Theory Biosci. (2010) 129:135–139
DOI 10.1007/s12064-010-0100-1

ORIGINAL PAPER

How symbiogenic is evolution?

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Received: 10 December 2009 / Accepted: 14 January 2010 / Published online: 15 June 2010

Abstract When new entities are formed by the integration of individual organisms, these new entities possess characteristics which go beyond the sum of the individual properties of each element of the association, resulting in the development of new attributes and capacities as an integrated whole. In this process, these new entities also agglutinate and dynamize synergies not present in the individual organisms. In this sense, evolution is a dynamic process that evolves not in the way of perfection or progress, but in the way of adaptation to new conditions. Symbiogenesis, as an evolutionary mechanism, allows a coherent conceptual rupture with some evolutionary ideas of the past and, at the same time, shows and builds a new approach to life, based on solid evolutionary ideas, expanding evolution to an adequate level of integration with the more recent data in biology. These ideas and concepts should be integrated in a post-neodarwinian approach to evolution that needs further attention from the scientific community. The development of a Symbiogenic Theory of Evolution could contribute toward a new epistemological approach of the symbiotic phenomenon in the evolutionary context. This, in our point of view, could be the beginning of a new paradigm in science that rests almost unexplored.

Keywords Evolution · Symbiogenesis · Symbiosis · Post-neodarwinism · Synergies

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Introduction

Even symbiosis has been overlooked by the scientific community, the more recent data on the biological field point out that it is a worldwide phenomenon in nature, and consequently, one of the main characteristics of biological systems is to establish associations and connections with other organisms. These connections can be made through language, chemical communication, or other types of liaisons involving molecular, physical, or psychological relations. This manifestation is one of the key characteristics of life and its diversity. In a way, life has not established itself or developed to exist alone. Since the introduction of the symbiosis concept by De Bary in 1878, and currently accepted in biology, as "the living together of unlike named organisms" and the theoretical formulation of symbiogenesis by Constantin Merezhkowsky, in 1909, as "the origin of organisms by the combination or by the association of two or several beings which enter into symbiosis," this field of science has been a place of controversy and discussion (Sapp et al. 2002; Carrapiço 2010). The symbiogenesis concept was a landmark for the further development of studies on biology and evolution, even if it was not well understood, nor received proper attention from the scientific community, at the time it was formulated. Throughout the twentieth century, biologists have generally considered symbiosis as a curiosity, a strange and exceptional phenomenon and nothing more than a residual aspect of the evolution problem (Sapp 2003). Its study fell largely outside the conceptual and technical framework of biology, namely that of classic neodarwinism.

Nevertheless, symbiosis is a widespread phenomenon with great biological relevance that has a fundamental role in the organization and evolution of life, as it is discussed in this paper. It has, however, neither received proper attention from the neodarwinian scientific research community nor from the science education community. This reality demonstrates the need for conceptual changes in the traditional vision which has been transmitted on the organism's structure and function. The latter has had profound consequences for the biological, medical, and social domains, which have remained practically unchanged until now.

The importance of being symbiont

Most living forms have symbiotic relationships with microorganisms, and, in that sense, symbiosis seems to play a very important role in the origin, organization, and evolution of life. Symbionts and their role in the association are among the key actors in this interplay.

Historically, the symbiont was seen as a supporting actor, who had some kind of a secondary role in the play, where the host was the protagonist and the main character. However, bacteria and, more recently, viruses are seen as an important element of these symbiotic relationships and as a key factor in evolution (Margulis and Sagan 2002; Villarreal 2005; Roossinck 2008; Sapp 2009). As an example, the Gram-negative bacteria of the genus *Wolbachia* form permanent intracellular symbioses with many invertebrates, namely insects and nematodes (Sapp 2003). This common prokaryote (*Wolbachia*) can manipulate the development of the host, and is inherited through maternal transmission and has the ability to prevail over chromosomal sex determination. It selectively kills males and induces parthenogenesis, which can convert genetic males in breeding females. It can also influence sperm competition and generate cytoplasmic incompatibility between strains and species, causing profound morphological and behavioral changes and making this hereditary symbiosis a mechanism for rapid speciation (Sapp 2003, 2004, 2009).

The discovery, that part of the human genome and other mammals are of viral origin (Villarreal 2005), lead us to consider the importance of horizontal gene transfer in evolution and in consequence the shape of life on Earth. Since viruses can adapt rapidly to new environments, it allows for the host to adapt faster and efficiently to these new environmental conditions through the introduction of new genomic elements (Roossinck 2008). Viruses are equally important in the development and evolution of plants, including flowering plants, and this was recently emphasized by Villarreal (2005) and Roossinck (2008). Also, virus–host symbiogenesis can result in virus speciation through the acquisition of new genes, from the host by the virus and also by symbiotic viruses when exchange of genomic elements can lead to the formation of new viral species (Roossinck 2008), opening new ways to understand the dynamics of the tree of life.

Another good example of the importance of the symbionts in the host development can be found in the symbiotic relation between the marine gastropod *Elysia chlorotica* and the chloroplasts of the alga *Vaucheria litorea*. These chloroplasts are acquired by the algal food of the gastropod and stay functional for several months in the mollusk's digestive epithelium cells where they produce photosynthesized products that are used by the animal (Rumpho et al. 2008). The chloroplasts are dependent on proteins that are normally encoded by the nuclear genome of the alga. Its maintenance in the gastropod cells, however, results from a previous horizontal transfer of genes from the alga to the host, through a virus incorporated by the mollusk in its genome (Rumpho et al. 2008).

Azolla, an example of hereditary symbiosis and novelty

Azolla is a worldwide heterosporous free floating or semi-aquatic pteridophyte, presenting overlapping scale-like bilobed leaves covering a rhizome that floats horizontally on the water surface of freshwater environments. Each leaf has an immersed, thick, greenish or reddish and photosynthetic dorsal lobe and a very thin, immersed hyaline ventral lobe. The chlorophyllous dorsal lobe (floating) has an ellipsoide cavity with 20–25 simple hairs, which develop during the differentiation of the leaves, one primary branched hair and one secondary branched hair. Inside this cavity exists a permanent and specific endosymbiotic prokaryotic community of a filamentous nitrogen-fixing cyanobacterium, usually referred to as *Anabaena azollae*, and several genera of bacteria, embedded in a mucilaginous fibrillar network that immobilizes all of them, and which fills the peripherical area of the cavity. The center of this cavity is apparently empty, devoid of mucilage, cyanobacteria, and bacteria, and is probably filled by gas or liquid (Carrapiço 2002, 2006). This leaf cavity behaves as both the physiological and dynamic interface units of this symbiotic association where the main metabolic and energetic flows occur. In this sense, it can be considered as a natural microcosm, a special self-organized microsystem, with a well-defined ecological structure. This symbiotic association can also be considered a successful co-evolved system, with the symbionts always present in the fern's life cycle (including the sexual structures), suggesting a phylogenetic parallel evolution of the associated partners, and can be considered as a typical example of a hereditary symbiosis (Carrapiço 2002, 2010).

The presence of the prokaryotic community, which lives in permanent symbiosis with *Azolla*, plays a relevant role in the plant's survival skills in face of new conditions (Carrapiço 2010). This is related to the fact that this community constitutes a source of evolutionary innovation, where symbionts are the means through which a rapid renovation and adaptation of the entire symbiotic system occurs, as a response to new environmental challenges and with consequences at the metabolical, physiological, and even genetic levels.

The *Azolla* leaf cavity can also be considered as the basic physiological unit of this symbiotic association (Grilli Caiola and Forni 1999), where complex ecological communities of permanent microorganisms co-exist with the fern to maintain the whole. Novel metabolic and organic capabilities are acquired and developed by the partners to establish a new level of organization, extending beyond the capability of each individual forming the association. This information is supported and agrees with the concept introduced by René Dubos and Alex Kessler in 1963 related to the creative manifestations of symbioses, where the nutritional effects of symbiosis are not its most interesting manifestation. Most important is the fact that many symbiotic systems produce substances and structures that neither one of the two components produces when growing alone (Dubos and Kessler 1963). The later is emphasized by Angela Douglas in her book "Symbiotic Interactions" wherein she argues that the common denominator of symbiosis is not mutual benefit but a novel metabolic capability, acquired by one organism from its partners (Douglas 1994), and more recently as a type of biological alliance where all the participating organisms benefit (Douglas 2010) Also, Douglas Zook reinforced these principles and underlines that symbiosis is the acquisition and maintenance of one or more organisms by another that results in novel structures and metabolism. Some symbiotic evolution may involve partner genetic exchanges (Zook 1998).

These ideas can be clearly exemplified by the metabolism of nitrogen associated to *Azolla-Anabaena's* symbiotic system shared by the host and partners. The atmospheric N_2 fixed by the cyanobacterium through the heterocysts is converted into ammonia and released into the leaf cavity. It has been shown that intracellular ammonia pools of symbiotically associated *Azolla* are five times greater than those of endophyte-free *Azolla* (Braun-Howland and Nierzwicki-Bauer 1990). The activities of ammonia-assimilating enzymes in the isolated trichomes of the dorsal leaf cavity were much higher than those in *Azolla* leaves, while these activities in the *Anabaena* filaments were repressed to very low levels. For example, it was shown that the host accounted for at least 90 and 80% of the total glutamine synthetase and NADH-dependent glutamate dehydrogenase activities, respectively. These results suggest that hair cells play an important role in the assimilation of the nitrogen, fixed and released into the cavity by the cyanobiont. The nitrogen is transferred to the pteridophyte (Uheda 1986), after having been acquired during the development of the symbiotic process. Recent data published by Papaefthimiou et al. (2008) indicate the existence of different cyanobacteria strains or ecotypes inhabiting the fern species. These results reinforce our idea that the leaf cavity behaves as a micro-ecosystem or as a natural microcosm (Carrapiço 2002, 2010) with a self-organization and an ecological defined structure, where natural selection acts to evolve different cyanobacterial ecotypes.

All this information reinforces the concept of superorganism. More specifically, in ecological terms, each plant and animal must be considered as "superorganism"—symbiome, which includes its own genes, those of cellular organelles (mitochondria and, or chloroplasts), as well as the genetic information of symbiont bacteria and viruses living within the organism (Sapp 2003). It is also important to take into consideration the relevance of the fitness and how we validate it in terms of symbiotic prevalence. It goes beyond the reproductive view of each individual and reinforces the ecological behavior of the symbiotic system as a whole (Bouchard 2007).

These ideas and concepts, especially the concept of the superorganism, can be applied to *Azolla* and its symbiotic association, which is a good example of a synergistic biological system (Carrapiço 2010). In this association, complex ecological communities of permanent microorganisms co-operate along with the fern in the maintenance of the whole. New metabolic and organic capabilities are acquired and developed by the partners, which establish a new level of organization that goes beyond the individual capabilities of any individual partner, suggesting that the synergies associated to symbiosis had and have a leading role in the morphological, reproductive, physiological, and metabolical complexification of the organisms (Corning 2005).

When 1 + 1 does not equal 2

At the annual meeting of the Swiss Natural History Society held in 1867 in Rheinfelden, Simon Schwendener introduced the dual hypothesis to explain the nature of lichens, indicating that those are an association of two organisms, a fungus and an alga, behaving as "master and slave." This innovative idea can be seen in detail in the proceedings of the meeting where it is written that

> Prof. Schwendener from Basel explains in his presentation, referring to his earlier publications on the anatomy of the lichen thallus, that during his recent investigations he became more and more convinced that … the gonidia and fibres of a large group of lichens were not genetically related; instead the latter had to be interpreted as growth of fungal hyphae on

> algae. According to the current view of the speaker lichens have to be seen not as autonomous plants, but as fungi in connection with algae (Honegger 2000).

It can be considered a classic example of what could be a new approach to the organism concept. It was not easy for the scientific community to accept it, as shown by the statement of William Nylander, a well-known lichenologist of his time. In the preface of an 1896 publication entitled "Les Lichens des Environs de Paris," he points out, almost 20 years after Schwendener formulated his dual hypothesis, that "On sait bien aujourd'hui que la formule 'les lichens sont des champignons vivant en symbiose avec des algues' est une assertion de pure fantaisie ou une calomnie." Such statement reveals the difficulty of the scientific community to accept the new ideas on the structure of lichens, especially the idea that an organism could be formed by two genetically separate organisms living in symbiosis. Currently, the taxonomical classification of lichens still remains a difficult task, given the division of opinions on whether they are algae, fungi, or both. In our point of view, the only way to solve this problem is to consider symbiosis not only as a factor of evolutive change, but also as a valid classificative criterium in the organization of the biological world.

When new entities are formed by the integration of individual organisms, these new entities possess characteristics which go beyond the sum of the individual properties of each element of the association, resulting in the development of new attributes and capacities as an integrated whole. In this process, these new entities also agglutinate and dynamize synergies not present in the individual organisms. The result of this process can be shown in Fig. 1.

Following these findings, we may consider evolution as a complementary process of divergence and integration. Divergence in the production of new life forms, and integration when entities join to form new ones (Sapp 2003). In this context, evolution is a dynamic and a synergistic process that evolves and responds not in the sense of perfection or progress, but in the sense of adaptation to new conditions.

As Nathalie Gontier argued in 2007, in her paper on universal symbiogenesis,

> besides the obvious application of the universal scheme in micro-evolutionary symbiosis studies and the origin of eukaryotic beings, it will be argued that universal symbiogenesis can also include the study of viruses and their hosts, hybridization, and even extra-biological phenomena such as culture and language (Gontier 2007).

This idea that follows Sapp's symbiome concept (Sapp 2003) reinforces the approach to a new field of knowledge in biology, which goes beyond the domain of the life sciences, incorporating the fact that all individuals of a species contain associated bacterial populations that can act in the determination of the phenotype. In this sense, the eukaryotes are not genetically unique entities, and the concept of the individual must be seen as a complex biological ecosystem, composed of multiple interdependent parts living symbiotically (Sapp 2003, 2009). It is at the level of the symbiome, that integrates a multigenomic genetic pool, that natural selection acts.

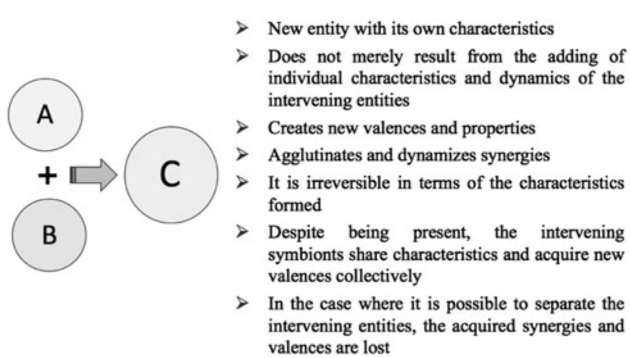

Fig. 1 Characteristics of new entities when formed by the integration of individual organisms

Concluding remarks

Symbiogenesis must be considered as an evolutionary mechanism which implies that evolution should be understood in a broader context, where symbiosis plays an essential role in the organization and structuring of the biological world. Symbiosis should also be perceived as a valid epistemological tool to understand the biological and evolutionary phenomena, a type of biological alliance, where natural selection often favors co-existing forms of life. In this perspective, symbiosis does not imply a strict compartimentation of interspecific relationships. Rather it should be regarded as a continuous and dynamic equilibrium process of different relations, such as mutualism, parasitism, and commensalism, where the acquisition of new genes through lateral transfer plays an important role. This idea also implies the central role of interactions, in which individuality (new entity) emerges through incorporation. It involves horizontal mergers, which can be rapid, and, usually, discontinuous, creating permanent and irreversible changes, the ground for evolutionary novelty. Something new arises through merging, such as a unique or new metabolism or structure(s), which was not present before symbiosis. In this context, we can consider this entity as a new taxonomic novelty or even as a new level of biological organization which can be included in a Symbiogenic Theory of Evolution.

Acknowledgments I am grateful to Nathalie Gontier and Maria Helena Costa for the encouragement in the development of this work and to Helena Carrapiço for the English revision of the text.

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