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EVOLUTION OF PLANT-ANIMAL INTERACTIONS

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1. Introduction

The evolution of plant-animal interactions goes back to the Early Archean, where the first signals of photosynthesis may have been detected in the Isua Peninsula in Greenland, a phenomenon that is related to the isotopic anomalies of carbon. The first evidence of reliable fossils of photosynthetic microorganisms has been identified by micropaleontologists in the Late Archean and Early Proterozoic. A closely related topic in this geologic time interval is the evolution of trophic relations and metabolic diversification in the microbial world. In the context of the three domains of life, Archaea, Bacteria and Eucarya, the bifurcation of multicellular organisms into plant and animals becomes evident only during the Paleozoic. Cell evolution also leads up to the unicellular dichotomy of autotrophs and heterotrophs. Symbiosis has a strong role to play in the transition to plants and animals in the Phanerozoic. It is timely to focus on details of evolution in the Cretaceous and Tertiary, where detailed pathways of evolution have been gathered in many geographical regions, including the Karst region of northern Italy. Various experimental techniques have contributed to elucidate the coevolution of plants and animals. A special case of plant-animal interaction is the evolution and dispersal of hominins, including their impact on the ecosystems. A significant development in understanding the evolution of plant-animal interactions is based on the possibility of identifying reliable biomarkers that can characterize its different stages, from the earliest microbes to the extant plant and animals. Such identification of biomarkers labeling different stages of evolution may orient the search for life in the exploration of the Solar System.

2. Evolution of the microbial world in the Proterozoic-Early Phanerozoic

2.1. THE TREE OF LIFE AND LAST UNIVERSAL COMMON ANCESTOR

The dichotomy between plants and animals evolved from the microbial world that lasted for the major part of Earth's history (Archean and Proterozoic). The microbial cellular

plan consists of prokaryotes, lacking a central nucleus and eukaryotes that evolved later containing a central nucleus enveloping the genetic material. Eukaryotes were earlier considered similar to eubacteria and archaeobacteria and diversified from a universal last common ancestor (Fig. 1).

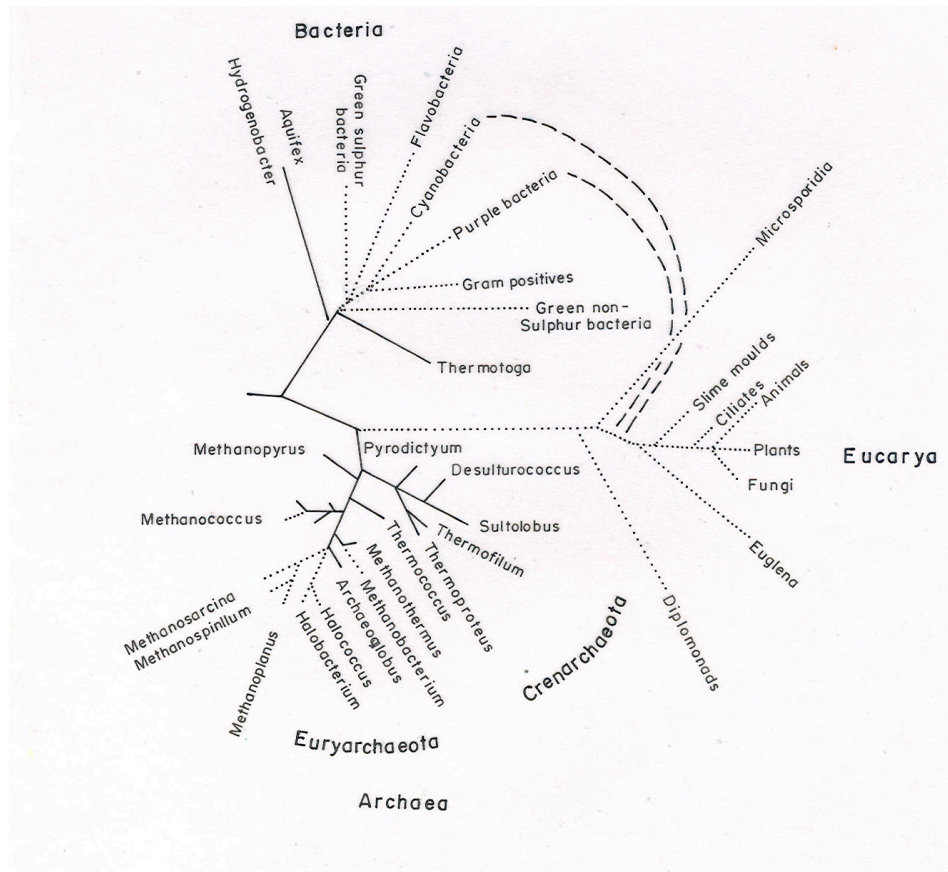


Fig. 1. The relationship between the three domains of life. We have followed the original proposal and terminology used by Carl Richard Woese and co-workers, where the nucleated cells are grouped in the domain Eucarya, while the microorganisms themselves are called eukaryotes (Woese *et al.*, 1977).

In the Archean biosphere, the microbial communities were dominantly marine, however, unicellular eukaryotes must have been present. The unicellular organisms that can survive in extreme conditions are very close to the eukaryotes evolved in Paleoproterozoic around 2.1 billion years (Ga) before the present (BP). Cyanobacteria were the main prokaryotic microbial fossils reported from rocks dating from 3.5 Ga BP. Life on Earth has been classified in three domains Archaea, Bacteria and Eucarya (eukaryotes), (Woese *et al.*, 1990). The evolution of eukaryotes is a debatable subject. The Archean oceans may have colonized by prokaryotes and proto-eukaryotes. The prokaryotes are single-celled microorganisms and can be easily distinguished by their DNA structure that is simple and not bounded by membrane and nucleus. The mitochondria, chromosomes and chloroplasts are also lacking in prokaryotic cells.

The symbiosis between prokaryotes and eukaryotes resulting in modern mitochondria and chloroplasts are well established. This remark lies at the basis of the eventual separation of multicellular organisms between plants and animals (cf., Sec. 3.1). It is believed that the modern eukaryotic cell resulted from symbiosis of eubacterial organelles into an Archaea-like rootstock (Margulis and Cohen, 1994). The Archaea domain includes, amongst others, hot spring bacteria and methanogens. The Bacteria domain includes cyanobacteria, anoxygenic photosynthetic bacteria and the mitochondria and chloroplasts of the eukaryotes (Wheeler *et al.*, 1992). Archaea and Eucarya shared a common ancestor that was not shared by Bacteria. Hyperthermophiles diverged from the Archaea-Eucarya domains (Woese *et al.*, 1990, Wheeler *et al.*, 1992).

Paleobiological evidence indicates that eukaryotes evolved more than 2 Ga BP. Modern unicellular organisms surviving in the extremely hot conditions may be their modern analogues (Tewari *et al.*, 2008). This view differs from the earlier view that the eukaryotes share a common ancestry with Archaea. This evidence was based on genes that code for RNA of ribosomes in Eucarya, Archaea and Bacteria. The nuclear genome contains genes that are of specific eubacterial origin, and some genes are specific to eukaryotes (Woese, 1987). The first appearance of endosymbiont eukaryotes is not clear, however, they must have originated in late the Archean. Multicellular eukaryotes evolved later, around 1.5 - 1.0 Ga BP. These metazoans appeared as a major eukaryotic radiation in the Mesoproterozoic period around 1.0 - 0.8 Ga BP (Knoll, 1984). Terminal Neoproterozoic (0.65-0.57 Ga BP) was the time of Ediacaran explosion of diploblastic animals (Conway Morris, 1989; Narbonne and Hofmann, 1987). Their possible modern analogues are coelenterates like jellyfish, corals and sea anemones. The Cambrian explosion of triploblastic animals with three germ layers is the major event of multicellular metazoans. In the present paper we discuss physical and paleobiological evidences of prokaryote to eukaryote evolution on Earth. The presence of microorganisms in Antarctic lakes including eukaryotic diatoms and cyanobacteria not only supports the idea that life can survive in extreme environments, but also that life may thrive on Mars and Europa (Chela Flores 1998; Chela Flores *et al.*, 2008; Tewari, 1998, 2001a).

2.2 EVIDENCE FOR THE PRECURSORS OF PLANTS AND ANIMALS

Life is generally accepted to have evolved on Earth between 3.8 and 3.77 Ga BP, but an earlier onset of life cannot be excluded. This would have important implications for the current debate on the possibility of temporal overlap of earliest life with a bolide impact scenario terminating at ~3.8 Ga BP (Whitehouse *et al.*, 1999, Moorbath, 2005). The early organisms probably formed around submarine hydrothermal vents. Prokaryotes are the oldest known organisms on Earth and constitute about 70% history of life till eukaryotes appeared around 2.1 Ga BP. The fossil bacteria are found well preserved in black cherts associated with stromatolitic carbonates in most of the Archean and Proterozoic period (Schopf, 1993, 1994; Knoll and Walter, 1992, Tewari, 2004, Shukla *et al.*, 2006). Westall (1999) has shown experimentally that earlier eukaryotes were identified under thin sections mainly by internal spots as the supposed remains of organelles, tetrahedral tetrads of cells, scars indicative of meiotic cell division, large cell size, and mitotic cell division; however, these features are reported from silicified bacteria. Tewari (1989, 2004) has also reported these features from the Deoban cherts of

the Lesser Himalaya. Thus it is difficult to recognize the exact time of the first appearance of eukaryotes in fossil records. Cyanobacteria from the early Archean rocks of Australia and South Africa have been reported from the hydrothermal environment (Schopf, 1993; Westall *et al.*, 2001).

The microbial assemblage was photoautotrophic, but the oxygen level was rather low in the Achaean period. The Proterozoic microbiota is highly diversified and well preserved in subtidal to intertidal cherts associated with microbially mediated stromatolites (Tewari, 2004, 2007, Schopf *et al.*, 2008). Meso- to Neoproterozoic Deoban and Buxa stromatolitic carbonates of the NW and NE Lesser Himalaya are classical example of preservation of these microfossils (Tewari, 1989, 2004, 2008, Shukla *et al.*, 2006, Schopf *et al.*, 2008). The atmospheric oxygen increased during Paleoproterozoic to Mesoproterozoic around 1.9-1.6 Ga BP and perhaps eukaryotes started appearing as leiosphaerid acritarchs in China (Zhang, 1984). Eukaryotic algae are known from 2.0 Ga BP Duck-Creek Dolomite of Western Australia. The diversity in fossil microorganisms is also environmentally controlled in some classical sections like Draken Formation of Spitzbergen (Knoll and Swett, 1987) and Deoban-Blaini-Krol-Tal Formations of the Lesser Himalaya (Tewari, 1989, 2001b, 2004, 2007). Subtidal to intertidal stromatolites and microbiota of Deoban Group include: *Kussiella kussiensis*, *Colonnella columnaris*, *Baicalia nova* and *Tungussia (stromatolites)* and eukaryotic algae *Kildinosphaera* along with *Eomycetopsis robusta*, *Siphonophycus kestron*, *Oscillatoropsis media* and others (Tewari, 1989, 2004 and references therein). Eukaryotic algae diversified in open marine tidal flat environment.

The discovery of *Vendotaenia* (and a new genus *Krolotaenia gniloskayi*) from the Lesser Himalaya shows that eukaryotic alga evolved during Ediacaran or Vendian/Terminal Proterozoic period and the Vendotaenid assemblage coincides with Ediacaran assemblage in the Krol Formation of the Lesser Himalaya, India, Canada and elsewhere (Tewari, 1988, 1989, 1993, 1999, 2004, 2007; Hofmann, 1994). The Krol Formation also contains Neoproterozoic diverse organic walled microfossils, stromatolites, and Ediacaran metazoans (Tewari, 2004 and references therein). *Vendotaenia* is also found in Dengying Formation of China in association with eukaryotic sphaeromorphic acritarchs. This is a coevolution of plants and animals during Ediacaran/Vendian times (650 – 540 million years before the present, Ma BP, after the Neoproterozoic glaciation best known as ‘Snow Ball Earth, Tewari’, 2001b). Recently, we have discussed the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ during Meso- to Neoproterozoic period in the Lesser Himalaya with reference to global paleoclimatic events, paleobiological evolutionary changes and their global correlation (Tewari (2007; Tewari and Sial, 2007).

Ediacaran metazoans were diploblastic animals and related to the increase in the atmospheric oxygen of about 4 — 8 % of the present atmospheric level (PAL). Triploblastic organization of these metazoans is further increased by atmospheric oxygen level up to 10 % PAL. Cambrian radiation event of multicellular life is linked with 40 % PAL around 0.57 — 0.5 Ga BP. Prokaryotic to eukaryotic cellular evolutionary change has taken place during one billion years of Earth’s early history. Additional micropaleontological discoveries combined with the use of recent instrumental techniques like Laser Raman Spectroscopy and Confocal Laser Scanning Microscopy (three dimensional imaging) of microfossils (Schopf *et al.*, 2002; 2008, in press) is important to establish the biogenicity of early life and the presence of organic matter (biomolecules) in them. Further detailed Carbon isotope chemostratigraphy of the key stratigraphic boundaries like Proterozoic—Cambrian (Tewari and Sial, 2007,

Tewari, 2007) and Cretaceous—Tertiary boundary in the Karst region of northern Italy (Tewari *et al.*, 2007) will eventually shed more light on evolution, diversification and catastrophic extinction of life on Earth.

2.3 THE EVOLUTION OF TROPHIC RELATIONS SINCE THE ARCHEAN

A closer and more detailed understanding of the origin of life on Earth has forced upon us a more significant view on the Late Archean and Early Proterozoic evolution of trophic relations in the microworld.

Presently we are more aware that hydrothermal vents in the Earth oceans may have played a role in the origin and evolution of the three domains of life (cf., Fig. 1). Indeed, it is possible that throughout evolution entire ecosystems depend on geothermal, rather than solar energy. This is not only evident on the Earth, but this is also likely to be the case on the other oceans of the solar system, as for example on the moons of Jupiter: Europa and Ganymede. On these satellites this particular hypothesis for the origin of life may be tested in the foreseeable future. The Europa-Jupiter System Mission is now being seriously considered by the main space agencies of Europe, the United States, Japan and Russia, after the initial proposal of the LAPLACE mission (Blanc *et al.*, 2009).

The primary sources of organic matter for the microbial autotrophs are photosynthesis, methanogenesis and sulfate reduction. In the limited space available it is most important to single out and highlight that the group of sulfate reducers may contain some of the earliest forms of life on Earth. Thus, this special form of metabolism may hold the key to understanding the primordial state of life, since sulfate-reducers are in deeply rooted branches of the phylogenetic tree of life (Shen and Buick, 2004).

The morphological simplicity of the primitive sulfate reducers is one drawback in probing the fossilized remains of these microbes. Instead we must rely on the science of biogeochemistry when our objective is to enquire on the antiquity of life and its trophic relations. From the early papers of Manfred Schildowski and co-workers the stable isotope geochemistry of sulfur and the other biogenic elements (H, C, O, N) has been reviewed extensively (Schildowski, 1983; Strauss and Beukes, 1996).

For a proper understanding of the Archean S-isotopic record we should first realize that the abiotic fractionation of carbon isotopes can produce effects comparable to geomicrobiological effects, as described in detail elsewhere (Horita, 2005). However, the situation is more favorable for sulfur. Indeed, microorganisms mediate the reduction of sulfate to sulfide. The resulting fractionations can be reliably taken as good markers for the geological record, especially for the Archean S-isotopic record, where we have hinted that the oldest signatures for life are to be retrieved. The biology that underlies this significant aspect of our quest for the evolution of trophic relations is as follows: the preferential use of ^{32}S over ^{34}S by microorganisms deplete the sulfide in the environment of ^{34}S with respect to the original sulfate. Several species of bacteria and Archea can make this happen via the metabolic pathway known as dissimilatory sulfate reduction. Sulfur itself is not incorporated into cell, but it ends up in the oxidation of organic matter. In normal marine sediments of sulfate, the fractionation can range from 10 to 49 ‰, but this effect can be as large as 70 ‰. On the other hand, unlike the case of carbon described above, abiotic isotope fractionations can yield fractionations in the range 15-20 ‰, for example in the magmatic reduction of gaseous sulfate to hydrogen sulfide

(Rollinson, 2007). This leaves an ample margin for distinguishing the microbial activity in rocks at a hydrothermal vent and the abiotic fractionations.

The S-isotopic record of sulfide and sulfate in Archean sedimentary rocks ranges from Isua of ~3.8 Ga BP (pyrite in banded-iron formations) and ~3.47 Ga BP (barite deposits). In these early times the sulfate reducers were beginning to leave measurable traces, but some difficulties have still to be fully understood, as to their sources and the role of the atmospheric contributions. In the more recent pyrites in black shales of ~2.7 Ga BP (Shen and Buick, 2004, Fig. 6), where the traces are better understood. In conclusion the stable isotope geochemistry of the ~3.47 Ga barite deposit suggests that reactions mediated by microorganisms were already fractionating sulfur much in the same way as present day sulfate-reducing microorganisms.

2.4 METABOLIC DIVERSIFICATION

In the previous section we have sketched some of the most ancient microbes lying deepest at the root of the tree of life. We proceed to discuss briefly how the evolution of the Earth atmosphere repressed these Archaea into distinct niches. The main driving force was the gradual oxygenation of the atmosphere after the discovery of photosynthesis. Ecosystems adopted a layered mat-morphology and sediments. Such ecosystems are well known in present day environments, such as the dry valley lakes of Antarctica.

The microbial mats found today in these environments are composed primarily of cyanobacteria, heterotrophic bacteria, protozoan cysts, eukaryotic algal cells and minerals associated with microbial activity occurring throughout much of the benthic regions of the dry valley lakes (Wharton *et al.*, 1983; Mikell *et al.*, 1984; Vincent, 1988). In the Archean the photosynthesizers were distributed in the upper layers, while the anaerobic microorganisms, such as the sulfate-reducing Archaea were relegated to the lower layers. The eventual consequence of this atmospheric factor was not only to segregate the sulfate reducers (and others such as the methanogens) to lower layers of the mat formations, but to these microorganisms were further relegated to restricted niches. Consequently it was inevitable that evolutionary diversification would follow. Indeed, today we have a large number of obligate anaerobes, not only Archaea, but also mesophilic bacteria. The microfossil record testifies that as oxygenation was gradually driven towards PAL by ~2.1 Ga BP, sulfate reducers and methanogens left their imprint supporting the general outline of Darwinian evolution of the three main domains of the tree of life (Fig. 1). Beyond the consequences of natural selection and adaptation, a new force in evolution, symbiosis, emerged that was to play a crucial role in the eventual dichotomy of multicellular life: plants and animals as we shall discuss in the following sections.

3. Cellular evolution and the dichotomy plant-animals.

3.1 THE ROLE OF SYMBIOSIS IN EVOLUTION

We may separate living organisms in two large groups: prokaryotes (Bacteria and Archaea) and eukaryotes (Encarta, cf., Fig 1 and Sec. 2.2), the first group consists

exclusively of unicellular organisms, considered vegetal organisms since immemorial times (Gerona, 1988) and the second, on the contrary, consists of all multicultural plants and animals inhabiting the Earth. On the base of this division we assume that bacteria and unicellular algae, in their quality of autotrophic organisms are considered vegetal organisms, and we focus our attention on the importance of the interactions of these organisms among themselves, with eukaryotes, and with the multicultural organisms. Frequently these interactions have developed along the history of life a sort of dependence, which very often comes into a real co-evolution for both organisms. The interactions between unicellular autotrophic organisms among them, and between prokaryotes and eukaryotes (unicellular and multicultural), play a major role in speciation and evolution.

In general, the close ecological relationship between the individuals of two (or more) different species is defined as symbiosis. Ecologists use a different term for each type of symbiotic relationship: mutualism is defined as the symbiotic relationship between the individuals of two (or more) different species, where both species benefit; commensalism implies only one of the interacting species benefits, the other is unaffected; finally, parasitism consists in one species benefits as the other remaining harmed. Microbial symbiosis is known today to be a ubiquitous aspect of life.

According to contemporary conceptual consensus, the mitochondria of all eukaryotic cells and the chloroplasts of plants and protists were once free-living bacteria (alpha-proteobacteria and cyanobacteria, respectively) that became incorporated in a primitive host cell (Gray, 1992; Margulis, 1992). It was first suggested that chloroplasts originated as symbionts (Schimper, 1883). The concept was further developed by Merezhkowsky (Sapp, 2005), who coined the word “symbiogenesis” for such a synthesis of new organisms. He maintained that nucleus and cytoplasm had originated by symbiogenesis. Microbial evolutionists also consider whether the cell nucleus may have also arisen by some sort of fusion of symbiosis between two different kinds of bacteria.

Neodarwinism considers the Cambrian explosion as the “big bang” of biology. During this period, between 560 and 495 Ma BP, many now-extinct plants and animals burst onto the scene. Why the Cambrian explosion occurred is not fully understood, but at the cellular scale the real “big bang” for plants and animals occurred some 1800 Ma earlier, with the first appearance of eukaryotic cells (Sapp, 2005). With its membrane-bound nucleus and all the associated features, such as mitosis, meiosis, and multiple chromosomes to package tens of thousands of genes per cell, it provided the material and the conditions for the differentiation of tissues, organs, and organ systems of plants and animals. Symbiosis is at the foundation of our being. Multicellular organisms, humans included, probably evolved and were maintained by bacteria.

Symbiosis has not only played a principal role in the emergence of eukaryotes, it has been vital throughout eukaryote evolution. Although eukaryotes are the most morphologically complex microbes with the largest biomass on earth, have the greatest biochemical complexity (Whitman *et al.*, 1998). Microbial symbionts perform many chemical reactions that are not possible for their hosts. Collectively they can photosynthesize, fix nitrogen, metabolize sulfur, digest cellulose, synthesize amino acids, provide vitamins and growth factors, and ward off pathogens.

The fact that microbial symbiosis is a fundamental aspect of life was first suggested by botanists of the late 19th century. The dual nature of lichens, nitrogen-fixing bacteria in the root nodules of legumes, fungi in the roots of forest trees and orchids, photosynthetic algae living inside the bodies of protists, hydra, and the flat worm

Convolvula roscoffensis, suggested a temporal continuum of dependency of microbe and host from transient to permanent interdependence. When these phenomena were considered together with cytological evidence for reproducing organelles within the cells of plants and animals, they led several biologists of the late 19th century to a conception of the cell itself as a symbiotic community (Sapp, 1994). Speciation induced by parasitic or mutualistic symbionts has been suggested for taxa ranging from plants to insects to monkeys (Thompson, 1987). Models for symbiont-induced speciation have been proposed based upon hybrid inferiority and selection for reinforcement genes. However, taken on their own, such models have severe theoretical limitations and little empirical support. Thompson highlighted the importance of the environment on the symbiont-induced speciation. He established two conditions for symbiont-induced speciation: firstly, interaction norms in which the outcomes of host/symbiont interactions differ between environments; and secondly, differential coadaptation of host and symbiont populations between environments or along an environmental gradient. Thompson defined symbiont-induced speciation as a form of ‘mixed-process coevolution’, namely, reciprocal evolution in which adaptation of a population of one species to a population of a second species (or co-adaptation of the populations) causes the population of the second species to become reproductively isolated from other populations.

The term ‘symbiosis’ was introduced from the Greek language (“living together”) into biology by Anton de Bary in 1879 (Darlington, 1951). He was discussing the presence of algae in the leaves of the aquatic fern *Azolla* and the stunning discovery that lichens were double organisms, combinations of algae and fungi. Today we know that all of the 15,000 species of lichens are made of fungi and either algae or cyanobacteria. A whole new structure, the thallus, emerges out of their association. For de Bary, lichens and other examples of symbiosis offered proof of evolution, and they indicated a means of macroevolutionary change in addition to Darwinian gradualism, based today on gene mutations and recombination.

Frank (1885), who had used the term *symbiotismus* a year before de Bary, reported another important symbiosis between fungi and the roots of forest trees, an association that he named “mykorrhizen” (fungus root). Although the discovery of the mycorrhiza and its ubiquity is generally attributed to Frank, the claim that root fungi may be beneficial to plants had been made a few years earlier for the Indian pipe, *Monotropa hypopitys* (Kamienski, 1881; Trappe and Berch, 1985).

Mycorrhizas are known to occur in practically all terrestrial plants: the plants benefit from receiving essential nutrients, and the fungi benefit by receiving organic compounds from the plant (Allen 1991; van der Heijden and Sanders, 2002). This symbiosis is thought to have been instrumental in the colonization of land by ancient plants some 450 Ma BP (Malloch *et al.*, 1980; Redecker *et al.*, 2000).

Mycorrhizas are also at the very foundation of the world’s most complex and biodiverse ecological systems. Trees of tropical rain forests, in nutrient-poor soils, rely on these fungi to bring them precious nitrogen and minerals (Went and Stark 1968).

Nitrogen-fixing bacteria in the root nodules of legumes have also been central to discussions of symbiosis since the 19th century. Because plants cannot metabolize the molecular nitrogen so abundant in the atmosphere, they require nitrogen in the form of nitrate converted to NH_4^+ . Legumes overcome this problem by their symbiotic union with a group of N-fixing bacteria, rhizobia, which live in specialized root nodules, where excess NH_4^+ is exchanged for sugars exuded by the plant. Symbiotic N fixation underpins the whole global N cycle. During the production of the nodule there is gene

transfer between host and bacteria: some of the plant DNA is transmitted to the bacteria (Pirozynski, 1991). It is not known whether the reverse is also true (Quispel, 1998).

For plants, the associations with fungi and bacteria were the innovative key in the colonization of land and of specific habitats. The eukaryote-associated microbes act as metabolic partners for accessing limiting nutrients and also as protectors, producing toxins that ward off herbivores or pathogens. Similar associations have arisen with animals, allowing colonization of diverse niches, such as the specialized feeding on plant or animal tissues, and the use of deep-ocean hydrothermal vent habitats. Often, the associations are persistent for the hosts, frequently being transmitted vertically across generations, from mother to progeny. The symbionts may be sufficiently fused that they cannot live apart, or be easily recognized as distinct entities (Moran, 2006).

3.2 EFFECTS OF SYMBIOSIS BETWEEN AUTOTROPHS AND HETEROTROPHS

The physiological and morphological effects of symbiosis between photosynthetic autotrophic organisms (plants) and heterotrophic organisms (animal) are varied. Sea anemones, hydra, giant clams, sponges, and tropical corals capture algae from the ocean, harbor them in their cells, and are nourished by their symbionts' photosynthetically produced carbon compounds. Corals acquire up to 60% of their nutrition from *Symbiodinium*, which in return obtain from the coral polyp nitrogenous compounds that are scarce in the crystal-clear tropical waters. A worldwide catastrophe for tropical corals has resulted from disrupting this delicate balance (Hughes *et al.*, 2003). Prolonged high sea-surface temperatures, sedimentation, and inorganic pollutants cause coral "bleaching": corals lose their algae, leaving their tissues so transparent that only the white calcium carbonate skeleton is visible. Without the algae, corals starve to death.

Lichens are intimate and long-term symbioses of photosynthetic algae or cyanobacteria and heterotrophic fungi. As intimate symbioses where the photosynthetic partner is inhabiting its heterotrophic partner (Law and Lewis, 1983; Ahmadjian, 1993), lichen symbionts are often hypothesized to have undergone long-term coevolution, especially when one or both symbionts appear obligate and specialized (Ahmadjian, 1987). However, coevolution has not been rigorously tested for lichen associations (Michele *et al.*, 2001).

To demonstrate coevolution directly requires an assessment of increased fitness resulting from reciprocal genetic change (Thompson, 1994), although coevolution could be demonstrated indirectly by showing parallel cladogenesis or cospeciation between symbiont lineages (Page and Hafner, 1996). A hypothesis of parallel cladogenesis would be accepted with highly specific associations between algal and fungal partners, especially if there is strict vertical transfer of inhabiting algal partners throughout a fungal lineage. In contrast, this hypothesis would be rejected in the case of horizontal transfer of algal partners among fungal lineages. This phylogenetic process is here called algal switching.

When reciprocal evolution leads to cospeciation, coordinated speciation events, equal numbers of species among symbiont partners should evolve—a situation not predicted from morphological studies of these algae (Tscheramak-Woess, 1988) and their fungal partners (Hawksworth *et al.*, 1995).

3.3 THE ROLE OF THE ENVIRONMENT IN COEVOLUTION

Evidence is accumulating that interactions of animals with environmental microbes have resulted in the coordinate evolution of complex symbioses, both benign and pathogenic (McFall-Ngai, 1998; Henderson *et al.*, 1999; Hooper *et al.*, 2001), and that coevolved animal-bacterial partnerships represent a common, fundamental theme in the biology of animals. Most environmentally transmitted symbioses in animals, such as the alimentary canal and squid–vibrio associations, are extracellular and often remain open to the environment throughout the life history of the host. Thus, the host/symbiont community must not only develop mechanisms by which to achieve a balanced, functioning population ratio but it must also ensure specificity of the interaction from the inception of the relationship throughout its persistence. Available evidence suggests that such controls are mediated by: the direct interaction of the bacterial cells with the host cells that are colonized; and by the immune system, both innate as well as adaptive (when present), which samples the population and keeps the host informed of the state of the interaction (McFall-Ngai, 2001). For example, interactions with the gut microbiota induce the mammalian intestinal mucosa to produce mucins and alpha- defensins, which inhibit the symbionts from invading host tissues (Hooper *et al.*, 2001).

We remark, then, that humans are also engaged in several mutualistic relations with other species (cf., Sec.5). These relations range from our gut flora (without which we would not be able to digest food efficiently) and our immune system, to domesticated animals like dogs or horses. These interactions provide protection, or other services in return for food and shelter. Furthermore, man takes benefits from mutualism of other species (second degree mutualism): in traditional agriculture men exploits the mutualistic beneficial functions of many plants as companion plants, providing each other with shelter, soil fertility and the repelling of pests, for example, the cultivation of beans, which may grow up cornstalks as a trellis while fixing nitrogen in the soil for the corn. Therefore, symbiosis is recognized to be a ubiquitous aspect of life. The mechanisms at the origin of these relationships and how and why species might cooperate is a question that has been raised by a number of writers who philosophically question the conception of evolutionism and the notion of linear historical progress.

4. Evolution in the Cretaceous and Tertiary of the Trieste Karst

4.1 EXAMPLES OF PLANT-ANIMAL INTERACTIONS

The geological history of the Karst area encompasses a time spanning from the early Cretaceous to the mid-Eocene (Cucchi *et al.*, 1987; Pugliese and Tunis, 2006). This stratigraphic sequence includes carbonate deposits (from the early-Cretaceous to the mid-Eocene partim) overlaid by mid-Eocene torbiditic beds (marls and sandstones of the flysch). Most carbonate beds record shallow water marine-to-lacustrine settings where the development of the relationships between plants and animals took place. Considering the carbonate sequence, paleontologists may propose examples of these relationships and reconstruct some hypothetical environmental scenarios through the analysis of well-defined fossil taxa. The most representative taxon was the calcareous microalgae (especially Cyanophyta, Dasycladaceae, Charophyta, Corallinaceae), organic builders (including rudists and corals) and microfaunae (benthic foraminifers).

Calcareous algae populated several shallow substrates of the depositional environments recorded in the Karst stratigraphic sequence. The main taxa correspond to Cyanophyta, Charophyta, Dasycladaceae and Corallinaceae. The calcareous algae may represent a favorable basis for a food chain and a good house for the animals.

Rudists and subordinately corals were the organic builders (especially requienids, radiolitids and hippuritids). Organic builders are usually symbiont-bearing taxa. Symbiosis consists of a host-symbiont interdependence between them and the microalgae. This interdependence is advantageous for both: a symbiont gets nutrients and place to live; the host gets food and oxygen. Moreover, photosynthesis induces calcification in settings that are poor in carbon dioxide. However, there is no data about the symbiosis between the Cretaceous rudists and microalgae. Corals were sporadically present in both Cretaceous and Tertiary beds and produced small build-ups, especially during the Tertiary. Similar to the modern corals, they can be considered symbiont-bearing taxa (cf., for example, Pearse and Muscatine, 1971).

Foraminifers are usually representatives of the most abundant taxon in the fossil record. They show several kinds of relationships. Like corals, the larger foraminifera are a symbiont-bearing taxon (cf., for example Hohenegger, 1999). Moreover, following Langer (1986, 1993), who studied the recent foraminifers from some Mediterranean sectors, we focus on the adaptation of the epiphytic foraminifers to a well-defined microenvironment:

- A. Permanently immobile (flat, trochospiral, conical) taxa, that live attached to plants; some arborescent taxa live on shaded parts (rhizomes) of plants.
- B. Temporarily mobile conical, trochospiral taxa that glide over flat substrates (algae, sea-grasses) extending their pseudopodia toward moving direction.
- C. Mobile taxa living within algae that build three-dimensional meshworks; some plane-spiral, or annular species, suspend their tests and, like the spiders, produce a pseudopodial network to capture their food.
- D. Mobile ovate, elongate or planispiral taxa living over the plants.

4.2 HYPOTHETICAL ENVIRONMENTAL SCENARIOS

Considering the previously described examples of relationships plants-animals, some environmental scenarios can be interpreted as follows:

4.2.1 Low-hydrodynamism lagoons (inner lagoons)

More or less restricted lagoons, mostly occurred in the Cretaceous and Paleocene beds, evidencing anoxic, or oxygen-deficient conditions. In particular:

- Early Cretaceous dark limestones, are usually present an oligotypical biota, consisting of opportunistic taxa and limestones with calcareous algae (Cyanophyceae, *Thaumatoporella parvosiculifera*, rare Dasycladaceae). Also stromatolitic layers suggest shallow vegetated bottoms, where animals grazed. Thin-shelled both vegetarian and predator gastropods might have lived in these environments. Vegetated bottoms may be suggested by foraminifers that were mostly represented by morphotypes D (mobile small Miliolidae) and A (arborescent Nubeculariidae, and rare small cone-shaped species). The organic builders activity is sporadic (and is due to the requienids) maybe in relation with an increase of the hydrodynamism and the related temporary improvement of oxygenation. Caffau *et al.* (2000) described the corresponding biota that was

characterized by the requienids giving rise to thin banks. Moreover, a relatively rich biodiversity emerged: foraminiferal morphotypes A (Nubeculariidae), D (small Miliolidae) suggests possible vegetated bottoms, in the presence of grazing gastropods.

Just above the early Cretaceous limestones, a Cenomanian dolomitic unit testifies very restricted conditions, in relation with a sea-regression. It represents extreme life-conditions (innermost lagoon, tidal pool), where only gastropods and the agglutinated foraminifer *Glomospira* have been found. It is presumable that these very restricted settings might have been occasionally vegetated, as also demonstrated by sporadic stromatolitic layers that may have been colonized by grazing taxa.

The boundary between this unit and the overlying late Cenomanian dark limestones is marked by an interval characterized by paleosoils. The dark limestones correspond to a renewal of the benthic life that was linked to a transgressive phase. The community showed opportunistic taxa very similar to those already observed in the early Cretaceous unit.

Algae (Cyanophyceae and *T. parvosiculifera*) might have provided a favorable substrate for prey/predators life, also in the presence of the most opportunistic radiolitids, which gave rise to small patch-reefs. Within a better-diversified biota, the foraminifers showed possible morphotypes A (Nubeculariidae), B (Nezzazatidae), C (*Biplanata peneropliformis*, *Biconcava bentori*, *Broeckina balcanica*) and D (*Spiroloculina*, small Miliolidae), suggesting a vegetated nature of the substrate.

During the Senonian, some levels were characterized by poor-oxygen conditions showing opportunistic biota. They consisted of taxa that were similar to those found in the early Cretaceous. Brackish water wetlands occurred during the Danian and Selandian. Most beds yield small foraminifers represented by the morphotypes B (discorbids), C (*Bangianna hanseni*) and D (small Miliolidae). In relation with the fresh water influence, the scenario also included Charophyta, mostly thin-shelled gastropods and ostracods, together with very rare foraminifera (morphotype B, discorbids). Charophyta were represented by their girogonites, encrusted stems and *Lagnophora* that probably produced meadows, where animals grazed.

4.2.2 High-hydrodynamism lagoons (open lagoons)

These settings are represented mainly by Senonian (partim) and Tertiary limestones with a high fossiliferous content. They present a very rich life including algae, foraminifera and mollusks.

The late Cretaceous beds include the alga *T. parvosiculifera* and a rich rudist fauna (radiolitids and, subordinately, hippuritids and requienids). Foraminifers were dominated by mobile morphotypes C and D (*Moncharmontia apenninica* and *Spiroloculina*, and Miliolidae, respectively) with rare morphotypes B (*Stensioina surrentina*). Among the mollusks, the gastropods were sometimes abundant, maybe grazing on the vegetated substrates, as also suggested by the presence of epiphytic foraminifers. In addition, radiolitids and requienids provided a builder activity scenario.

The limestones from the Danian to Thanetian contain abundant algae that were mostly represented by Dasycladaceae. Foraminifers were constituted by abundant miliolids (morphotype D) and agglutinated and rotaliid species. Moreover, isolated and colonial corals were present during the Danian. They were rare in some thin beds of the Padriciano section (Pugliese *et al.*, 2000) and common in Slovenia beds (Drobne *et al.*, 1988). Thus, it is presumable that a zooxanthellae/corals symbiosis occurred.

4.2.3 Peritidal settings

Their most common findings are signaled just below and above the K/T boundary. Their sequences were repeated several times, denoting changes of intertidal/subtidal/supratidal biota. Concerning the aqueous assemblages, the remnants obviously testify unstable environmental conditions, where an opportunistic biota of lagoon occurred. In particular, lagoon layers contain foraminifers represented by mobile morphotypes (*B. hansenii* and small Miliolidae, in Tertiary beds) and grazing thin-shelled gastropods and ostracods. Wherever present, the stromatolites testified a substrate favorable for grazing gastropods.

4.2.4 Open marine settings

Ramps and in general coastal settings are common during the Senonian and after the Thanetian. The limestones often present a high bioclastic content, where only rare corallinean algae were found.

Senonian biota was well diversified. In agreement with Caffau *et al.* (2004), the Senonian ramps included organic builders consisting of hippuritids, sometimes together with radiolitids and requienids. Moreover, rare zooxanthellate thamnasteroid corals occurred (Russo, personal communication, 2004). Among the foraminifers, the mobile lenticular porcelaineous macroforaminifer *Keramosphaerina tergestina* was present. This scenario corresponds to an oligotrophic scarcely vegetated bottom. Moreover, corallinean algae may have highlighted an epibiosis: they might have encrusted shells of other taxa.

Tertiary ramps and coastal marine settings were characterized by limestones with a more or less high muddy content. Corallinean algae and larger foraminifera were very common. Foraminifers were probably mobile morphotypes (large Miliolidae, Alveolinidae, Nummulitidae and *Orbitolites complanatus*). Thamnasteroid zooxanthellate corals (Turnšek and Drobne, 1998) and rare sea-urchins were present. This scenario suggests oligotrophic conditions.

4.3 SYMBIOSIS SCENARIOS

Karst limestones yield a host-symbiont system in several horizons of its stratigraphic sequence. The existence of these systems may be confirmed, in agreement with the taxonomic uniformitarian (*sensu* Dodd and Stanton, 1990). Actually, modern corals and larger foraminifera are symbiont-bearing taxa (cf., for example: Pearse and Muscatine, 1971; Hohenegger, 1999). Obviously, skeletal parts of the symbiont-bearing taxa can be easily fossilized.

This manner of plant-animal interaction took place during episodes of environmental stability. This stability corresponds to environments populated by a well-diversified biota, consisting of several species in equilibrium. Following Travé *et al.* (1996), Hottinger (1998), Hottinger and Drobne (1988) and Drobne *et al.* (2000), this biota was k-strategist (*sensu* Dodd and Stanton, 1991). This biota is well recorded in the deposits of ramps and, in general, in the coastal settings during the Senonian, the Thanetian and after the Thanetian. Senonian ramps included mainly hippuritids, together with radiolitids, thamnasteroid corals, some Corallinaceae and foraminifers, such as the lenticular porcelaineous larger foraminifer *K. tergestina*. Thus, corals and larger foraminifera might testify a symbiosis zooxanthellae/corals and zooxanthellae (or other

algae)/larger foraminifera. Tertiary ramps-coastal marine settings confirm plant-animal interactions that are described for the Senonian. Corals (Turnšek and Drobne, 1998; Hohenegger, 1999) larger foraminifera (Alveolinidae, Nummulitidae, Orbitolites) were in symbiosis with algae.

If the open lagoons, ramps and shallow marine settings represented stable environments with a well-diversified biota, the more or less restricted lagoon testified unstable settings. The former settings showed interactions based upon a K-strategist biota, together with the possible and subordinate coexistence with r-strategists. The latter settings evidenced r-strategist opportunistic taxa, only. In this regard, data concerning possible r-strategists as symbiont-bearing taxa are not confirmed.

Larger foraminifera/microalgae systems may also provide detailed paleo-depth data. Hohenegger (1999) proposed a precious synthesis of the extensive literature on larger symbiont-bearing foraminifera. In general, foraminifera should protect the symbionts from the intense illumination in a shallow-water environment.

Moreover, the presence of zooxanthellate hermatypic corals constitutes a geological thermometer. In fact, the system zooxanthellae-corals is active if the sea-water temperature is at least 18°C., in relation with the presence of an anhydrase enzyme, that inhibits the system for temperature less than 18°C. The existence of the host-symbiont system was surely inhibited during oxygen-poor conditions (inner lagoon, wetlands, peritidal settings). Moreover, within the paleo-environmental evolution recorded by the Karst rocks, the impact of the meteorite in correspondence of the K/T transition produced remarkable effects. Among the evidence of this event, δC^{13} showed a negative shift, denoting a decrease of the photosynthesis activity (Pugliese et al., 1995) and the possible occurrence of host-symbiont systems.

5. Interaction of plants and animals since the appearance of hominins

5.1 THE FIRST HOMININS

Hominins emerge in the animal kingdom around 6-7 Ma BP. The hominin clade includes the humans and all their ancestors back to the human-ape separation. They are identified by two main traits from the great apes: bipedalism, (with the associated differences in pelvis, spine, knee and foot) and skull shape (with the associated change in brain size and shape), both probably induced by evolutionary effects that can be classified as animal-plant interaction.

Australopithecins are the hominins preceding the *homo* species and showing their presence in the fossil record between 4 and 1 Ma BP. They have characters common to apes, such as the small 300-400 g brain, and to humans, such as bipedalism and small teeth. During times when australopithecines were in their hey day, around 3.5 - 3 Ma BP, the Sahara desert did not exist and these hominins could move freely in a vast land of forests and savannahs extending from the Cape of Good Hope to Java. In fact, already 3.5 Ma BP, australopithecines were present not only in southern and eastern Africa, but also 2500 kilometers west, in Chad. One of the oldest *Australopithecus* Little Foot the 4 million-year old hominin found in Sterkfontain (Clark, 1999). The anatomy of the ankle joint shows Little Foot was bipedal but able to climb in trees by virtue of a divergent big toe. He felt comfortable walking upright, but he might have escaped back into the trees for safety or foraging. Australopithecines were well adapted to bipedalism, but were not

consistent bipeds. A nearly juvenile complete *A. afarensis* (nicknamed Selem), was found in 2005 at Dikika, 4 km from where Lucy, the most famous 3.5 million years old Australopithecus was found. Its body confirms the mosaic evolution patterns found in other australopithecines (Alemseged *et al.*, 2006). The passage from quadrupedalism to bipedalism started in the lower limbs and pelvis, keeping the primitive characters in arms and shoulders.

One of the differences between *Homo* and *Australopithecus* is the smaller size of *Homo*'s jaw muscles. Australopithecines needed powerful muscles for processing nuts and other hard foods that became necessary after the environmental change in Africa during the Pliocene. The recently discovered human gene MYH16 is responsible for encoding the protein myosin heavy-chain 16 that has a key role in human muscles (Stedman, 2004). They are much weaker when this protein is absent or altered. For example, in both humans and macaques, MYH16 is encoded by a particular gene that is expressed in the temporalis muscle. At some point the human gene had a mutation that strongly reduced the function of the protein. After that mutation the muscle mass become 8 times smaller than that of the macaque. But *when did this mutation occur?* By comparing the number of changes in the human gene with that in other species one can infer that the mutation happened 2.1 to 2.7 Ma BP, exactly at the time the genus *Homo* originated. Also living primates such as chimps have strong jaw muscles and in fact they have the MYH16 gene that fully activates the function of the corresponding protein.

On the other hand, muscle size can influence bone growth. Reduction of the jaw muscle attenuates the stress on skull bones. The larger and thinner skull of humans could be the effect of smaller jaw muscles. In conclusion there could be a strong connection between size of jaw muscles and size of the brain. In addition, a less massive jaw muscle could have allowed a better coordination of the mandible function, improving speech capabilities. The first *Homo* species is *H. habilis* that emerged around 2 - 2.5 Ma BP. At that time, the climate was becoming drier and cooler, inducing dramatic changes of vegetation and fauna. This is a challenging moment for hominins in Africa, who needed to change diet and way of life in order to survive.

5.2 THE INFLUENCE OF CLIMATE

At that time not only Africa, but also the whole planet was going through dramatic environmental changes. A progressive 50 million years of slow cooling during the Tertiary had brought the planet into the Pleistocene, when the Arctic ice cap also developed. Despite being referred to as 'Ice Age', the Pleistocene was characterized by glacial advance, as well as recess. Long periods of glaciations were punctuated by briefer warm periods (interglacial - lasting less than 20,000 years). Between 2.6 and 1.1 Ma BP, a full cycle of glacial advance and retreat took about 41 thousand years. During the past 1.1 million years, the cycle lasted 100 thousand years. The paleo-temperature record is being obtained with increasing detail measuring the oxygen isotopes in ice cores from Antarctica and Greenland. The recent EPICA project has provided a detailed temperature record for the last 700 thousand years (EPICA, 2004).

Homo with a larger brain and stone tools suddenly appears in the African record following an evolutionary selection induced by environmental change. He was selected for survival, while all the other small brained were not, except for *Paranthropus Robustus*, who stayed around for a while and then also was extinct about 1 Ma BP.

Between 2.5 and 1.5 Ma BP, *H. habilis* and *H. ergaster*, were sharing the African savannas with robust australopithecines. This long coexistence was made possible due to the different strategies of adaptation for coping with increasing seasonality. Some Australopithecines, such as the *Paranthropus robustus*, with their powerful chewing muscles anchored to the characteristic sagittal crest and their large molar teeth with thick enamel, were well adapted to eat tough fibrous plant foods. Carbon and oxygen isotope analysis using laser ablation mass spectrometry show that the australopithecines adapted their diet to seasonal and interannual variations [Sponheimer et al. 2006]. *H. ergaster*, with his smaller jaw muscle and smaller molar teeth, had a diet of softer food, including fruits and meat. It is generally believed that the carnivorous diet provided the energy needed for the increase of brain in *Homo*. The species *Australopithecus* survived until about 1.5 -1.3 Ma BP, when the African savannas were fully inhabited by *H. ergaster*, capable of controlling fire. We know little on the details of the extinction of australopithecines and on the role that *H. ergaster* and environmental change had on their demise. *Who was ergaster? Was he really the product of evolutionary events in Africa as maintained most paleoanthropologists?*

The details of the true ancestor remain uncertain. Some say *habilis* was the ancestor of all species once called *erectus* and now called *ergaster* in Africa, *erectus* in Asia and *heidelbergensis* in Europe. Other claim *rudolfensis* and not *habilis* was the ancestor of *erectus*. According to Meave Leakey, kenianthropus was its ancestor. In any case, *erectus* suddenly appeared in Africa about 1.8 Ma BP. The best representative of this species is the Nariokotone skeleton, 1.75-m tall (twice the size of australopithecines), modern body proportions, only 20% size difference between males and females. A carnivorous creature with a brain of 900 cc and armed with advanced stone tools, he was the first species to migrate out of Africa, conquering India, China, Sudan, eventually reaching Europe. It is generally believed that meat was the key source of energy for the growth of *Homo*'s brain that led to the bifurcation of this species from other hominids at the end of the Pliocene.

Less orthodox viewpoints have been gaining ground recently (Dennell, 2005). It has been suggested that the speciation event that produced *Homo* could have occurred in Asia. This is based on a number of assumptions. Firstly, *Australopithecus* and/or *habilis*-like hominins were living in the vast savannas going from western Africa to China 3.5 - 2.5 Ma BP. There are several sites with stone tools that have chronologies older than the date assumed for the 'out of Africa 1' event. 'Taphonomic circumstances and lack of field work' are possible reasons explaining the absence of australopithecine fossils in Asia. Secondly, Asia was the centre of global climate change around 2.5 Ma BP with the arising of monsoon seasons that caused the expansion of grassland in a more dramatic way than in Africa. Climate change was also due to the collision between Indian and Eurasian Plates that formed the Tibetan plateau. If climate change was the cause of the speciation that originated *Homo ergaster/erectus*, one should remember that according to recent paleoclimatic studies, Asia and not Africa was at the center of the cooling event at the Pliocene/Pleistocene boundary. Third, the more advanced *Homo* species that developed in Asia as a response to the cooling event disperses back to Africa. This would explain the sudden appearance in Africa, about 1.8 Ma BP, of *H. ergaster*, a species apparently without immediate ancestors.

The main morphological effects of the evolution from australopithecines to *H. ergaster* had been an increase in body size and brain volume (from 300 - 400 cc to 1100-1200 cc). The size of the brain is also linked to the linguistic aptitude and paleo-

anthropologists believe *H. ergaster* had already developed some capacity to communicate in a rudimentary manner through spoken language. According to recent hypotheses, brain size is related to jaw structure that was smaller in *H. ergaster* than in earlier hominids. This was probably the effect of a diet rich in cooked meat, more available thanks to the sophisticated stone tools with sharp blades, very efficient for butchering large animals.

Climate variability must have been the key catalyst in the evolution of the *H. sapiens* genome in Africa, possibly from *H. ergaster* and *H. heidelbergensis*, and its dispersal into Eurasia. The climate change witnessed by modern humans in the last 100 - 200,000 years is recorded, as we said before, in polar ice cores, deep-sea sediments, corals and varves. These terrestrial archives show with great detail the dramatic variability of climate during this period, a feature that is common to the whole Pleistocene epoch. Isotopic analyses provide high-resolution information on the average temperature of the planet, dropping nearly 10 degrees below the present mean values during glacial times and reaching higher values than the present during interglacials. The movement of modern humans must have been seriously conditioned by the corresponding changes in landscape, vegetation and sea level. The Sahara was a critical area influencing the diffusion North-South of modern humans. Its occupation was favored during the warm and wet interglacial times, while aridity produced by cold and dry glacial periods would have made life more difficult for humans. Climate change increased the flux of people in direction of North Africa and the Levant during glacial times, facilitating Middle Paleolithic/Middle Stone Age cultural interactions between these areas.

Cultural and social progress during the Middle Paleolithic/ Middle Stone Age was very slow. Rock shelters and caves became the home base for relatively large groups, which would occasionally break into small bands for hunting and foraging. The first use of seafood and the grinding of plant seeds were introduced, probably during the Last Interglacial. This diversified the diet for modern humans, introducing new elements of flexibility, useful during their geographic spreading.

5.3 ISOTOPIC SIGNATURES OF PLANT-ANIMAL INTERACTIONS

DNA analysis in extant human populations suggests that anatomically modern humans (AMHs) left Africa around 70 thousand years (ka) BP, reaching Australia around 50 ka BP and Europe 40 ka BP (Wells, 2005). Archaeological sites with human presence need to be dated in the relevant areas to confirm these predictions. A precise chronology is also required to evaluate the impact of AMH on the ecosystem they were invading, including their possible involvement in the extinction of the ice-age megafauna and of 'less evolved' human species they were encroaching (Barnosky, 2004).

As an example, Australia lost 85 % of its terrestrial animals with body mass exceeding 44 kg during a short period of time, around 50,000 ka BP, in coincidence with the arrival of humans. One of these animals was the large flightless bird *Genyornis newtoni*. Its eggshells can be found across Australia, together those of an extant large bird, *Dromaius*, the emu. The chronology related to the presence of these birds during the late Late Pleistocene have been determined using radiocarbon dating by accelerator mass spectrometry, amino acid racemization, and thermal ionization mass spectrometry U-series analysis on eggshells and luminescence dating on associated sediments (Miller, 1999). More than 700 dates have been obtained, showing the continuous presence of

Genyornis between 100 - 50 ka BP, when it suddenly disappears from the archaeological records. On the other hand, the eggshells of emu show their continuous presence until the present. The diet of the two birds was reconstructed using the $\delta^{13}\text{C}$ ratio of the eggshells, which reflects the plants that were part of their diet. The results show that Genyornis ate mainly C3 plants, whereas emu had a broader diet including both C3 and C4 plants. Hence, the emu could adapt its feeding strategy to different ecological conditions and this might have been critical for surviving through environmental changes 50 ka BP, probably caused by the arrival of the first humans in Australia (Miller, 2005).

6. Discussion and perspectives

6.1 WHAT HAVE WE LEARNT SO FAR?

The bicentenary of Charles Darwin's birth in 2009 as well as the 150th anniversary of the publication of *The Origin of Species* seems an appropriate time to raise questions that Darwin was unable to do, since he was constrained to the world-view that was typical of the 19th century. For this reason it seems timely to review, as exhaustively as possible, the main outline of what we now have learnt so far from the evolution of life, especially the evolution of plant-animal interactions. Within the limitations of a short review, we have attempted to sketch a comprehensive view of evolution of life on Earth spanning from bacteria to humans. We pointed out some insights that have already been gained from the earliest traces of life in the Lower Archean, due to the significant combined progress of stable isotope geochemistry and micropaleontology (Sec. 2). The ascent of multicellular life can by now be documented convincingly (Sec. 3). There is robust evidence for evolution of multicellular life over a billion years just before the Ediacaran fauna (Sec. 2.1) till the origin of the primates (Sec. 5.1). This is especially convincing in the coevolution of plants and animals that is known in a given geographical region. In such cases evolution can be followed up in exhaustive detail, as we have done in our region for the sake of illustration (Sec. 4). With the fast progress of technology, especially the instrumentation of the physical sciences, we have been able to present some microfossil evidence for both the precursors of plant and animals (Sec. 2.2). and how the trophic relations evolved from solar energy to geothermal energy (Sec. 2.3) is also dwelt upon. The transition from these two form of energy that are available for living processes led to an inevitable layered segregation that even led to new niches for anaerobes. These events left a geochemical record that testifies, convincingly, as to the general lines of Darwinism (Sec. 2.4). New instrumentation has also enabled us to demonstrate how plant-animal interactions have influenced the origin of humans (Sec. 5). This is relevant, since stable isotope variations have shed insights in the climate and the diet of our ancestors. The relevant parameters are $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (Ambrose, 2006).

6.2 NEW PERSPECTIVES ON EVOLUTION

Chemical evolution has been able to sketch successfully the pathway from molecules to the first cell. But research in astrochemistry has also outlined the pathway from nucleosynthesis in stellar interiors to the spread of the biogenic elements (C, H, O, N, S)

through supernova explosions into interstellar gas, leading to the inevitable synthesis of organic molecules that will eventually be the building blocks for the origin of life on Earth and eventually elsewhere in the solar system and around other stars. This is the way that evolution from the Archean, the world of Archaea and Bacteria, to the Holocene, the world of the humans, should be viewed. Some questions that could not be formulated during Darwin's lifetime now begin to be forced upon us. Answers might be within our grasp in the foreseeable future. This short review only allows us to touch on one of them, namely, *What can be learnt from the evolution of life on Earth that might contribute to the search for life elsewhere?*

This question is especially relevant for understanding the intelligibility of nature (Chela-Flores, 2009). Many space missions are currently testing the laws of physics, the geophysical conditions of planets and satellites of the Solar System, and searching for evidence that evolution may have taken place elsewhere. Probing carefully the different stages of evolution that are available to us in great detail, as we have described in the present review, will contribute to identify useful biosignatures.

Other topics of current interest for the evolution of life are beyond the scope of our work. The reader is referred to excellent reviews elsewhere. Amongst such questions we should mention: *Can the Modern Synthesis of Darwin's evolution and Mendelian genetics be enlarged to take into account present-day insights of developmental biology?* (Pennisi, 2008).

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