From one – many

By Yuriy Pichugin

Life is a miracle, and the history of life is largely a mystery. However, what appears to be a mystery and a miracle may be entirely explicable as science collects more and better evidence, allowing us to piece together the history of Earth's biology. It strikes us with the ability to invent novelty and complexity over and over again. In the Archean, 3.7 billion years ago, there were only prokaryotic (bacterial) cells. About 2 billion years ago, in the Proterozoic, eukaryotic cells evolved with a cell nucleus and other organelles. Shortly thereafter, the first multicellular species emerged.

Then, the Cambrian started 540 millions years ago, and is marked by the appearance of the first hard skeletons. This means teeth and claws for the predators as well as shells and carapaces for the prey. The range of opportunities for life to adapt skyrocketed, and a great diversity of species suddenly emerged. We know this event as Cambrian explosion. There, the very first spine emerged, marked the beginning of the most complex animals -- chordates. The development of skeleton made it possible for life to exit from oceans to land in the Silurian period 100 million years later. About 250 millions years ago, in the Triassic period, warm-blooded animals emerged, which allowed colonization of harsh environments with low or rapidly changing temperatures. Also, about 2 million years ago, the brain development in previously unremarkable branch of apes allowed them to use stones as tools. In some sense, that was the beginning of humankind.

These evolutionary processes have never stopped and should never stop, not as long as life exists. How did we get from the Archean primitive life to the present state, with all the complexity and diversity of life forms? This question still remains the subject of scientific investigation.

We know that the driving force behind this history is evolution by natural selection.

For this to happen, three conditions must be fulfilled. First, there must be a population, which members can multiply in numbers (reproduction). Second, the population should be diverse: different individuals produce different numbers of offspring, possessing different traits from one another (variation). Finally, the offspring should resemble their parents (heredity). If so, then the population composition will inevitably change because individuals with beneficial traits will simply outgrow, outcompete, and outreproduce the others.

While the abstract idea making natural selection possible is simple, the actual inner mechanisms of evolution are not. Everything starts with a single molecule: DNA. This molecule is long, complex and, most importantly, pretty resilient against external hazards. It mostly serves as a huge archive of all possible instructions. DNA is surrounded by the fine reading machinery, constantly producing a stream of smaller molecules of RNA – individual blueprints for the proteins. Each protein does some job in the cell, and while they operate in concert, the cell is kept alive. Cells comprise the organism, which has to survive in an environment and compete with other life forms for limited resources. Individuals, more successful in these actions, have the opportunity to produce more offspring carrying the genes of their ancestors, which help them to propagate further. There are further complications here: there are instructions in cells not encoded into DNA code (epigenetics), DNA-sequences not translated into proteins (non-coding DNA), encoded proteins not contributing to the cell well being (apoptosis), offspring genes deviating from parental ones (recombination and mutations), and many other fascinating phenomena. All these factors weave together and are commonly responsible for the evolution of life in Earth, even including the development of complexity.

A great example of the complexity of evolution is an emergence of multicellularity. We know about around 25 independent instances of this event. The oldest one dates back to Precambrian era, while the last one happened just 200 million years ago. Therefore, there must be some common driving force behind this. What could motivate the independent cells to adopt a collective living? A simple answer is: bigger is better. No doubt, the raw size matters, larger organism is more resistant to threats from external environment. But then, why is having two cells together superior to having just one cell of double size? The answer to this question is more delicate.

First, while a living cell can do many things, it cannot do all of them well simultaneously. Therefore, unicellular organisms are subjected to a number of restrictions. Multicellular species can delegate different tasks to different cells, i.e. employ a division of labor. Consider cyanobacteria, ones of the oldest photosynthetic bacteria. In order to grow, they are able to extract carbon and nitrogen from inorganic compounds and fixate them into organic molecules. The process of carbon uptake is called photosynthesis and as a side effect it produces a very active chemical – oxygen. Indeed, the oxygen we breathe was originally a toxic byproduct of resource extraction operations. Oxygen is especially harmful for the fragile nitrogen fixation procedure. When modern unicellular cyanobacteria face the necessity to combine both processes, their growth rate drops in half. Multicellular cyanobacteria in such case develop cell specialization. Some cells develop a thick coating isolating them from the atmosphere and totally focus on the nitrogen fixation, delivering the products to neighboring cells, which continue with photosynthesis alone.

Second, being inside the colony allows an organism to create its own, internal environment, potentially to a more preferable one from the external environment. For example, the warm-blooded animals are able to keep the temperature of their bodies, independently on the weather conditions. Therefore, penguins and polar bears are able to be active under temperatures below zero, where cold-blooded animals have to go dormant, and therefore successfully live in the polar regions of Earth.

A good example of such environmental changes on a much smaller scale is demonstrated by bacteria called *Pseudomonas fluorescens*. Normally, put in liquid nutritious media, they grow as unicellular creatures, absorbing nutrients from media and breathing oxygen diffusing from the surface. While the population grows, mutations emerge. Usually, these mutations have no effect, since the genetic code is rather robust with respect to errors. However, when the mutation has an effect, it generally means that something gets broken. And one of such mutations can disrupt the system, which prevents a cell from producing cellulose. Cellulose is necessary for a normal functioning as it provides a protective coating to the cell. However, not much of it is needed in the life of bacteria, so cellulose production is generally suppressed. If the system doing so malfunctions, the cell will produce cellulose all the time and will be unable to stop. Instead of a thin coating, the cell gains a thick shell. As an immediate consequence, cells born upon a division of such a mutant are both embedded in the cellulose produced by the mother cell and cannot split apart from each other. Cellulose's role turns from a protective layer into the glue keeping cells together and the mutants grow in colonies unlike to solitary "normal" cells.

This changes the way the organism behaves at a fundamental level. Instead of independent cells, which freely move up and down in the liquid, these colonies float in liquid and form a biofilm on the surface. There, mutant cells suddenly get a reliable access to both needed resources: liquid nutrients from below and oxygen from above. This way, living as a collective enabled the organism to create environmental conditions that are inaccessible to the unicellular beings. Furthermore, with time, these colonies occupy the whole surface and prevent oxygen to go into the depth. Thus, everything living beneath the surface dies of suffocation. Only a thick bacterial biofilm remains.

As with any radical innovation, the emergence of multicellularity leads to a number of problems unforeseeable in advance. A great advantage of multicellularity is having many cells working together towards the common good. But what if not everyone does it? Whenever there is cooperation, there is a problem of free riders exploiting the benefits of collective living without contributing to it. Returning to the bacterial example, cells in the biofilm continue to accumulate mutations. One of them turns a glue-producing cell into its original form, either by repairing cellulose suppression mechanism or by breaking something in the mechanism producing cellulose. But now such a cell is already embedded into the biofilm. Double-mutant cells still share an exclusive access to all resources with the rest of the biofilm but do not invest anything into cellulose glue generation. As a consequence, these cells contribute to the load but do not contribute to the biofilm's durability. Furthermore, double mutants grow faster than the glue producers since they save on the glue production. At some point, the biofilm breaks under the load of these cells and sinks down. Unfortunately, *Pseudomonas fluorescens* fails to establish a long-term multicellular community. Still, its story illustrates many aspects of opportunities and challenges of the evolution of multicellularity.

Let's look at another challenge. The evolution of multicellular organisms means also that they have to reinvent a life cycle. The raw reproduction of cells contributes only to the growth of an organism from being small to being large. But how will the organisms themselves reproduce? Generally, multicellular creatures detach a part of them to be the seed of a new organism. But how do these seeds come back to the parent state? Interestingly, there are not one but two ways how it can be done. First, is doing so by raw growth. One cell can divide into two, then turn into four and so on until the mature organism is reconstructed. But there is another option as well. Many smaller seeds (which may come from different parents) come together and fuse into a new mature organism. Social amoeba *Dictyostelium discoideum* does exactly this. While resulting organisms look like a shapeless slug missing any structure, one might expect it to be very primitive and incapable of performing very many biological functions. Surprisingly, it is potent enough to navigate the surface and even find a way through the maze to reach a desirable (and well earned) piece of food.

Some times, one problem solves another. Remember these non-producing "cheater" cells emerging in the bacterial biofilm? They turn out to be a perfect in spreading *Pseudomonas fluorescens* around the world. These cells freely float and do not stick to the same place, and also are more suited to live in liquid environments. After spreading to a new location suitable for starting a new colony, they are ready to switch back to the glue producing type, where they begin a colony anew in a better location. Thus, this creates a life cycle switching between glue-producing and non-producing cell types. From this point of view, the emergence of glue producing mutants and non-producing double mutants was not just beneficial mutations. It was essential steps in the organism development. The collapse of the biofilm under the load of double-mutants was not the end of the transient multicellular being. Instead it was the act of reproduction and releasing seeds into the nature.

Independent of the group formation mode, the existence of multicellular organisms invokes the concept of development, totally absent in unicellular species. After birth, some time is needed to pass before an organism will be, in principle, able to reproduce again. Given that multicellular organisms are generally larger than unicellular species, their developmental times tend to be much longer than the period between cell division times. For instance, for human species, the generation time is about 20 years, while the typical bacteria is able to divide every 20 minutes. As a consequence, the response to natural selection is much slower in the multicellular species, as it is directly connected to the number of generations passed. For our species, the typical time scale of gaining adaptations is hundreds of thousands of years: two orders of magnitude larger than the length of recorded history of humankind. At the same time, bacteria can develop novel features, such as antibiotic resistance, within a matter of years.

What makes the evolution of multicellularity so important is that it is not a unique outstanding process in the history of life. There are other, very similar events commonly named as major evolutionary transitions. They all share the feature of lower-level entities discarding their independence and selfish interests to the community. At the same time the community itself obtains individuality and becomes the unit of higher-level. Long before the emergence of multicellularity, the first genes assembled into chromosomes and started to replicate together. An early endosymbionts of an ancient prokaryotic cell turned into mitochondria and chloroplasts forming the first eukaryotic species. The major transitions resulted in the emergence of larger units and opened the way to increase the complexity of life forms. Formerly independent entities became sub-units, which can be further specialized in different tasks, so the evolution can push the higher-level unit to the capacities, which were simply unthinkable before. Each major transition was a huge step forward in the evolution of life, so they are among the most fascinating phenomena investigated by the modern biology.

The emergence of multicellularity is not restricted to bacterial colonies, either. Many millions of years afterwards, some insect species like ants and bees gave up their individual independence for the common benefit. They formed eusocial colonies, where only the queens are able to reproduce, while other individuals serve to the well being of the hive. Among all major transitions, the emergence of multicellularity is the most accessible: there are organisms both unicellular and multicellular; sometimes we even find closely related species at different degrees of multicellularity evolution. Also, these organisms usually have very fast evolutionary rate, so some hypotheses of what can drive evolution of multicellularity could be experimentally tested within a time frame of few months. And once we decipher the riddle of the evolution of multicellularity, we will also come closer to solve a number of other great puzzles of life.

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