cambrian is not a direct outcome of an increase in animal diversity at the base of the Cambrian, but rather an outcome of several pulses of increased diversity, throughout the early and middle Cambrian. This increase in morphological complexity and in disparity was made possible by an increase in the complexity of the central nervous system, which in itself was a selective response to the ecological complexity of the biosphere, which had been increasing from the late Ediacaran. The molecular fingerprints of this process can probably still be found in the most diverse animal groups that exist today.

Text Box 1 – Defining and quantifying complexity

Since we are exploring the evolution of complexity, it seems prudent to put down a definition of complexity. This point has been discussed and debated extensively for many years[92,96-99], and it is beyond the scope of the current contribution to cover this debate in sufficient detail. Most approaches to quantifying complexity have to do with the number and diversity of constituent parts, be it cell number or difference between segments in a segmented organism[99]. Within the framework of the current hypothesis, the increase in complexity I am discussing involves an increase in the number and diversity of differentiated organs, in the diversity of serially repeated organs and in the adaptive specializations of different organs. Under this practical definition, a centipede is more complex than a nematode and a crayfish is more complex than a centipede. At the level of individual organs, a multicellular metanephridium is more complex that a single-celled protonephridium. Different organisms can display different levels of complexity and different aspects of complexity in a modular fashion. Nonetheless, organisms with higher degrees of complexity (e.g. arthropods, vertebrates) tend to display complexity in several organ systems and characteristics at once.

Complexity is an inherent characteristic of biological systems in general and of the Earth's biosphere in particular. However, complexity is expensive in terms of genetic and energetic resources, and there is no a-priori reason to assume that complexity per se has any inherent evolutionary advantage. There have been many attempts to model the conditions what would lead to an increase in complexity over time[92,96,100], and these are often based on emergent phenomena or regulatory complexity, rather than on positive selection for complexity.

There is probably no single metric that can quantify the overall complexity of an entire organism in such a way that one can map it on a phylogenetic tree as though it were a quantitative character. Nonetheless, it is possible to identify lineages that are generally more complex than other lineages, and to identify lineages wherein complexity increases over time, based on some version of the practical definition given above. There are undoubtably many cases of increase in complexity over evolutionary time in specific lineages. However, consistent directional increase in complexity is not a universal phenomenon[73]. The Brain-First hypothesis aims to explain the directional increase in complexity in three phyla at a crucial stage in the early evolution of the biosphere.

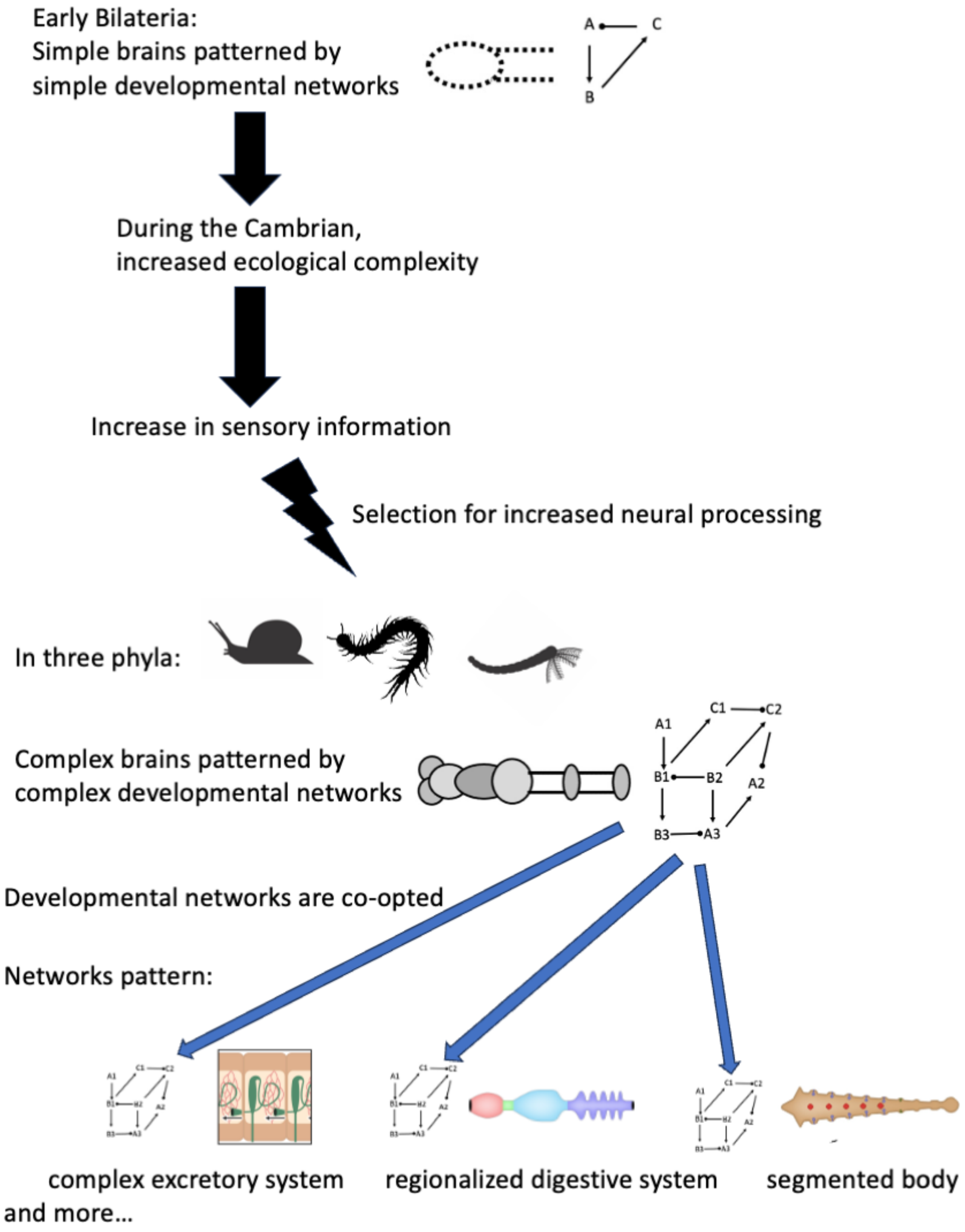

Figure Legend

Figure 1 – Schematic representation of the main points of the Brain First Hypothesis.

References

[1] Gould, S. J. (1989). *Wonderful Life - The Burgess Shale and the Nature of History*. New York: Norton.

[2] Conway Morris, S. (1998). *The Crucible of Creation - The Burgess Shale and the Rise of Animals*. Oxford: Oxford University Press.

[3] Briggs, D. E. (2015). The Cambrian explosion. *Current Biology, 25*(19), R864-868. doi: 10.1016/j.cub.2015.04.047

[4] Erwin, D. H., & Valentine, J. W. (2013). *The Cambrian Explosion - The Construction of Animal Biodiversity*. Greenwood Village, Colorado: Roberts & Company.

[5] Caron, J.-B., & Jackson, D. A. (2008). Paleoecology of the Greater Phyllopod Bed community, Burgess Shale. *Palaeogeo. Palaeoclim. Palaeoeco., 258*(3), 222-256. doi: 10.1016/j.palaeo.2007.05.023

[6] Erwin, D. H., & Tweedt, S. (2012). Ecological drivers of the Ediacaran-Cambrian diversification of Metazoa. *Evol. Ecol., 26*(2), 417-433. doi: 10.1007/s10682-011-9505-7

[7] Budd, G. E., & Jackson, I. S. (2016). Ecological innovations in the Cambrian and the origins of the crown group phyla. *Philos. Trans. R. Soc. Lond. B, 371*(1685), 20150287. doi: 10.1098/rstb.2015.0287

[8] Erwin, D. H., Laflamme, M., Tweedt, S. M., Sperling, E. A., Pisani, D., & Peterson, K. J. (2011). The Cambrian conundrum: Early divergence and later ecological success in the early history of animals. *Science, 334*(6059), 1091-1097.

[9] Servais, T., Cascales-Miñana, B., Harper, D. A. T., Lefebvre, B., Munnecke, A., Wang, W., & Zhang, Y. (2023). No (Cambrian) explosion and no (Ordovician) event: A single long-term radiation in the early Palaeozoic. *Palaeogeog. Palaeoclimat. Palaeoeco., 623*. doi: 10.1016/j.palaeo.2023.111592

[10] Conway Morris, S. (2000). The Cambrian 'explosion': slow-fuse or megatonnage. *Proc. Natl. Acad. Sci. USA, 97*(9), 4426-4429.

[11] Zhuravlev, A. Y., & Wood, R. A. (2018). The two phases of the Cambrian Explosion. *Sci. Rep., 8*(1), 16656. doi: 10.1038/s41598-018-34962-y

[12] Cong, P. (2023). The early animal radiation: insights from interpreting the Cambrian problematic fossils. *Front. Earth Sci., 11*. doi: 10.3389/feart.2023.1120118

[13] Parry, L. A., Boggiani, P. C., Condon, D. J., Garwood, R. J., Leme, J. M., McIlroy, D., . . . Liu, A. G. (2017). Ichnological evidence for meiofaunal bilaterians from the terminal Ediacaran and earliest Cambrian of Brazil. *Nat. Ecol. Evol., 1*(10), 1455-1464. doi: 10.1038/s41559-017-0301-9

[14] Chen, Z., Chen, X., Zhou, C. M., Yuan, X. L., & Xiao, S. H. (2018). Late Ediacaran trackways produced by bilaterian animals with paired appendages. *Sci. Adv., 4*(6). doi: 10.1126/sciadv.aao6691

[15] Darroch, S. A. F., Smith, E. F., Laflamme, M., & Erwin, D. H. (2018). Ediacaran extinction and Cambrian explosion. *Trends Ecol. Evol., 33*(9), 653-663. doi: 10.1016/j.tree.2018.06.003

[16] Evans, S. D., Hughes, I. V., Gehling, J. G., & Droser, M. L. (2020). Discovery of the oldest bilaterian from the Ediacaran of South Australia. *Proc. Natl. Acad. Sci. USA, 117*(14), 7845-7850. doi: 10.1073/pnas.2001045117

[17] Schiffbauer, J. D., Selly, T., Jacquet, S. M., Merz, R. A., Nelson, L. L., Strange, M. A., . . . Smith, E. F. (2020). Discovery of bilaterian-type through-guts in cloudinomorphs from

the terminal Ediacaran Period. *Nat. Commun., 11*(1), 205. doi: 10.1038/s41467-019-13882-z

[18] Howard, R. J., Giacomelli, M., Lozano-Fernandez, J., Edgecombe, G. D., Fleming, J. F., Kristensen, R. M., . . . Pisani, D. (2022). The Ediacaran origin of Ecdysozoa: integrating fossil and phylogenomic data. *J. Geol. Soc., 179*(4). doi: 10.1144/jgs2021-107

[19] Evans, S. D., Hughes, I. V., Hughes, E. B., Dzaugis, P. W., Dzaugis, M. P., Gehling, J. G., . . . Droser, M. L. (2024). A new motile animal with implications for the evolution of axial polarity from the Ediacaran of South Australia. *Evol. Dev., 26*(6), e12491. doi: 10.1111/ede.12491

[20] Servais, T., Owen, A. W., Harper, D. A. T., Kröger, B., & Munnecke, A. (2010). The Great Ordovician Biodiversification Event (GOBE): The palaeoecological dimension. *Palaeogeo. Paleoclim. Paleoecol., 294*(3-4), 99-119. doi: 10.1016/j.palaeo.2010.05.031

[21] Saleh, F., Vaucher, R., Vidal, M., Hariri, K. E., Laibl, L., Daley, A. C., . . . Lefebvre, B. (2022). New fossil assemblages from the Early Ordovician Fezouata Biota. *Sci. Rep., 12*(1), 20773. doi: 10.1038/s41598-022-25000-z

[22] Davidson, E. H., & Erwin, D. H. (2006). Gene regulatory networks and the evolution of animal body plans. *Science, 311*(5762), 796-800. doi: Doi 10.1126/Science.1113832

[23] Peterson, K. J., Dietrich, M. R., & McPeek, M. A. (2009). MicroRNAs and metazoan macroevolution: insights into canalization, complexity, and the Cambrian explosion. *Bioessays, 31*(7), 736-747. doi: 10.1002/bies.200900033

[24] Budd, G. E. (2015). Early animal evolution and the origins of nervous systems. *Philos. Trans. R. Soc. Lond. B, 370*(1684), 20150037. doi: 10.1098/rstb.2015.0037

[25] Yang, D., Guo, X., Xie, T., & Luo, X. (2018). Reactive oxygen species may play an essential role in driving biological evolution: The Cambrian Explosion as an example. *J. Environ. Sci., 63*, 218-226. doi: 10.1016/j.jes.2017.05.035

[26] Sperling, E. A., Frieder, C. A., Raman, A. V., Girguis, P. R., Levin, L. A., & Knoll, A. H. (2013). Oxygen, ecology, and the Cambrian radiation of animals. *Proc. Natl. Acad. Sci. USA, 110*(33), 13446-13451. doi: Doi 10.1073/Pnas.1312778110

[27] Budd, G. E., & Jensen, S. (2015). The origin of the animals and a 'Savannah' hypothesis for early bilaterian evolution. *Biol. Rev.* . doi: 10.1111/brv.12239

[28] Droser, M. L., Tarhan, L. G., & Gehling, J. G. (2017). The rise of animals in a changing environment: Global ecological innovation in the late Ediacaran. *Annu. Rev. Earth Planet. Sci., 45*, 593-617. doi: 10.1146/annurev-earth-063016-015645

[29] Murdock, D. J. E. (2020). The 'biomineralization toolkit' and the origin of animal skeletons. *Biol. Rev. Camb. Philos. Soc., 95*(5), 1372-1392. doi: 10.1111/brv.12614

[30] Holland, P. W. H. (2015). Did homeobox gene duplications contribute to the Cambrian explosion? *Zool. Lett., 1*(1), 1. doi: 10.1186/s40851-014-0004-x

[31] Marshall, C. R. (2006). Explaining the Cambrian "explosion" of animals. *Annu. Rev. Earth Pl. Sci., 34*, 355-384. doi: 10.1146/annurev.earth.33.031504.103001

[32] Parker, A. R. (1998). Colour in Burgess Shale animals and the effect of light on evolution in the Cambrian. *Proc. R. Soc. B, 265*(1400), 967-972. doi: DOI 10.1098/rspb.1998.0385

[33] Erwin, D. H. (2015). Novelty and innovation in the history of life. *Current Biology, 25*(19), R930-940. doi: 10.1016/j.cub.2015.08.019

[34] Dunn, F. S., Liu, A. G., & Donoghue, P. C. J. (2017). Ediacaran developmental biology. *Biol. Rev. Camb. Philos. Soc.* doi: 10.1111/brv.12379

[35] Fedonkin, M. A., & Waggoner, B. M. (1997). The Late Precambrian fossil *Kimberella* is a mollusc-like bilaterian organism. *Nature, 388*(6645), 868-871.

[36] Chen, J. Y., Bottjer, D. J., Oliveri, P., Dornbos, S. Q., Gao, F., Ruffins, S., . . . Davidson, E. H. (2004). Small bilaterian fossils from 40 to 55 million years before the Cambrian. *Science, 305*(5681), 218-222. doi: 10.1126/science.1099213

[37] Jacobs, D. K., Hughes, N. C., Fitz-Gibbon, S. T., & Winchell, C. J. (2005). Terminal addition, the Cambrian radiation and the Phanerozoic evolution of bilaterian form. *Evol. Dev., 7*(6), 498-514.

[38] Evans, S. D., Droser, M. L., & Gehling, J. G. (2017). Highly regulated growth and development of the Ediacara macrofossil *Dickinsonia costata*. *Plos One, 12*(5), e0176874. doi: 10.1371/journal.pone.0176874

[39] Xiao, S. H., & Laflamme, M. (2009). On the eve of animal radiation: phylogeny, ecology and evolution of the Ediacara biota. *Trends Ecol. Evol., 24*(1), 31-40. doi: Doi 10.1016/J.Tree.2008.07.015

[40] Darroch, S. A., Sperling, E. A., Boag, T. H., Racicot, R. A., Mason, S. J., Morgan, A. S., . . . Laflamme, M. (2015). Biotic replacement and mass extinction of the Ediacara biota. *Proc. R. Soc. B, 282*(1814). doi: 10.1098/rspb.2015.1003

[41] Chen, Z., Zhou, C., Yuan, X., & Xiao, S. (2019). Death march of a segmented and trilobate bilaterian elucidates early animal evolution. *Nature, 573*(7774), 412-415. doi: 10.1038/s41586-019-1522-7

[42] Mángano, M. G., & Buatois, L. A. (2020). The rise and early evolution of animals: where do we stand from a trace-fossil perspective? *Interface Focus, 10*(4). doi: 10.1098/rsfs.2019.0103

[43] Arendt, D., Technau, U., & Wittbrodt, J. (2001). Evolution of the bilaterian larval foregut. *Nature, 409*(6816), 81-85.

[44] Arenas-Mena, C. (2017). The origins of developmental gene regulation. *Evol. Dev., 19*(2), 96-107. doi: 10.1111/ede.12217

[45] Erwin, D. H. (2009). Early origin of the bilaterian developmental toolkit. *Phil. Trans. Roy. Soc. B, 364*(1527), 2253-2261. doi: Doi 10.1098/Rstb.2009.0038

[46] Erwin, D. H. (2020). The origin of animal body plans: a view from fossil evidence and the regulatory genome. *Development, 147*(4). doi: 10.1242/dev.182899

[47] Evans, S. D., Droser, M. L., & Erwin, D. H. (2021). Developmental processes in Ediacara macrofossils. *Proc. R. Soc. B, 288*(1945), 20203055. doi: 10.1098/rspb.2020.3055

[48] Guo, J., Parry, L. A., Vinther, J., Edgecombe, G. D., Wei, F., Zhao, J., . . . Cong, P. (2022). A Cambrian tommotiid preserving soft tissues reveals the metameric ancestry of lophophorates. *Current Biology, 32*(21), 4769-4778 e4762. doi: 10.1016/j.cub.2022.09.011

[49] De Robertis, E. M., & Sasai, Y. (1996). A common plan for dorsoventral patterning in Bilateria. *Nature, 380*, 37-40.

[50] Kimmel, C. B. (1996). Was *Urbilateria* segmented? *Trends Genet., 12*(9), 329-331.

[51] Balavoine, G., & Adoutte, A. (2003). The segmented Urbilateria: A testable scenario. *Integr. Comp. Biol., 43*(1), 137-147. doi: DOI 10.1093/icb/43.1.137

[52] Cornec, J. P., & Gilles, A. (2006). *Urbilateria*, a complex organism? *Medec. Sci., 22*(5), 493-501.

[53] Hejnol, A., & Martindale, M. Q. (2008). Acoel development supports a simple planula-like urbilaterian. *Phil. Trans. Roy. Soc. B, 363*(1496), 1493-1501. doi: 10.1098/rstb.2007.2239|ISSN 0962-8436

[54] Holland, L. Z., Carvalho, J. E., Escriva, H., Laudet, V., Schubert, M., Shimeld, S. M., & Yu, J. K. (2013). Evolution of bilaterian central nervous systems: a single origin? *Evodevo, 4*. doi: 10.1186/2041-9139-4-27

[55] Cribb, A. T., & Darroch, S. A. F. (2024). Geobiology: Machine learning puts bioturbation on the map. *Current Biology, 34*(13), R630-R632. doi: 10.1016/j.cub.2024.05.038

[56] Dornbos, S. Q., & Bottjer, D. J. (2000). Evolutionary paleoecology of the earliest echinoderms: Helicoplacoids and the Cambrian substrate revolution. *Geology, 28*(9), 839-842. doi: Doi 10.1130/0091-7613(2000)28<839:Epotee>2.0.Co;2

[57] Mángano, M. G., & Buatois, L. A. (2017). The Cambrian revolutions: Trace-fossil record, timing, links and geobiological impact. *Earth-Sci. Rev., 173*, 96-108. doi: 10.1016/j.earscirev.2017.08.009

[58] Budd, G. E., & Jensen, S. (1998). Trace fossils and the Cambrian explosion. *Trends In Ecology & Evolution, 13*(12), 507-507. doi: Doi 10.1016/S0169-5347(98)01503-1

[59] Dzik, J. (1994). Evolution of "small shelly fossils" assemblages of the Early Paleozoic. *Acta Pal. Pol., 39*(3), 247-313.

[60] Dzik, J. (2007). The Verdun Syndrome: simultaneous origin of protective armour and infaunal shelters at the Precambrian–Cambrian transition *The Rise and Fall of the Ediacaran Biota* (Vol. 286, pp. 405-414). London: Geological Society, London, Special Publications.

[61] Bicknell, R. D. C., Campione, N. E., Brock, G. A., & Paterson, J. R. (2025). Adaptive responses in Cambrian predator and prey highlight the arms race during the rise of animals. *Current Biology, 35*(4), 882-888. doi: 10.1016/j.cub.2024.12.007

[62] Gilbert, P. U. P. A., Bergmann, K. D., Boekelheide, N., Tambutté, S., Mass, T., Marin, F., . . . Knoll, A. H. (2022). Biomineralization: Integrating mechanism and evolutionary history. *Sci. Adv., 8*(10), eabi9653. doi: 10.1126/sciadv.abl9653

[63] Budd, G. E., & Jensen, S. (2000). A critical reappraisal of the fossil record of the bilaterian phyla. *Biol. Rev., 75*(2), 253-295.

[64] Davidson, E. H., & Erwin, D. H. (2009). Evolutionary innovation and stability in animal gene networks. *J. Exp. Zool. B, 314B*, 182-186. doi: 10.1002/jez.b.21329

[65] Daley, A. C., Antcliffe, J. B., Drage, H. B., & Pates, S. (2018). Early fossil record of Euarthropoda and the Cambrian Explosion. *Proc. Natl. Acad. Sci. USA, 115*(21), 5323-5331. doi: 10.1073/pnas.1719962115

[66] Chen, H., Parry, L. A., Vinther, J., Zhai, D., Hou, X., & Ma, X. (2020). A Cambrian crown annelid reconciles phylogenomics and the fossil record. *Nature, 583*(7815), 249-252. doi: 10.1038/s41586-020-2384-8

[67] Harper, D. A. T., Hammarlund, E. U., Topper, T. P., Nielsen, A. T., Rasmussen, J. A., Park, T.-Y. S., & Smith, M. P. (2019). The Sirius Passet Lagerstätte of North Greenland: a remote window on the Cambrian Explosion. *J. Geol. Soc., 176*(6), 1023-1037. doi: 10.1144/jgs2019-043

[68] Zhao, F., Zhu, M., & Hu, S. (2010). Community structure and composition of the Cambrian Chengjiang biota. *Sci. China Earth Sci., 53*(12), 1784-1799. doi: 10.1007/s11430-010-4087-8

[69] Fan, J. X., Shen, S. Z., Erwin, D. H., Sadler, P. M., MacLeod, N., Cheng, Q. M., . . . Zhao, Y. Y. (2020). A high-resolution summary of Cambrian to Early Triassic marine invertebrate biodiversity. *Science, 367*(6475), 273-+. doi: 10.1126/science.aax4953

[70] Kiontke, K., & Fitch, D. H. (2013). Nematodes. *Current Biology, 23*(19), R862-864. doi: 10.1016/j.cub.2013.08.009

[71] Wills, M. A., Gerber, S., Ruta, M., & Hughes, M. (2012). The disparity of priapulid, archaeopriapulid and palaeoscolecid worms in the light of new data. *J. Evol. Biol., 25*(10), 2056-2076. doi: 10.1111/j.1420-9101.2012.02586.x

[72] Murdock, D. J. E., Bengtson, S., Marone, F., Greenwood, J. M., & Donoghue, P. C. (2014). Evaluating scenarios for the evolutionary assembly of the brachiopod body plan. *Evol. Dev., 16*(1), 13-24. doi: 10.1111/ede.12059

[73] Chipman, A. D. (2024). *Organismic Animal Biology - an evolutionary approach*. Oxford: Oxford University Press.

[74] Brusca, R. C., Giribet, G., & Moore, W. (2022). *Invertebrates 4th edition* (4 ed.). Oxford: Sinauer Associates.

[75] Edgecombe, G. D., & Legg, D. A. (2014). Origins and early evolution of arthropods. *Palaeontology, 57*(3), 457-468. doi: 10.1111/pala.12105

[76] Chipman, A. D., & Edgecombe, G. D. (2019). Developing an integrated understanding of the evolution of arthropod segmentation using fossils and evo-devo. *Proc. R. Soc. B, 286*, 20191881. doi: http://dx.doi.org/10.1098/rspb.2019.1881

[77] Edgecombe, G. D. (2020). Arthropod origins: integrating paleontological and molecular evidence. *Ann. Rev. Ecol. Evol. Syst., 51*(1), 1-25. doi: 10.1146/annurev-ecolsys-011720-124437

[78] Edgecombe, G. D., & Legg, D. A. (2013). The arthropod fossil record. In A. Minelli, G. Boxshall & G. Fusco (Eds.), *Arthropod Biology and Evolution* (pp. 393-415). Berlin Heidelberg: Springer.

[79] Parry, L., Vinther, J., & Edgecombe, G. D. (2015). Cambrian stem-group annelids and a metameric origin of the annelid head. *Biol. Lett., 11*(10). doi: 10.1098/rsbl.2015.0763

[80] Nanglu, K., & Caron, J. B. (2018). A new Burgess Shale polychaete and the origin of the annelid head revisited. *Current Biology, 28*(2), 319-326 e311. doi: 10.1016/j.cub.2017.12.019

[81] Parry, L., & Caron, J. B. (2019). *Canadia spinosa* and the early evolution of the annelid nervous system. *Sci. Adv., 5*(9). doi: 10.1126/sciadv.aax5858

[82] Parkhaev, P. Y. (2008). The Early Cambrian radiation of Mollusca. In W. Ponder (Ed.), *Phylogeny and Evolution of the Mollusca* (pp. 33-69): University of California Press.

[83] Wanninger, A., & Wollesen, T. (2019). The evolution of molluscs. *Biol. Rev. Camb. Philos. Soc., 94*(1), 102-115. doi: 10.1111/brv.12439

[84] Chen, Z., Baeza, J. A., Chen, C., Gonzalez, M. T., Gonzalez, V. L., Greve, C., . . . Sigwart, J. D. (2025). A genome-based phylogeny for Mollusca is concordant with fossils and morphology. *Science, 387*(6737), 1001-1007. doi: 10.1126/science.ads0215

[85] Blieck, A., & Turner, S. (2003). Global Ordovician vertebrate biogeography. *Paleogeo. Paleoclim. Paleoeco., 195*(1-2), 37-54. doi: 10.1016/s0031-0182(03)00301-8

[86] Dearden, R. P., Lanzetti, A., Giles, S., Johanson, Z., Jones, A. S., Lautenschlager, S., . . . Sansom, I. J. (2023). The oldest three-dimensionally preserved vertebrate neurocranium. *Nature, 621*(7980), 782-787. doi: 10.1038/s41586-023-06538-y

[87] Martin-Durán, J. M., Pang, K., Børve, A., Lê, H. S., Furu, A., Cannon, J. T., . . . Hejnol, A. (2018). Convergent evolution of bilaterian nerve cords. *Nature, 553*(7686), 45-50. doi: 10.1038/nature25030

[88] Chipman, A. D. (2010). Parallel evolution of segmentation by co-option of ancestral gene regulatory networks. *Bioessays, 32*(1), 60-70. doi: Doi 10.1002/Bies.200900130

[89] Helm, C., Beckers, P., Bartolomaeus, T., Drukewitz, S. H., Kourtesis, I., Weigert, A., . . . Bleidorn, C. (2018). Convergent evolution of the ladder-like ventral nerve cord in Annelida. *Front. Zool., 15*, 36. doi: 10.1186/s12983-018-0280-y

[90] Davidson, E. H., McCay, D. R., & Hood, L. (2003). Regulatory gene networks and the properties of the developmental process. *Proc. Natl. Acad. Sci. USA, 100*(4), 1475-1480. doi: Doi 10.1073/Pnas.0437746100

[91] Feigin, C., Li, S., Moreno, J., & Mallarino, R. (2023). The GRN concept as a guide for evolutionary developmental biology. *J. Exp. Zool. B, 340*(2), 92-104. doi: 10.1002/jez.b.23132

[92] Hagolani, P. F., Zimm, R., Vroomans, R., & Salazar-Ciudad, I. (2021). On the evolution and development of morphological complexity: A view from gene regulatory networks. *PLoS Comput. Biol., 17*(2), e1008570. doi: 10.1371/journal.pcbi.1008570

[93] Rudel, D., & Sommer, R. J. (2003). The evolution of developmental mechanisms. *Developmental Biology, 264*(1), 15-37.

[94] Arthur, W., Jowett, T., & Panchen, A. (1999). Segments, limbs, homology, and co-option. *Evol. Dev., 1*(2), 74-76.

[95] Moczek, A. P. (2008). On the origins of novelty in development and evolution. *Bioessays, 30*(5), 432-447. doi: 10.1002/bies.20754

[96] Adami, C., Ofria, C., & Collier, T. C. (2000). Evolution of biological complexity. *Proc. Natl. Acad. Sci. USA, 97*(9), 4463-4468.

[97] Minelli, A., & Valero-Gracia, A. (2022). Spatially and temporally distributed complexity-a refreshed framework for the study of GRN evolution. *Cells, 11*(11), 1790. doi: 10.3390/cells11111790

[98] Alvarez-Ponce, D., & Krishnamurthy, S. (2025). Organismal complexity strongly correlates with the number of protein families and domains. *Proc. Natl. Acad. Sci. USA, 122*(5), e2404332122. doi: 10.1073/pnas.2404332122

[99] Rock, T., & Wills, M. A. (2025). Quantifying the configurational complexity of biological systems in multivariate 'complexity space'. *J. R. Soc. Interface, 22*(222), 20240558. doi: 10.1098/rsif.2024.0558

[100] McShea, D. W. (2005). The evolution of complexity without natural selection, a possible large-scale trend of the fourth kind. *Paleobiology, 31*(2), 146-156. doi: Doi 10.1666/0094-8373(2005)031[0146:Teocwn]2.0.Co;2