

University of Massachusetts Amherst

From the Selected Works of Lynn Margulis (1938 - 2011)

December, 2010

**Symbiogenesis. A New Principle of
Evolution Rediscovery of Boris
Mikhaylovich Kozo-Polyansky
(1890-1957)**

Lynn Margulis, *University of Massachusetts - Amherst*



Available at: https://works.bepress.com/lynn_margulis/10/

Symbiogenesis. A New Principle of Evolution Rediscovery of Boris Mikhaylovich Kozo-Polyansky (1890–1957)¹

Lynn Margulis

University of Massachusetts Amherst, USA

e-mail: celeste@geo.umass.edu

Received February 28, 2010

Abstract—The following is a heavily edited transcript of my illustrated lecture, that included our 14 minute video (with a 2 minute animation model) that shows each step in live organisms hypothesized in the origin of nucleated cells from bacteria (“eukaryosis”). New observations presented with modern examples of live phenomena make us virtually certain that B.M. Kozo-Polyansky’s “new principle” (1924) of the importance of symbiogenesis in the evolutionary process of at least 2000 million years of life on Earth is correct. The widely touted but undocumented explanation of the origin of evolutionary novelty by “gradual accumulation of random mutations” will be considered an erroneous early 20th century hunch proffered primarily by Englishmen, North Americans and other anglophones. They (Neodarwinist “explanations”) will be replaced by the details of symbiogenesis: genetic mergers especially speciation by genome acquisition, karyotypic fissions (neocentromere formation, related chromosome change) and D.I. Williamson’s larval transfer concept for animals. Although ignored and dismissed in his life time, Kozo-Polyansky’s brilliant work will be lauded for symbiogenesis in the same style that Gregor Mendel’s studies of inheritance of “factors” in peas was for recognition of his establishment of diploid organism genetic principles by the beginning of the 20th century. My talk, photographs and moving pictures were presented at the Darwin conference, St. Petersburg, on September 23, 2009 introduced by E. Kolchinsky.

Keywords: symbiogenesis, eukaryosis, Kozo-Polyansky, speciation, genetic mergers, genome acquisitions.

DOI: 10.1134/S0031030110120087

In most of these sessions it is customary only at the end of one’s lecture to thank those who do the relevant work or have helped. I prefer to thank my predecessors or colleagues here at the beginning. I want to list the Russian literature that has led me to certain conclusions that I will mention. In my talk I plan to use few words, as I’d rather show you live organisms in color videos and photos, especially electron micrographs. Let us begin with Boris Perfiliev (1891–1969), does anyone know his name? He is the father of microbial ecology, some say. He made capillaries, tiny sedimentary cores, minicores, from square glass capillaries. He used them to study layered sediment, stratified microbial communities. From Perfiliev and his student Galina Dubinina we mention to Academician, Prof. Armen Takhtajan, who is still here. The great botanist is 99 years old, as you know. From Takhtajan I continue on to laud a scientist I knew well: Igor Raikov. Raikov (1932–1998) was one of the few modern scientists who recognized the importance of the protists for understanding the origin of genetic systems. Raikov was a person who actually did what Charles Darwin had suggested. Darwin admonished, if one wants to understand the evolution of any process or structure

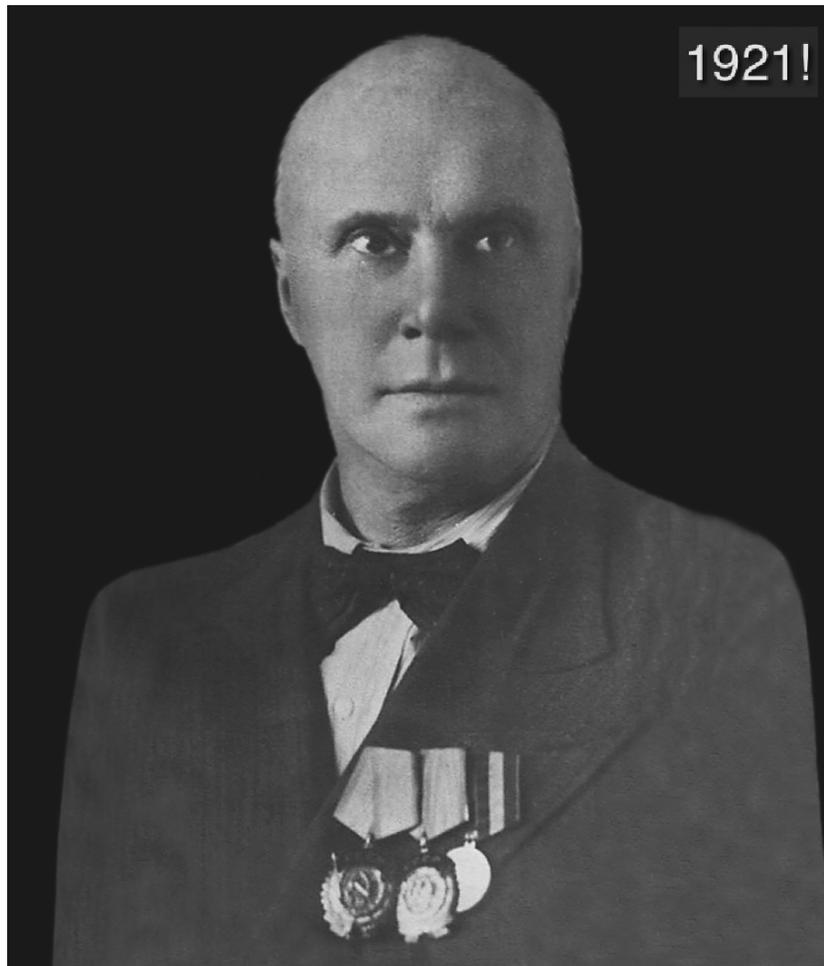
one should follow the oddities and the peculiarities, he called them “oddities and imperfections” in life, because otherwise one may be deceived into thinking that the way life is now is the way it always has been. But the way life forms are now is not the way they have always been. Raikov was excellent at reconstruction of ciliate evolution.

From Raikov we come to Liya Nikolayevna Kha-khina. It was her book (1992) that led me to Kozo-Polyansky (1924, 2010). Victor Fet, our translator, without whom this work would not have been possible, now lives in West Virginia. He was raised in Novosibirsk. His extremely literate father taught computers and informatics in Siberia. Unfortunately, Victor could not attend this evolution meeting. But his wife, Dr. Galina Fet, who comes from St. Petersburg, is here now. She has helped us enormously in our visit here. We thank her.

Who here knows the work of Dr. Galina Dubinina, microbiology professor from Moscow State University? Nobody! Amazing and unfortunate. Dubinina’s work, along with that of her team and her predecessor, Professor Boris Perfiliev, is crucial to our research, crucially important.

So I want to say, yes, Charles Darwin, in general, was correct. But Neo-Darwinism, fundamentally is a

¹ The article is published in the original.



Boris Kozo-Polyansky, 1921.

religion. It is very bad. It is very limited. It enters realms where it does not belong. It is reductionist. It does not even study life. Perhaps I should simply claim that Neo-Darwinism took the life out of biology. I do want to declare that Neo-Darwinism has had a stultifying effect on the study of the evolution of life on Earth. The Neo-Darwinists removed life itself, they extracted the life out of “Biology”, the science of life.

Now let us speak about hidden evolution, the arcane narrative of life on Earth through time.

So what is symbiosis? And what is symbiogenesis? What “symbiosis” is not “cost-benefit” analysis, or “mutualism”. Such terms are not biology. They are not science, “cost” or “benefit” of life forms can not be measured. But symbiosis and symbiogenesis are science. “Symbiosis” refers to long term, permanent, sometimes cyclical, for example, seasonal, physical association between members of different species or other different taxa in general. To define “symbiosis” one ought to follow the original definition of the German scientist Anton de Bary (1831–1888). Symbiosis refers to a physical association between “differently

named” partners, at least two kinds, that endures for most of the life history of the organisms. Or for at least most or all of the life history of one of them. Symbiosis is not a social relationship. Why not? Because social relationships refer to associations between members of the same species. To be symbiotic a relationship must refer to members of different taxa. Symbioses are ecological relationships that, over a long period of time, may become symbiogenesis. In cases where new behaviors, structures or taxa, i.e., new tissues, new organs, new species, new genera, or even new phyla emerge, new relationships at many different levels can be identified as the consequence of symbiosis, then symbiogenesis has been demonstrated. I argue that first of all, symbiogenesis is not ever random. Random DNA mutations, of course, do occur, but they have very little directly to do with morphological or taxonomic innovation in evolution.

As the brilliant young Boris Mikhaylovich Kozo-Polyansky (1890–1957) recognized in his abstract of 1921 (Fig. 1), symbiogenesis is the major source of innovation in evolution. Kozo-Polyansky’s work was

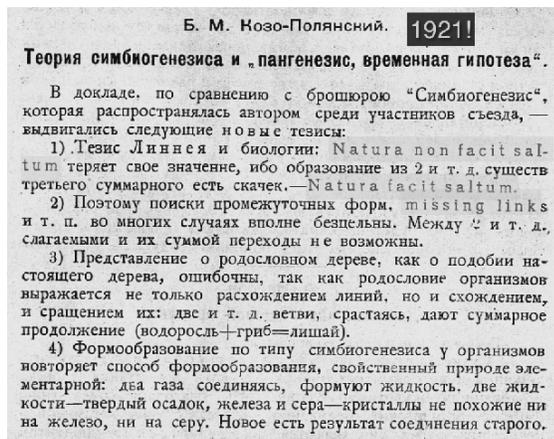


Fig. 1. Boris Kozo-Polyansky abstract, All-Russian Botanical Congress, 1921 (Russian; black & white).

not just recognition and collection of scientific literature, including English and German language literature. Rather, unlike his Russian predecessors K.S. Mereschkovsky (1855–1921) and A.S. Famintsyn (1835–1918), Kozo-Polyansky recognized and did not reject the Darwin-Wallace concept of natural selection (Guerrero, Margulis, Fet, 2010). Kozo-Polyansky showed symbiogenesis generated the original novelty, whereas Darwin-Wallace natural selection followed, maintained and perpetuated it. Natural selection, an elimination process, perpetuates symbiogenesis but does not generate innovation in the evolutionary process. Kozo-Polyansky realized that it was not possible for evolution to establish new species without all three evolutionary phenomena taken together. First “biotic potential,” the tendency of all species, all kinds of organisms, every group, every population, always to grow at a rate unsupportable by its immediate environment. The growth rate of all populations exceeds the capacity of the local environment to maintain the rate of flow of matter and energy required to sustain it. Second, whence the appearance of inherited novelty? The generation of inherited novelty, according to Kozo-Polyansky, was by symbiogenesis followed by natural selection. “Evolution” is a complex process, a system that requires at least these three components: the immense reproductive capacity of live beings called “biotic potential”, the generation of inherited novelty by several modes that include symbiogenesis, karyological alterations such as polyploidy in plants and karyotypic fissioning in mammals. Maintenance of inherited changes in communities is by natural selection but variation itself is not generated by Darwin-Wallace natural selection.

In the language of “systems theory” evolution is a system and like in the processes of any system one cannot privilege one over other of the parts; all parts are required for the system to function. Kozo-Polyansky understood this when, in 1921, he was 30 or 31 years old.



Fig. 2. Ivan Emmanuel Wallin (1883–1969). Photograph courtesy of the Denison Memorial Library, University of Colorado (black & white).

Yes, Konstantin Sergeyevich Mereschkovsky and Andrey Sergeyevich Famintsyn, and Kozo-Polyansky are recognized as “symbiogeneticists.” These scientists are listed and described in the Russian encyclopedia as symbiogeneticists. They are unknown in the anglophone literature.

In our country we have Ivan Emmanuel Wallin (1883–1969) who, of course, did not read Russian (Fig. 2). But he had an immense knowledge of biology, so he invented his own words for the same phenomenon as Kozo-Polyansky’s “new principle.” Wallin came to the same conclusions of the importance of symbiosis in evolution as did Kozo-Polyansky, but entirely independently. And so Ivan Emmanuel Wallin, son of Swedish immigrants to the United States, wrote his wonderful book. He knew nothing about Mereschkovsky, Famintsyn, or Kozo-Polyansky. He knew no or very little Russian literature. Wallin’s idea (1927) was called “symbiogenicism.” He invented this neologism in the title of his great book on the origins

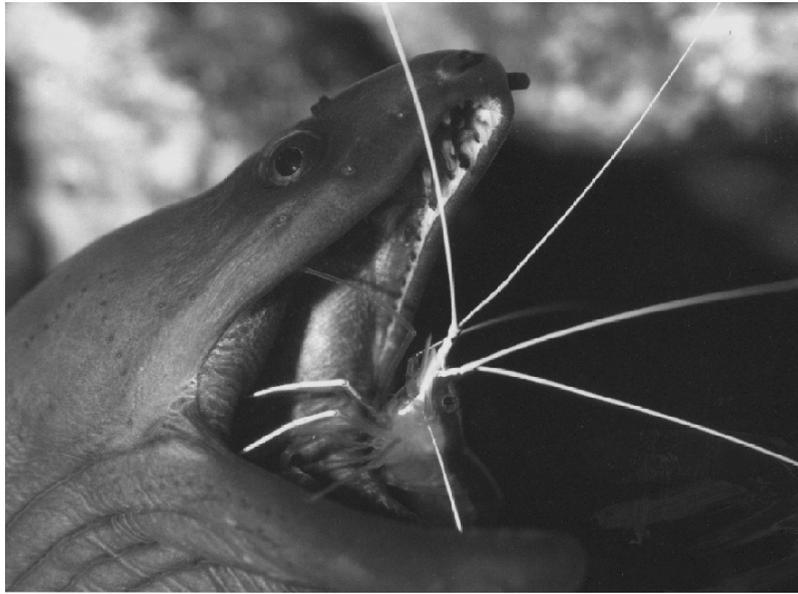


Fig. 3. Moray eel and tooth-cleaning shrimp (color).

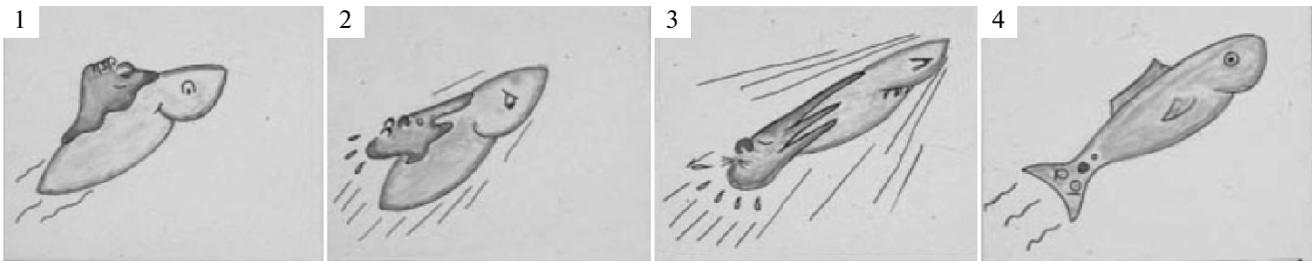


Fig. 4. Stressed “fish”, symbiogenesis solves their long-term problem (black & white; illustration by Steven Alexander).

of species. Wallin claimed that mitochondria evolved from symbiotic oxygen-respiring bacteria and that chloroplasts originated from blue-green algae, cyanophytes (called today cyanobacteria). Wallin had the same ideas basically as did Kozo-Polyansky. So these concepts came from two geographically independent sources. The ultimate geographical sources of symbiogenesis concepts came from the study of live organisms in Russia, St. Petersburg, the Mediterranean coast (Villefranche-sur-Mer, Laboratoire Arago, Banyuls-sur-Mer, Stazione Zoologica di Napoli), the marine laboratory at Roscoff and the Channel Islands of the Atlantic, the Marine Biological Laboratory at Woods Hole on Cape Cod, Wallin's studies in New York and in the Colorado Rockies and later symbiosis studies on lichens, in the Caribbean and on corals at the Great Barrier Reef in Australia.

Here we see a shrimp that cleans the teeth of a moray eel (Fig. 3). This is symbiosis, but is it symbiogenesis? No. Why? This shrimp picks the teeth, eats debris in the toothy mouth and lives with the eel. Although this particular species of shrimp is not

ingested by that eel, the eel eats other kinds of shrimp. It is symbiosis, but not symbiogenesis. Both partners grow and reproduce separately. Both shrimp and eel can live separately. One sees no obvious novelty generated by this symbiosis; i.e., symbiotic physical association. The relationship between the shrimp and the eel is still a behavioral one. Symbiogenesis starts with at least two independent different taxa. Life is difficult, as I don't have to tell you, sometimes it is exceedingly difficult. Often life is intolerably hard and difficult. And occasionally symbiogenesis provides the solution (Fig. 4). Symbiogenesis is evolution, not just ecology.

It is estimated that on Earth today are 250000 protocist species. Among them are representatives of the nucleated ancestors of animals, fungi and plants. My thesis, Kozo-Polyansky's thesis, and Wallin's thesis, persistently and ignored, for the most part, by Anglophone biologists, is that your ancestors and mine evolved from bacteria by symbiogenesis in the Proterozoic eon 2500 to 542 million years ago. Why do we claim that the first nucleated cells evolved in the Proterozoic? Because of the presence of fossils called acri-

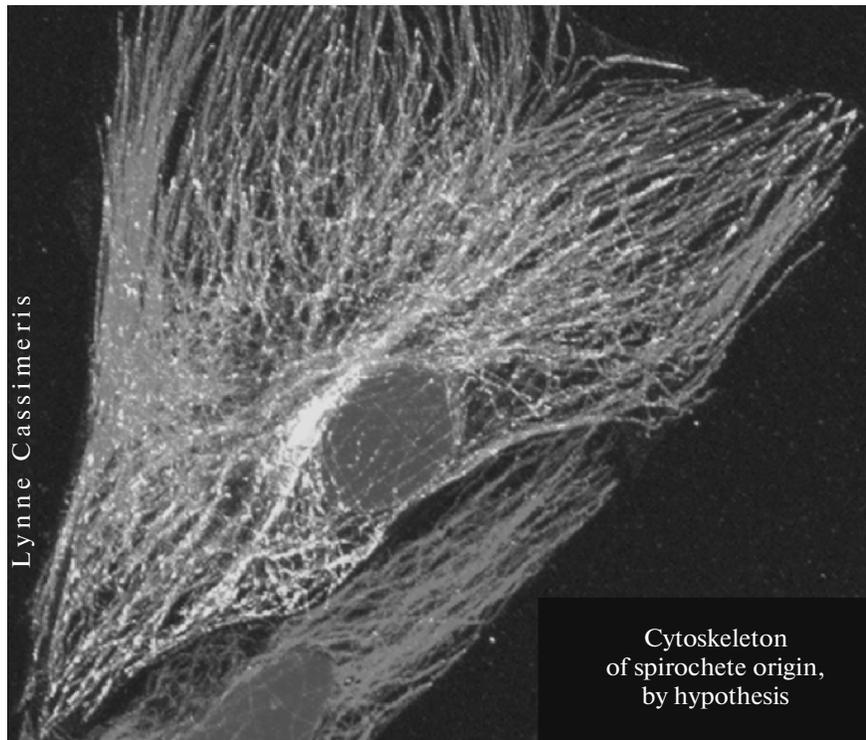


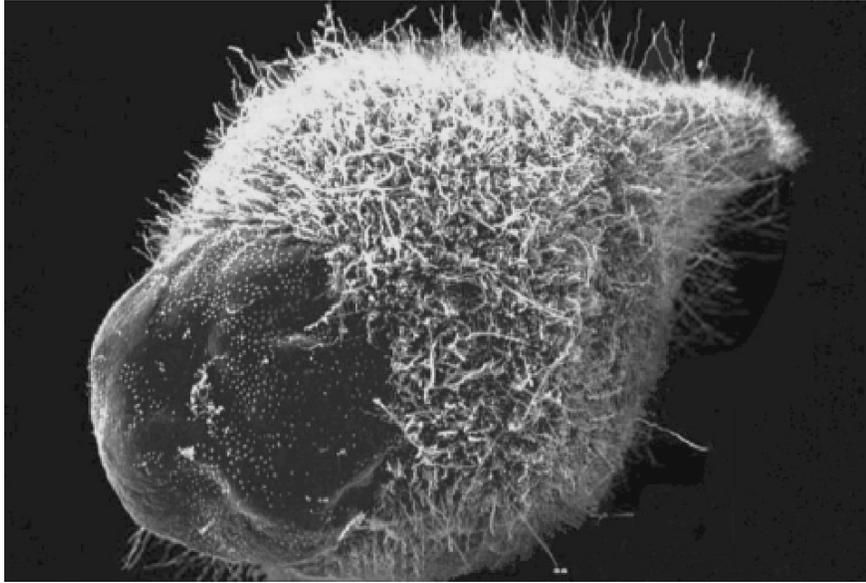
Fig. 5. Mitosis in an animal cell (Courtesy of Professor Lynne Cassimeris, Lehigh University, Bethlehem PA, USA) (color).

tarchs, from a few microns to some over 500 micrometers in diameter. No one knows exactly what they are, but everyone who studies these robust microfossils, mainly by palynological techniques, agrees that microfossils called acritarch fossils are eukaryotes. They are fossil nucleated organisms that began to appear in the geological record more than 1000 million years ago.

Now, this cell, very familiar to all of you, is an animal tissue cell in mitosis. Mitotic cells contain at least one nucleus and the proteinaceous microtubules of the spindle and other cytoskeletal structures. These features are entirely absent in prokaryotes, both bacteria and so-called “Archaea”. Here we see labeled in blue through fluorescence microscopy the microtubular “end-binding protein” (Fig. 5). The reddish-yellow and yellow signal is from microtubule protein absolutely typical of nucleated cells. This class of protein underlies cell motility seen at the level of the light microscope. Through the optical microscope, one observes intracellular movement. Some prokaryotes, that is certain bacteria may be huge, 80 micrometers in diameter, but inside them one never sees what I call “anima”, “animation”, or any movement like this.

I want to show you now the power of symbiogenesis. We begin with amber, which under certain environmental conditions fossilizes very well. Here is a fossil termite, *Mastotermes electrodominicus*, that preserved so well that in the amber fossil record David Grimaldi and his colleagues (American Museum of

Natural History, New York City) saw fossil muscle tissue at the light microscopic level! Fossil termites in amber were thin sectioned with a microtome. The investigators visualized the 20-million-year-old muscle tissue of the *Mastotermes* insect. So, Grimaldi said to us, since we see details of muscle tissue, why can we not also seek the termite’s intestinal protists? And we did! We not only see protist fossils in *Mastotermes*, but we found the oldest spirochetes (and other bacteria, for example bacillus spores) in the fossil record embedded in ancient amber. This had been collected from a mine in Central America, Dominican Republic by the museum director, Dr. Jorge Wagensberg and others (Wier et al., 2002). This specimen I show you here of *M. electrodominicus*, because it is the best preserved of any mastotermitid in the world, is on exhibit at the Museum of Science in Barcelona, Spain. However, in less valuable specimens of amber of *M. electrodominicus*, when cut and imaged the termite microbes are seen. Now let’s speak about *Mixotricha paradoxa*, a unique termite protist that lives only inside *Mastotermes darwiniensis* in the southern hemisphere. We take a trip to Kakadu Park in northern Australia, near Darwin. The climate there is very hot. The extremely hot climate may have persisted for 15 to 20 million years in this region, continuously unbearably hot. And here we see *Mastotermes* termites. Inside those termites, in 1956, Professor L.R. Cleveland photographed the organism—*Mixotricha paradoxa* “protozoan”, which both cilia and flagella on its same cell (Cleveland and



Mixotricha paradoxa

Fig. 6. *Mixotricha paradoxa* SEM (black & white).

Grimstone, 1964). He did not believe that. After years of study he had never before seen this phenomenon, “cilia and flagella” on one-and-the-same cell.

We re-photographed *Mixotricha paradoxa* many years later. From the 1970s until this century we intermittently studied this paradoxical protocyst. And we are now sure that these beating “hairs” are not cilia. They are spirochetes, as Cleveland and Grimstone (1964), first reported on the basis of Grimstone’s superb electron micrographs. The tiny surface spirochetes of *Mixotricha* are morphologically indistinguishable from *Treponema pallidum*, spirochetes of syphilis (Margulis et al., 2009). This, in other words, is a motility symbiosis. The large protist is fringed with 250000 ectobiotic spirochetes embedded in its cortex by attachment sites. Toward the cell’s posterior a different, medium-sized spirochete, is present. It is morphologically indistinguishable from *Borrelia burgdorferi*. And the cell surface also has the little teeny ones. The moving fringe here, as seen with the electron microscope, on nearly all the anterior surface of *Mixotricha*, is covered by the treponemes, and by other symbiotic bacteria. There are thus 250000 treponemes, just after cell division, and before the next division. Perhaps 500000 treponemes per protist cell. Some half-million attached spirochetes confer motility on the *Mixotricha paradoxa*. A scanning electron micrograph of *Mixotricha*’s surface is seen in Fig. 6. Five different kinds of spirochetes comprise the cell (Wenzel et al., 2003). In the posterior ingestive area are another set of endosymbiotic bacteria. Termites can eat wood, but they cannot digest it. Therefore the one *Mixotricha* cell equals approximately a total of nine different kinds of prokaryotic microbes comprising a

single protist. One *M. paradoxa* cell is comprised of half a million individuals. Without the symbiotic associates, no termite digests wood. Here you see the large spirochete *Canaleparolina*, the treponema spirochetes, the protist with a large piece of wood inside. This “single, individual cell” is composed of at least nine different kinds of cells, each with its own genome. This, of course, is a group. And the animal in which this group lives is a termite worker. The termite insect starves within about two weeks if we remove its bacteria and protists. The isopteran dies on the second or third week. But Cleveland showed that if bacteria and protists are injected back into the intestine of the moribund termite the starving insect recovers completely. What, then, is the “individual” here? It is a community. Of course “group selection” occurs!

We have reconstructed the entire evolutionary story of eukaryosis. We have now observed every step in the origin of the cilium (Wier et al., 2010). Our hypothesis is that cilia, undulipodia in general, evolved from living spirochetes. Spirochetes donated many genes for crucial metabolic proteins to eukaryotic nuclei (Hall, 2010). The spirochetes form attached round-body (RB) propagules (Brorson et al., 2009). Spirochetes, of course, are gram negative eubacteria (Margulis and Chapman, 2010). They constitute a phylum; they are pleiomorphic and are totally viable not only in the typical “cork screw” (spiral or helical) swimming stage but also in the slower round-body (RB) form. The RBs (also called vesicular, cystic stage induced by penicillin and other “unfavorable conditions.” Although environmental changes i.e., quantity of free oxygen, elevated temperatures, cold, desiccation, altered salt concentrations and many chemical or medium alter-

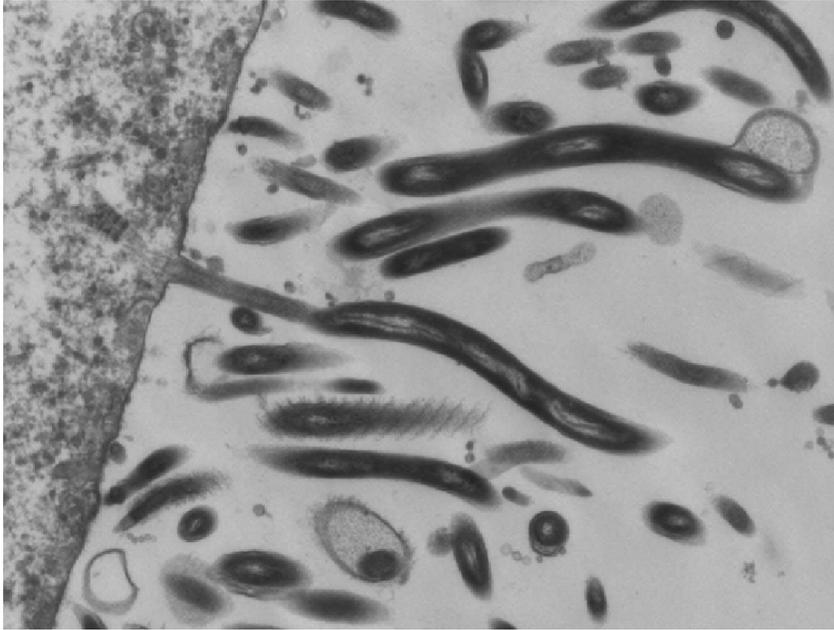


Fig. 7. Spirochete attached to eukaryotic cell from the intestine of *Mastotermes darwiniensis*, reminiscent of a cilium. (Electron micrographs by Professors Andrew M. Wier & Luciano Sacchi).

ations (e.g., notably in carbohydrate and other fermentable food source, amino acid composition, antibiotics induce the spirochete **RB** resistant stage. The proof that **RBs** are entirely viable and reversible to the more active typical swimming helices is the discovery of *Spirochaeta coccoides* in the intestine of the kalotermitid (dry and dampwood-ingesting termite) from *Neotermes castaneus* (Dröge et al., 2006). *S. coccoides* feeds osmotrophically, its population maintains and grows in the anoxic or micro-oxic paunch (specialized hindgut) of this subtropical damp wood termite from Florida, Caribbean islands and coasts and other warm, humid North American localities. Nitrogen-fixing bacteria abound in *Neotermes castaneus*, a fact that may be related the fact that *Spirochaeta coccoides*, in culture does not convert to the typical helical swimmer stage. It has not, to our knowledge, been reported elsewhere.

Please let us look again at Figs. 6 and 7. The epibiotic spirochetes seen here on this trichomonad *Mixotricha paradoxa* are not cilia even though they look remarkably like cilia and other undulipodia of eukaryotes. Nor are they any other kind of undulipodial homologue. They are analogues as discussed by A. Wier (Wier et al., 2010).

Why are so many termite microbes motile? Because if the microbes do not continuously move up the intestine, anteriorly, towards the mouth, they will be excreted with the feces. Many amitochondriates, mostly parabasalids seem to avoid and escape oxygen by remaining centrally located in the insect gut. Many must be motile all the time. They must swim up the intestine. They have to either be attached, or they have

to swim. Otherwise they will be flushed into the soil. So many, many times, spirochetes alone, spirochetes attached to others, or spirochetes attached to intestinal epithelia evolved the ability to remain inside the insect's anoxic, warm, wet, nutrient-rich intestinal habitat.

Although this resembles a cilium with a basal kinetosome, it can not be. In this micrograph the "n" is the nucleoid of the spirochete. There are no missing links here as Kozo-Polyansky said in his 1921 All-Russian Botanical Congress abstract. This is seen as an analogous step in the origin in cilia from spirochetes (Fig. 7, see Wier et al., 2010). We have overwhelming evidence that symbiogenesis is the basis of novelty in organisms composed of nucleated cells (Margulis, 1993). In the earliest symbioses of eukaryotes we hypothesize that intracellular motility, "anima," was acquired by an archaebacterium (Hall and Margulis, 2010).

"Eukaryosis" refers to the process of evolution that form the earliest eukaryote. The earliest eukaryotes are represented by archaeamoebae, metamonads, and parabasalids. None have mitochondria. They still live in anoxic environments today. Most amitochondriates dwell in environments with low concentrations of oxygen, often with high amounts of sulfur.

A bacterial consortium in sulfur-rich environments was discovered by Boris Perfiliev in the Crimea by 1932. It was later studied by his student Prof. Galina Dubinina. She is my age. When first observed Perfiliev called what he thought was one single organism, the genus "*Thiodendron*". The name in Greek means "sulfur tree." Dubinina discovered that "*Thiodendron*" is a spirochete consortium. The spirochete



Fig. 8. *Fucus* with “*Thiodendron*” (color) (Courtesy of Professor Galina Dubinina, Moscow).

components swim towards sulfide, or they die. They must have sulfide. These partner organisms produce and release sulfide. They are “sulfidogenic.” They make sulfide. Although described by Perfiliev and his students in work between the 1930s and the 1970s, they never realized that they had a consortium of two kinds of bacteria at once. The earliest eukaryotes evolved in anoxic, sulfidic, organic-rich marine or estuarine conditions that were typical of the Proterozoic eon. The “ancestral spirochete” co-descendant is now alive in culture in the Moscow collection, and has recently been deposited in the Braunschweig, Germany culture collection. The multi-authored paper (Dubinina et al., 2010) is finally accepted and published. The first of these spirochetes, attracted by sulfide, is from Staraya Russa, Novgorod Oblast, Russia, is named *Spirochaeta perfilievii*. The Dubinina team has been working over 20 years on them. They have other related spirochetes to name.

Sulfurous environments are key. The Dubinina spirochetes swim toward sulfur, they are chemotactic. They use O₂, ambient oxygen, oxygen in the air, to protect themselves from oxidation damage. They are oxygen-tolerant to the extent that they always need sulfide. They oxidize the required sulfide to elemental sulfur. I’ve seen them for 30 or 40 years, without knowing what they were; here they are in nature (Fig. 8). The white scum is what they first called “*Thiodendron latens*”. The seaweed is *Fucus vesiculosus*. The spirochete-sulfidogen consortia that Perfiliev discovered is in white in this photograph. Dubinina’s team has found them in at least six places: in Pacific Ocean islands, in the White Sea, and a sulfur springs resort, Staraya Russa. Dubinina has found them there, and in

other sulfurous environments. They apparently occur all over the world in massive, geochemically important quantities. When Moscow University received their Japanese electron microscope for the first time, Dubinina studied these organisms under strict anoxia, no oxygen: fewer than ten to the minus ten parts oxygen. The long stringy filaments broke up. Out swam classical “1 : 2 : 1” and “2 : 4 : 2” spirochetes. In higher amounts of ambient oxygen (0.001–2%) spirochete this grows longer and longer and longer with no change in width. Apparently *S. perfilievii* waits for anoxia in which the sulfide causes the oxygen-induced filament, with no change in width, to break up again to its typical 10–15 μm length. This spirochete or its very close relative, if we are correct, shares a common ancestor with the sperm tails of half the people in this room.

Here I explain you the animation part of our video called “eukaryosis”.² The atmosphere concentration of oxygen rose because of cyanobacterial photosynthesis. The association between motile spirochetes and sulfidogenic archaeobacteria became permanent. A merger occurred, and a stable association followed when the eubacterium DNA recombined with the spirochete’s DNA. This “permanent conjugation” of Dubinina’s spirochete occurred with *Thermoplasma acidophilum* (Dennis Searcy’s thermoacidophilic archaeobacterium). The association of the eubacterial sulfide oxidizer and the archaeobacterial sulfidogen evolved to become karyomastigont system of eukaryotes called the “last eukaryotic common ancestor

² “*Eukaryosis*” film animation was made by James MacAllister.

(LECA), (Margulis et al., 2006). The karyomastigont itself-nucleus "connector" and undulipodia evolved into what became the mitotic spindle. Oxygen concentration was still rising in the mid-Proterozoic eon, and sulfide was decreasing in many local habitats. Mitochondria were symbiotically acquired after "eukaryosis." The karyomastigont-mitotic spindle was already present in anoxic conditions in amitochondriate. The mitochondriate began as an oxygen respiring delta- or alpha-proteobacteria. The cyanobacteria are still laughing. Cyanobacteria can survive nearly everything except they cannot live under acidic conditions. They "bleach" as their chlorophylls are destroyed.

Now let us look at these cilia to see what these spirochetes have become. We do not know all the details, just most of them. I do not know Y.A. Vinnikov (1910–1987), but he wrote this wonderful book (1982) on the structure of vertebrate sensory systems. A constant in all of cell biology, the nine plus two [9(2)+2] microtubules comprise the undulipodia, they are always 0.25 µm in diameter. Why? Because they are evolutionary homologues. "Undulipodium" is the generic term for the eukaryotic structure that is usually motile. The bacterial flagellum is unrelated. The eukaryotic undulipodium, intrinsically motile, should never be called a "flagellum." There are many, many different kinds of undulipodia. The flagella are bacterial structures only. They are not ever intrinsically motile. Motility in bacterial flagella is restricted to the basal rotary motor embedded in the cell (plasma) membrane. The bacterial flagellum is passive. The shaft is composed of a single, sometimes two proteins. Undulipodia are intracellular bounded by the eukaryote's plasma membrane. They are always inside the cell. Undulipodia include all cilia and many other organelles of motility. They are active along their length. They undulate. Intrinsically motile they are composed of at least 350 different proteins, many detailed in the cell biology literature. Perhaps undulipodia have as many as 1000 proteins, not including membrane proteins. Tubulins, tectins, dynein, ATPase and many others are consistent components of all standard undulipodia. "Undulipodium" is a word from W. Hartmann's German text book. The word "undulipodium" was used in A. P. Shmagina's crazy Russian book (1948). We foreigners learned about the term "undulipodia" from Polish protozoologists especially via by the California scientist and author: Theodore Jahn. He wrote the popular book on how to identify the "protozoa". Shmagina opened the throats of dogs, studied their tracheal epithelia to watch their cilia. As the dog died, she watched the ciliary movement slow down.

The ciliated retinal rod cells of the eyes of vertebrates have "nine two plus two" microtubular undulipodia, underlain at the base by the "nine three plus zero", i.e., the [9(3)+0] kinetosomes. The retinal rod ciliary bases, the sperm tails, the epithelial cilia, the

algal, the fern and *Ginkgo* sp. tree sperm tails, all are entirely homologous. They are all examples of undulipodia. They are also present as extensions in cone cells of vertebrate eyes. The "rods" and "cones" are simply infoldings of the ciliary membranes. The olfactory (smelling) processes are also undulipodia. Here, too, the mechanoreceptor cells of lobster antennules are composed [9(2)+2] pattern of microtubules. The kinocilium of the inner ear and most other sensory cells are modified cilia, as Vinnikov realized. The term undulipodium was introduced into Russian literature but our Anglophone literature refuses to use it. Most people still call these cell sensitive structures and sperm tails "flagella", but they are not flagella. They are undulipodia.

"Undulipodium" is singular, undulipodia is originally a mixed Latin and Greek word: *undula* (wave); *podium* (foot). The so-called "flagella" of eukaryotes, sperm tails, nasal epithelial cilia, gravitational sensors, balance organs, taste bud cilia, fish lateral lines, mechanoreceptors of insects and the antennules of lobsters that show microtubules at their distal ends (nine times two = eighteen plus two = 20 microtubules) in total; these are derived from undulipodia. The antennules evolved as amplification systems based on the microtubules of undulipodia. The sperm tails of aquatic plants such as mosses and liverworts are also [9(2)+2] microtubule motile structures. Scientists agree, based on electron microscopy, that undulipodia, limited to eukaryotes, are all evolutionary homologues.

I have hypothesized that all undulipodia are homologues modified from the original ancestral attached spirochete. I continue to say they are evolutionary homologues whose ancestor was and still is a descendant of a free-living spirochete bacterium that very much resembled Dubinina's *Spirochaeta perfilevii*. The spirochete was water and salt sensitive, oxygen-tolerant but anaerobic, chemosensitive in general, mechanosensitive, perhaps even light sensitive. It was especially sulfide sensitive.

At night, when the sulfide is high, and the oxygen is gone from the estuarine water's surface this can be seen. What is all that white scum? This slide picture was taken at night just past Eel Pond beyond the School Street bridge at the marsh at Woods Hole, Cape Cod, Massachusetts. Only from extremely early in the morning, about 01:00 are these white patches seen. They represent massive quantities of actively motile spirochetes that probably avoid the oxygen released as waste in cyanobacterial photosynthesis.

The same spirochetes, we surmise from their morphology and behavior, are related to Dubinina's *Spirochaeta perfilevii*. What do they do? They take in sulfide to protect themselves against oxygen by transformation of that sulfide into elemental sulfur globules. The second component of the natural "Thiodendron" consortium is a sulfidogenic bacterium. The ancestor in eukaryosis may have been a thermoplasma like those studied by Dennis Searcy. They may have been

another kind of sulfidogenic archaeobacterium such as a "*Sulfolobus*" another candidate for the archaeobacterial ancestral component in all nucleated cells. We don't know. But the concept is clear. One plus one equals one, not two, in biology. One sperm plus one egg equals one fertile egg. But one sulfide-oxidizing eubacterium, a spirochete plus one sulfidogenic archaeobacterium, a thermoplasma, makes one eukaryote: $1 + 1 = 1$. He was correct, no missing links exist, just as Kozo-Polyansky understood this concept clearly by the time he submitted his abstract in 1921.

Here we see spirochetes put in culture from the Islas Canarias, the Canary Islands of Spain, that we collected from microbial mat muds. We first saw them in sulfide-rich organic mud beneath the photosynthetic layers in the delta of the Ebro River in Catalunya, Northeast Spain. Monica Sole and I placed them in various growth media. And this unidentified protist grew in with the fermenting spirochetes. The media, if not anoxic, was certainly severely depleted in free oxygen. This protist swimmer looks like the earliest tiny eukaryote we can imagine. It is an has no mitochondria: the medium in which it grows here is anoxic. It has undulipodia. It is a eukaryote with a single nucleus as part of the karyomastigont system and it grows here in a culture of spirochetes. Why? Because both this tiny mastigote protist and the spirochetes thrive in low oxygen, rich organic mud with an abundance of sulfide gas. We posit that this was the early environment for eukaryotes. Today the same marine habitats support spirochetes and similar protists. In this tube from sulfide-rich organic mud nearly all swimmers are spirochetes of different kinds. Except this mastigote—it is a eukaryote, living as a "contaminant" in the mixed spirochete culture. Everywhere in the world 2000–1000 million years ago, most likely, this sort of habitat easily could be found.

On the anterior portion of this next protist (*Deltatrichonympha*, also from mastotermitid termites) are "waving hairs". They are [9(2)+2] undulipodia, whereas on the posterior portion, here they are spirochetes. Without electron microscopy the details of this *Deltatrichonympha* cell are hard to discern. The undulipodia and the free-swimming spirochetes are difficult to distinguish. Spirochetes became undulipodia. They began as separate organisms. Sometimes they attach to each other or to different other life forms without modification. Sometimes they are modified. We hypothesized the steps in the attachment of spirochetes as they evolved into cilia before we found them. Sometimes spirochetes enter the cells to which they attach. Sometimes they are inside other cells permanently. These associations still occur now. Some became permanent attached forms over and over again. Only one type probably is our ancestor. They are fermentors. So each of the steps in evolution exists today in environments low in oxygen, usually high in sulfide, not always, but always high in organic

food. Here we have one of those wood-ingesting insects, termites.

Let us look closer at the same insect protist association (*Staurojoenina* in *Neotermes*) a little later in the day. We videographed that ten years ago but only recently found in electron micrographic sections that here are five different kinds of spirochetes living inside the protist. We don't know what they're doing, but we know that they are spirochetes living inside a vacuole in a single protist cell in an intestine of a termite (Wier et al., 2007). The cilia, nee spirochetes evolved to become first sensory cilia after they permanently attached to the sulfidogen in the formation of the karyomastigont. The term "karyomastigont" was invented by C. Janicki in 1915. The karyomastigont comprises the nucleus, the proteinaceous nuclear connector, the kinetosome-centriole and its undulipodium. The karyomastigont is an organellar complex. We claim it is the evolutionary product of the DNA of both sulfidogenic archaeobacterium and spirochetal eubacterium partners. The karyomastigont became the mitotic spindle, as it still is in *Ochromonas* (Fig. 9).

Edouard Chatton's drawings show the karyomastigont with its nucleus, nuclear connector, three forward, one trailing undulipodia. During division, the karyomastigont becomes the mitotic spindle. No one thinks of karyomastigont-to-spindle transformation as an evolutionary phenomenon even though this organellar system permeates the protistological literature. Now, Chatton could not publish these charts, the ones he made for his students. Some are on display in the City Museum of Perpignan in southwest France. The gorgeous charts show too many different types of organisms for an professional journals. But Chatton very well knew the relationship between the karyomastigont nucleus and doubling of the kinetosomes and the formation of the thin mitotic spindle (called a paradesmos). Chatton depicted the relationship between the motile undulipodium and mitotic motility as a process. He knew they were intimately involved and related to the evolution and taxonomy of protist groups. I just added modern terminology to his teaching chart (Fig. 10). They are called "course boards" in the literature (Soyer-Gobillard, Schrével, 1986).

The relation between motility, mitosis and symbiogenesis underlies the phyla of the protoctista. We have estimated that there are 36 extant phyla in Kingdom Protoctista (Margulis and Chapman, 2010). That motility, locomotion, and mitosis as cell processes are examples of the same kind of intracellular motility was recognized perfectly well by Chatton. A study of these Darwin's "oddities and imperfections", not at all typical of animals, show how evolution must be reconstructed from clues. Karl Belar in 1933 in the alga of genus *Ochromonas* showed how, when the cell divided in mitosis, the karyomastigont became the mitotic spindle.

Here is a surf clam egg. Watch it carefully, please. Here is its "germinal vesicle," that means "nucleus". Let

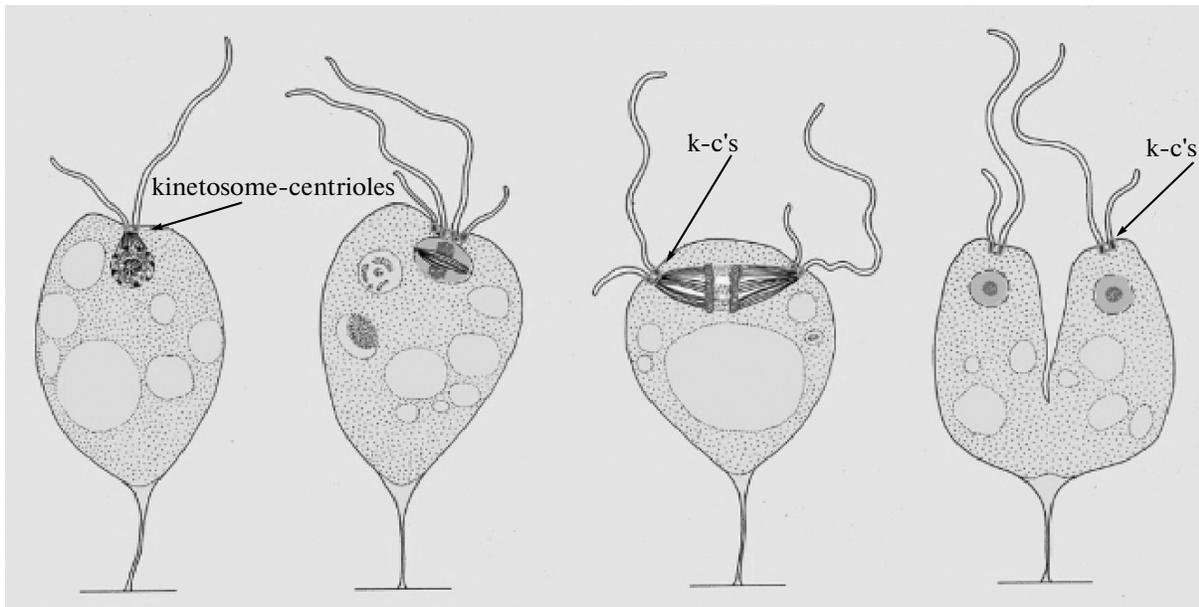


Fig. 9. *Ochromonas* in division (black & white).

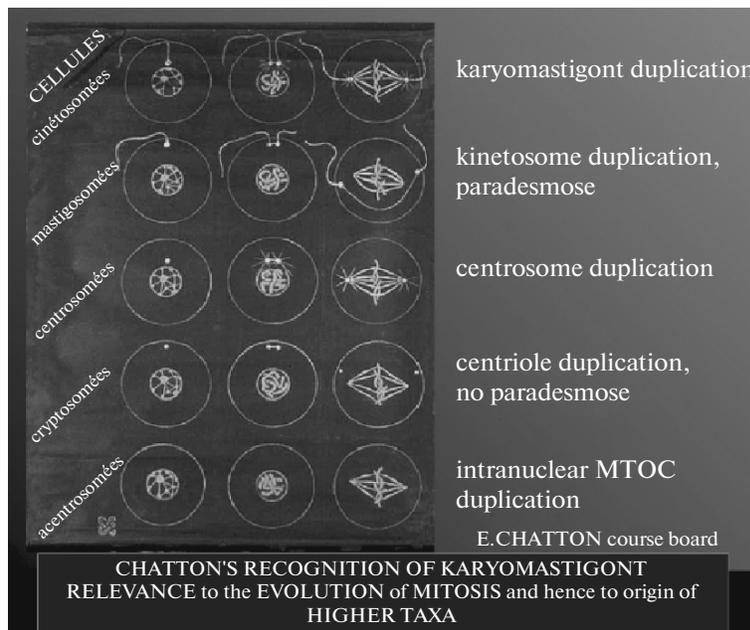


Fig. 10. Edouard Chatton's mitosis related to protist phylogeny (color).

us see it again. Germinal vesicle, nucleus, nucleolus, and polar body (Alliegro and Alliegro, 2008, 2010). Please wait. Nucleus, nucleolus and the little sphere. The little sphere is the nucleolinus. What is a nucleolinus?

The “nucleolinus” is an organelle that contains nucleic acids of the kinetosome-centriole centrosome

system. The nuclear membrane breaks down. The nucleolus in the surf clam (*Spisula*) also breaks down periodically. And the nucleolinus is interpreted by Alliegro (2010) to be the portion of nuclear DNA that in the cytoplasm that releases centriolar RNA. If we are correct, the legacy should be of the original spirochetes. The recent work on surf clam egg centrosomes

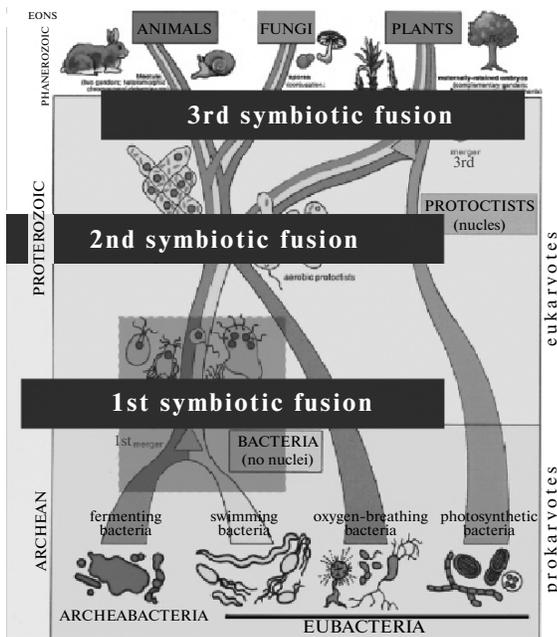


Fig. 11. Origin and evolution of nucleated organisms from bacteria by symbiogenesis (color; illustration by Kathryn Delisle).

by Mark and Mary Anne Alliegro and their colleagues is spectacular (Alliegro et al., 2010).

Each gray triangle represents a symbiotic merger (Fig. 11). Some species of amitochondriates are meiotic. Not many. Mitosis evolved before meiosis. Meiosis evolved as a variation in mitosis probably in response to seasonal environmental cycles. These processes came after the origin of the karyomastigont, i.e., after eukaryosis. Both evolved at least once before the symbiotic acquisition of mitochondria.

Now I explain you how free-swimming spirochetes act. Just as undulipodia do in amoebomastigote transformation, they actively pull themselves in beneath their membranes. Spirochetes produce viable round body (RB) propagules. If we change the growth medium to “unfavorable conditions,” they can form RBs, survive for at least two years in this state, and later revert. First their outer membranes swell. Then they draw in their protoplasmic cylinders. The RBs are totally viable. They reform helical swimmers later when “favorable environmental conditions” are restored. At least twelve different types of spirochetes, free-living and symbiotic, show this behavior (Brorson et al., 2009). Here, once inside, is a spore-like structure produced by *Spirosymplokos deltaeiberi*. The “spore-like structure” is not heat resistant, but it is desiccation resistant. They are totally viable. Some spirochetes are light sensitive. We hypothesize the cytoskeleton evolved from free-living spirochetes like those of Dubinina (Dubinina et al., 2010). “Cytoskeleton” implies anima, animation, movement, and intracellular movement generally.

Only the bacterial groups that enter the evolutionary story of nucleated cells are depicted at the bottom of Fig. 11. A whole series of protist “oddities and imperfections” in the Proterozoic eon evolved as the oxygen concentration rose. Oxygen-breathing bacteria become the mitochondria, and almost all other eukaryotes evolve from mitochondriate protists. The gray triangle, upper right, indicates that photosynthesis that evolved in the bacteria was acquired by algae that evolved into plants. The triangles from bottom to top represent the first symbiotic union, the second symbiotic union, and the third symbiotic fusion. Fusions abounded: undulipodia first, mitochondria next, cyanobacteria precursors to photosynthetic plastids third. Undulipodia confer intracellular motility, mitochondria confers oxygen respiration, plastids provide internal cellular photosynthesis in eukaryotes.

And so where does Kozo-Polyansky come in? The abstract before he wrote his book was shown in Fig. 1. He wrote that nature does make jumps, because the two or more components of a symbiosis are very different from the combined partnership. Apparently no one read his book. Or maybe they read it but it was not understood. It certainly was not translated. Apparently, from the quote written by Nina Bazilevskaya in 1959 two years after he died, the Russian botanists did not think it was worth translating. He was very young. He went on to become a successful professional botanist. This plant, *Androsace kozo-polyanskii*, is a primula, a primrose. You can see the arctic plant on our Harvard University Press flyer for the translation of his book (Fig. 12). He did much other work in botany, but as far as I know, he never returned to symbiogenesis after publication of this prescient, unique little book in 1924. Nobody listened to him. Nobody fought for his ideas. Nobody, perhaps in part because he did not write or speak German or English. How can anyone publish about evolution if he knows no English? One cannot pit oneself against all Neo-Darwinists.

Here we return to Voronezh, to the botanical garden named after Boris Mikhaylovich. Here we see high water in spring. Victor Fet and I explain Kozo-Polyansky’s work but we didn’t change it. We think he was correct in nearly all his statements. We added a glossary and most importantly we show new photographs of live organisms and new electron micrographs to which he referred. We say it will be obvious to anyone who loves life on Earth that Boris Mikhaylovich Kozo-Polyansky correctly interpreted the evidence for symbiogenesis as source of innovation in evolution (Fig. 12). The acquisition of genomes, especially of bacteria and other microbes, we show is the most important positive source of evolutionary novelty. The new illustrations show how prescient and correct was his work.

You may wonder how the birches survive the Russian winter. Perhaps bacteria make antifreeze compounds. Kozo-Polyansky did not use the word “prokaryote.” He referred to bacteria by many names:

SYMBIOGENESIS

A New Principle of Evolution

BORIS KOZO-POLYANSKY

Edited by Victor Fet and Lynn Margulis

Introduction by Peter H. Raven

Translated by Victor Fet

"How could this book not have been a part of evolutionary biology since its publication in 1924? What a difference it would have made in the 'evolutionary synthesis' if this book were easily in the hands of biologists in German or English translation!"

—William Provine, Cornell University

More than 80 years ago, before we knew much about the structure of cells, Russian botanist Boris Kozo-Polyansky brilliantly outlined the concept of symbiogenesis, the symbiotic origin of cells with nuclei.

It was a half-century later, only when experimental approaches that Kozo-Polyansky lacked were applied to his hypotheses, that scientists began to accept his view that symbiogenesis could be united with Darwin's concept of natural selection to explain the evolution of life. After decades of neglect, ridicule, and intellectual abuse, Kozo-Polyansky's ideas are now endorsed by virtually all biologists.

Kozo-Polyansky's seminal work is presented here for the first time in an outstanding annotated translation, updated with commentaries, references, and modern micrographs of symbiotic phenomena.

available in spring 2010
new in cloth

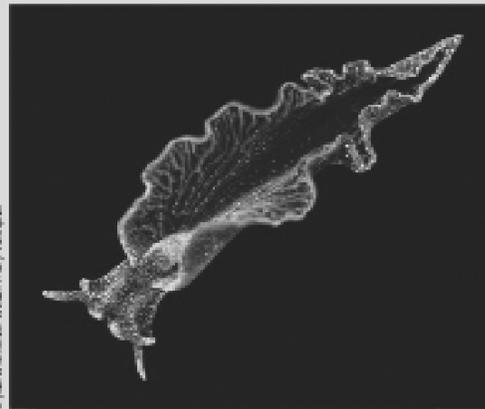


Photo of Kozo-Polyansky's work: Viktor Burzhabo

Boris Mikhaylovich Kozo-Polyansky (1890-1957) graduated from Moscow University and in 1918 joined a Soviet university in his native Veronezh where he eventually became a vice-president, as well as director of the local botanical garden.

Victor Fet is Professor of Biology at Marshall University, Huntington, West Virginia.

Lynn Margulis is a Distinguished University Professor in the Department of Geosciences at the University of Massachusetts-Amherst.

Peter H. Raven is President of the Missouri Botanical Garden.



HARVARD UNIVERSITY PRESS

WWW.HUP.HARVARD.EDU
FACEBOOK | TWITTER | YOUTUBE
BLOG: HUPRESS.TYPEPAD.COM
TEL: 800.485.1619

Fig. 12. Kozo-Polyansky English translation book flyer, Harvard University Press, 2010 (color).

bioblasts, cytodes, flagellated cytodes, nephaloid and consortia. Chatton invented the useful term “procariotique.” He did not define it, he used it in a table and

placed “cyanophytes” with prokaryotes and algae, including dinoflagellates, under the column “eucariotique”.

However, it was B.M. Kozo-Polyansky who explicitly recognized that the tiny life forms were elementary units of the living. He knew well that they have all the properties of life: growth, reproduction, mechano-, chemo-, and photo-sensitivity, motility, photosynthesis. He understood what today we call bacteria as a group. He realized that bacterial elements are recombined and interact. They generate saltations, punctuated change in evolutionary lineages. Yes, he claimed, even in his 19 line abstract. Yes, “*Nature does make jumps*”. Evolution is not gradual as Darwin insisted. Species do not change by accumulation of random mutations as Neo-Darwinists insist.

Here, what we don't see is that in this Voronezh Lake Ugolnoe are the bacteria that run most of the biogeochemical system, as V. I. Vernadsky's work in his book *Biosphere* suggests.

Thank you all very much.

QUESTION: Is symbiogenesis important to prokaryotic evolution, to prokaryotic speciation?

MARGULIS. Fine question. Yes, random mutation and gradualism does occur in prokaryotes. Some symbiogenesis happened, for example in *Thioplaca*. But this was nothing like the rampant symbiogenesis in eukaryotes. Speciation itself, as a phenomenon, first evolved in eukaryotes. In prokaryotes overnight one rids the cells of plasmids, and the act of loss changes a species name (e.g., *Bacillus anthracis* to *Bacillus cereus*), or even, with the loss of plasmids for nitrogen fixation, induces a name change at the level of genus (e.g., from *Rhizobium* to *Azotobacter*!) Plants, animals, fungi or protoctists don't change species and genera by overnight treatments in ethidium bromide or a simple increase in temperature. Of course not.

New species in eukaryotes evolve primarily because ancestral ones acquire entire new genomes that, by many steps, integrate over geological periods of time. They generate closely related descendants as new lower taxa radiate. The best contemporary work on this now is by microbiologists and agriculturally-oriented entomologists.

Perhaps you know the older book of Professor Sorena Sonea “Une nouvelle bacteriologie.” His new book is called “Prokaryotology” (Sonea and Mathieu, 2000). He's a Romanian who has lived in Canada, in Quebec, for his entire professional life. He and his colleagues show that the bacterial genetic system is worldwide. When a bacterium is cultured in the laboratory, one deals with the “terminals” of the worldwide system of bacteria—the “terminals”, not the “computer” itself. In Sonea's metaphor the computer is outside, the “mainframe computer” dwells in nature.

Bacteria have access to huge numbers of bacterial genes. Indeed they may access any gene of another bacterium by means of transforming-principle DNA extruded into the environment, transduction by phage, conjugation, plasmid transfer and other “small replicon” or “genophore” unidirectional bacterial

gene transfer. So we either have one worldwide species of bacteria or no bacterial species at all.

The phenomenon of speciation, even though the words “species” and “speciation” are used as if they meant the same phenomenon, is a process *only* of eukaryotes. The jumps, the saltations, from prokaryotic components to composite “individuals” is a property of all eukaryotes. All have evolved symbiogenetically. Symbiogenesis is fundamental to all eukaryotic taxa from species to phyla (Margulis and Chapman, 2010).

Gradualism, the gradual accumulation of random mutations, ironically is more characteristic of prokaryotes. No one has ever shown, in the laboratory, field or fossil record, the veracity of that the Neo-Darwinist mantra. That “gradual accumulation of random mutations” results in a passage from one to another, new and different, species has never been documented. By contrast, the phenomenon, rampant in eukaryotes: the symbiogenetic origin of new species has been detailed in much literature (Margulis and Sagan, 2002) ever since the tradition was begun in 1921 and ignored. It is mandated now, that those who wish to understand the evolution of life on Earth, even at this late date, start to read the wise words of the young Boris Mikhlayovich

Kozo-Polyanski, native son of Voronezh (Kozo-Polyansky, 2010; Fet and Margulis, 2008). He knew, and we know now, that we live on an Earth more alive and more complicated than the Neo-Darwinists (or even the more humble of us) can describe or explain. Our world is a prodigy of its grand and profound past, its millions of years of natural history.

ACKNOWLEDGMENTS

Drs. Mark A. and Mary Anne Alliegro, Celeste Asikainen, Dr. Andrej Victorovich Bakhtiarov, Øystein Brorson, Judith Herrick Beard, Dr. Michael J. Chapman, Kendra Clark, Prof. Michael F. Dolan, James MacAllister, Melishia Santiago, Dr. Galina Fet, Dr. Anna Gorbushina, Prof. Ricardo Guerrero, Dr. John Hall, Dr. Liya N. Khakhina, Prof. Eduard I. Kolchinsky, Prof. Wolfgang Elisabeth Krumbein, Prof. Jan Sapp, Dr. Bruce Scofield, Prof. Alfred I. Tauber, Prof. Andrew Wier, Dr. Marina V. Loskutova, Prof. Michael L. Williams, and especially Prof. Victor Fet (Marshall University, West Virginia). I express my deepest gratitude for the indispensable (but only partial) financial aid that came from the Alexander von Humboldt Stiftung (Berlin), The Eastman Distinguished Professorship (Balliol College of Oxford University), The Tauber Fund, the NASA Planetary Biology Internship (Woods Hole, MA) and The Graduate School and the College of Natural Science at the University of Massachusetts-Amherst.

REFERENCES

1. M. C. Alliegro and M. A. Alliegro, "Centrosomal RNA Correlates with Intron-Poor Nuclear Genes in *Spisula oocytes*," in *Proceedings of the National Academy of Sciences of USA, 2008*, vol. 105, pp. 6993–6997.
2. M. A. Alliegro and M. C. Alliegro, "The Rediscovery of the Nucleolus and Its Role in Spindle Formation," in *Proceedings of the National Academy of Sciences of USA, 2010* (submitted).
3. Ø. Brorson, H. S. Brorson, J. Scythes, J. MacAllister, A. Wier, and L. Margulis, "Destruction of Spirochete *Borrelia burgdorferi* Round-Body Propagules (RBs) by the Antibiotic Tigecycline," in *Proceedings of the National Academy of Sciences of USA, 2009*, vol. 106, pp. 18 656–18 661.
4. L. R. Cleveland and A. V. Grimstone, "The Fine Structure of the Flagellate *Mixotricha paradoxa* and Its Associated Microorganisms," in *Proceedings of the Royal Society London, Ser. B, 1964*, vol. 159, pp. 668–686.
5. S. Dröge, J. Fröhlich, R. Radek, and H. Koenig, "*Spirochaeta coccoides* sp. nov., a Novel coccoid Spirochete from the Hindgut of the Termite *Neotermes castaneus*," *Applied and Environmental Microbiology* **72**, 391–397 (2006).
6. G. Dubinina, M. Grabovich, N. Leshcheva, F. A. Rainey, and E. Gavrish, "*Spirochaeta perfilievii* sp. nov., Oxygen-Tolerant, Sulfide Oxidizing, Sulfur and Thio-sulfate-Reducing Spirochete Isolated from a Saline Spring," *International Journal of Systematic and Evolutionary Microbiology* **60**, 2002–2050 (2010).
7. V. Fet and L. Margulis, "Symbiogenesis and B.M. Kozo-Polyansky," *Sovremennoe sostoyanie, problemy i perspektivy regional'nykh botanicheskikh issledovaniy* [Current Status, Issues, and Perspectives of Regional Botanical Studies], *Materials of the International Conference, 2008*, Voronezh State University, Voronezh, Russia, 2008, pp. 6–9 [in Russian].
8. R. Guerrero, L. Margulis, and V. Fet, "Darwin-Wallace Paradigm Shift. Ten Days That Failed to Shake the World," *Studies of the History of Biology* **2** (1), 85–90 (2010) [in Russian].
9. J. L. Hall, "Spirochete Contribution to the Eukaryotic Genome," in *Proceedings of the National Academy of Sciences of USA, 2010*, MS no. 2009-08564 (in press).
10. J. L. Hall and L. Margulis, "From Movement to Sensation. Chapter 13," *Chimera and Consciousness, Evolution of the Sensory Self*, Eds. by L. Margulis, C. Asikainen, and W. E. Krumbein (MIT Press, Cambridge (Mass.), 2010) (in press).
11. L. N. Khakhina, *Concepts of Symbiogenesis: A Historical and Critical Study of the Research of Russian Botanists* (Yale Univ. Press, New Haven, 1992).
12. B. M. Kozo-Polyansky, *Novyi printzip biologii. Ocherk teorii simbiogeneza* (Puchina, Leningrad; Moscow, 1924) [in Russian].
13. B. M. Kozo-Polyansky, *Symbiogenesis: A New Principle of Evolution*, Translation from the Russian by V. Fet, Eds. by V. Fet and L. Margulis (Harvard Univ. Press, Cambridge (Mass.), 2010) (in press).
14. L. Margulis, *Symbiosis in Cell Evolution: Microbial Communities in the Archean and Proterozoic Eons*, 2nd ed. (W. H. Freeman, New York, 1993), vol. XXVII.
15. L. Margulis and M. J. Chapman, *Kingdoms & Domains. An Illustrated Guide to the Phyla of Life on Earth* (Elsevier, San Diego; London, 2010).
16. L. Margulis, M. J. Chapman, R. Guerrero, and J. L. Hall, "The Last Eukaryotic Common Ancestor (LECA): Acquisition of Cytoskeletal Motility from Aerotolerant Spirochetes in the Proterozoic Eon," in *Proceedings of the National Academy of Sciences of USA, 2006*, vol. 103, pp. 13080–13085.
17. L. Margulis, M. Dolan, and J. Whiteside, "Imperfections and Oddities" in the Origin of the Nucleus," *Paleobiology (Special Issue in Memory of Stephen J. Gould: Macroevolution: Diversity and Disparity)*, Eds. by E. S. Vrba and N. Eldredge, 2005, vol. 31, suppl. 2, pp. 175–191.
18. L. Margulis, A. Maniotis, J. MacAllister, J. Scythes, Ø. Brorson, J. L. Hall, W. E. Krumbein, and M. J. Chapman, "Position Paper: Spirochete Round Bodies (RBs), Syphilis, Lyme Disease & AIDS: Resurgence of "the Great Imitator"?", *Symbiosis*, **47**, 51–58 (2009).
19. L. Margulis and D. Sagan, *Acquiring Genomes: A Theory of the Origins of Species* (Basic Books, New York, 2002), vol. XVI.
20. A. P. Shmagina, *Mertsat'noe dvizhenie [Ciliary Movement]* (Medgiz, Moscow, 1948) [in Russian].
21. S. Sonea and L. G. Mathieu, *Prokaryotology: A Coherent View* (Les presses de l'Université de Montreal, Montreal, 2000).
22. M.-O. Soyer-Gobillard and J. Schrével, *Oral Presentations of Work of Edouard Chatton* (at Laboratoire Arago, Banyuls-sur-Mer, France, 1986) (unpublished).
23. Y. A. Vinnikov, *Evolution of Receptor Cells, Molecular Biology, Biochemistry and Biophysics* (Springer Verlag, Berlin, 1982), vol. XII.
24. I. E. Wallin, *Symbiogenesis and the Origin of Species* (Williams & Wilkins Co., Baltimore, 1927), vol. XI.
25. M. R. Wenzel, R. Radek, G. Brugerolle, and H. Koenig, "Identification of Ectosymbiotic Bacteria of *Mixotricha paradoxa* Involved in Motility Symbiosis," *European Journal of Protistology* **39**, 11–23 (2003).
26. A. M. Wier, M. F. Dolan, D. Grimaldi, R. Guerrero, J. Wagensberg, and L. Margulis, "Spirochete and Protist Symbionts of a Termite (*Mastotermes electrodominicus*) in Miocene Amber," in *Proceedings of the National Academy of Sciences of USA, 2002*, vol. 99, pp. 1410–1413.
27. A. M. Wier, J. MacAllister, and L. Margulis, "Hibernacular Behavior of Spirochetes Inside Membrane-Bounded Vesicles of the Termite Protist *Staurjoenina assimilis*," *Symbiosis* **44**, 75–83 (2007).
28. A. M. Wier, L. Sacchi, M. F. Dolan, C. Bandi, J. MacAllister, and L. Margulis, "Spirochete Attachment Ultrastructure: Implications for the Origin and Evolution of Cilia," *The Biological Bulletin* **218**, 25–35 (2010).