

Review article

Endosymbiosis in Sponges: Relevance for Epigenesis and Evolution

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Abstract

Symbiosis of bacteria and algae with protoctists and fungi, plants and animals may involve the coexistence of two different genomes in the same cell or organism. Even if gene transfer between the symbiotic partners is restricted or absent, symbiotic integration involves signal transduction systems and specific epigenetic processes of reciprocal gene modulation that occur through a complex molecular cross-talk between the symbionts. Although especially studied for the *Rhizobium*-Leguminosae association the fine tuning of symbiont recognition and regulation occurs as well in other symbioses. New morphological, physiological and behavioural adaptations arising in symbiotic associations, as the light organ of squids, result from morphogenetic and biochemical constraints driven by epigenetic processes. In this context, we consider the secondary acquisition of photosynthesis due to symbiosis with cyanobacteria, zoochlorellae and zooxanthellae, which occur in several sponges. According to recent observations on the Mediterranean sponge *Petrosia ficiformis* this kind of symbiosis induces relevant morphological and physiological adaptations in the sponge. Symbiosis with heterotrophic bacteria, as occurs in the sponge *Chondrosia reniformis*, seems also to influence the physiology and the behavior of the sponge significantly. The evolutionary significance of the

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new genetic-epigenetic mechanisms arising from the symbionts interaction is examined in the light of recent views on the epigenetic inheritance systems. It is suggested that symbiotic innovations can follow a two-step (epigenetic and selective) gradualist evolutionary model. This model states that the epigenetic processes due to the symbiont interaction, induce phenotypic changes which, because inherited, are submitted to selection in the course of generations.

Keywords: Symbiotic adaptations, epigenetic mechanisms, sponge endosymbiosis

1. Introduction

Symbiosis represents a basic process for the evolution of life. It is now recognized (Maynard Smith and Szathmàry, 1995) that a major transition in evolution involved the transformation of bacteria to organelles (mitochondria, plastids) of the eukaryotic cell (Margulis, 1970, 1993). These organelles conferred to the eukaryotic cell new fundamental metabolic properties as aerobic respiration and photosynthesis.

Many other innovations in physiological and morphological evolution of protocists, plants and animals are due to symbiosis (Margulis and Fester, 1991). Cyanobacteria, other types of bacteria and algae have conferred new metabolic pathways upon plants and animals (e.g. nitrogen fixation, chemosynthesis, methanogenesis, photosynthesis, vitamins, amino acids, sterols and luminescence). The body shape and the anatomy of organs and organ systems of the larger symbiotic partner have been in some cases remarkably influenced by harbouring the symbionts and by their metabolites. Fish and squid photophores, enlarged guts of ruminant mammals, insect mycetomes, root nodules of leguminous plants and lichen thalli are examples. At the cell level endosymbionts are often enclosed in a novel organelle, the symbiosome (Roth et al., 1988).

As demonstrated in particular for the *Rhizobium*-Leguminosae association, the metabolic and morphological innovations produced in the host by the symbiont require changes in gene expression and regulation through a molecular dialogue between the partners (Douglas, 1994). This implies that the symbiotic association has an evolutionary history which is associated with the emergence of new physiologies that originate from the coexistence of two different genomes in the same organism and often in the same cell. They require specific epigenetic processes to produce the novel phenotypic features due to symbiosis. Thus, we consider, in spite of the lack of data, that symbiotic associations represent a favourable field for the study of epigenetic cell processes and their significance in evolutionary mechanisms.

Epigenetic changes, which can be inherited through cell lines but also intergenerationally, include extranucleic as well as epinucleic systems (EIS).

Epinucleic processes are those in which DNA expression is changed through chromatin markers, as DNA methylation, but without changes in the DNA sequences. The epinucleic changes can be inherited through non-Mendelian inheritance mechanisms as epialleles (Jablonka et al., 1992; Jablonka and Lamb, 1995). Inherited epigenetic changes, accompanied also by changes in the pattern of the transcription complexes, may be important for associations – such as the *Rhizobium*-Leguminosae or those of zooxanthellae and cyanobacteria with animals as cnidarians or sponges – which are based chiefly on a molecular cross-talk between the symbionts without gene transfer. On the other hand, as indicated by Landman (1991, 1993), even nucleic processes as gene transposition and gene curing may be considered among the non-Mendelian processes for the inheritance of acquired characters through symbiosis.

These aspects should be taken into consideration to explore if novelties can arise into the symbionts after new gene insertion and genome integration, besides the well-known case of mitochondria and plastids. Interactions between the genetic and the epigenetic systems are now well documented. The introduction of new genetic material into the host genome induces changes in the chromatin marks-methylation pattern, as shown by the transposable elements of *Zea mays* (Fedoroff et al., 1989). The same occurs in the case of transgenes in plants as *Petunia* (Meyer et al., 1992) and mammals as *Mus* (Engler et al., 1991). Insertion of viral-like DNA segments may bring about rapid and dramatic changes in gene regulation and development (McDonald, 1990).

Symbionts can be transmitted vertically through the eggs, as in insects, but also acquired at each generation from the environment, as in flatworms, bivalve molluscs, many cnidarians and luminescence symbioses of marine fishes and cephalopod molluscs. The same occurs in most symbioses in plants as those with rhizobia and mycorrhizal fungi. Therefore, highly evolved symbioses may have originated through the reiterated invasions of the host by endosymbionts coming from the environment. This feature seems consistent with an important role of epigenetic processes in driving the coevolutionary events of symbiosis. Horizontally transmitted symbiosis has produced, through complex molecular mechanisms, evolutionary novelties such as the nodules of legumes (Douglas, 1994), the feeding site of root knot nematodes (Opperman et al., 1994) and the light organs of sepiolids (Montgomery and McFall-Ngai, 1994; McFall-Ngai, 1994).

Epigenetic interactions, involving gene expression and regulation, control also the pattern of symbiont recognition and proliferation, e.g. in *Chlorella-Hydra* association (McAuley, 1987, 1990) and in the bacterial symbiosis of the insect *Sitophilus orizae* (Nardon and Wicker, 1981; Nardon, 1995). Experimental studies on the association between bacteria and *Amoeba proteus* (Jeon, 1995) show that the newly infected amoebae become dependent on the

symbionts within 200 cell generations following infection. Endosymbionts avoid digestion first by being resistant to lysosomal enzymes when exposed to them within phagolysosomes and second by preventing lysosomal fusion with symbiosomes. The presence of these bacteria causes various physiological changes in host amoebas.

Sponge symbioses with bacteria and microalgae are varied and widespread. These kinds of symbioses have led to significant changes in the physiology and morphology of several sponges and they represent a clear example of the influence of symbionts on the host morphology and physiology, thereby furnishing a favourable field for the study of the epigenetic processes that lead to these adaptations.

In this paper we illustrate the phenotypic effects of symbioses on the sponges with special regard to new data on the Mediterranean sponges *Petrosia ficiformis* and *Chondrosia reniformis*. We also discuss how novelties arising from symbioses in sponges and other organisms are consistent with evolutionary mechanisms which take in account the inheritance of acquired characters (Landman, 1991, 1993) and the epigenetic inheritance systems (Jablonka and Lamb, 1995).

2. Endosymbiosis in Sponges

There is circumstantial evidence that some prokaryotes established symbiosis in ancestral sponges, probably during the late Precambrian (Wilkinson, 1983a). A recent and attractive suggestion is that sponges are the outcome of the symbiosis of colonies of choanoflagellates with bacteria and that this symbiosis led to the formation of the mesohyl collagenous matrix which should be the basic structure of the metazoan phylum of Porifera. Another suggestion (Wilkinson, 1983a) on an ancient evolutionary role of sponge symbiosis is that the alternate dominance between corals and calcareous sponges (particularly Stromatoporoids and Sphinctozoans) in the Paleozoic and early Mesozoic reefs was due to the acquisition of photosynthetic symbionts of increasing efficiency.

At present, symbiosis with bacteria and protoctists occurs in the large majority of marine and fresh-water Demospongiae as well as in Calcarea and Hyalospongiae. Different types of symbionts, often in large populations, have been found in sponges. Heterotrophic bacteria (Bertrand and Vacelet, 1971) and cyanobacteria (Sarà, 1971) are very common in marine sponges, zoochlorellae are important for fresh-water spongillids (Trigt, 1919), zooxanthellae in marine boring clionids (Sarà and Liaci, 1964a), and diatoms in Antarctic sponges (Gaino et al., 1994). Sponges can also host fungi (Roth et al., 1962). Different types of symbionts may occur in the same sponge. Bacterial symbionts

may be located in the mesohyl matrix or in symbiosomes inside specialized sponge cells (bacteriocytes). It is currently held that a vertical transmission of sponge bacteria occurs between generations in the sponge-bacteria symbiosis. Indeed, Levi and Levi (1976) described the transmission of bacteria from the follicular bacteriocytes enveloping the egg of *Chondrosia reniformis* into the ensuing larvae and young sponges after metamorphosis. In some cases (Gaino et al., 1987) phagocytosis and occurrence of bacteria in the sponge eggs have been observed, but their role and survival after phagocytosis is still unclear.

Symbiotic associations are regarded as a very important source for sponge metabolism, especially when represented by autotrophic symbionts. In fact, the transfer of significant amounts of photosynthate, in particular glycerol, from the symbionts to their hosts is a well known process (Wilkinson, 1979, 1980, 1983; Arillo et al., 1993), which allows symbiotic sponges to occupy oligotrophic habitats (Wilkinson, 1987). Cyanobacteria can contribute to the sponge feeding through metabolite transfer which can also follow their fragmentation: *Aphanocapsa feldmanni* and *A. raspaigellae* are subjected to a massive process of disintegration in the sponge tissue, producing a large amount of dispersed algal substance in the form of droplets and of minute fragments (Sarà, 1971). They contribute to the sponge pigmentation and to the production of secondary metabolites, as defense substances. The zoochlorellae contribute to the feeding and gemmule production of fresh-water sponges. The presence of zooxanthellae in clionids seems related to the boring activities of these sponges (Hill, 1996). As far as bacteria are concerned electron microscopical studies gave evidence that many morphologically and physiologically different bacteria occur in the mesohyl and in cells of marine sponges. Difficulties arise to state which bacteria are true symbionts of the sponge and which come as a diet from the surrounding environment.

Cyanobacteria are particularly abundant in the Indo-Pacific tropical sponges, thus affecting their feeding physiology. Sponges as *Phyllospongia* show characteristic shape adaptations for light uptake. Because of the biomass of symbiotic cyanobacteria which can sometimes approach that of the sponge cells, several Indo-Pacific coral reef sponges may behave as phototrophic organisms, deriving at least 50% of their energy from their photosynthetic symbionts (Wilkinson, 1983).

3. Sponge Adaptations to Cyanobacterial Symbiosis: The Case of *Petrosia ficiformis*

Cyanobacteria are usually present in temperate shallow-water sponges. In the body parts exposed to the light, these microorganisms confer a colour that shifts from dark brown to violet and pink in relation to the density of the

cyanobacterial population. One example is given by *Petrosia ficiformis* which shows some conspicuous morphological and physiological changes in relation to the cyanobacterial symbiosis. This species represents a useful model for the study of sponge adaptation to the cyanobacterial symbiosis because it may live both in full light as an intensely pigmented symbiotic form and in complete darkness as a white aposymbiotic form. Between these two extreme conditions there is a series of different morphs adapted to intermediate light conditions.

In addition to cyanobacteria of the species *Aphanocapsa feldmanni* (Feldmann, 1933; Sarà and Liaci, 1964 b) the microflora of *Petrosia ficiformis* includes also different types of other bacteria (Vacelet and Donadey, 1977). The microorganisms live inside vacuoles, true symbiosomes, of specialized bacteriocytes. The vacuoles, which are often interconnected, contain cyanobacteria or other bacterial types, depending on the light conditions. Superficial bacteriocytes of light exposed animals have only cyanobacteria, bacteriocytes of white cave-living animals and of deeper parts of the choanosome contain other bacteria. Mixed populations can be seen in the same cell in intermediate situations. Some bacteriocytes have outward-opening vacuoles, with bacteria apparently passing in or out of the vacuole (Vacelet and Donadey, 1977). Bigliardi et al. (1993) showed that the large vacuoles occupying most of the cell contained several distinct symbionts randomly distributed. Freeze-fracture replicas confirmed that the symbionts were contained in large membrane-bound vacuoles lined by a thin layer of cytoplasm and that sometimes a close association between the vacuole membrane and the symbiont envelope was evident. According to Bigliardi et al. (1993) these features, similar to those existing in insect bacteriocytes, may be interpreted as the morphological basis of specific interactions between the eukaryotic and prokaryotic cells involved in endocytobiosis.

In *Petrosia ficiformis* cyanobacteria are located in a superficial layer of the sponge called the symbiocortex. Light-exposed specimens show dense cyanobacteria populations and a tabulate shape. Light-sheltered specimens have a lower symbiont concentration and the sponge becomes cylindrical in shape. These changes affect the surface/volume ratio of the sponge whose values increase when chlorophyll concentration decreases. This allows sponges to maintain a constant quantitative relationship between its biomass and the photosynthetic symbiont population (Bavestrello et al., in press). The recent acquisition that opaline sponge spicules may act as optical fibers channeling light inside the sponge body (Cattaneo-Vietti et al., 1996) confirmed previous observations on the penetration of *Ostreobium* green algae along the spicular sponge fibers into the internal part of the body of the sponge *Tethya seychellensis* (Gaino and Sarà, 1994). Recent observations on the ectosomal

skeleton of *P. ficiformis* showed that its arrangement reflects sponge light exposure (Bavestrello et al., in press). Indeed, in the large and tabular specimens living on the exposed cliff the ectosomal skeleton is composed of a network of large monaxonic spicules, located tangentially to the surface. Inhalant pores are very rare. In the cylindrical, anastomized, creeping specimens, with reduced cyanobacteria populations, a dense coat of vertical spicules is overlapped on the tangential spicular network. This arrangement could be tentatively interpreted as a light channeling system, improving light transfer in semi-dark habitats. In these specimens the number of pores increases considerably, suggesting a more important role of the pumping system to support the feeding request. The specimens living in darkness are small and rounded. They do not show the coat of vertical spicules and the percent of surface covered by inhalant pores increases (Fig. 1).

As a consequence, *P. ficiformis* shows striking changes in its morphology due to the cyanobacteria. These changes, clearly adaptive, regard the size (much larger in the light exposed than in the dark cave specimens), the shape, the surface skeleton and the density of pores.

Metabolic activity also shows different adaptations to the symbiotic and aposymbiotic conditions. The cyanobacterial symbionts release fixed carbon in the form of glycerol and organic phosphate to the sponge tissue (Wilkinson, 1979). Arillo et al. (1993) suggested that the reducing equivalent transfer from the autotrophic symbiont to the heterotrophic host occurs through a mechanism similar to the glycerol 3-phosphate shuttle functioning between chloroplasts and cytoplasm in plant cells (Heber, 1974). This mechanism appears to be a biochemical adaptation that arose in the ancestral endosymbiotic system between plastids and the eukaryotic cell. In dark conditions *P. ficiformis* reacts to the absence of cyanobacteria by activating metabolic pathways able to maintain the cell reducing power. Whereas, *Chondrilla nucula*, a sponge harbouring cyanobacteria and living only in illuminated habitats, undergoes morphological changes (Gaino et al., 1977) and metabolic collapse (Arillo et al., 1993) when transplanted in dark caves. The metabolism of *P. ficiformis* when cyanobacteria are lacking is exclusively linked to the pumping activity. It is interesting to note that the catabolites released (as $\mu\text{g}/\text{l}$ of NH_3) are lower in symbiotic specimens, thus supporting the hypothesis of a nitrogen utilization by the cyanobacteria populations (unpublished data).

Another biochemical difference between the symbiotic and aposymbiotic morphs of *P. ficiformis* regards the secondary metabolites: the two morphs show different terminal units on one side of the molecule of the corresponding carbinols (Cimino et al., 1980).

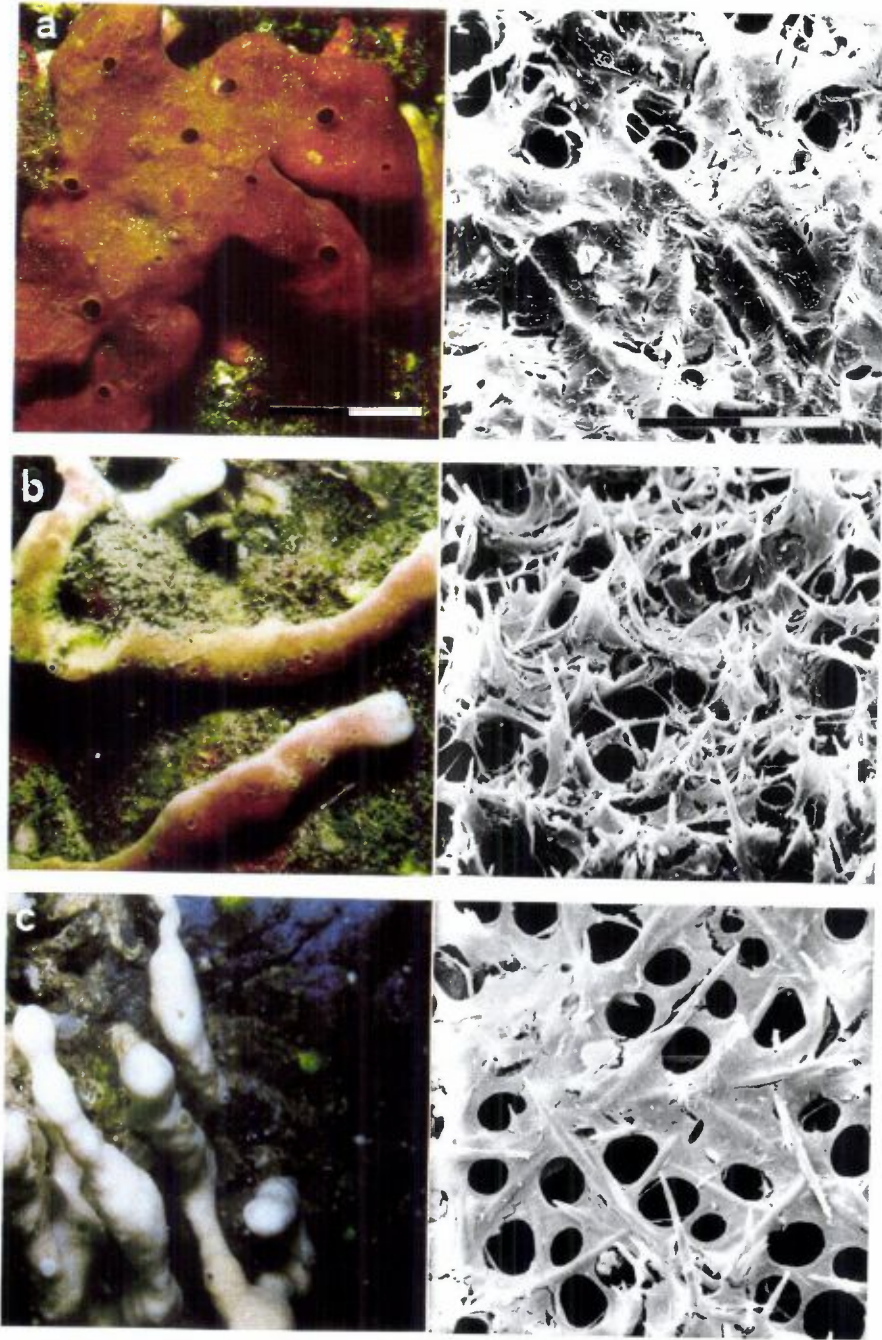


Figure 1. *Petrosia ficiformis* phenotypic variations of the general aspect (left) and of the ectosome structure (right) of three specimens living on a lighted cliff (a), in a semidark (b) and dark cave (c). Scale bars: left, 1 cm; right, 50 μm .

4. Sponge Adaptation to Heterotrophic Bacteria Symbiosis: The Case of *Chondrosia reniformis*

Symbiotic bacteria, living in the sponge mesohyl and cells, belong to different categories, but their function is often far from being understood (Wilkinson et al., 1981). Different roles have been hypothesized, such as digestion of material not available to the host sponge (Vacelet, 1975; Wilkinson, 1978), direct incorporation of dissolved organic matter from the seawater (Wilkinson and Garrone, 1980) and digestion and recycling of insoluble sponge collagen (Wilkinson et al., 1979). Wilkinson et al. (1981) suggested that the high production by bacteria of mucous matter is involved in the sponge structural rigidity. Recently, a category of nitrifying bacteria has been identified (Diaz, 1996) and Vacelet et al. (1996) described a symbiosis between methane-oxidizing bacteria and a deep-sea carnivorous cladorhizid sponge.

The sponge *Chondrosia reniformis* shows a rich population of symbiotic bacteria (Levi and Levi, 1976). This sponge lacks an autochthonous spicular skeleton but may incorporate foreign material to strengthen its collagenous ectosome. The sponge ectosome is covered by mucus affecting adhesion, translocation and incorporation of particles (Bavestrello et al., 1996).

The particle translocation and uptake is quickly stopped when to the experimental medium antibiotics, as erythromycin and chloramphenicol are added (unpublished data). This feature indicates a possible involvement of bacteria in the complex mechanism of the incorporation of minerals by the sponge, thus stressing its physiological and behavioral integration with the prokaryotic symbionts.

Bacteria play a remarkable role also in the sponge behavior and reorganization. Indeed, the behavior of *C. reniformis* is intimately linked with its collagenous tissue. The sponge can move on the substratum by changing the consistency of the body. We may speculate that bacteria intervene with the system signaling for changes in the collagenous matrix structure.

5. Discussion

Sponge endosymbiosis, especially that with cyanobacteria which led to a "secondary photosynthesis" in sponges, stresses the role of a coupled epigenetic-genetic integration in the symbiosis evolution (Fig. 2).

Symbiotic interactions, in sponges as well as in other host organisms, involve: i) recognition and regulation of the microbial symbionts; ii) morpho-functional changes. Both require epigenetic mechanisms with a cross-talk between the endosymbiont and the host affecting gene expression through

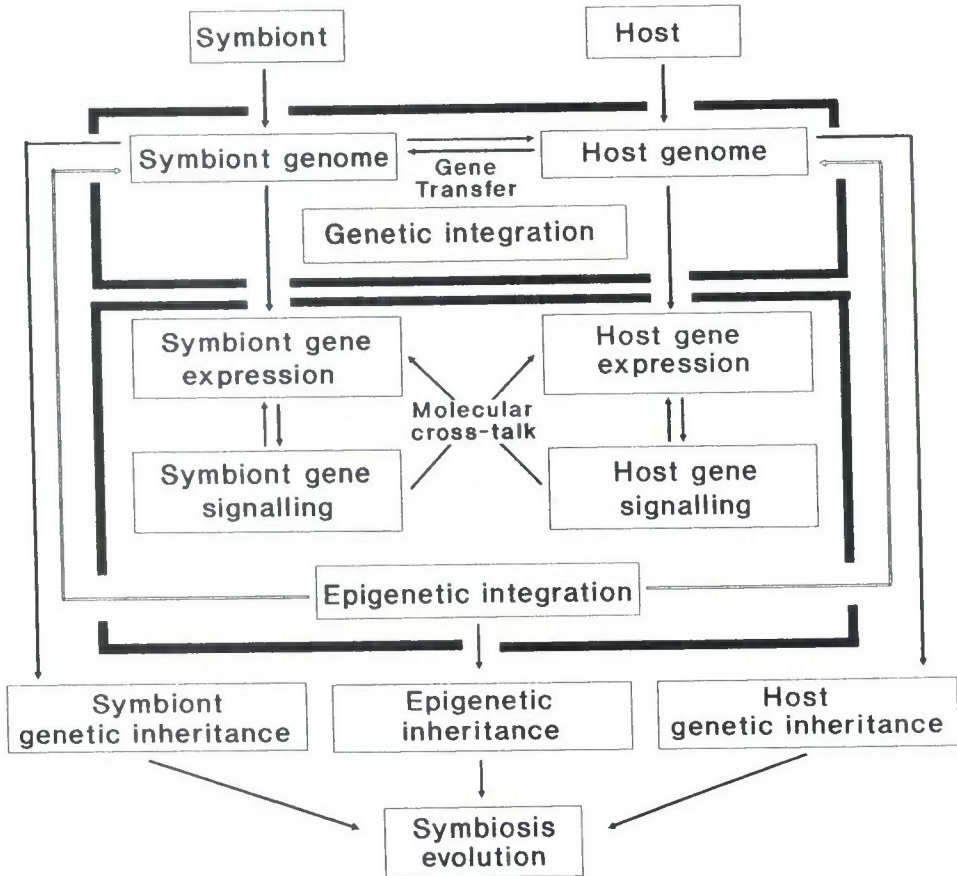


Figure 2. Schematic view of genetic and epigenetic processes in the evolution of endosymbiosis (syntrophogenesis). Empty arrows (from epigenetic integration to symbiont and host genomes) represent the genetic assimilation hypothesis. Other explanations in the text.

activation and inhibition of specific genes. The epigenetic integration is linked to a genetic integration due to the coexistence in the same host cell and/or organism of the endosymbiont and host genomes. Gene transfer between the genomes can enhance the genetic integration producing in turn other epigenetic effects (Fig. 2).

The existence of an epigenetic host/endosymbiont integration is crucial since epigenetic inheritance mechanisms, linked to the "sensitive" system of receptors and transducers (Sarà, 1996), are often carriers of environmental information. In symbiosis this information is represented by the reciprocal

signal exchange between the endosymbiont and the host, which influences their coadaptation and coevolution. Environmental and genetic information interact epigenetically allowing morphological, physiological and behavioral novelties to arise (Sarà, 1993). The ability of the sponge *P. ficiformis* to elicit, when symbiotic, new metabolic pathways, along with morphological adaptations to photosynthesis, is an example of the evolutionary relevance of these epigenetic-genetic mechanisms.

The new physiological and morphological patterns can be firmly established only progressively in the course of generations. At each generation the effects of the epigenetic processes on the phenotype are controlled and optimized, through the work of natural selection. A two-step gradualistic evolutionary model, with the two alternate but interacting epigenetic and selective phases (Sarà, 1993), fits well with the evolutionary changes in endosymbiotic associations. According to such a model, selection acts essentially as a filter while the set-up of the phenotype is entrusted into epigenetic processes through auto-organization and constraints. We may speculate that symbiosis evolution could be accelerated through the genetic assimilation mechanisms, which was demonstrated by Waddington's (1959) experiments on *Drosophila*. These experiments show that morphological changes, induced by some environmental stress and initially not inherited, can be inherited if these flies are submitted to the same stress for many generations.

According to Williamson (1996) gene transfer between lineages due to both symbiosis and hybridization can result in evolutionary saltations. Yet gene transfer between the symbionts may be restricted or absent and symbiont integration may occur through a complex molecular cross-talk. As a consequence, symbiosis evolution requires also the gradualistic epigenetic selective mechanism.

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