
ENDOSYMBIOSIS
AND SELF-ORGANIZATION

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INTRODUCTION

In biology, the full range of mutualistic phenomena is drawing renewed interest. Their role in the functioning of organisms and ecosystems, and in the evolution of living beings is increasingly better known and recognized, despite some risks of idealization inherent to images conveyed by the concepts of symbiosis, mutualism and commensalism. In this paper we will consider the closest and most intense of all the associative phenomena, endosymbiosis. Thus, in the first part, we will examine the meaning of endosymbiosis as a biological phenomenon.

Endosymbiosis is an association of two partners belonging to different species, in which one of the partners lives inside the other. The microorganisms forming the intestinal flora of vertebrates provide one example. However, we will limit ourselves to the case of endocytosymbiosis in which one of the partners is a microorganism living inside one of the host's cells, the closest form of interspecific association; it is also called intracellular endosymbiosis. The mode of approach and the progress in molecular biology allow us to reflect, more particularly, upon the meaning of the new functional unit consisting of the respective genomes of the host and symbiont. What creates this new unit? This question forms the framework of the second part. We will start with the existence of reciprocal actions between the two partners (for example, the immune-defensive response to the invasion of the symbiont by the host and the production

of stress proteins by the symbiont, or the complementarity of the two genomes in the host's nutrition.) Whether that basis corresponds to the level of biochemical mechanisms or to that of physiological mechanisms in the host-symbiont relationship, our hypothesis will be that such interactions first occur at the genetic level, this despite the limited contemporary scientific data on the matter. Biological data seem to indicate that evolution through endocytosymbiosis leads to a coadaptation of genomic potentials in a new unit subject to selection. The new cellular organization would emerge from that co-adaptation. All this raises questions as to the existence of a self-organization process in endocytosymbiosis. After having established the relation of the above issues to the overall problem of self-organization, we will attempt to validate a model of genomic interactions between the two partners involved in intracellular endosymbiosis.

I. EPISTEMOLOGICAL ANALYSIS OF THE CONCEPT OF SYMBIOSIS

A) SYMBIOSIS AND MUTUALISM

From the standpoint of its meaning, symbiosis first appears as a particular case of mutualistic systems. Indeed, one speaks of mutualism when several species draw a mutual benefit from their presence in a given ecosystem. There are mutualisms in which plants and animals mutually benefit from the other's presence at a certain time in their life (as in pollination by insects seeking nectar). These forms of mutualism are not really symbioses, insofar as they require neither permanent association nor cohabitation. Symbiosis implies a greater proximity in the "living together" of two partners, the host and the symbiont. Since the concept of symbiosis was created around the expression "living together", there cannot be symbiosis without proximity and duration. It truly distinguishes itself from mutualism through the notion of common life, of permanent interaction of vital operations within a physical area defined by the host. The difficulty and ambiguity found in some authors come from the fact that they give symbiosis a wider definition, so as not to exclude the loosest forms while emphasizing the closest. We will see below that another distinction between symbiosis and mutualism focuses on the nature of interrelations. In symbiosis, interrelations do not necessarily benefit both partners. As long as "living together" is included in parasitism, i.e. as long as the symbiont does not kill its host, parasitism can be considered an extreme case of symbiosis.

As to different sorts of mutualism, Addicott (1984), like many authors, clearly integrates "cost/benefit" calculations ¹. From this viewpoint, an appreciation of their diversity must be taken into account in the understanding of mutualistic interactions. This author, however, recognizes that the benefit may tend to be unidirectional (a phenomenon we will also

find in endosymbioses). In the same way, Axelrod and Hamilton (1981) point to both cooperation and exploitation as sources of benefits in mutualistic systems². There is then a sort of game (the famous prisoner's dilemma) in which cooperative and non-cooperative strategies toward the other one alternate. "The problem is that while an individual can benefit from mutual cooperation, each one can also do even better by exploiting the cooperative efforts of others³." The fact that the behavior of the two partners is never certain explains the complexity in modeling. In the area of symbiosis, Axelrod and Hamilton cite the example of *Rhizobium* strains. Some live free in the soil and others live in the nodules of legumes, which they supply with the nitrogen they fixed. The authors continue: "In the light of theory to follow, it would be interesting to know whether these parasitized legumes are perhaps less beneficial to free living *Rhizobium* in the surrounding soil than those in which the full symbiosis is established⁴." Furthermore, if the establishment of a cooperation based on reciprocity is not evident, where it does exist there is a strong probability that it will perpetuate itself. Axelrod and Dion (1988) write: "Once cooperation based upon reciprocity is established, no player can do any better than to cooperate as well, provided the chance for future interaction is high enough⁵." Endosymbiosis then would be, from the viewpoint of cooperation, a phenomenon which, *a priori*, could lead to this type of modeling (cf. I, d).

B) FROM MUTUALISM TO INTERDEPENDENCE

Lynn Margulis attempted to redefine symbiosis at the International Conference of Bellagio in 1989 by asserting her will to go back to De Bary's original intent, i.e., to a definition of the phenomenon under study that would be the most extensive possible but also the most specific. This is why she considers that symbiosis concerns "a set of ecological interactions between non-human organisms" and that it implies an association in the sense of physical proximity between organisms of different species, as well as a significant, prolonged time span in the history of life⁶. Such a definition seems cleared of all mutualistic connotations and refers to an ecosystemic approach. Further, symbiosis may include a more or less intense integration at the metabolic, genetic and behavioral levels. However, this does not mean that it lacks precision. It should be noted that the author gives great importance to the analysis of the biological phenomenon based on the elements of the definition⁷. She writes: "Symbiosis analysis elucidates the following: (1) chemical and behavior recognition of organisms of different species, (2) initial contacts between prospective symbiotic partners, (3) selection pressures leading to the establishment and disestablishment of associations, and (4) genetic, metabolic and behavioral aspects of partnership integration." The last point concerns

interactions between the host and its symbiont, when symbiosis is established. Until about 1990, these interactions were mainly seen at the levels of nutrition and reproduction, in cellular biology, biochemistry and physiology. Since 1990, the genetic and molecular aspects of the symbiont life began to be better known, although the functional area of molecular interactions in the symbiont DNA as well as between the host DNA and the symbiont DNA, is still almost entirely unexplored.

Margulis (1991) seems to consider symbiosis primarily as a dynamic phenomenon taking place in a specific time frame and susceptible to evolve by creating a new unit of integration, more complex in structure and in function. This corresponds to what Nardon (1995) calls "symbiocosm" and can be described as: the symbiocosm, resulting from the association of the two partners, is therefore a new biological entity in evolution, subjected to natural selection and at this level in line with the theories of Neodarwinism⁸. Corning (1995) also establishes a link between the alleged role of endosymbiosis in biological evolution and in complexity⁹. However, he has a tendency to simplify the meaning of symbiosis phenomena. On the one hand, he considers them as being fundamentally, in evolutionary history, some fusion of cells "with complementary functional specializations," leading to more complex cells. Yet, in the establishment of an endosymbiosis, each partner first tries to protect its autonomy and maintain its structure. Far from merging with the host, the endocytosymbiont must control its defensive reactions¹⁰. On the other hand, for Corning, symbiosis is only the expression of a synergetic principle, which is far from being certain, as we will see again later. Corning sees symbiosis within the framework of integration, considering that, along with differentiation, it is one of the mechanisms of increase complexity. Whether symbiosis is tied to notions of mutualism and synergy (Corning) or is unconnected to them (Margulis), seeking the link between the phenomenon itself and the resulting integrated structure cannot be avoided.

Scott (1969) and Smith (1990) also tried to determine the nature of the symbiotic relationship. Scott (1969) thinks that symbiotic association is "a permanent feature of the life cycle of organisms¹¹." His definition replaces direct physical contact with the notion of physiological interdependence. This paves the way for D.C. Smith (1990) for whom symbiosis is a state of balanced physiological interdependence between two or more organisms, without involving a permanent stimulation of defensive reaction mechanisms¹². This implies that defensive reactions still exist potentially as, for example, in the hosts of symbiotic bacteria. Symbiosis consists of an association of two or more organisms living together in a state of mutual interdependence. It is interesting to note that this formulation synthesizes the "living together," which corresponds to observations and is the basis

for De Bary's initial approach, and mutual interdependence, a concept which clears the collective imaginary of the idealized notions of mutual assistance and synergy. Recourse to the concept of mutual benefit must be avoided, states Smith, because it is very difficult to evaluate the reality of a profit for the two partners. In fact, the cost of the nutrition of the host by the symbionts is generally not estimated and neither is the cost for the host of supporting symbionts¹³. At the present time, some studies in molecular biology (see Lai *et al.*, 1994) begin to mention a slowing down in the growth of symbionts because of the alteration of the host genome and the genetic control exercised by the host (absence of a regulating structure, selective induction of certain genes by the host...) ¹⁴. On the other hand, if the host's fitness is favored (in the case of *Sitophilus oryzae*, the doubling of fertility and the acceleration of individual development greatly influence population dynamics), the host itself tends to become dependent on symbiosis¹⁵. Smith (1990) declares that coelenterates suffer an increased death rate in the absence of their symbiotic algae¹⁶.

Smith (1990) views the existence and physiological organization of the two partners as interdependent. On the nutrition level, he considers the beneficial effect produced by the interrelation as unilateral. For him, the host-symbiont interrelation results in a form of exploitation. This point of view is upheld by Nardon *et al.* (1997) at the metabolic as well as genetic levels¹⁷. They write: "In integrated symbioses, as in the *Sitophilus oryzae*, the symbiont has lost its autonomy and the host paradoxically appears as a parasite of its symbionts. The host exploits the symbionts on the metabolic level and controls their localization and density¹⁸." In another area, Charles *et al.* (1997) have shown that the symbiont's genome is reduced by about 36 per cent as compared to the *Escherichia coli*'s genome¹⁹. This reduction would be due to a series of deletions in the symbiont's genome and of gene transfers to the host during co-evolution. Nardon *et al.* (1997) conclude, "If this hypothesis of gene transfer is accepted, symbiosis could be interpreted as a sophisticated mechanism of genic predation" (which would not be inconsequential in the matter of intergenomic interactions in the organization of the new living entity²⁰). Despite our emphasis on the situation of dependence affecting the symbiont, it must be remembered that the host exerts a protective action on symbiotic populations. Marine symbioses are a good example. The polyp *Zoanthus australiae* protects the symbiont against light and the destructive action of salinity²¹. Furthermore, the host also nourishes the symbiont. The cost of this is difficult to assess. Finally, Jeon (1995) showed that, in the particular case of endosymbiosis of bacterium X in amoebae, the symbiont induced the host's dependence toward it²². Therefore, there would really be mutual dependence for survival.

C) INTERDEPENDENCE OF THE PARTNERS
AND UNIT OF EVOLUTION

Corning (1996) treating the subject of cooperative interactions in general, makes reference to the “dependence paradox”, according to which the more the benefits of cooperation increase, the more the parts become dependent on the whole²³. Contrary to Smith, Corning mostly refers to the dependence on the new unit of selection created by the symbiosis. But he takes as basis cooperative interactions producing synergetic effects, which would be advantageous for all partners in the symbiosis at the selection level—a hypothesis not necessarily demonstrated. Blackstone (1995) refers to “the units of evolution framework”, which is particularly useful for assessing the evolutionary implications of the endosymbiotic theory²⁴. This new living unit can harbor antagonistic as well as synergetic relationships; it is this unit that is subject to the pressure of selection. Corning (1996) also states that natural selection allows us to characterize a causal dynamics that involves changes in some functional interactions within an organism²⁵. In symbiosis, the unit of survival may become obligatory, “a decrement in the performance of the whole might result in the demise of the parts.” Jeon (1972, 1983) infected a strain of *Amoebae proteus* with parasitic bacteria²⁶. In ten years, the partners developed complete interdependence and could no longer live separately; although in many cases, it is not possible to apply, as Corning does, the obligatory character of symbiosis to the new unit. Indeed, sometimes symbiosis is obligatory for the symbiont and not for the host. Many strains of coleoptera endosymbionts could never be cultivated in an artificial medium without the influence of the host, while exist aposymbiotic strains of *Sitophilus oryzae* even if their fertility is lower and their development time longer²⁷.

Considering the particular case of an intracellular endosymbiosis (part of the overall case of endocytosymbiosis), we noted in the *Sitophilus oryzae* the complexity of organization and metabolic and genetic interrelations favoring the host²⁸. This approach to endosymbiosis seems close to that of Buchner (1953), despite the difference in scientific data and techniques used²⁹. In a general presentation of mutualistic relationships and symbiosis in plants, titled *War and Peace in the Plant Kingdom*, Boullard (1990) follows up on Buchner’s approach (1953)³⁰. Boullard includes endosymbiosis in the concept of close symbiosis based on Buchner’s definition (1953) which he quotes as, “Regulated and apparently trouble-free cohabitation, between two partners of different species, one being included in the other’s body (usually at a higher level of organization) and whose mutual adaptation has reached such a degree of intimacy that it justifies the hypothesis of a beneficial rearrangement for the host³¹.” Such a definition allows the retention of physiological interdependence as the source

of multiple relationships of mutual adaptation between the two partners, as well as of the constitution of a new unit of selection, while emphasizing the utilization and domestication of the symbiont by the host. At the end of this analysis, the issue of the real nature and limits of intracellular endosymbiosis reappears. Theoretically, one could argue about the connection of parasitism to symbiosis. Nevertheless, there are cases of very integrated symbiosis in which the host paradoxically behaves as a parasite of the symbiont. It seems difficult, then, to draw the line! If we examine the interrelations between the two partners in an endosymbiosis, the problem of equivalence between endosymbiosis and an ecosystem may also be discussed. The issue is of great importance for modeling interactions between the two individuals of two distinct species. The point is to know whether endosymbiosis may be treated as an ecosystem of two co-evolving species, as far as genetic regulation is concerned. For Schwemmler and Gassner (1989), "an ecosystem is generally understood to be a well-defined area (biotope) containing different kinds of organisms which form an interdependent community (biocenosis) ³²."

D) FROM ENDOSYMBIOSIS TO SELF-ORGANIZATION

We are facing several approaches toward intra-cellular endosymbiosis. Firstly, we share the point of view adopted by L. Margulis (1991) as well as Schwemmler and Gassner (1989), i.e., a group of ecosystemic interactions in which endosymbiotic interactions conform to very precise criteria: a significant *association in time and space*, a "*living-together*", in accordance with De Bary's early intuition (1879) ³³. The symbiont lives inside and with the host: therefore we think that both entities are *biologically interdependent* insofar as the most fundamental elements of the definition are not sufficient to imply a notion of mutual benefit. Furthermore, the benefit received by one of the partners seems to be difficult to measure and is constantly changing ³⁴. Secondly, a new unit is formed from complementary entities. This new unit is complex, orderly, hierarchical and subjected to selection. However, progress in experimental knowledge allows us to speak either of two co-evolving entities or one unit integrating two sub-systems. Thirdly, the matter of understanding endosymbiosis as a complex system is addressed. According to Corning (1995), the issue of complexity covers the relationship between the parts and the whole ³⁵. This relationship refers back to numerous "subtle forms of interdependency" provided by experimental results.

Let's return to the issue of co-evolution between the two entities. De Bary's definition, which considers symbiosis as an association, presupposes the distinction between these two entities. For Schwemmler and Gassner (1989), the associative proximity achieved in endosymbiosis implies a mutual dependence between individual units of the intracellular

ecosystem³⁶. This mutual dependence does not require a complete functional specialization of each entity, but it does imply that normal biological functioning is not possible in the absence of the partner. In such model, the symbiont would evolve into a particular organelle within the whole cell; thus, the intracellular ecosystem allows a more accurate definition of the nature in interactions between entities. This is not the case with models of co-evolution in ecosystems based on game theory. Indeed, game theory models a cooperation between species supposing that each one acts in turn and can mutate on a gene (Kauffman 1993) in order to increase its own fitness and thus its adaptation, depending on the partner³⁷. Yet, in the case of endosymbiosis, the host's control over the symbiont does not permit such autonomy. Furthermore, symbiosis involving physical associative proximity between two specific species is more intense in the area of interdependence than all of the interrelations taking place inside an entire ecological community formed of very diverse species. In view of the often-dominant position of the host, as in the case of *S. oryzae*, this would mean a particular type of ecosystem in which interactions between the two partners are not necessarily reciprocal. Likewise, the progressive integration of the endosymbiont inside the host creates difficulties in evaluating the fitness of the symbiont's species and renders unrealistic suppositions linked to its autonomy. From the standpoint of the model, treating endosymbiosis as one system of co-evolution among others risks being illusive.

The above considerations prompt questions about the specific nature of the endosymbiosis phenomenon. Raising the hypothesis of interactions that may involve cooperation as much as competition, amounts to considering the state of the organism as a consequence of a dynamic process. Brian C. Goodwin (1993) writes that adaptation means no more and no less than the stability of a life strategy within a dynamic process³⁸. To speak of epistatic interactions is to locate these interactions beyond something static and stable (the genome), but also to make them relative to that stable state which can really exist only as a terminal phase. In the case at hand, interactions may result in a new state starting from a certain state of the two genomes. In such a process, "causality becomes immanent rather than contingent³⁹." Setting the organization of the living in the continuity of a "sequence of processes in a loop" instead of merely understanding its elements, is in fact to enter into the framework of self-organization⁴⁰. Self-organization, then, constitutes the living system within the interactions that continually regenerate the system occur, as well as being capable of broadening and transforming it⁴¹. However, it is true that self-organization and complexity must not remain just "buzzwords" issuing from a kind of intuition based on a body of scientific conclusions. As for any living system, the ultimate teleonomic justification of the

processes of cooperation and interdependence would come from their best aptitude at creating a thermodynamic stability (as wrote Prigogine in 1978 and 1980) expressed by the construction of a certain order ⁴².

II. INTERACTIONS BETWEEN HOST AND ENDOSYMBIONT. EXPLANATORY HYPOTHESES

A) KAUFFMAN'S POSITION

According to Kauffman, the starting point in reviewing Darwinian positions in biology is the existence of a spontaneous, non-accidental order. "Laws of complexity spontaneously generate much of the order of the natural world ⁴³". Such an assertion is supported by a certain number of biological facts—which can be cases-in-point for the verification of these laws: the existence of auto-catalytic molecular systems within the cell; activation and suppression circuits of the genes; the co-evolution of ecosystems ⁴⁴. Kauffman's whole purpose in *The Origins of Order* (1993), *At Home in the Universe* (1995) and *Investigations* (1996), is to begin to discover the laws of complexity which would explain the capacity of life to forge an order, thus reversing the natural slope of entropy. For Kauffman (1995), the words "self-organization", "order" and "emergence" are associated most of the time. Each is a part of the definition of the others. "Much self-organization may have made the emergence of life well-nigh inevitable ⁴⁵". "In this book, I propose that much of the order in organisms may not be the result of selection at all, but of the spontaneous order of self-organization ⁴⁶". One must therefore go further to define self-organization in biology, other than by the general terms of "emergence of life" or "spontaneous order," which are, in fact, the effects of self-organization. For Kauffman, self-organization is a property of networks. The genome could function as an information system—hence its possible modeling as a Boolean network. In 1993, Kauffman wrote : "In the genomic computer system, many genes and their products are active at the same time; hence, the system is a parallel-processing chemical computer of some kind. The different cell types of the developing embryo and its trajectory of development are, in some sense, expressions of the behavior of this complex genomic network ⁴⁷". The network of interactions between the various sites of the same genome could produce different cellular types within the embryo, through the intermediary of metabolic and physiological networks. Because of the lack of realism in the supposition of an independent contribution of each gene to the fitness of an organism, Kauffman (1993) establishes the hypothesis of intra-genomic interactions ⁴⁸. He thus considers that in a system of N genes, the contribution to the fitness of one allele of a gene depends on the alleles of the remaining $N-1$ genes. These dependence interrelations are epistatic interactions that we have already

defined (I-d) as conditioning the state of the genome. According to Kauffman (1993), although one knows nothing of the nature of the interactions and their complexity, one can nevertheless create a model of the network of epistatic interactions by attributing to the interactions random fitness values. This is the NK model, where N represents the number of genes and K the number of other genes in epistatic interaction with each gene. A characteristic property of the model, which we will address again (II-c), is that for $K=2$, the length and number of Boolean attractor cycles is \sqrt{N} . Kauffmann (1993) also established a model NKC , applicable to two species in co-evolution, where N represents the number of genes of each species, K the number of interactions per gene within each genome, and C the number of interactions of each gene with a gene of the other species⁴⁹. This model is based on the game theory and assumes that each species can attempt a random mutation in response to a mutation by the other species.

The main issue with the Boolean network model adopted by Kauffman to account for self-organization is the relationship between the qualitative and the quantitative. What is the validity of such a transition between, on the one hand, biological results accounting for organic operation including qualitative and quantitative aspects, and, on the other hand, a purely quantitative functional scheme? In other words, according to Corning (1995) do mathematical relationships reveal qualitative functional interactions within and between living systems⁵⁰? In view of the inability to answer this question of isomorphism between the model and reality, it appears appropriate to start with reality as translated by the experience and research of the biologist. In our case, it is a question of what we know of the interactions between the genome of the host and that of the symbiont, and above all, of the hypotheses that we can construct at this level based on acquired knowledge (of DNA structures, regulating mechanisms, identification of proteins...). Then, it is a matter of stating anew the appropriate question: To the extent that there is a selection of a new living functional organization through intracellular endosymbiosis, is such organization to be related to the whole of interactive phenomena, and how to validate these interactive phenomena at the genetic level? Whatever model is used, its effectiveness and validity can come only from the results obtained and from measurable characteristics of endosymbiosis in terms of system stability, the number of attractors (in the case of Boolean networks), the rate of growth of the symbiont, the length of time required to obtain the necessary interdependence, and so forth. In the case of the NKC model, Kauffman supposes that the number of interactions inside the genome of each species (K) and with the genome of the other species (C) is the same for each gene⁵¹. *A priori*, this appears to be an hypothesis which is never fully supported, and risks, therefore, to taint the results.

Kauffman rarely addresses symbiosis in his work. He does not mention it in *The Origins of Order* (1993), and briefly mentions the example of the evolution of endosymbiotic bacteria into mitochondria in *At Home in the Universe*. He speaks of the stability of the host and of the mitochondria genotypes in terms of "mutual consistency", and thus makes reference to a state of mutual equilibrium, analogous to the equilibrium of Nash⁵². In such a case, it would not be advantageous for a player to change as long as the other did not. This is the "defect-defect" strategy. But the author views the symbiont as autonomous at the genetic level and evokes the mutual advantage for both partners^{53,54}. His position in this study is not, therefore, sufficiently detailed, and he visibly prefers concentrating all his attention on the co-evolution of ecosystems.

B) EXPERIMENTAL ARGUMENTS AND HYPOTHESES OF INTERACTIONS

The first example will make clear the difficulty of the problem faced. It is the endosymbiosis between the sea anemone, *Anthopleura elegantissima* (Cnidaria) and the photosynthetic dinoflagellate, *Symbiodinium californium*. What is known in these marine endosymbioses is the carbon nutrition of the host by the photosynthetic symbiont. Veis and Levine (1996) clearly state that the molecular control and maintenance of these associations are still largely unknown⁵⁵. Yet, for these authors, it is a matter of starting this research program by attempting to describe the proteins specific to the symbiosis so as to subsequently achieve a better knowledge of the two genomes and their regulation. The results give two proteins specific to symbiotic animals and six proteins whose synthesis is highly increased among aposymbiotic animals. Nevertheless, the authors specify that the majority of proteins resemble each other (and about 60 per cent have exactly the same profile in the two strains). In that case, a model having the same interactions (intra-genomic or with the host) in all the genes of the symbiont, is obviously not applicable. The two specific proteins (including an enzyme required for photosynthesis) are probably encoded by the genome of the symbiont. They could be regulated by the genome of the host. For the six proteins having differential synthesis, the results suggest the action of regulation tributary of a product of the symbiont or a rearrangement of genes by transfer of genetic material to the host. The construction of a model with so small a number of known interactions does not appear realistic. We are faced here with the typical case where interactions appearing upon the implementation of the association have as yet never been described⁵⁶.

An example of endocytosymbiosis of significance in evolutionary history is the association of one cyanobacterium with one eukaryotic cell, being the cyanobacterium the supposed ancestor of chloroplasts. Sugita

et al. (1995) report the discovery of a ribosomal protein (regulating the translation stage), very close to proteins discovered in chloroplasts⁵⁷. The corresponding *rps1* gene has been localized, and may be dependent on a single promoter. One of the genes identified in the proximity of *rps1* could be interacting with *rps1*. The advantage of this type of study resides in the similarities of structure and expression between the gene of the cyanobacterium and the gene of the chloroplast. According to the authors, endosymbiosis and transformations of the genome have left about one third of the proteins encoded by the genome of the chloroplast. In terms of interaction, in addition to the transfer of genes, endosymbiosis could include the replacement of certain interactions internal to the genome of the symbiont by one or more of the interactions with the host genome. It would be necessary to test, with a small number of genes, the consequences of passage from $K=2$ to $K=1$, $C=1$. This would correspond to a control of the host cell DNA over the endosymbiont, for example—through the intermediary of a single promoter. McFadden *et al.* (1994a) discovered that the endosymbiont of the alga *Cryptomonas* produces its own transcription and translation apparatus, independent from the host's apparatus, even if its nucleus has been apparently reduced⁵⁸. This means that transfers of genes are far from being the general rule. The endosymbiont of *Cryptomonas* retains an autonomy that can qualify it as "cell within a cell"⁵⁹. This photosynthetic endosymbiont could also encode certain proteins for the host chloroplasts. Apparently, these results suggest the existence of unknown interrelations of activation or repression between two structurally autonomous genomes. The same research team (McFadden *et al.*, 1994b) emphasizes, on the contrary, that in amoeboid algae (coming from endosymbiosis of an alga in an amoeba), the nuclear genome contains vestiges of the endosymbiont genome, because the amoebae may use them for the structure and function of the chloroplast⁶⁰. However, the nucleus of the endosymbiont (which has evolved into a chloroplast) could keep the elements essential to self-replication and synthesis of its proteins. It would thus appear that the transfer of genes is a stage more or less prominent, as the case may be, in the evolution of the partnership and progressive transformation of the endosymbiont into a cellular organism. The last type of endosymbiosis, in which the host exploits the photosynthetic capacities of the symbiont, resembles so much a form of exploitation and assimilation of the partner, that the authors (1994b), wonder about the *raison d'être* of the endosymbiotic genome⁶¹.

Among insect endosymbionts, the molecular mechanisms of endosymbiosis are just beginning to be better known. The metabolism of amino acids of plant lice makes the association with bacterial endosymbionts obligatory (cf. Febvay *et al.*, 1995)⁶². Lai *et al.* have examined the gene amplification of the gene coding for the tryptophane in the symbiont

Buchnera aphidicola ⁶³. The protein DnaA has two fixation sites before the genes *trpE* and *trpG*. The authors do not specify if the protein acts as a repressor of transcription. On the other hand, they highlight in this endosymbiont the absence of regulation structures, which have been found in other organisms. One can conceive that two regulation sites are associated with each unit and that the protein in question expresses an interaction specific to the genome of the symbiont. This would correspond to the model $K=1$. But the authors say nothing of the initiation of the transcription (promoter). One finds the classic model of two inputs (one promoter, one operator) in other studies, but it is not possible to generalize. Charles and Nardon (1997) state that the model with two promoters appears to dominate among many endocytosymbionts ⁶⁴. According to this result, if we advance the hypothesis that the promoters, are tributaries of an induction by the host (at the present state of knowledge, it is pure conjecture), we could have, for example, $K=1$ or $K=2$ with $C=2$.

In the symbiont of the cereal weevil *Sitophilus Oryzae*, Charles and Nardon (1997) evoke the existence of two genes for the expression of stress proteins *hsp 10* and *hsp 60* (proteins produced in response to a thermal shock, or to the stress generated by symbiosis within the two partners): the operon *groE*, analogous to the one of the colibacillus and the encoder for these proteins, and the gene *rpoH* ⁶⁵. This gene encodes the subunit 32 of RNA polymerase, which attaches itself on the first promoter of *hsp 60*. If one adopts a scheme close to that of the colibacillus and in line with experimental results, one would have:

- for the gene *rpoH*, two promoters and two attachment sites of the protein DnaA, which is encoded by another gene of the symbiont and acts as a repressor of the transcription.
- for the operon *groE*, two promoters, of which one is activated by RNA polymerase with subunit 32, and the other by RNA polymerase with subunit 70.

The protein DnaK, encoded by another gene of the symbiont, regulates the translation of the RNA messenger. All this remains in the realm of the possible, to the extent that Charles et al. (1997) assert that the operon *groE* could be regulated by several promoters for the accumulation of stress proteins ⁶⁶. The same authors speak of selective induction in the condition of symbiosis. Charles and Nardon (1997) advance the hypothesis of the induction of the expression of the protein *hsp 60* by the host ⁶⁷. This hypothesis is in line with the fact that intra-cellular life can generate stress for endosymbiotic bacteria. The sum of these scientific conclusions and of the hypotheses that they allow, led us to the following system: the two promoters of the gene *rpoH* could be dependent on an induction by the host; the repressor proteins DnaA and DnaK express an intra-genomic interaction.

In the case of the endosymbiosis between the amoeba and the X bacterium, Jeon (1995) believes that the operon *groE* may also have two promoters⁶⁸. The second promoter would reflect an adaptation of the endosymbiotic bacterium for survival in a hostile cellular environment. However, research in endosymbiosis in the amoeba appears to lead to the conclusion that the host becomes dependent on the bacterium for its own survival. One may conclude that the interaction (whatever its nature) between the bacterium and its host functions in both directions.

C) EPISTEMOLOGICAL ANALYSIS OF THE MODEL

All these results tend to indicate that the symbiont genome models with ($C=1$ or $C=2$, $K=1$ or $K=2$) are not unrealistic and should be tested. It would be necessary however, to have indicators allowing validation of the hypothesis of the existence of these interactions in the experimental results of biologists. As we have stated, these indicators could be in the form of biological parameters evaluated by scientists and corresponding to the forecast values based on the hypotheses advanced for these interactions. It seems appropriate to us to select the parameters in the order of duration, i.e., length of time required for development or evolution toward establishment of interdependence. These parameters would validate the effectiveness of the system in the formation of a true unit, as well as of equilibrium between the two partners in the symbiosis.

Having defined an attractor as "a set of points or states in state space to which trajectories within some volume of state space converge asymptotically over time," Kauffman (1993) hypothesizes that cellular types are the attractors toward which genome activities converge⁶⁹. The biological foundation of this hypothesis resides in differentiation. This biological process puts into play different models of genetic activity with the same genes and arrives at different cellular types⁷⁰. If, in the case of an organism theoretically considered isolated, it appears relatively logical to view stable and interconnected cellular types as diverse expressions of the genome, what could be the attractors with regard to the genome of the symbiont? Should we look at the different endosymbiotic cells through the life cycle of the host or through the different types of the endosymbiont? If the cycles of attractor states of the network are cellular types, Kauffman has shown that their number can be predicted by the square root of the number of genes. According to this author, "in the human being, containing about 100 000 genes, one should expect to observe on the order of 317 different cellular types. However, the number of cellular types presently known in humans is 256⁷¹." Further, Kauffman himself acknowledges that the more complexity increases, the more accurately the "square root" function with $K=2$ can estimate the number of cellular types. Indeed, the conclusions of this author appear to show that the

number of cellular types is, above all, a linear function of genomic complexity, and that it can be well estimated by the “square root” function as one progresses toward higher-level organisms ⁷². The number of genes required to form cellular types in lower organisms may be overestimated (many non-transcribed sequences) and the author affirms that “in evolution, progressively more genes are required for each additional cell type ⁷³.” In other words, as one progresses toward more complex organisms, “more regulatory genes are needed to coordinate gene expression.” Thus, the whole of the genome is much more called-upon and it is logical that a power function of the number of genes can forecast the number of attractors. To state it differently: this question of cellular types is decidedly not a good criterion for our endosymbionts.

The issue of interactions and what they produce presupposes a minimum of reflection on the host-symbiont system as likely to create an order and a unit, which would be subject to the pressure of selection. The question “Does the unit of life created by endocytosymbiosis imply self-organization?” is all-the-more crucial as the problem of integration of a prokaryote in a eukaryotic cell amounts to the hypothesis of a qualitative jump creating a superior unit, capable of evolution, and with transformation of the prokaryote into an organelle of the eukaryotic cell. Interestingly, Blackstone (1995) thinks that the host-mitochondrion interaction occurred first between two units of evolution ⁷⁴. It is no less true that in this intercellular interaction, there is not only control of the mitochondrion by the cell, but also control of the cell by the mitochondrion. However, the mitochondrion is an excellent example of a situation where the genes of the nucleus regulate the transcription and replication of the DNA of this organelle... The very term, ‘unit of evolution’, and even more so the one of ‘unit of selection’, imply the Darwinian theory. Lewontin (1970) thought that in using an approach in terms of units of evolution for a whole set of organisms, the new classes of data would provide new grounds for demonstrating Darwin’s theory of evolution ⁷⁵. In fact, Darwinian postulates—variation and natural selection—apply to individualized organisms constituting specific units. The organism of a symbiotic individual is derived from an association of co-genomes and has acquired a new individual character. This new biological individuality is, in turn, subject to the selection-mutation equilibrium.

The only new element in endosymbiosis considered as a potential evolutionary mechanism is that a complex associative phenomenon functions upstream from the process of selection and gives birth to a new structure ⁷⁶. Darwinism does not reject this type of process. It is regarded as an evolutionary force subject to selection and providing an explanation of variation (between symbiotic and aposymbiotic individuals). It does not concern itself with providing a model for it, since it is not so which

determines changes in the species. The co-evolution of two partners in endosymbiosis involves the attenuation of reciprocally virulent mechanisms and the establishment of a state of equilibrium in the complementarity of functions. This occurs in spite of certain antagonisms, which manifest themselves in endocytosymbionts through the development of stress proteins. Kauffman (1995) explains this by the tendency of genotypes in co-evolution toward a stable, logical and optimal equilibrium⁷⁷. The strength of this type of reasoning is in the retention of natural selection and in coupling its functioning with self-organization. Kauffman recognizes the difficulty in the above:

In short, almost 140 years after Darwin's seminal book, we do not understand the powers and limitations of natural selection, we do not know what kind of complex systems can be assembled by an evolutionary process, and we do not even begin to understand how selection and self-organization work together to create the splendor of a summer afternoon in an alpine meadow flooded with flowers, insects, worms, soil, other animals, and humans making our worlds together⁷⁸.

From an epistemological viewpoint, this new approach invites us to reflect further on the nature of the new unit of selection. Sober (1985) has given much thought to the difficulties posed by the evolutionary forces within Darwinian theory⁷⁹. He proposes to transpose the idea of variation to a level of organization other than that of the individual organism⁸⁰. What interests us is, for instance, the variation between populations of aposymbiotic and symbiotic insects. This variation directs us to the potentialities absent in the insect but present in the symbiotic bacterium and contributed by it to the insect. This puts us in the framework of Sober's proposition to look at variations between the species. If we consider both species participating in the symbiosis as an object of selection, we are brought back to the concept of a unit of selection formed by association of co-genomes. MacLaurin (1998) writes:

The 'units of selection debate' concerns the ontology of systems undergoing natural selection (...) I think that the unit of selection is a functional kind called a replicator. A replicator is anything that is accurately reproduced over a large number of successive generations⁸¹.

This definition can apply once the new genome of the symbiotic individual has reached a state of functional equilibrium. Thus, it is a matter of analyzing the presence or absence of characters not as a result of a cause, but as a result of a transmission of information. The Markov chains or the Boolean networks can account for certain parameters of organic form resulting from genetic information and epistatic interactions⁸². This is what B. H. Weber writes about Kauffman's models: "The usefulness of Kauffman's models is that they are level independent and do not include

any causality beyond how elements of an ensemble can be related⁸³." The unit of selection thus formed by the host and symbiotic species, will be, first, a unit of replication, and thus of transmission of information to the next generation, and then a functioning and interrelations unit for the two associated co-genomes within the same generation.

In terms of the history of science, this new approach to the constitution of a unit subject to selection attempts a synthesis between the Darwinian trend and the older one of developmental biology which finds its sources among a number of 19th century French zoologists (Edwards and Perrier, among others). The developmental explanation is taken up by Sober (1985) who distinguishes it from the variational explanation⁸⁴. Feltz (1997) writes that "the developmental question (...) refers back to completely different mechanisms, which concern development modalities of the individual organism⁸⁵." These mechanisms, objects of research, are themselves subject to natural selection. Today, everything proceeds as if the models of self-organization were more effective than descriptions of the 19th century zoologists, a different method for understanding the endogenous causes accounting for an order in the domain of the living. Weber (1998) writes:

Kauffman states that his goal is to broaden the context of Darwinism by incorporating self-organization principles to evolutionary theory to reflect the fact that, while natural selection is always at work, it does not have to generate all the order of biological systems by itself. Implicit in this claim is the goal of achieving a synthesis of the Darwinian and developmentalist research traditions⁸⁶.

In fact, therein lies the ambitious nature of Kauffman's work. But if natural selection is always at work in the immediate environment of an organism, can one consider that self-organization will first furnish the structures upon which selection will then act? Is there not a still greater complexity in the constant interaction between the organism and its environment? Regarding this, an example which encompasses selection and organization in networks within the same process, is provided by Edelman (1992, 112)⁸⁷. This author believes that in the nervous system, selection acts upon the neuron networks. These networks present connections, which do not show a genetic character. One can thus speak of somatic selection in the building of these networks.

According to P. E. Griffiths (1996), the defenders of adaptationism frequently content themselves with the argument that no other research program has related the adaptive force of organisms to their environment⁸⁸. Reality, nevertheless, is not so simple and the controversy around adaptation can also be attributed to divergences in appraisals of the various evolutionary factors. The question of the existence of epistatic interactions

in the “host-symbiont” system and of an eventual induction by the host of certain mechanisms specific to the symbiont, is clearly a very delicate and little-known point. Griesemer (1998) seeks to define what can be qualified as epigenetic heredity⁸⁹. The word “epigenetic”, suggests a hereditary system logically and physically located above the genes (Griesemer, 1998). The author applies this idea to the existence of chromatin elements having a relation to heredity, but considers that this word is less adaptable to the type of intracellular regulation that we envisage—as the interactions and the symbiotic or mitochondrial DNAs are clearly dependent on the replication of nuclear DNA. Indeed, “epistatic” suggests a “dynamic” operation influencing the state of the “static” support, but totally dependent on it.

Kauffman (1993) admits having no idea of what the interactions between two loci may be, nor of the manner in which these interactions could influence the fitness of the individual⁹⁰. As far as he is concerned, essentially arbitrary interactions are possible. It is enough to define them as liaisons between the genes controlling the characters. This is a bit vague, and it illustrates once again that the only way to render the author’s statements plausible is to be found in the cohesion of his results with biological data. One of these matching points between results and data is the possibility of reaching a state of equilibrium, similar to the equilibrium of Nash, between two species which co-evolve within the ecosystem. With regard to endosymbiosis, the stability of that state of equilibrium would involve the establishment of a new association between the two genomes, with all that this implies (transfer of genes, interaction). This is measured in terms of simulations of the fitness values of the host. When these fitness values are stabilized, the equilibrium of the system is reached. It can also be said that this equilibrium supposes the establishment of attractor state cycles in the two partners. Kauffman (1996) declares that this equilibrium implies the compatibility of fitness peaks of the two partners and the impossibility of an invasion of the system by new variants⁹¹. However, as we stated at the end of the first part, there remains for each species in co-evolution the supposition of the capacity to mutate randomly on a character and in turn. Clearly, such a supposition appears to us as completely unrealistic in the case of endosymbiosis. We raise here the question of the possibility of adapting to this particular case a model conceived for ecosystems of autonomous species. The integration of one partner in the other and the genetic processes involved in this situation appear to us to completely distort the basic hypotheses of the model adopted by Kauffman (who, incidentally, speaks very little of symbiosis⁹²). Nevertheless, Stuart Kauffman (1996) sees the validity of this system for all types of co-evolution⁹³. He therefore supposes that there is a capacity within each agent, and between the two agents, to harmonize toward a self-organized

but critical state, that is, always evolving at the edge of chaos. An alteration in the structure of one of the partners can radically put in question the mutual adjustment process. Nevertheless, in endosymbiosis, it is the opposite that is seen: The alteration of the symbiont genome appears often to be the requirement for the establishment of a stable relationship.

From a stricter biological point of view, Von Sternberg (1996) elucidates the notion of "genomic shock." The genomic shock occurs when some environmental factors cause the genome to undergo stress⁹⁴. The stress which endosymbiosis involves can very well produce a genomic shock of this type. Von Sternberg believes that the genomic shock can imply a new dynamics of movement toward a new attractor. In our case, one could thus consider that endosymbiosis involves a genomic shock with reconstitution of a new genome, the product of the two associated co-genomes, and two new attractors which would be the bacteriome and the bacteriocyte (the two new cellular types). The same author considers genomic self-organization as a product of interactions between the molecular components or between subsystems, and believes that the constraints stemming from the ensemble of these interactions limit the "phylogenetic trajectory" of the genome and produce genetic alterations. Interactions, new constraints, genetic alterations, but also better adaptability—all this corresponds very well to endosymbiosis, which may then be seen as self-organization of a single genomic system.

D) VALIDATION ELEMENTS FOR THE SELF-ORGANIZATIONAL MODEL

In spite of all the caveats expressed with regard to the applicability of the Kauffman model, it is interesting to test the hypothetical functioning of a Boolean network in the case of endosymbiosis. Our starting point will be the existing data for the cereal weevil, *Sitophilus oryzae* and for amoebae.

We have emphasized the difficulty of applying the notion of cellular type in the case of the endocytobiont. Nardon and Grenier (1990) cannot claim to have cultivated endocytosymbionts⁹⁵. Their numerous attempts resulted, in the best of cases, in obtaining strains of bacteria apparently resembling endocytosymbionts, but without any proof as to their identity. A conclusion is drawn that endosymbiotic bacteria in coleoptera, even if they had lived freely before symbiosis, have become entirely dependent on their host. One cannot, therefore, speak *a priori* of the existence of two cellular types (normal and sporulated) as is the case for free-living bacteria. Tiivel (1990) states that there are habitually different types of endocytosymbionts in the insect—some two or three per species. As to associated cellular types in the *Sitophilus*, there are two: the bacteriomes and the bacteriocytes⁹⁶. With regard to time required to obtain a generation, the endosymbiotic bacterium takes much longer to reproduce itself. Accord-

ing to Nardon and Wicker (1981), the endosymbiont of the cereal weevil reproduces itself 7 times per month. Clearly, it would be good to find a model which would account for this slow-down⁹⁷. Kauffman (1993) established a stabilization of interactions model for $N=24$ genes, $C=1$ and $K=2$ ⁹⁸. One can wonder if it would not be more adequate to speak of a simulation. Indeed, modeling requires that all available data gathered through experiments and corresponding to the problem under study, be included in the model, be it a mathematical one or not. Anyhow, in the present case, we introduce data and constraints that are fictitious (although in line with the known biological phenomena). These data and constraints are tied to the hypothesis of the existence of interactions. Their reality is measurable only through comparison of the results they enable us to obtain with available biological data. Such an activity tallies more to a simulation than to a model⁹⁹.

In the case of the cereal weevil, one cannot evoke the establishment of a symbiotic relationship, since symbiont does not exist (or no longer exists) outside the host. If one does, however, use this model (with knowledge that Nardon, 1990, considers the symbiont itself to have at least 20 genes usable by the host), then one sees that at the most 700 symbiont generations are required to arrive at an equilibrium¹⁰⁰. This represents 8.33 years. However, some pairs of species can stabilize themselves relatively rapidly—after about 200 generations or, in our case, 2.4 years. Jeon (1983) succeeded in establishing a mutual relationship “host-symbiont” for amoebae in the space of 200 amoebae generations, or over 18 months¹⁰¹. At the end of the 18 months, Jeon (1995) emphasizes that it is the amoeba that is dependent on its symbiont. 8.5 years later, or about 1130 amoebae generations, the endosymbiosis reached a state of equilibrium¹⁰². Thus, we get the same order of magnitude between the simulation and the duration of the Jeon experiment. Regarding the supposed relation between cellular types and the number of genes, considering that it is the two or three cell types of the symbiont that interest us, it remains to choose between an estimate of the total number of symbiont genes, an estimate of the number of genes actually transcribed, or the number of genes useful to the host and in effective interaction with it. This varies greatly. The *E. coli* bacterium has between 2 000 and 3 000 structural genes. What can be said of the transcribed complexity? Kauffman (1991, 1993) considers that about 600 genes are involved in the bacterium regulation system¹⁰³. Given that the estimate of the number of attractor state cycles is much too high (24), the only explanation that can be advanced is that the number of genes involved in formation of bacterial cell types is smaller than believed and that the number of intra-genomic interactions (K) may be greater than two. This would allow, according to the model, to get closer to the number of cell types forecast. Finally, we must consider the

case of our endocytosymbiont. Charles et al. (1997), estimate that there is a 36 per cent reduction in its genome as compared to the genome of *E. coli*¹⁰⁴. Further, if we look at the number of genes useful to the host or effectively in interaction with the host, the prediction of cell types could become more realistic. In addition, we introduced the hypothesis of interaction with the host genome, an interaction that could also explain the control of cell types of the endocytobiont by the host.

According to Von Sternberg's (1996) description of genomic self-organization, it appears that one could consider the host-symbiont system, as constituting a single genome¹⁰⁵. It is necessary to look again at our data. One may consider that the genome of a symbiotic insect contains about 18 000 transcribed genes. The number of attractor state cycles will thus be on the order of 134 with the hypothesis of $K=2$. If one looks at the *drosophila*, the number of cellular types observed will be between 60 and 65. The order of magnitude is correct, especially if one considers the sum of constraints (all the genes do not necessarily have the same number of interactions, K could equal 3, etc). From this vantage point, the introduction of the symbiont does not fundamentally modify the relation between the number of genes and the number of cellular types of the insect.

CONCLUSION

All of the problems exposed in this work has brought us to better outline the essential characteristics of endocytosymbiosis (particularly interdependence and unit of evolution) in order to frame the question of the existence of a phenomenon of self-organization. While multiple interactions are actually real at the cellular level between the two partners of endosymbiosis, they are more difficult to specify at the genetic level. Everything remains to be discovered about epigenetic interactions between host and symbiont. In the absence of sufficient data, an interactive model specific to endosymbiosis and, allowing satisfactory interpretation of molecular phenomena in a "host-symbiont" ecosystem, has not yet been devised. Nevertheless, the role of interactions constitutes a research hypothesis, based on experimental results: The permanence of a state of shock in endosymbiosis reveals simultaneously a control and an adaptation of the endosymbiotic bacterium in a milieu which not only does not appear to be beneficial to it, but more often than not, is frankly hostile. This shows clearly that epigenetic interactions will be the central part of the interactions between the cells—interactions which do not exclude certain antagonisms. Awareness of them, and their study, may give a new meaning to the concept of mutual interdependence as the one being at the core of significance of symbiosis.

Further, the hypothesis that there exist regular interactions in constant numbers for each gene constitutes a generalization, even an extrapolation, without direct relation to experimental results. To suppose that in each gene two promoters are directly dependent on the host is already a hypothesis not founded on the general situation but only on some very specific cases. To further suppose that everything happens as if the genomic interactions functioned in a Boolean network is to put into operation a virtual machine, which is supposed to reproduce the succession of live generations. It is above all, the duration to stabilize of the system (in number of generations) and its form (in number of attractors) which allows the establishment of the connection with actual life. For endosymbiosis, the Kauffman model with $C=1$ and $K=2$ would correspond well to what has been experimentally observed by Jeon in the amoeba. This suggests a similarity in the stabilization of each of the interactive systems.

Our work has thus been that of a “clearing operation” and the epistemological analysis of the model (II c) has allowed the outline of the relation between unit of selection and self-organization in the case of intracellular symbiosis. To consider the interactions only at the genomic level is a form of reductionism. Nevertheless, results obtained by biologists authorize us to see in the host-symbiont system a unit of evolution where real co-adaptation occurs (attenuation and balancing of diverse antagonisms, control by the host over the symbiont, transfer of symbiont genes to the host) in line with natural selection. In that context, the symbiotic population can be considered as a favorable variant. The biological data illustrating the co-adaptation of the genomes correspond well to the explanatory paradigm of self-organization. But the biologist and the epistemologist must face one other problem: the nature of interactions—let us repeat this—remains largely unknown. This forces us to clearly separate biological functionality and the process employed in its simulation. From the biological angle, one comes up against the question of reorganization of the genomes after the onset of symbiosis. Two scientific facts constitute a paradox in co-evolution: the control of genetic mutations of the symbiont by the host and the necessary alteration of the symbiotic genome to form the new unit of evolution. These two facts plead in favor of a search for a model of self-organization specific to endosymbiosis.

Our work, on the whole, shows that there exist serious reasons to visualize self-organization in endosymbiosis in the sense of a new organization of relationships between the host genome and the symbiont genome, aiming at the constitution of a new unit of selection. To speak of self-organization for endocytosymbiosis is a sufficiently realistic hypothesis to warrant a

research program for epistemology in the framework of self-organization co-evolutionary systems.

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NOTES AND REFERENCES.

- 1 J.F. Addicott, "Mutualistic interactions in population and community processes". In: Price & al. , *A New Ecology : Novel Approaches to Interactive Systems*, New York: A. Wiley, 1984: 437-452.
- 2 R. Axelrod, W. Hamilton, "The evolution of cooperation", *Science*, 211 (1981), 1390-1396. Benefits are measured according to the effect of interactions between partners on fitness (survival and fertility).
- 3 R. Axelrod, W. Hamilton, (note 2), p. 1391. Christopher Stephens (1996) criticizes the position of Axelrod and Hamilton because, while there may be cases in which it is more beneficial for one partner to exploit the other rather than to cooperate, there may also be cases in which the best strategy for both is to exploit each other's possibilities in turn. Stephens views this strategy as a form of cooperation. See: C. Stephens, "Modeling reciprocal altruism", *The British Journal for the Philosophy of Science*, 47 (1996), 533-551, p. 538.
- 4 R. Axelrod, W. Hamilton, (note 2), p. 1392.
- 5 R. Axelrod, D. Dion, "The further evolution of cooperation", *Science*, 242 (1988) 1385-1389, p. 1386.
- 6 L. Margulis, "Symbiogenesis and symbiontism". In: L Margulis and R Fester (ed.), *Symbiosis as a Source of Evolutionary Innovation*, Cambridge Mass: MIT Press, 1991: 1-14, p. 4. In the same manner, P. Nardon (1995) takes up the same elements when he writes, "Symbiosis characterizes a state of permanent association during at least one part of the biological cycle between two or several organisms, specifically distincts and even most often phylogenetically very far apart". He is only making the meaning of significant duration and the distinction between organisms more specific. P. Nardon, "Rôle de la symbiose dans l'adaptation et la spéciation", *Bulletin de la Société zoologique de France*, 120, 4 (1995), 397-406, p. 398.
- 7 L. Margulis, (note 6), p. 8.
- 8 See: O. Perru, "Qu'est-ce que la symbiose?", *Revue des questions scientifiques*, 168, 2 (1997), 113-136.
- 9 P.A. Corning, "Synergy and self-organization in the evolution of complex systems", *Systems Research*, 12, 2 (1995), 89-121.
- 10 H. Charles, P. Nardon, "La protéine hsp60 : chaperon moléculaire de la symbiose intracellulaire", *Regard sur la biochimie*, 3 (1997), 17-23.
- 11 G. D. Scott, *Plant Symbiosis*, London: E. Arnold, 1969.
- 12 D.C. Smith, "Signification de la symbiose". In: P. Nardon et al. (ed.), *Endocytobiology IV*, Paris: I.N.R.A., 1990: 29-35.
- 13 Smith underlined the fact that some symbionts grow more rapidly in an isolated culture than in association, perhaps because the host would act more to limit than to supply nutrients to the symbiont.
- 14 C.Y. Lai, L. Baumann, P. Baumann, "Amplification of trpEG : Adaptation of Buchnera aphidicola to an endosymbiotic association with aphids", *Proc. Natl. Acad. Sci.*, 91 (1994), 3819-3823.
- 15 A.-M. Grenier, P. Nardon, G. Bonnot, "Importance de la symbiose dans la croissance des populations de *Sitophilus Oryzae* L. (Coléoptère, Curculionidae), Etude théorique et expérimentale", *Acta oecologica, Oecologia applicata*, 7 (1986), 93-110.
- 16 Without going as far as mortality, the dynamics of the compared populations of symbiotic and aposymbiotic strains of *Sitophilus oryzae*, Nardon and

Grenier (1990) underline wide differences among them in the duration of development and fertility: The absence of symbiotic bacteria causes a 26% increase in the duration of development and a 30% decrease in fertility (defined as the number of adults obtained during one week for a given group of males and females). See: P. Nardon, A.-M. Grenier, "La symbiose, facteur important dans la croissance et l'évolution des populations de *Sitophilus oryzae* (Coleoptera, Curculionidae)". In: P. Nardon et al. (ed.), *Endocytobiology IV*, Paris: INRA, 1990: 369-372.

17 See: P. Nardon, H. Charles, A. Heddi, "Un modèle de symbiose : le charançon *Sitophilus oryzae*, Implications évolutives". In: Exbrayat et Flatin (ed.), *L'évolution biologique, science, histoire ou philosophie*, Paris: Vrin, 1997: 297-305.

18 P. Nardon, A.-M. Grenier ("Symbiose et évolution", *Annales de la Société entomologique de France*, 29 (1993), 113-140, p. 116.) speak of the supplying of 5 vitamins by the symbiont to the host, which corresponds to « the functioning of 15 - 20 genes acquired all at once and transmitted to all the descendants.» (P. Nardon, H. Charles, A. Heddi, (note 17)) Finally, the host seems to control not only the symbionts habitat and their trans-ovarian transmission but also their number. (See: * T.Tiivel, "L'endocytobiologie chez les cicadelles en tant que modèle d'adaptation cellulaire et d'évolution". In: P. Nardon et al. (ed.), *Endocytobiology IV*, Paris: INRA, 1990: 373-376.

* T.Tiivel, "Cell symbiosis, adaptation and evolution : Insect-bacterial examples". In: L Margulis and R Fester (ed.), *Symbiosis as a Source of Evolutionary Innovation*, Cambridge Mas: MIT Press, 1990: 170-177.

* P. Nardon, (note 6).

* P. Nardon, H. Charles, A. Heddi, (note17)).

It was demonstrated that the genomes of different strains of *S. Oryzae* determine a given number of bacteria per larval bacteriome, whatever the cytoplasm is. Therefore, there is indeed control on the part of the host DNA over the multiplication of symbionts. Whether this control is exerted toward an increase or toward curtailing the normal rate of development of a bacterial colony, it is nonetheless a control.

19 H. Charles, G. Condemine, C. Nardon, P. Nardon, "Genome size characterization of the principal endocellular symbiotic bacteria of the weevil *Sitophilus oryzae*, using pulsed field gel electrophoresis", *Insect Biochem. Molec. Biol.*, 27, 4 (1997), 345-350.

20 P. Nardon, H. Charles, A. Heddi, (note 17), p. 304.

21 L. Van Thinh, "Ultrastructure, photosynthèse, et bilan journalier du carbone fixé photosynthétiquement dans la symbiose des zooxanthelles *Zoanthus australiae* dans le port de Darwin (Australie)". In: P. Nardon et al. (ed.), *Endocytobiology IV*, Paris: I.N.R.A, 1990: 289-292.

22 (a) K.W. Jeon, "Bacterial endosymbiosis in amoebae", *Trends in Cell Biology*, 5 (1995), 137-140.

(b) K.W. Jeon, "The large, free-living amoebae: wonderful cells for biological studies", *Journal of Eukaryotic Microbiology*, 42, 1 (1995) , 1-7.

23 P.A. Corning, "The cooperative gene: on the role of synergy in evolution", *Evolutionary Theory*, 11 (1996), 183-207.

24 N.W. Blackstone, "A units-of-evolution perspective of the endosymbiont theory of the origin of mitochondrion", *Evolution*, 49, 5 (1995), 785-796.

25 See: P.A. Corning (note 23).

26 (a) K.W. Jeon, "Development of cellular dependence in infective organisms: Micurgical studies in amoebas", *Science*, 176 (1972), 1122-23.

- (b) K.W. Jeon, "Integration of bacterial endosymbionts in amoebae", *International Review of Cytology*, Suppl. 14 (1983), 29-47.
- 27 See: P. Nardon, A.-M. Grenier, "Genetical and biochemical interactions between the host and its endocytobionts in the weevils *Sitophilus* (Coleoptera, Curculionidae) and other related species". In: S. Scannerini et al. (ed.), *Cell to Cell Signals in Plant, Animal and Microbial Symbiosis*, Berlin: Nato Asi, 1988: 255-270.
- P. Nardon, A.-M. Grenier, "Endocytobiosis in coleoptera: biological, biochemical and genetic aspects". In: W. Schwemmler (ed.), *Insect Endocytobiosis Morphology, Physiology, Genetics and Evolution*, New York: CRC Press, 1989: 175-216.
- 28 According to Schwemmler and Gassner, the term "endocytobiosis" delineates space (endocytoto = interior of the cell) while leaving enough possibilities at the level of life forms (biosis). Indeed, it includes both, endocytobionts and endocytosymbionts, as well as cellular organelles susceptible of evolution from more autonomous life forms. Most authors quoted in here use "endocytobiosis" with the meaning of "endocytosymbiosis". See: W. Schwemmler, G. Gassner, "Endocytobiosis". In: W. Schwemmler (ed.), *Insect Endocytobiosis Morphology, Physiology, Genetics and Evolution*, New York: CRC Press, 1989: 4-8, p. 4.
- 29 P. Buchner, *Endosymbiosis of Animals with Plant Microorganisms* (1953), New-York: Interscience, 1965.
- 30 B. Boullard, *Guerre et paix dans le règne végétal*, Paris: Ellipses, 1990.
- 31 B. Boullard, (note 30), p. 184.
- 32 W. Schwemmler, G. Gassner, (note 44), p. 4.
- 33 L. Margulis, (note 6). W. Schwemmler, G. Gassner, (note 44). H.A. De Bary, (note 1).
- 34 Generally, it is possible to abide by the following: the host and symbiont live in interdependence, with a certain advantage for the host. The latter seems to exploit its symbiont nutritionally as well as genetically (this last remark seems more justified in the case of endosymbiosis than in that of ectosymbiosis).
- 35 See: P.A. Corning, (note 9).
- 36 W. Schwemmler, G. Gassner, (note 28), pp. 4-5.
- 37 S.-A. Kauffman, *The origins of Order, Self-Organization and Selection in Evolution*, New York: Oxford University Press, 1993, p. 245.
- 38 B.-C. Goodwin, "La genèse de formes dynamiques : l'organisme et l'esprit", *Intellectica*, 16 (1993), 45-60, p. 53
- 39 B.C. Goodwin, (note 38), p. 54.
- 40 L.-L. Salvador, "Pour un relativisme interactionniste conséquent – de Piaget à Darwin et retour", *Intellectica*, 16 (1993), 101-131, p. 107.
- 41 See: F. Varela, *Autonomie et connaissance*, Paris: Seuil, 1989.
- 42 I. Prigogine, "Time, structure and fluctuations", *Science*, 201 (1978), 777-84.
- I. Prigogine, *From Being to Becoming: Time and Complexity in the Physical Sciences*, San Francisco: W.-H. Freeman, 1980.
- 43 S.-A. Kauffman, *At Home in the Universe. The Search for the Laws of Self-organization and Complexity*, New York: Oxford University Press, 1995, p. 8.
- 44 S.-A. Kauffman, (note 43), p. 50.
- 45 S.-A. Kauffman, (note 43), p. 43.
- 46 S.-A. Kauffman, (note 43), p. 25.
- 47 S.-A. Kauffman, (note 43), p. 25.

- 48 S.-A. Kauffman, (note 43), p. 41.
- 49 See: S.-A. Kauffman, (note 43).
- 50 See : P.A. Corning, (note 43).
- 51 S.-A. Kauffman, (note 43), pp. 245-249. S.-A. Kauffman, (note 43), pp. 224-230.
- 52 S.-A. Kauffman, (note 43), p. 223.
- 53 S.-A. Kauffman, (note 43), p. 215.
54. S.-A. Kauffman, (note 43), p. 216.
- 55 V.M. Veis, R.P. Levine, "Differential protein profiles reflect the different lifestyles of symbiotic and aposymbiotic *Anthopleura elegantissima*, a sea anemone from temperate waters", *Journal of Experimental Biology*, 199 (1996), 883-892.
- 56 See: V.M. Veis, R.P. Levine, (note 55).
- 57 See: M. Sugita, C. Sugita, M. Sugiura, "Structure and expression of the gene encoding ribosomal protein S1 from the cyanobacterium *Synechococcus* sp. strain PCC 6301: striking sequence similarity to the chloroplast ribosomal protein CS1", *Molecular Journal of Genetics*, 246 (1995), 142-147.
- 58 G.-I. Mac Fadden, P.R. Gilson, C.J. Hofmann, G.J. Adcock, U.G. Maier, "Evidence that an amoeba acquired a chloroplast by retaining part of an engulfed eukaryotic alga", *Proc. Natl. Acad. Sci.*, 91 (1994a), 3690-3694.
- 59 See: G.-I. Mac Fadden, P.R. Gilson, C.J. Hofmann, G.J. Adcock, U.G. Maier, (note 58).
- 60 G.-I. Mac Fadden, P.R. Gilson, S.-E. Douglas, "The photosynthetic endosymbiont in cryptomonad cells produces both chloroplast and cytoplasmic-type ribosomes", *Journal of Cell Science*, 107 (1994b), 649-657.
- 61 See: G.-I. Mac Fadden, P.R. Gilson, S.-E. Douglas, (note 60).
- 62 G. Febvay, I. Liadouze, J. Guillaud, G. Bonnot, "Analysis of energetic amino acid metabolism in *Acyrtosiphon pisum*: a multidimensional approach to amino acid metabolism in aphids", *Archives of Insect Biochemistry and Physiology*, 29 (1995), 45-69.
- 63 See: C.Y. Lai, L. Baumann, P. Baumann, (note 30).
- 64 See: H. Charles, P. Nardon, (note 26).
- 65 See: H. Charles, P. Nardon, (note 26).
- 66 H. Charles, A. Heddi, J. Guillaud, C. Nardon, P. Nardon, "A molecular aspect of symbiotic interactions between the weevil *Sitophilus oryzae* and its endosymbiotic bacteria: over-expression of a chaperonin", *Biochemical and Biophysical Research Communications*, 239 (1997), 769-774.
- 67 See: H. Charles, P. Nardon, (note 10).
- 68 See: K.W. Jeon, (note 22b).
- 69 S.-A. Kauffman, (note 37), p. 177. According to Weisbuch (1989), attractors represent an ensemble of states of the system forming a loop. If each cellular type corresponds to a cycle of attractors, this means that differentiation results in reproducing indefinitely all the cellular types of the organism, the different cycles being regulated and, in some way, showing "solidarity" among themselves. Certain cell types are prone to differentiation, others are rather final results. As B. Feltz points out, the boolean model can help in interpreting the phenomenon of cellular differentiation. See: G. Weisbuch, *Dynamique des systèmes complexes, Une introduction aux réseaux d'automates*, Paris: Editions du CNRS, 1989. B. Feltz, "Auto-organisation, sélection et émergence dans les théories de l'évolution". In: B. Feltz, M. Crommelinck, P. Goujon (ed), *Auto-organisation et émergence dans les sciences de la vie*, in printing, Bruxelles: Ousia, p. 332.

- 70 S.-A. Kauffman, "Antichaos and adaptation", *Scientific American*, 265 (1991), 78-84.
- 71 B. Feltz, (note 69), p. 331.
- 72 S.-A. Kauffman, (note 37), p. 488.
- 73 S.-A. Kauffman, (note 37), p. 462.
- 74 See: N.W. Blackstone, (note 24).
- 75 R.-C. Lewontin, "The units of selection", *Annual Review of Ecology and Systematics*, 1 (1970), 1-18.
- 76 The epistemologist must analyze biologists' understanding of this new structure and associated characters. According to R. N. Brandon (in printing, 265-279), one can apply either the selected effect (ES) model or the causal role (RC) model. The selected effect (ES) model will give:
- (1) A bacterium has begun to live in intracellular symbiosis with the cereal weevil.
 - (2) This new genetic heritage represents something of a «gene kit» which can be transmitted to descendants.
 - (3) Selection acts on the physiological advantage brought by the symbiont (in the case of the cereal weevil, the supply of 5 vitamins).
 - (4) The «selected» interrelation between host and symbiont results in an interdependence, where the symbiont can no longer survive outside the host. The RC model can be described as follows: The eukaryotic cell functions through the ability to integrate a bacterium in the physiological mechanisms of growth and reproduction of the host (s). The functioning of this model is relative to an analytic explanation (A) (here showing how the eukaryotic cell receives new genetic aptitudes). A is relative to the capacity of s to accomplish G (to transmit the endosymbiont to descendants). The eukaryotic cell functions in that manner because A adequately elucidates the aptitude of s to accomplish G, drawing on the aptitude of the eukaryotic cell to effect the integration of the endosymbiotic bacterium.
- These two models are borrowed from Brandon, who himself borrowed the second one from Armundson and Lauder (1994). The first model involves a teleological view. The second is totally void of the idea of finality and aims only to analyze a complex system as such. It can be interpreted only in terms of capacity, functionality and the resulting structure: the integration of the endosymbiotic bacterium in the eukaryotic cell. See: R. Armundson, G.V. Lauder, "Function without purpose : the uses of causal role function in evolutionary biology", *Biology and Philosophy*, 9 (1994), 443-469.
- R.N. Brandon, "La téléologie dans les systèmes auto-organisés". In: B. Feltz, M. Crommelinck, P. Goujon (ed), *Auto-organisation et émergence dans les sciences de la vie*, in printing, Bruxelles: Ousia.
- 77 S.-A. Kauffman, (note 43), p. 223.
- 78 S.-A. Kauffman, (note 43), p. 163.
- 79 E. Sober, *The Nature of Selection, Evolutionary Theory in Philosophical Focus*, Chicago: Chicago University Press, 1985, p. 154.
- 80 E. Sober, (note 79), p. 155.
- 81 J. Mac Laurin, "Reinventing molecular Weismanism: Information in evolution", *Biology and Philosophy*, 13 (1998), 37-59.
- 82 The limits to reasoning coming obviously from the extreme simplification of biological data by use of the values of «0» or «1».
- 83 B.H. Weber, "Origins of order in dynamical models", *Biology and Philosophy*, 13 (1998), 133-144, p. 142.

- 84 E. Sober, (note 79), pp.147 sq.
- 85 B. Feltz, "Pertinences et limites de l'explication par sélection". In: Exbrayat et Flatin (ed.), *L'évolution biologique, science, histoire ou philosophie*, Paris: Vrin, 1997: 415-428, p. 426.
- 86 B.H. Weber, (note 83), p. 142.
- 87 G. Edelman, *Biologie de la conscience*, Paris: Odile Jacob, 1992, p. 112.
- 88 P.E. Griffiths, "The historical turn in the study of adaptation", *The British Journal for the Philosophy of Science*, 47 (1996), 511-532.
- 89 J. Griesemer, Review: "Turning back to go forward. A review of epigenetic inheritance and evolution. The lamarckian dimension, by Eva Jablonka and Marion Lamb", *Biology and Philosophy*, 131 (1998), 19-24.
- 90 S.-A. Kauffman, (note 37), p. 41.
- 91 S.-A. Kauffman, "Investigations, The nature of autonomous agents and the world they mutually create", *Lecture 1*, Santa-Fe Institute, 1996.
- 92 S.-A. Kauffman, (note 43), pp. 215-223.
- 93 See: S.-A. Kauffman, (note 91).
- 94 R. Von Sternberg, "The role of constrained self-organization in genome structural evolution", *Acta Biotheoretica* 44 (1996), 95-118.
- 95 See: P. Nardon, A.-M. Grenier, (note 16).
- 96 See: T. Tiivel, (note 18).
- 97 P. Nardon, C. Wicker, "La symbiose chez le genre *Sitophilus* (Coléoptère, Curculionide). Principaux aspects morphologiques, physiologiques et génétiques", *Annales de Biologie*, 20 (1981), 327-373.
- 98 S.-A. Kauffman, (note 37), p. 247.
- 99 A computer simulation was made for $N = 30$, $K = 2$, $C = 1$, and seems to show the establishment of cycles after several hundred repetitions based on random connectivity for interactions of the types «K» or «C». Indeed, in simulating an induction on the part of the host on the symbiont, then two intra-genomic interactions, and a retro-action of the symbiont on the host (reciprocity of interaction of type «C»), one obtains at first a chaotic result (from t to $t+1$, the number of activated sites varies up to 100% without there being really any order). When the simulation runs about 250 to 300 times, one gets a certain regularity in the sequences with periodicity of 5 to 6. Cycle length on the order of 5 to 6 corresponds well to Kauffman's forecast (1993) which gives the root result (N) for the number of cycles as well as for their length. It appears that a certain equilibrium is reached before the value of 700 generations, the maximum forecast by Kauffman. In any case, one finds the same order of magnitude.
- 100 P. Nardon, "Symbiosis and evolution : the weevil model (*Sitophilus Oryzae*, Coleoptera, Curculionidae)". In: R. Lesel (ed.), *Microbiology in Poecilotherms*, London: Elsevier Science Publishers, 1990.
- 101 See: K.W. Jeon, (note 26b).
- 102 See: K.W. Jeon, (note 22a).
- 103 See: S.-A. Kauffman, (note 37) et (note 70).
- 104 See: H. Charles, G. Condemine, C. Nardon, P. Nardon, (note 19).
- 105 See: R. Von Sternberg, (note 94).

ABSTRACT

From the beginning, symbiosis has been viewed as an association of two specifically distinct organisms living together. Firstly, symbiosis may suggest an idea of mutual benefit, but partners become dependent on symbiosis. Such dynamical phenomenon between two organisms in physiological interdependence is liable to evolve by creating a new life unit. This notion implies a reorganization of genomes and seems especially adapted to describe endosymbiosis. Putting forward a hypothesis of interactions leads us to consider the organism as a consequence of a self-organization process. In this paper, we refer to Kauffman's works and to various cases of endosymbiosis. Considering the symbiotic genome, some biological results tend to indicate that models with ($C=1$ or $C=2$, $K=1$ or $K=2$) are not unrealistic and should be tested. But different epistemological questions prompt us to reflect further on the role of natural selection, the reality of the modeled interactions, the nature of the process of mutual adjustment. Moreover, regular interactions cannot exist in constant numbers for each gene. In spite of all these caveats regarding the applicability of Kauffman's model concerning the establishment of a symbiosis in amoebae, simulation and experimentation yield results included in the same order of magnitude.

RESUMEN

ENDOSIMBIOSIS Y AUTORGANIZACIÓN

Desde un inicio, se ha considerado a la simbiosis una asociación entre dos organismos específicamente distintos que viven juntos. En primer término, la simbiosis puede sugerir una idea de beneficio mutuo, pero los organismos implicados se vuelven dependientes de ella. Es posible que este fenómeno dinámico entre dos organismos en interdependencia fisiológica evolucione para dar origen a una nueva unidad de vida. Tal noción implica una reorganización de genomas, y parece especialmente adecuada para describir la endosimbiosis. Formular una hipótesis de interacciones nos lleva a considerar al organismo como la consecuencia de un proceso de autorganización. Con base en el genoma simbiótico, algunos resultados biológicos tienden a indicar que los modelos con ($C=1$ o $C=2$, $K=1$ o $K=2$) son realistas y deberían explorarse. Sin embargo, diferentes cuestiones epistemológicas nos invitan a reflexionar más a fondo sobre el papel de la selección natural, la realidad de las interacciones modeladas, la naturaleza del proceso de adaptación mutua. Más aún, no pueden existir interacciones regulares en números constantes para cada gene. Pese a todas estas limitaciones en cuanto a la aplicabilidad del modelo de Kauffman relativo al establecimiento de una simbiosis entre las amebas, la simulación y la experimentación brindan resultados en el mismo orden de magnitud.