A sign-theoretic approach to biotechnology

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A sign-theoretic approach to biotechnology

Ph.d. thesis by

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To the memory of my father,

Lucio Mario Bruni Celli,

Who always supported me in these kinds of endeavours.

A sign-theoretic approach to biotechnology

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Introduction

One of the great debates that came out of the 20th century was about the existence or not of an ecological crisis. Some outstanding thinkers pointed out that if there was a crisis, it was not ecological but epistemological. It is not the ecosystem that is in crisis (although it manifests the consequences of such a crisis) but many of our cultural premises, including some within the modern social legitimising agent, scientific understanding. At the beginning of the 21st century it is very hard to find any scientific, technological or industrial reality that is not, one way or the other, concern with these matters. The most visionary industries are well aware of the steps forward that have to be taken in order to survive in this context, in which the "public" has become very sensitive towards the ecological record.

As the importance, and economic benefits, of environmentally conscious engineering have become apparent, research into methods and techniques in this area has grown tremendously. Biotechnology maybe even more than many other industrial sectors cannot afford to withdraw from this background. For many experts, the bottleneck for transferring biotechnologies from the laboratories to the productive realities is, in most cases, not technical but normative in nature. This may be due to two concomitant reasons:

a) the real ecological and social impacts that some of these techniques may have (looked upon the background of the benefits that they may bring about);

b) the unselective blind opposition (due to lack of proper information) from vast sectors of society towards any kind of gene technology application.

As Scannerini (1994) has pointed out, the real enemies of biotechnology and genetic engineering are the claims of omnipotence of the "biological revolution", the "everything is allowed" attitude, the reduction of humans to their molecular biology, the "idolatry of megaprograms", in which a great deal of the scientific results are constituted by the data and the techniques to maintain the program. These attitudes misinform society and generate a vicious circle in which always more farfetched demagogical promises (like for example "feeding the third world") are put forward in order to justify research and development and to gain acceptance from the general public. So, from the biotechnologist point of view, Scannerini argues that a return to modesty in the objectives (as the search for techniques to solve practical problems), the abandoning of Faustian dreams (even unconscious ones), a respect for ethics,

humans and the environment, constitute the passport that should be requested from a biotechnologist and that will guarantee the development of really serious and useful biotechnologies (Scannerini, 1994: 136).

When I started approaching some of these problems, in particular the relation between biotechnology and biodiversity, I was wandering about which could be a suitable theoretical framework to address the problem of causality in biology when dealing with this kind of issues. For more than 15 years I had been studying Gregory Bateson's contributions which had served me as an inspiration in different aspects of my work. In particular I was "hunting" for a framework that would take into consideration communication processes in living systems, from molecules to ecologies. This is how I "discovered" and entered into contact with the Biosemiotic Group at the University of Copenhagen and the rest of the biosemiotic community.

The departing point for this project was an attempt to analyse what does it mean that the general concept behind biotechnology is that of biological information, which is normally ascribed to the so-called informational molecules, mostly DNA or RNA. This analysis immediately pointed out to the limits of identifying "biological information" exclusively with DNA, a substance. When trying to understand how organisms "treat" biological information, one realises that DNA is not the exclusive physical support for biological information. Therefore the question had to be reformulated into how do organisms treat biological information in general and what bearings does this have for biotechnology? This is where biosemiotics becomes quite a valuable epistemological tool. Nature produces signs that we re-elaborate metabolically and culturally, becoming ourselves signs for the rest of the natural realm. Organisms constantly produce signs and protoplasm. And within this context we produce "cultural signs" that enable us to steer natural protoplasm production in a given direction. We can do that at many different levels, as the history of agriculture and medicine may show, from breeding up to gene technology.

Whether we get involved in assessing the nature of the ribosomal mutation that produces the "right" change to make a bacterium resistant to certain antibiotic, or in the design of the optimal "architecture" for a set of bacteria colonies to maximise the efficiency of a toxic waste treatment bioreactor, or in the selection and insertion of beneficial endophytic bacteria within a plant, or in the genetic modification of an organism so that with its new protein it enhances its service to us, we count with numerous tools to follow the essential trophic and energetic pathways of such

processes. Not so for the intrinsic production (and interpretation) of signs. Therefore it becomes easy to neglect the context in which these artefacts are supposed to work.

Finally the question became: what impact would it have to our understanding of biotechnology complementing the existent and growing knowledge of the efficient mechanisms for "protoplasmic manipulation, design and production" with a better understanding of the corresponding sign-processes that take place along with growth, differentiation and biological function at different hierarchical levels? What would be the impact on efficiency? On precision? On biosafety? On ecology?

During the formulation of this project the existence of a growing trend in biology that puts the emphasis on communication processes at all levels of the biological hierarchy became clear. This trend is also manifest in the present stage of biotechnology development. Anything that has to do with signal transduction and regulation in living systems has to do with communication processes.

I started with a preliminary definition of what Jesper Hoffmeyer had called *biosemiotic technology*: the use of biological semiotic controls for technological purposes (Hoffmeyer, pers. comm., 2001). Initially, there were two ways in which I could approach the issue: is all biotechnology biosemiotic by definition? I.e.: does biosemiotic technology emerge as the increasing consciousness of biotechnology being of semiotic nature? Or, does biosemiotic technology begin with the application or the combination of biosemiotic knowledge with current technology? In this work I tend to privilege the second point of view, although it has to be acknowledged that they are two sides of the same coin.

My interest in relating what we called biosemiotic technology to biosafety and sustainability - as a way of providing a useful framework for organising and integrating knowledge, in the form of "maps", about the myriad of multi-trophic and multi-semiotic interactions in Nature, by exploring the emergence of patterns in codes, contexts and *interpretants* - may come from the bias of my previous education in environmental engineering. But I hope that some of the ideas developed here have a more general relation to the epistemology of biology in general.

There are different categories of sustainable biotechnology. There is for instance "environmental biotechnology" which is practically the use of biotechnology in environmental engineering. There is biotechnology for sustainable crop systems. There is biotechnology for production of biodegradable materials. And, last but not least, there is biotechnology for human health.

In a sense all biotechnology, theoretically, could be classified as sustainable technology (leaving aside, of course, the use of biotechnology for war purposes, crimes, the creation and spread of disease, the "denaturalisation" of Nature or the creation of abnormalities). But in order to do so, biosafety should be considered in its wider sense as a guiding principle. Given the semiotic connectivity of the different spheres in which biotechnology operates - human health, food and crop security, environmental protection - an approach to biosafety as a guiding principle and as a kind of biosemiotic technology can be of great help. So as biotechnology will become aware of its shift towards biosemiotic technology, this awareness could play a major role in portraying the intricate interplay between the vertical (genealogical) semiotic system and the horizontal (ecological) semiotic system that have to be simultaneously considered when manipulating "biological information" with the bio-techniques.

The "language" of bacteria may be very primitive compared to other languages that emerge at higher hierarchical levels. However they share some of the same sophistication and principles behind the communication "devices" of our own cells, whose communication and co-operation gives rise to emergent semiotic levels such as our own natural languages and cultural realities.

Information is not only "transduced" linearly through dyadic cascades of reactions. It is also "transduced" by the creation of complex *logical products*. There is a non-deterministic aspect in this. Causality within, between, and around living systems may, at certain levels, be more or less independent from the underlying physical dynamics, although always based on it. To the study of genetic codes we have to connect the study of metabolic, ethologic and ecological codes.

At whatever level of living matter, the processes of communication are operating within a frame of *triadic causality*. Whatever particular level we may choose for inspection is in communication with other hierarchical levels. No level of the biological hierarchy is incommunicated from other levels. Triadic logic is a "built-in" tautology in the whole system of living matter, and it shares with the tautology of the physical world (dyadic logic) the fact that it is based on it. But it is one step further, in terms of "freedom", with respect to the physical tautology.

If the promising future of biotechnology lies in the use of "biological information", *a sign-theoretic approach to biotechnology* seems to be highly pertinent. I hope that the present work gives a contribution in this direction. Allow me to finish this introduction by providing a brief overview of its structure.

In the first chapter I give a personal overview of the issue of causality in biology. The important point that I want to stress there is the conceptual relation between quantity and quality and between form and substance. I make a connection between our understanding of these conceptual relations and the limits to reductionism in biology. I review the historical challenge that the concept of "biological information" has posed (and still poses) to molecular biology in particular and to biology in general. Another issue that comes forward is the recurrence in the history of biology of notions related to "goal-oriented-behaviour" and "emergence". The chapter ends by identifying a very strong and spread trend in life sciences that sees biology at different hierarchical levels as "the science of sensing". I point out that this is something that the biosemiotic paradigm has been claiming throughout its development in the last three decades.

In chapter two I make a short and instrumental introduction of the biosemiotic paradigm and of the logic behind triadic causality. I present the integrative concept of biological information that has been developed in biosemiotics, based on Gregory Bateson's "biological cybernetics" and on Charles Peirce's semiotics. I also provide a brief overview of current knowledge about genome architecture, evolution and communication. I stress the importance of not neglecting the fact that there is no simple linear relation between genotype and phenotype. By introducing the notion of "global phenotype", inseparable from the "genome space", I emphasise their mutual semiotic constitutivity. In section 2.5, I present a "toolbox" of concepts for "mapping" semiotic networks across hierarchical levels and for relating the different emergent codes in living systems. I consider this an important part of the work because there I define some of the main concepts that will help me to analyse different codes and semiotic processes in living systems in order to exemplify what is the relevance of a sign-theoretic approach to biotechnology. In particular, I introduce the notion of *digital-analogical consensus* as a semiotic pattern for the creation of complex logical products that constitute specific signs. The chapter ends with some examples of conspicuous semiotic interactions that come forward in different kinds of empirical studies at different hierarchical levels.

Given the central role that the elucidation of signal transduction networks has acquired in the "integrative agenda" in biology, in chapter three I go into some of the details of these networks in order to exemplify how a semiotic approach can be of help when organising the knowledge that can lead us to understanding the relevance,

the role and the position of signal transduction networks in relation to the larger semiotic networks in which they function, i.e.: in the hierarchical formal processes of mapping, translation, transformation and transmission of information. The idea is also to investigate how this debate may influence the "integrative agenda" in biology, especially at a time in which biotechnology is considered to be the industrial use of "biological information". I introduce concepts such as the "signalome" and the "embryonic signalome" and I use the "Ca^{2+} code" to advance a hypothesis of how a cellular system achieves the necessary categorial perception that allows it to avoid undesirable cross-talk by using the semiotic patterns that I have called digital-analogical consensus. After having exemplified some of the "applications" of the toolbox for mapping semiotic networks, I proceed towards a general definition of biosemiotic technology. I furnish some examples of current technological developments that qualify as biosemiotic technology including some of their possible future developments. Finally I make a connection between a sign-theoretic approach to biotechnology and sustainability, with a glimpse into the future.

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Chapter 1
Causality in biology

1.1 Limits to reductionism and the material-mechanical causation

It seems that any attempt to understand the significance of a scientific endeavour is intrinsically related to the understanding of causality. Any of the goals advocated within science have to do with understanding how things work in "this world". Whether the goal of the scientist is to improve human life, intelectual curiosity, sustainability, health, or economic profit, the scientist will be involved in understanding the causal links in the specific cases with which he or she becomes involved. However, for the most part, this understanding of causality is taken for granted and many students and even professional scientists ignore the premises or presuppositions on which their work and their assumptions are based. Bateson summarised this state of affairs by saying that "Those who lack all idea that it is possible to be wrong can learn nothing except know-how" (Bateson, 1979: 28).

It is widely accepted that biology, and in particular empirical biology, operates within a framework of reductionism, although for the most part practitioners may be unaware of what does this really mean. According to Nagel (1998: 3) the reductionist idea claims basically that "all of the complex and varied and apparently disparate things and processes that we observe in the world can be explained in terms of universal principles that govern the common ultimate constituents out of which, in many different combinations, those diverse phenomena are really composed". He points at two aspects of reductionism, constitutive and explanatory: "The constitutive thesis is that everything is made of the same elements; the explanatory thesis is that everything that happens can be given an ultimate explanation in terms of the laws governing those elements" (Nagel, 1998: 3).

In science in general, and in biology in particular, the common ultimate constituents are material and therefore the universal explicative principles are those of physics, which in turn are based on a material-mechanical conception of causality. This "express-link" between reductionism, materialism and mechanicism is not always explicitly acknowledged.

While it is usually admitted that the first product of rationalism in the scientific field was the Cartesian mechanicism, materialism was only a later product (which was to become explicit in the XVIII century). The mechanicist thesis basically says that everything is explainable based solely on the principles of matter and local movement. To the scientists of mechanicist orientation any concept lacks explicative value if such concept cannot be analysed in terms of the dynamical possibilities inherent to the material structures, by reason of the configurations and movements of the component particles.

In opposition, Leibniz, (who according to Norbert Wiener (1955/1948: 18) was the last man to have had full command of all the intellectual activity of his time!), claimed the insufficiency of mechanicist physics, as an explanation, because it can only account for the exterior appearance of things, and is incapable of saying anything about their essence. In this sense it can be said that mechanicism has only a "representative" value and not an "explicative" one (Guénon, 1995/1945: 97).

It has been customary to ascribe the label of mechanicism to the ancient atomistic conceptions of Democritus and Epicurus (the only two real precursors of the modern epistemology), and very often it has also been claimed that these two Greek thinkers were also forerunners of materialism, although the notion of matter of the modern physicists was yet to be born. To Democritus the principles of all things are the atoms and the vacuum. The necessary movement of atoms gives rise to visible bodies through aggregations and disgregations. Even our knowledge is constituted through material pathways, when the "fluxes" of atoms coming from existing bodies strike our sense organs (Di Luciano, 1993: 702). On the other hand, the vacuum, not being a possibility of manifestation, could not have a place in the manifested world, leading the atomists to a paradox: not admitting by definition any other positive existence than that of the atoms and their combinations, the atomists are directly led to suppose that between the atoms there exists a vacuum in which the atoms can move (Guénon, 1995/1945:37).

In modern times materialism was reborn with the revival of mechanicism in the XVII and XVIII centuries. The materialistic theory advanced by Locke about the possibility that matter could "think", and the way the materialists assumed "empirical gnoseology", gave rise to what was called "sensism". Sensism designates the philosophical doctrine that reduces any content and act of cognition exclusively to a transformation process of sensations (there were already such conceptions in the work

of some sophists like Protagoras) (Di Luciano, 1993: 703). This thesis represents the arrival point of materialism to the higher levels of complexity in biological systems, like for instance the human mental process. Later, materialism would get new vigour in the XIX century within the framework of positivism and Darwinian evolution.

As we know, Decartes did not feel like proposing his "animal-machine" theory at the human level giving in this way rise to his famous dualism, (mind and matter), within which he decided to consider one term and consciously neglect the other, as opposed to his successors who negate the existence of one of the parts altogether. In other words, Descartes could not be considered a material reductionist himself, he was a dualist. It was up to his followers to neglect one of the components of the dualism, considering only the part that was amenable to the mechanicist conception in order to reduce the entire reality in a way that was naturally going to lead to materialism (Guénon, 1995/1945: 98). If they had the intention of explaining mechanically every phenomenon that is produced by and within animals, including those manifestations that have a more evident "psychic", "mental" or "cognitive" character, why could not the same be done with regard to human beings, why not neglect the other term of the dualism altogether also in this case, as something unnecessary for the explanation of things? From here to the consideration that it is a useless complication and treating it as in fact inexistent, to finally simply and absolutely negate its existence, the step is a small one. In this way Descartes's mechanicist physics paved the way to materialism (Guénon, 1995/1945: 98).

In our times there is almost consensus about materialism given the fact that the philosophical approaches related to natural sciences have almost unanimously adopted an "anti metaphysical" stand which considers materialism as a universal, coherent and sufficient system. In this framework the reduction of psychology to biology and of biology to physics becomes an ideal which is only limited by the progress of science (Di Luciano, 1993: 703). Therefore we can say that in general when we refer to reductionism we are referring to reduction to material-mechanical causality. This leads to the ultimate reductionism which is the idea and the tendency to reducing everything to the quantitative point of view. To the materialisation of everything corresponds the quantification of everything.

Quality and quantity are usually considered as two complementary terms, although the nature of such a relation is very seldom made explicit. The common belief about the complementary of these two terms already says something about the

"real" existence of qualitative aspects in scientific phenomena (Guénon, 1995/1945: 19). In order to talk about quantity and quality one has to talk about unity and multiplicity since the quantity refers to the multiplicity of units (independently of their qualities) while the quality refers to the "identification" of a unit. In this sense we can speak of a quantitative or arithmetic multiplicity (the quantity itself separated of any quality) and we can also speak of a qualitative multiplicity (the set of qualities or attributes that constitute the essence or nature of a being or thing) (Guénon, 1995/1945: 18).

We can define the quality as the constitutive synthesis of all the attributes belonging to a being or thing and which make such a being or thing that which it is. This corresponds closely to the sense Aristotle gave to the word "eidos" which he used also to designate the concept of "species": the nature or essence which is common to an indefinite multitude of individuals. Such a nature is of a purely qualitative order, i.e.: it is not "numerable", it is indivisible and integral in everyone of the individuals belonging to such a species. It is not modifiable by the number of individuals and it is not susceptible to variations of the "more" or "less" type.

The "quality" implicit in the Aristotelian notion of "species" is equivalent to the scholastic notion of "form" which to the schoolmen was complementary to the notion of "substance". The medieval schoolmen identified "substance" with matter, but they made a distinction between *"materia prima"* (a universal substance, indistinct and undifferentiated) and *"materia secunda"* (a substance in relative sense, i.e.: the substance of a particular existence). Later, this difference was lost and substance became simply matter as it is understood by modern physics (therefore for simplicity I will refer to the "materia secunda" as simply "matter").

In the conceptual relation of "form and substance" it is interesting to notice that the word "substance" comes from *substantia*, from *substare*, literally "that which is under", and from here come the notions of "support" and "substrate". The implication being that the substance is hierarchically under the form, it constitutes its passive physical support or substrate. In a sense it is unintelligible with respect to the kind of intelligibility that we could find in explanations at the level of the *form*. (Guénon, 1995/1945: 24).

Matter (substance) must be determined in accordance with the special conditions of the existing world. What is the nature of such determination? With the expression *"materia signata quantitate"*, Aquinas wanted to emphasize that that which is

inherent to matter, that which makes it be what it is, is not the quality but the quantity, which is so found *ex parte materiae*. In this sense, the quantity is *one of* the conditions for existence in the sensible or corporeal world, and it would not be possible to ascribe to matter properties which do not belong to it. The quantity lies in the substantial part of the "form-substance" relation. It is a basic or fundamental condition, but one could not expect to find there all the explanations of "our world", e.g.: the mere existence of the foundations does not guarantee the existence of the building, although the foundations are indispensable to it. Quantity is a necessary prerequisite, but it does not by itself explain form (Guénon, 1995/1945: 25-26).

Quantity can be manifested in two different modes:

1) discontinuous quantity, which is the one we directly associate with the number, since the succession of the integer numbers constitutes a discontinuous series (being all the extensions to this notion - i.e.: fractional numbers, incommensurable numbers, etc. - derivatives of the integer series, conceived in an effort to reduce, for as much as possible, the intervals of the numerical discontinuum, in order to make less imperfect its application to measurements of continuous magnitudes).

2) continuous quantity, which refers to magnitudes of a spatial and temporal order.

Guénon (1995/1945: 26) asks which of these two modes can be defined as pure quantity. Since Descartes defines matter by means of the extension - and he puts this at the base of his quantitative physics (which was already mechanicist although not yet materialistic) - one could be tempted to conclude that it is the "extension" (given that it is directly inherent to matter) which represents the more fundamental mode of the quantity. On the contrary, Aquinas suggests that it is the number that constitutes the substantial base of the world, and therefore it can be considered as the pure quantity (Guénon, 1995/1945: 26).

Guénon (1995/1945: 27) argues that in the Cartesian definition of matter there could have been some mistake or confusion, and that there has been introduced, may be without the knowledge of the author, an element which is not strictly quantitative. In fact, although the "extension" evidently has a quantitative aspect, it cannot be considered like pure quantity. It is also noticeable that the most advanced theories for quantitative reduction are generally "atomistic" in one way or the other, that is, they introduced in their notion of matter a discontinuity that puts them closer to the nature of the number than to that of the extension: and the very fact that the corporeal matter

can only be conceived as an extension, remains for every "atomist" a strong contradiction.

According to Aquinas, it is the number that must be considered the fundamental mode of the pure quantity. Other modes (e.g. the continuous quantities) are only derivatives, i.e.: they are quantities because they participate to the number, something that is implicitly recognized when it is commonly claimed that everything that is quantifiable must be possibly expressed numerically. Although the quantity is the predominant element in these other modes (e.g. continuous magnitudes), these modes always appear more or less combined with the quality.

In spite of the fact that the number could never be perceived directly in its "pure state" in the sensible world, it must be considered the constitutive fundamental mode of the quantity (Guénon, 1995/1945: 27). In other words, a magnitude, before "being quantified", already presents some qualitative aspects by virtue of its continuity. This is equivalent to the cybernetic analogical mode, to which I will be referring later in this work. I think it was in order to reduce the continuous to the discontinuous that integral calculus was originally devised. By integrating a bi-dimensional shape, or a volume, do we not get the illusion of quantifying a form? Another complication comes from the fact that our measurements of magnitudes are never absolute, but based on relative *differences* of comparable magnitudes.

It is also worth noticing that the word "matter" derives from the Latin verb "metiri" which means "to measure", i.e.: to introduce a determination. Measurement refers principally to the sphere of the continuous quantity, that is, things that have a spatial character (being time equally continuous but measurable only indirectly through relative movement in space) (Guénon, 1995/1945: 29).

Accordingly, we can talk about two types of measurement:

1) a "geometric" measurement, which is abstract and serves to define the extension, and

2) a "physical" measurement, which can be applied to matter by virtue of its extensive character. This does not mean that its "nature" can be reduced exclusively to the extension, as suggested by Descartes, a fact that becomes more than evident when we are dealing with "living matter"(Guénon, 1995/1945: 30).

In reality, the second case is traceable to the first one because it is by virtue of its situation in the extension and the occupation of a certain portion of it that bodies can be actually measured, while the rest of their properties could not be susceptible of

measurement if they were not somehow re-conductible to the extension (Guénon, 1995/1945: 31). Since the continuous is not pure quantity, a measurement will always present some imperfection in its numerical expression because the discontinuity of the numbers makes impossible a totally adequate application of it to the determination of continuous magnitudes (Guénon, 1995/1945: 31). This means that the extension itself is not exclusively reducible to the quantity (one of the defects of the Cartesian mechanicism). In order to be purely quantitative the space would have to be entirely homogeneous in a way that its parts could not be distinguished among them by any other characteristic than by their respective magnitudes. This would be like saying that the space is a container without content. Theoretically one could say that the geometrical space can be conceived as having such homogeneity. But this supposition is harder to accept when it comes to the physical space, which contains bodies whose very presence is enough to determinate a qualitative difference between the portions of space that they occupy (Guénon, 1995/1945: 37). This is what Bateson implied with the following rhetorical question: " 'What is it in the territory that gets onto the map?' We know the territory does not get into the map. That is the central point about which we here are all agreed. Now, if the territory were uniform, nothing would get onto the map except its boundaries, which are the points at which it ceases to be uniform against some larger matrix" (Bateson, 1972: 451).

Since mechanicism reduces the entire nature of bodies to their extension, it must consequently suppose that their presence does not effectively add anything to what it is already expressed by the extension. As we will see later, this is something absolutely far from being true, at least, in the "biological world". For the mechanicist the different properties of a body are simply modifications of its extension understood as purely quantitative. This means claiming that the surface and the volume of a given body, measuring the portion of extension it occupies, *are* the body itself, with all its properties. Otherwise one would have to admit that the extension itself has some qualitative aspect, in which case it cannot serve as the base of a purely mechanicist theory (Guénon, 1995/1945: 38). Similar reasoning can be done in relation to other physical determinations which are of purely spatial order (and therefore derivatives of the extension), as for example the "situation" of a body, which may be shown to be not exclusively quantitative since the distance between two bodies is not enough to define the (relative) situation of one of them: the "direction" is also needed, introducing a further qualitative element to the nature of space (Guénon, 1995/1945:

38). If that is the case, asks Guénon (1995/1945: 38), where can these properties come from if they were not somehow inherent to the extension itself? And how could they be inherent to the extension itself if the nature of this latter is not allowed to have any qualitative elements?

In elementary geometry there is not only the magnitude of the figures to be considered but also the *form.* Could a geometrician claim, for example, that a triangle and a square with the same surface are two identical things? He or she could only say that these two figures are "equivalent", tacitly implying "from the point of view of their magnitude"; but she would have to acknowledge that in relation to another "parameter", the form, there is something that distinguishes them, and if the equivalence in magnitude does not imply the similitude in form, this is because the latter is not reducible to the quantity (being the form definable by a set of direction tendencies determined by the tangents at each point of the continuum) (Guénon, 1995/1945: 39). Similar considerations about the qualitative nature of time are even easier to accept than the "qualification of space", given the continuous nature of the former.

At the extreme of this "dichotomy", quantity-quality (and, is it really a dichotomy?), the quantity becomes the residue of an existence which has been emptied of everything that constituted its essence, that which identifies such an existence as belonging to the species to which it belongs by virtue of its nature (Guénon, 1995/1945:14). Taken to its lowest point, this reductionism leads to a normative ideal of pure quantity, deprived of any qualitative distinction, which is of course unattainable, but it constitutes a limit towards which we would like to tend for scientific legitimacy. Such a limit is impossible to achiev because it would be placed below, or outside, of the actual manifestations under study.

This is what one has to bear in mind when one advocates for reducing "everything" to material-mechanical causality. One could very easily advocate for epistemological quantification, but is there really someone out there ready to negate altogether the idea of an ontological qualification, i.e. the idea that qualities do exist? I.e., the negation of the existence of qualities in our world, a world which of course is out there, I hope, but which we are constitutively obliged to reduce (construct) in our limited non-Laplacean mind?

In fact, the XVII century's mechanicism reformulated the atomist idea according to which flavours, odours, colours, etc., were not properties inherent to things

themselves, but characters that we project upon things by virtue of the subjective structure of our perceptive apparatus: only the geometrical-mechanical properties belong to the things themselves. This transfer of the quality to the perceptual apparatus may on the surface look like the "Umwelt" theory postulated by Uexküll (1982/1940), but here we have to face two complications: 1) would the perceptual apparatus have itself its own (built-in) qualities? 2) do the geometrical-mechanical configurations, regularly "translated" into qualities by equivalent perceptual apparatus (of, for example, individuals of a same biological species), constitute a quality by virtue of that regularity?

Does all this mean that strict materialists are idealists (Berkeley *sensu*)?

Does it mean that when I am talking to you, in reality you are just a huge bunch of mixed hexagons, pyramids, donuts, spheres and rhomboids, but the huge bunch of mixed hexagons, pyramids, donuts, spheres and rhomboids of my perceptual apparatus, tricks me into believing that I am talking to you?

The conception in which the entire nature of a body is reduced to its extension, and in which the extension is considered only in its quantitative aspect is meant to be applied to both "inorganic" bodies and living beings. Kant takes to the extremes the modern negation of any possible autonomy of the qualitative: the sensible qualities are only a particular genre of quantity. Similarly, Hegel advances the necessity of reducing any qualitative aspect to a more complex system of determinations, being the quality a poorer category with respect to quantity.

Descartes made the half of the world which he considered the most important entered into the quantitative realm. Later, materialism worked to push the entire world into this realm, eliminating in fact the Cartesian dualism. The reduction had to be presented as a reduction of "form" to "substance", of "mind" to "matter". It was just a matter of effectively implementing such a reduction through ever more appropriate theories and of establishing the goal to which the totality of science should dedicate itself: being able to bring everything down to the quantity, i.e.: being able to include everything in only one of the terms of the dualism (Guénon, 1995/1945: 99). In general, we can conclude that the modern tradition has tried by all means to devaluate the heuristic power of the "quality" category. One could legitimately ask: has it been successful?

1.2 The Laplacean dream

Positivism added a new dimension to the express-link between reductionism and material-mechanical causation: the quest for an exhaustive science, the idea of an asymptotic approximation to total knowledge. For many centuries the notion of omniscience has been highly influential and operative in the elaboration of multiple cognitive and heuristic strategies as well as in the representation of science and human knowledge, their possibilities and their limits (Ceruti, 1985: 25). This ideal has been so deeply established in common sense that it is considered as the necessary and natural way of scientific reason. One of the resulting premises that comes with this ideal is the belief that each increment in knowledge produces a correspondent withdrawal of ignorance. Therefore at the roots of modern science we find the idea of a knowledge that grows as an asymptotic approximation towards an infinite point of view that represents complete knowledge (Ceruti, 1985: 26).

In biology, the ideal to which we asymptotically thrive can be imagined as a huge mosaic with countless empty holes that, as science advances, are going to be filled in. From the reductionist point of view this mosaic is a huge taxonomy of constitutive species from which structures and functions can be derived with the help of some established physical laws. This taxonomy is not just a list of categories and species, it would need to be also an address-book, a (quantitative) census of every individual of every species since its exact location in space and time is what would allow us to derive, and control, the structure and function of higher categories.

In this view, the notion of "limit" is defined in relation to parameters such as "completeness", "exhaustiveness", "exactness", "precision" and "resolution", and these limits are (im)posed upon us only by defects in our epistemological tools which result in, or by, constraints to current technology itself. In this sense, the development of new technology and the filling-in of the exhaustive mosaic are mutually constitutive processes.

Today, as the advent of complex paradigms shows, this relation seems to be inverted: each gain of consciousness produces a shadow zone, and "the shadow is not anymore just what is outside light but, even less visible, it is produced in the very heart of that which produces light" (Ceruti, 1985: 32). In other words, to each increment of knowledge there corresponds an increase of ignorance, and to new types

of knowledge there correspond new types of ignorance. As stated by Morin (1984), complex ways of thinking can be formulated only from the moment in which a rupture with the idea of perfect knowledge is operated. Complexity is the contrary of completeness, argues Morin, and not its promise like many think. Complex thinking integrates the procedures of simplifying modes, which are desegregating and analytical "... the problem of complexity did not arrive arbitrarily or as the product of the vices of a tormented mind; it arose from the inevitable developments of modern scientific knowledge".

The normative goal of an exhaustive filled-in mosaic, a sort of exhaustive biospheric global genome and trophic web periodic chart, may seem as a naive interpretation of the reductionist research strategy. It may be argued that no serious scientist would believe in such a normative goal. However, a look into to the better financed and more influential international research programs makes the existence of such an ideal evident.

The mosaic matrix into which the identified components are to be filled in, ideally should cover all the constituents of the biosphere that exist at the present moment, so they can be fed into the "theory of everything" model that strict reductionism advocates. But this Laplacean dream is not only concerned with the horizontal (ecological) aspects, e.g. the present, and hereon initial conditions. When it comes to evolution, the mosaic matrix is just a state in a process. Laplacean exhaustiveness would seek to fill-in the mosaics of all the states since at least 4 billion years ago, a comprehensive full mosaic of the present being just a page, a state, of a long book, the process. Although no serious person would admit it, it would be nice to know exactly what happened, i.e. what were the constituents of the horizontal mosaic, say, on the 19th of April two billion years ago. The more time separates us from that moment, the more it seems compelling and necessary to know the details of such remote event, and, paradoxically, with everyday that passes by, in which we are supposed to be asymptotically closer to the exhaustive knowledge that will allow us to finally fill-in past gaps of the mosaic, we are also farther apart from the gaps we want to fill in.

In biology, the principle of analysing complex things into simpler more basic constituents has lead to the view that living processes can be explained (only) in terms of the material composition and physicochemical activities of living things (Nurse, 1998: 93). For example, ecology seeks to understand the structure and dynamics of

individual populations of plants and animals, of communities of interacting populations, and of ecosystems. May (1998) points out that ideally - the reductionist dream - it would be nice to build such understanding of how individual populations respond to disturbance upon a fundamental understanding of the behaviour and physiology of the constituent individuals (May, 1998:193). And in turn, the behaviour and physiology of organisms can be further reduced to its constituents.

The reductionist approach adheres to a methodology that recently, with the genome programs, has been renamed "discovery science". Most of the work is concerned with discovering the different molecular components, and there is hope that very soon we will come to know who all the actors are in biology. Knowing the structure will often provide the answer to other questions (Henderson, 1998: 37). If we start with the structure of biological macromolecules, we are interested in the size of the molecules, their behaviour in terms of aggregation or self-assembly, the secondary structure and atomic structure of the various molecular components. Some will go on "to attempt a full atomic simulation, using the energy functions to describe the inter-atomic interactions: the hydrogen bonds, the van der Waals forces and electrostatic forces. Once you are at this stage you can go on and on making increasingly precise calculations. If the explanation you are looking for depends on a balance between the energies of two states (e. g. an equilibrium between two conformations, or a folded versus an unfolded protein), and the energy difference between the two states is very small relative to the full summation that you are doing, it can be that you are caught out in a situation where the accuracy of measurement is insufficient". Once we know what all the components are and what they might do, we can then start looking for explanations and levels of answers. Once you have a structure and know the function you can make hypothesis without doing any more experiments (Henderson, 1998:37).

The next step would be to enquire about macromolecular assemblies and "machines" such as the ribosome carrying out protein synthesis, the replisome carrying out DNA synthesis, the macromolecular assembly which forms a phage and signalling pathways communicating signals from the cell surface to other components within the cell. The different macromolecules making up these assemblies and machines are usually studied *in vitro* as single components or as subsets of components in dilute solution in classical biochemical analysis (Nurse, 1998: 94).

The reductionist ideal in relation to the highest hierarchical levels of emergence, as for example the human "mental process", is still believed to be the most promising strategy as exemplified by the following statement:

> "New neuroanatomical components that one had no idea about are being described simply by looking at where specific proteins are distributed in the brain. My guess is [M. Raffs'] that the reductionist approach, even where it is just a fishing expedition, will lead to real understanding in unpredictable ways, and that the molecular and cellular basis of memory, learning and other higher brain function could well emerge bit by bit, until the mystery gradually disappears, just as has been happening in developmental biology" (M. Raff, in the discussion of a symposium paper by W. G. Quinn, 1998: 124).

Commenting upon the kind of refined details about muscle contraction found in the work of Ken Holmes (1998), Lewis Wolpert (1998: 1) remarked: "When Ken told me how much he knew about muscle, the thought occurred to me: how much more does he want to know? Does he want to know about muscle action at the level of quantum mechanics?" In the same vein he comments a lecture in which it was stated that 5% of the genes in our body encode tyrosine kinase receptors and that half the genes that we have are involved in intracellular communication: that is, membrane transduction. At this points Wolpert asks himself "how are we going to understand what is going on? And do we really want to know all that is going on? In other words, to what level do we want to reduce all this complexity?" (Wolpert, 1998: 1).

These questions brings to the forefront the issue of "levels of organisation" and with it the problem of how different levels can be related or causally integrated. In relation to the details already known about muscle contraction, Steven Rose (1998) suggests considering the relationship between physiology and biochemistry.

> "Physiology studies muscle contraction, biochemistry the molecular processes that occur during this contraction. The biochemistry of this process is pretty well understood down to some of the minutest molecular details. So why can't we just replace the physiologist's statement about muscle contraction with a statement about actin, myosin, etc.?
> If the purpose of doing so is to claim that the biochemistry is causally responsible for the physiological event, this is a very different use of the word

> 'cause' from the way it is normally employed to describe a relationship in time between cause and effect, in which the proximal cause of the muscle twitch is provided by the physiological description of impulses travelling down a motor nerve to the muscle. The biochemical process does not *precede* the muscle contraction; it *describes* the muscle contraction. We are really making not a causal but an *identity* statement ... The *meaning* and *function* to the organism of the muscle twitch is apparent in the physiology and anatomy, but quite absent from the biochemistry." (Rose, 1998: 177).

Rose asserts that this kind of reduction can very often lead to a "misplaced sense of causality", like the one that very often operates in neurogenetic determinism. This example can be easily generalised to many instances in which higher level processes are meant to be reduced to molecular dynamics, where the biochemical picture of the process is a necessary picture but hardly a full explanation of it. It is in this sense that in many cases mechanicism in biology may have more a "representative" value than an "explicative" one. That the "meaning and function" of complex molecular interactions can be found at higher levels of complexity hints to the fact that a material-mechanical causality (dyadic causality) is enough to characterise a given process at a mechanical level, but its relevance at a higher emergent level entails a different kind of causality, which later in this work will be defined as "triadic causality".

Besides "misplaced causation", Rose (1998: 179) also refers to some other steps in the reductionist strategy which pose problems or limits to it. He speaks of "reification" by which he means the conversion of a dynamic process into a static phenomenon, like for example a phenotype, especially when considering behaviour as a phenotype. Reification transforms the process into a fixed thing which can be abstracted from the interactive system in which it appears and studied in isolation, i.e.: the process is isolated from its *context.* By agglomerating different reified interactions we may tend to reduce the process to monotonic variables. This leads in some instances to "improper quantification", which argues that reified and agglomerated characters can be given numerical values in the belief that to mathematicise something is in some way to capture and control it (Rose, 1998: 180).

Talking about the integrative role of physiology with respect to genomics, molecular biology and evolutionary biology (and acknowledging a polemic and

"purple" prose, which may be necessary to "restore the balance in a debate that has swung a pendulum far too far in one (reductionist) direction"), Noble (1998: 66) echoes Sir James Black's remark that the future lays in the "progressive triumph of physiology over molecular biology" and he attempts to turn the selfish gene argument on its head "to depict genes more as 'captives' of the successful physiological systems that carry them from one generation to another". He then attenuates his remark by asserting that of course physiology cannot 'triumph' over anything, least of all over a valuable tool like molecular biology; "it can only interpret it". In this sense he asserts:

> "... if the genome contains all the 'information' for physiological function to emerge, then physiology contains all the 'interpretation' necessary to understand the genome. Some have even gone so far as to emphasize this symbiotic relation by calling the quantitative description of physiological function the 'physiome'... The genome, molecular biology and modern evolutionary biology have all injected new life into physiology. Integration is much more powerful than it could ever have been without these tools and the vast databases of information that they have created. And powerful computing has arrived at just the right time to enable us to exploit this opportunity" (Noble, 1998: 66-67).

It has to be observed at this point that very commonly integration is imagined as a problem of more powerful algorithms and computational tools, and very seldom it is thought in logical terms. The logic remains invariably the addition of complex dyadic relations. Of course there are many things that could not conceivably be done without computers, but when integration becomes a logical problem more computer power becomes hopeless. This issue is a recurring motif in contemporary biology and it could be interpreted as a sign of the pervadingness of the Laplacean dream.

According to Nurse (1998: 98) it will be an immense if not impossible task to adequately describe cellular phenomena in terms of a precise description of all the molecular interactions involved. There is already an information overload in cellular and molecular biology with many molecules identified but with the underlying processes much less understood. He suggests some interesting possible conceptual approaches to provide alternative levels or types of description which may produce adequate explanations of cellular phenomena without a full molecular characterisation.

The same things about the integrative role of the "physiome" can be said about other hierarchical levels, for instance, the ecosystem. For example, Ulanowicz (1997: 6) sees ecology as the ideal domain in which to pursue the study of organisational principles since in ontogeny, organisational influences per se are overshadowed by the mechanisms of transcription from genome to phenome. He suggests that the search for organisational principles in ecology may bring conceptual insight into evolutionary phenomena, developmental biology, the rest of the life sciences, and even physics. He points out that ecologists, as biologists, relegate the generation of cause to the netherworld of molecular phenomena; once at the scale of cells, organisms, and populations, they imagine themselves again in Newton's realm of strict determinism. To follow the process of neo-Darwinian evolution we are continually forced to shift perspectives abruptly from the stochastic world of Ludwig Boltzman, where new genetic combinations arise, to the deterministic arena of Isaac Newton, where only those organisms with the fittest genes can be counted on to survive. At this point he asks: is causality in nature really that schizoid? And he asserts that we try to overcome this schizophrenia by pushing the machine analogy deeper into developmental biology, relying on "molecular machines" and "mechanisms" in ontogeny with the desire of being able to arrive to a strict mapping between genome and resulting phenotype (Ulanowicz, 1997: 4).

1.3 The reductionist goal of exhaustive "material information"

There were two concomitant major trends in 20th century biology. On the one hand there has been the strategy of molecular and genetic reductionism giving rise to the molecular biology revolution and its well known successes, and on the other there has been a less apparent trend that could be characterised as "the semiotisation of nature" (Hoffmeyer, 1997b). Since the birth of molecular biology this latter trend has evolved for the most part in a spontaneous manner by adopting a whole new vocabulary which was being imported from the concomitant rising discipline of cybernetics (or information theory). The problem was that the way the term "information" was being understood in biology had little, if anything, to do with the original sense ascribed to it in information theory. However, the importance of these concepts was such that contemporary historians and philosophers of biology contend

that the notion of "information" has allowed us to mark the limits between molecular biology and biochemistry (Segal, 1998).

After fifty years of "informational talk" we find an entangled set of concepts and terms such as gene expression, information transfer, recognition, signalling factors, quorum sensing, analogues, replicons, functional mimicry, decoding, cross-talk, non-trophic interactions, etc. All these terms imply an understanding of causality which has had difficulties in conforming to the view of nature established in classical physics and dynamics because the "information" implied in biological context entails a semiotic process. The implementation of semiotic vocabulary by scientists without realising its semiotic nature is what Emmeche (1999a: 274) has called the process of "spontaneous semiotics" in molecular biology. Emmeche (1999a: 276) points out that these concepts keep reappearing in molecular biology not just because of their metaphorical qualities which make them convenient devices for exposition of existing knowledge, but because a great deal of molecular biology has to do with communication and sign interpretation within and between cells.

Both trends, molecular reductionism and spontaneous semiotisation, have had an ongoing influence in many branches of biology, from cell biology to ecology. However, very often, these trends have not been assumed explicitly and therefore theoretical confusion and epistemological problems may ensue. In other words, biologists operating at all hierarchical levels of organisation, from cell biology to ecology and biocoenology, should be aware of how these trends are influencing their disciplines and should therefore be able to count with a theoretical framework that does not make the process the mere result of spontaneity.

It is not by chance that spontaneous semiotics has been stronger in molecular biology than in other subdisciplines. This is the case because molecular biology is hierarchically considered the level where the informational processes begin (by DNA-centric genetic determinism). As molecular biology (through the reductionist strategy) exerts its influence on the rest of the subdisciplines, it brings along the epistemological ambiguities that result from the ongoing process of spontaneous semiotisation. It may turn out that physical reductionism is not sufficient to tackle the "informational" processes in living systems, and that the molecular level is not exclusively where these processes begin in a bottom-up sort of causality. The complexity of communication and semiotic processes give rise to emergent properties which in turn may exert a downward causality on lower levels of the hierarchy. Since

these semiotic processes do not act exclusively horizontally at a given level, but on the contrary conform semiotic networks of upward and downward causality, both in the ecological hierarchy and in the genealogical (evolutionary) hierarchy (Salthe, 1993), an explicit biosemiotic theoretic framework that deals with the "informational" processes along these hierarchies may be useful.

These two trends have also played a role in the recent development of technologies derived from the life sciences. First, we can perceive the influence exerted by molecular biology in many branches of the life sciences - technically and conceptually - as the natural outcome of the successes of the reductionist strategy in the last century. Second, in relation to this trend, we can also see the predominance of the notion of "biological information" and many other "semiotic" terms in the technological developments. The result of this state of affairs is, as we will see, a paradoxical notion of "material information" which is claimed to be the essential raw material for a broad technological control over living systems from cells to ecosystems.

The general concept behind most of modern biotechnology and particularly behind DNA technology allegedly has been that of biological information normally ascribed to so-called informational molecules, mainly DNA or RNA. But as biotechnology has reached new levels of complexity, that concept of biological information has become very narrow because any information besides the sequence of a single molecule remains unspecified. Yet, informational terms keep reappearing at all hierarchical levels and subdisciplines of biology. The capacity for specification of amino acid sequences cannot be a self-contained property because it will nearly always (i.e. when real living systems are involved and not only cell free systems) depend on context. There is no simple causality connecting genetic information to a given organismic output. This raises new problems for the fundamental concept of "information" behind these techniques. One of the basic claims of the present work is that the technological trend is moving away from a focus on information (as a material agent of causality) to a focus on signification and interpretation processes.

In this sense it will be useful to summarise some key issues of the epistemological status of the concept of "information" in the history of molecular biology. I take molecular biology as paradigmatic for the rest of biology because the notion of "information" has played a central role in its development and because molecular biology has acted a strong influence in most subdisciplines of biology. It is

in fact through this subdiscipline that the information notion is re-entering, for example, into ecology (with exceptions of early attempts of using information theory in ecology as for instance the pioneering work of Margalef (1968) or the more recent and original work of Ulanowics (1997)).

It can be said that this debate is as old as molecular biology itself. What I will put forward here is inevitably a very sketchy outline since the debate has been long and very varied and it would be impossible, and also unnecessary, to pretend to be exhaustive. (Some interesting historical-epistemological accounts can be found in Emmeche (1989), Emmeche and Hoffmeyer (1991), Heims (1991), Sarkar (1996), Segal (1998), Kay (2000)). It may be a curious anecdotic fact that it was Warren Weaver, an information theory scientist, who in 1938 coined the term "molecular biology" (in his yearly report as the head of the "Natural Sciences Division" of the Rockefeller Foundation) (Segal, 1998).

The debate on the concept of "biological information" has so far proceeded in an inductive manner, different concepts having been developed autonomously at specific levels and applications. The only epistemological tool that has been used across the different instances and subdisciplines is the mathematical theory of information. But the specific level that has received most attention is probably the genetic level instituting the long debated concept of "genetic information" in which the mathematical theory of information in the end showed up to have little application. Problems arise when dealing with the specification of the emergent levels that proceed from, and simultaneously surround, the genetic one. In a "scalar" view, the next step is that of regulation, in which different kinds of "information" enter into the scene and interact with the genetic level (and will necessarily have to interact with other emergent levels).

Perhaps one of the main organising hypotheses at the beginning of molecular biology was the "one gene - one enzyme" hypothesis supported among others by John B. S. Haldane since 1937 (Segal, 1998). By the end of the 1930s the principle of "specificity" was well established. What mediated biological interactions was a precise "lock-and-key" between the shapes of the molecules. This gave rise to the notion of "information" as specificity. In a thorough analysis Sarkar (1996: 190) implies that after 50 years of debate on the "information" concept in molecular biology what in reality has survived is the stereochemical specificity as suggested by

Pauling and others at the end of the 1930s (though with many antecedents; see Kay, 2000: 43).

In 1941, as Oswald T. Avery and his co-workers where searching for a molecular explanation for the transformation of pneumococcus, they found out that "genetic information" was not in proteins. The "transforming information" was carried by DNA. Caporale (1998: xiii) points out how remarkable it is that DNA was discovered as *something pneumococcus took up from its environment*, and changes its descendants. A detail that for the most part remained as secondary but which has acquired especial relevance in the present debate about the "dynamic genome space".

In 1944 Erwin Schrödinger made a conceptual connection between information theories and knowledge about cellular macromolecules into what many think was the basis of molecular biology (Gros, 1989: 31). However, he did not participate in the genesis of information theories. Here I use information theories in plural to stress the existence of an epistemological movement that was enquiring into the concept of information, and not just to one theory, although the Shannon/Weaver theory and the thermodynamics and statistical mechanics related to it were at the forefront (see Heims, 1991).

In the 1950s the "coding problem" was of central importance in molecular biology research. Georges Gamow lead a multidisciplinary group that emphasised the distinction between the abstract coding problem, "that of translating a four letter code to a twenty letter code", from that of finding the mechanism of translation (Sarkar, 1996: 193; Kay, 2000).

As stated by Francois Gros (1989: 33), biology, that initially had been a science of inventories and classification in the XIX century, then a statistical science with the first genetists, and finally a powerful analytical discipline with the biochemists before and during World War II, became after the war a science of codes and circuits. Since then it "became strangely related to micro-electronics and informatics" (Gros, 1989: 33-34).

In 1953 Ephrussi and his co-workers suggested to replace the terms "transformation" and "transduction" by the term "inter-bacterial information" in what seems to be the first modern use of "information" in genetics. This approach emphasised that information "does not necessarily imply the transfer of material substances, and recognise the possible future importance of cybernetics at the bacterial level" (Sarkar, 1996: 191). In 1958 when the term "information" was already

accepted as part of the standard conceptual framework of molecular biologists, Francis Crick (1958) pointed out that three separate factors were involved in protein synthesis: “the flow of energy, the flow of matter, and the flow of information”. This was in accordance with the famous statement made 10 years earlier by the founder of cybernetics, Norbert Wiener, who argued that information is information, not matter, nor energy. However, this crucial statement was not - and maybe has not yet been - taken to its consequences in life sciences.

Since then, according to historian and philosopher of biology Sahotra Sarkar (1996), there have been three main ways in which “information” has been construed in molecular biology which can be summarised as follows :

1) Codes, templates, and the Central Dogma

2) Cybernetics and gene regulation

3) Information theory

(Please notice that these three ways have not developed independently from each other).

The first approach produced what, together with recombinant techniques, is the essential tool of modern biotechnology: “the look up table” of triplets and amino acids. The net result of this view has been that biological information is equal to base order in DNA. Information is DNA. (For a comprehensive account of the history of “the code” see Kay, 2000).

The second one has played a relevant role in the explanation of “inducible” and “inhibitable” metabolic processes and enzyme systems. In this case information is said to be *that which provides feedback*. Although this gets closer to the definition of information to which I want to refer to, it still remains limited to enzyme systems. The cybernetic approach is an essential part for the explanations of enzyme systems’ dynamics (feedback regulation, control, induction and inhibition) as described for example by the “operon” model. The resulting picture is one in which the organism is viewed as a hierarchically organized cybernetic system. Sarkar also points out that more recent attempts to overcome a trivial definition of cybernetic systems attribute internal states to these systems and give them mechanisms of self-regulation and make all of these externally accessible.

In Jacques Monod’s view there is an acknowledgement that chemical interactions determine the behavior of the operon, but these interactions do not explain the behavior of the system responsible for control. Such controls “confer heightened

coherence and efficiency upon the cell... the very gratuitousness of these systems... enabled it to elaborate the huge network of cybernetic interconnections which makes each organism an autonomous functional unit, whose performances appear to transcend the laws of chemistry if not to ignore them altogether" (Monod, 1971; in Sarkar, 1996: 207). Monod's definition of "gratuity" - "the independence, chemically speaking, between the function itself and the nature of the chemical signals controlling it" (Sarkar, 1996: 206) - is exactly what is implied by the emergence of a code, or one of its main characteristics, i.e.: its (logical) independence from the chemical nature of the substrate. This concept is very much related to the existence of a superimposed logic to the dyadic logic of material-mechanical causality. Plainly said, "gratuity" implies triadic logic.

The third approach, although still present in theoretical discussions, has been said to be of little value, so that even in the 1950s its proponents had begun to question its use (Sarkar, 1996). Nevertheless it played an important social and inspirational role in the development of molecular biology. It has more to do with a mathematical measurement of uncertainty than with information in the way it is commonly thought about in biology, which instead implies a "semantic value" of information and not just the "quantity" of information. In fact, one of the problems in the application of information theory to biology has been that the theory has been used mainly to quantify "contents" of information in a given process or entity (e.g.: in a protein, in a fertilized egg, in the entire process of organic evolution) and has not served as a logical framework for the informational interactions within and between those entities in those processes. An alternative view to information theory could reveal that the definition of "information" has more to do with the characterisation of the context than with the quantified "content" of information, which becomes in this way reduced to a monotonic value in a given entity or process. It is this monotonic value that has had difficulties in finding applications in biology. However, in the last decade information theory has proved to be a very sharp tool for bioinformatic applications.

The important point is that information is not matter and therefore DNA per se is not information, it is a substance, i.e.: matter. And as pure matter, DNA does not contain the key to its own interpretation. In a way the molecule is hermetic (Hoffmeyer, 1997b). As mentioned above, problems arise with signal transduction networks and regulation, where we can see the unconscious emergence of a concept of "natural regulation", analogue to "natural selection", i.e.: selection without a

selector, regulation without a regulator, with the same problems and conceptual ambiguities. In the same way natural selection is something that exists but is not (physically) there, "regulation", as the mechanism that orchestrates and directs (i.e. interprets) the signals represented by molecules that bind to each other in specific ways when their concentrations are statistically relevant, starts to look as something that exists, whereas nobody knows *where* it exists. May be it was in this sense that Rene Thom claimed that "... information [if not considered in its semiotic context, I may add], is the obscure form of causality" (quoted in Segal, 1998). This represents a big challenge, not only to molecular biology but also to a semiotic explanation of it: the emergence of the interpretant, a problem to which we will necessarily return.

When it was thought that the information "problem" was solved and put aside with the cracking of the "genetic code", biologists began talking again about cracking other "codes". In this spontaneous inductive strategy (within the "spontaneous semiotics" in the life sciences described by Emmeche, 1999a: 274), different types of "information" keep emerging, which may not have a clear conceptual link with previous concepts of biological information. So the need for unifying concepts prevails together with the lack of proper interfaces to couple the different "codes" that are being inductively "cracked" and defined at the different emergent and "de-emergent" levels of the hierarchy. The informational terminology continues its exponential growth, but now, as biosemioticians had foreseen, we perceive an incipient trend that moves away from a focus on information to a focus on signification.

After deciphering what came to be known as the "genetic code", new problems challenged the "information" concept. In 1962 for instance, the Austrian-American biochemist Erwin Chargaff noticed that in spite of the fact that biological information might explain the highly specific relations between nucleic acid and protein, there was skepticism that it may give any insight into the equally specific relations between cells and multicellular communities:

> "If there was no continuous 'chain of information' from the lowest level to the highest, he argued there was no justification in claiming that 'DNA is the repository of biological information' " (Sarkar, 1996: 199).

Although this argument was raised to rebut the usefulness of the notion of "biological information", in reality it only, and very strongly, rebuts the exclusivity of DNA as biological information (or more precisely as the physical support for information). The argument also poses a very interesting and central challenge to contemporary biology: how can we conceive the "continuous chain of information from the lowest level to the highest" and perhaps from the highest to the lowest? I consider this the guiding question for a sign-theoretic approach to biotechnology.

In spite of all the known exceptions and knowledge gaps of the "code" (during transcription, translation or editing processes) that have been reported (e.g.: the non-universality of the code, the effects of frameshift mutations, the boundaries of coding and non-coding regions, the different types of mRNA editing), it still gives, together with bioinformatics, a very sharp tool for biotechnological applications. But as pointed out by Sarkar, the (genetic) "code" deals with a static context, it does not say anything about *dynamics*, the temporal progress of gene expression, that is, control and regulation. Sarkar claims that if the actual prediction of biological behavior through an interval of time is to be considered, the "code" theory would need to be supplemented by some theory of dynamics. And to the question "can considerations about information provide such a theory?" his answer tends to be skeptical. According to him a dynamical account, whether it is "physicalist", "informational", or whatever, will eventually be necessary if even approximate accounts of gene expression, interaction, cellular behavior and development of complex organisms, are to be pursued at the molecular level. But his conclusion is that the failure of explanations involving codes and information, and the success of the usual reductionist explanations in molecular biology suggest to abandon the former and pursue physicalist reductionist accounts of the interactions between DNA, RNA and protein systems as a network of chemical reactions using systems of linear differential equations to describe the process. But have explanations involving codes and information really been such a failure? The dialogue between theoretical biologists, mathematicians and cryptologists may sometimes has appeared as aimed at itself and many times did not lead anywhere, but that debate was probably indispensable in order to arrive at the solutions of the "coding-problem" that constitute modern biotechnology. So abandoning the informational domain in biology due to the lack of theoretical tools does not seem fruitful if you consider the results of that rich debate

so far, especially now that the notions of codes and information are again spreading throughout most subdisciplines and hierarchical levels of biology.

Nevertheless, Sarkar makes a good survey of the influence and fruitfulness of the informational concepts in the development of molecular biology. He arrives to his suggestion of abandoning the information language after surveying many points of view which mostly treat information as mechanical instructions (for amino acid residues), or pose questions about coding, or equate information to feedback, or ultimately insist on Shannon's mathematical information theory. However he does not mention any approach to the information concept that addresses the context-dependent nature of biological information in molecular and cellular processes, i.e.: biosemiotics, part of the epistemological landscape that throughout the 20th century has determined that trend in biology which has been characterised as the "semiotisation of nature" (Hoffmeyer and Emmeche, 1991). For instance, based on a research tradition of almost one century, in the last three decades biosemiotics has proposed a different and more integrative direction for the discussion about information within biology.

Approaching the complexity of "non-linearity", when attempting a passage from "one gene-one enzyme" approaches to developmental paths, with a complex (and complicated) account consisting of a huge set of interrelated differential equations, based on biochemical reaction rates and concentrations, does not necessarily have to be mutually exclusive with a cybernetic account that does not renounce the use of the "biological information" concept, but tries to improve its definition. In any case, the latter approach would surely give a different picture of that complexity, an overlapping picture, which may be useful for different purposes. But when dealing with biology, a cybernetic approach has to be by necessity linked to a biosemiotical approach. As we will see, biosemiotics supersedes the cybernetic account by considering the continuous mutual translation between digital and analogical information in living systems, while keeping track of the relation and dependence of those "signs" with their context.

According to Claus Emmeche (1999a), the skepticism that comes from Sarkar's remarks (about the usefulness of informational considerations that tend to somehow overcome the reductionist-physicist approach), pose a real challenge to biology and particularly to biosemiotics. Emmeche argues that if one cannot, from an old-fashioned positivist or physicalist point of view, see any legitimate use of or need for

semiotic concepts in the attempt to explain life's most basic mechanisms, biosemiotics could at least provide a more comprehensive view of the world than the one provided by physics and chemistry (when interpreted in a physicalist or an instrumentalist way). Otherwise it could point to certain fractures, paradigmatic anomalies and unsolved riddles within the fields of molecular, developmental and evolutionary biology in which complexity would block the way to the most optimistic hard-nosed reductionist explanations. But he also envisions a more moderate position that sees biosemiotics as a tool-box that in certain domains of biology may help to organise our knowledge better, pose more interesting questions, and make alternative testable hypothesis, even though it may not take the role of an alternative paradigm. It is this moderate role that I will be advocating in the present work in relation to complex systemic issues in biotechnology, such as for example biosensing, biosafety or biocontrol.

Through the path of "biological information" we arrive to the "genomics age" where genomes organisation, expression and interaction are the central issue. Whereas the 1990s have been characterised by genome projects that have called for massive data processing solutions, the next step will be understanding the results. Statements like the following are not uncommon: "It is estimated that biological knowledge is currently doubling every five years, and in the field of genetics, the quantity of information is doubling every twenty four months. The commercial possibilities, say the scientists, are limited only by the span of the human imagination and the whims and caprices of the marketplace." (Jeremy Rifkin, quoted in Tarcher and Putnam, 1998). From many sides arrive warnings of an "overflow" of scientific data and the difficulty of integrating such quantitative sum of details into meaningful frameworks.

> "The output from the molecular biology revolution has grown steadily and logarithmically from the first protein sequence, insulin, the first three-dimensional atomic structure of a macromolecule, myoglobin, the first DNA sequence, øX174 gene J, and the first genome sequence for a free-living organism, *Haemophilus influenzae*, to the current situation where the output rate is close to one new gene sequence every few minutes, several new three-dimensional structures a day and a new (bacterial) genome completed every few months." (Henderson, 1998: 36).

> "The unravelling of [the] genetic code, of molecular structure, of subcellular mechanisms, has been so breathtakingly rapid in creating a mountain of detailed information that integrative work has barely had time to define the problems let alone tackle them on the scale required. Nevertheless, the integrationist agenda is being defined" (Noble, 1998: 56).

What can we say about higher hierarchical levels? At the ecological level

> "the reductionist ... approach to reconstruction and assessment of the synchronic dynamic equilibrium of the biosphere (ecology) becomes experimentally impossible and conceptually insufficient. A more consistent model can only be obtained by postulating the molecular level as a component that does not exhaust the complexity and order of the higher levels, and accepting the possibility that the order of biological systems can increase by leaps and bounds following the appearance of emergent properties. In other words, the model is plausible whenever the genetic information does not exhaust all the information and co-information of the biological systems (cells, organs, organisms, populations)." (Scannerini, 1999).

It is becoming common currency to hear that "biotechnology is the industrial use of biological information" (leading biologist Lee Hood quoted in Pongor and Landsman, 1999). It has to be recognized that in this context the concept still remains elusive and therefore it continues to be subject of ontological and epistemological concern. However, "biological information" may turn out even to be a key element in the "integrationist agenda" or in any attempt to "operationalise sustainability".

How is "biological information" dealt with in the "genomic age"?

At the present stage of the debate, there is a huge sector in contemporary biology that advocates for interdisciplinary approaches in order to unravel complex biological codes. It is said that after having studied individual proteins and genes in isolation throughout the last four decades, the future lies in the study of the genes and proteins of organisms in the context of their informational pathways or networks. The nature of this interdisciplinarity reflects the ambiguous epistemological status of the concept of "biological information" on which these approaches are based. Honestly, one should admit there is not yet sufficient epistemological clarity in a such status. As

mentioned before, some very acute defenders of the reductionist strategy have suggested to totally drop any vestige of informational talk in biology since such lack of clarity does not add anything good to the explanations (see for example Sarkar, 1996).

From different directions - e.g.: novel research on "quorum sensing" (a transcription regulatory network link to inter-bacterial communication); the central role of signal transduction in molecular cell biology; the importance of non-trophic interactions in ecological studies - we can perceive how the concept of biological information is changing from its widely accepted and reductive meaning of DNA-based-genetic-information, to address new emergent levels in which the "context" becomes a priority. Here, we encounter complex cocktails of "bacterial pheromones" that researchers begin to consider like new codes to be "cracked", new languages to understand, metabolic codes at different integration levels of cells and organisms and semio-chemicals through which plants communicate. A more detail elaboration of these examples will be presented in the next chapter.

Besides these interesting developments some of the most successful approaches in terms of funding and acceptance in the mainstream include approaches in which the concept of "biological information" is treated in a very ambiguous way. May be the reason is, following Sarkar's arguments, that, in these cases, "information" is only loose talk and does not have any real bearing on the research strategy, which remains at the level of molecular reductionism. Let us take as example "systems biology", the influential approach advanced among others by Leroy Hood (one of the first scientists to advocate for the Human Genome Project and credited for having played a lead role in inventing automated DNA sequencers in the mid-1980s). In Hood's view, "systems biology" is interested in analysing whole systems of genes and proteins. Its central and most highlighted slogans are that "biology is an information science" and that "biotechnology is the industrial use of biological information" (Smaglik, 2000; Pongor and Landsman, 1999). There are several epistemological flaws in the way "biological information" is treated in this approach, but may be the most evident one is the confusion between information handled by organisms and information handled by the observer.

In a *Nature* interview (Smaglik, 2000) Hood defines "biological information" in a variety of ways that constantly interchange the different kinds of information involved in biological processes with the different kinds of data that a researcher must gather in

order to characterise a system (which is also information, but of a different logical type). He explains that “systems biology” uses tools for capturing information from all the different biological levels - DNA information, RNA information, protein information, protein interaction information, pathways and so forth, and this information has to be afterwards integrated. The ultimate objective is to write mathematical models that are capable of predicting something about the structure of the biological system under evaluation as well as predicting something about its properties, given particular kinds of stimuli or perturbations. In this sense information means simply data. It is not necessary here to invoke a concept of “biological information”, unless we agree that we mean “scientific information on biological facts” just like we would say “geographic information” or “public information”, all kinds of cultural information.

Hood asserts that biological systems encompass several different levels of information. On one level, a cell can be characterised by recording the genes that are expressed at any given time, but to understand the cell as an integrated biological system, you also need to study the structures of the proteins the genes encode, and the interactions of these proteins with each other as well as with other genes. And he acknowledges that "At the higher levels, things happen that you can't predict at the lower levels".

Without making explicit the equation “biological information” = “scientific data”, Hood asserts that there are three fundamental types of biological information:

1) First we have the one-dimensional language of DNA, with its four-letter alphabet. Here the fundamental units of information are the individual genes, most of which encode the second type of biological information: proteins.

2) Proteins are strings of letters derived from a 20-character alphabet. Proteins are synthesised as linear strings and the order of protein letters in each protein string direct how the string folds to generate three-dimensional molecular machines (although there is experimental evidence of the involvement of “other” proteins in protein folding that seems to conflict with this universal hypothesis, see for example Eder and Fersht, 1995). The hundreds of thousands different protein machines catalyse the chemistry of life and give organisms shape and form.

3) The third type of information arises from biological pathways and networks - groups of genes or proteins that work together to execute particular biological functions. These biological networks give rise to systems or emergent properties such

as memory, consciousness and the ability to learn. “Systems biology” requires that all of the gene or protein elements in a particular informational pathway be studied simultaneously to follow the informational flows - if we are ever to understand the system’s properties.

What is wrong with this concept, or concepts, of “biological information”? The first two types of information are two kinds of physical entities, genes and proteins, and as physical entities they are not more informative than lipids, ions, water, whole organisms, cars or sculptures. The third kind of information seems to be of a different logical type: particular informational pathways formed (structurally?) by the first two kinds of “information”, groups of genes and proteins “which have to be studied simultaneously to follow the informational flows - if we are ever to understand the systems properties”. So the third kind of information is the informational pathway itself in which information flows and which is structurally (i.e.: physically) constituted by other two kinds of (material) information. What is it that flows? Can the informational pathway itself be information to someone different than the researcher?

This kind of informational talk is a very common line of argument currently being advanced to extrapolate the successful reductionist-physicalist-mechanistic research strategy behind the HUGO project (and other whole genome projects) throughout the different emergent levels of the biological hierarchy up to ecosystems. Hood asserts that the idea that “biology *is* information” is a wonderfully integrating concept that theoretically should permit us to view biology, from molecules to ecologies, as an integrated whole. I agree totally with him on this, but are these contradictory concepts of information really suitable for such a necessary integrating role? Or are they inadvertently only a disguise to avoid a true integration and simply redefine the reductionist strategy in a massive quantitative search for exhaustivity? Is complexity once more being reduced to quantification?

Hood acknowledges that if one thinks about networks strictly in terms of biochemical mechanisms, things can get enormously complicated, but it is possible to ratchet it up to a higher informational level (again, in terms of data for the researcher) and look at how quantitative expressions of proteins or RNA change as you perturb systems. It is claimed that this would tremendously simplify the system and yet allow us to learn about it in very deep ways. The many different levels of information that ultimately must be integrated can be captured through the use of high-throughput

biological tools such as large-scale sequencing, genotyping, DNA arrays and proteomics analysis (novel mass spectrometry techniques for the relative quantification of proteins in complex mixtures and for the investigation of changes in protein expression profiles). Some systems biologists aim to characterise every aspect in a system as fully as possible, then construct the cellular equivalent of a wiring diagram — using differential equations to describe the interactions between each node. Others believe that, because cellular systems are constantly changing, such a linear approach will fall short of providing a full description of a biological system. Instead, they are putting their faith in analysing how different components, such as messenger RNA (mRNA) levels and protein expression, relate to each other under various conditions, and then using probabilities to recreate these relationships. The idea is that this better captures the dynamic, non-linear workings of a biological system.

It is argued that measuring both gene expression and protein production paints a clearer picture. Just measuring levels of mRNA, as is typical in gene-expression studies, tells scientists that a gene has been activated, but does not detail the amount of protein it encodes, or whether that protein is functional. For example, scientists can detect thousands of genes involved with the switch of a given metabolism (such as galactose metabolism in yeast). They then use a series of algorithms to group the mRNAs produced by these genes into families, based on common patterns of changes in the knockout experiments. Finally, they integrate the mRNA and protein data into a simple mathematical model that illustrates how the organism adapts to the changing condition (i.e. how yeast adapts to use galactose rather than other sugars) (Smaglik, 2000).

So far, no informational processes have really been considered in order to add something to the characterisation of the system. As things get more complex there is always the hope for developing better computer algorithms and more powerful hardware. This is the core of the interdisciplinarity that these approaches imply. So seeing biology as an informational science simply means seeing biology as a computational and data-management science, emphasising the convergence of what Hood rightly claims will be the two dominant technologies of the 21st Century, information technologies and biotechnology: “The tools and insights of one can increasingly be applied to the other”. It is in this sense that “systems biology” resorts to a multidisciplinary team to blend biology with the tools of mathematics and

informatics to provide a complete description of biological processes and systems, both before and after chemical or genetic perturbation, and to establish computational models that are predictive of the behavior of the system or its emergent properties in response to those perturbations.

The rise of "systems biology" comes from an epistemological approach that its proponents have named "discovery science", exemplified by genome sequencing projects. "Discovery science" is supposed to enumerate the elements of a system irrespective of any hypotheses on how the system functions. You start by defining all the elements in an object, irrespective of any questions you might want to pose about the object. That is, you sequence all of the bases in the genome or you describe all the proteins present in a cell. Or as stated by Henderson (1998: 37):

> "Much of our work is necessarily concerned with discovering the different molecular components, because we don't yet know who all the actors are in biology, although it won't be long until we do because of the various genome programs underway. Once we know what all the components are and what they might do, we can then start looking for explanations and levels of answers".

But, can we define the elements of an object without a previous conception of it, if no other a conception of its integrity as an object? Do we really have to wait or need to know what "all the components are and what they might do" in order to understand something? Should we wait until the whole mosaic is full to find the ultimate explanation? What about "ratcheting it up" to a higher informational level? Does it have to be by quantifying components at the molecular level or could we eventually rely on monitoring the emergence of patterns and habits at different hierarchical levels? But above all, would it be of help to know all the actors in a megaproduction if we do not understand the languages in which they play?

The idea seems to be to create an enormous infrastructure of information which then will enrich hypothesis-driven science. "As it generates data on scales of complexity and volume unprecedented in biological sciences, defying analysis by normal means of interpretation, presentation, and publication, discovery science depends on the integration of computational tools to store, model, and disseminate these exploding cascades of information" (Leroy Hood quoted in Smaglik, 2000), but

understood as "scientific information on biological facts" and not as "biological information".

1.4 Emergence and teleology

A good example of the reluctance often encountered in biology to consider something besides the linear (material) "cause and effect", which with no doubts is at the base of the successful empirical strategy of "knock-outs" (whether genes, species or other factors in the experimental set-up), is given by Patrick Bateson (1998: 169):

> "When told about all the things required to generate an observed piece of behaviour, many neuroscientists react with irritation at what seems to them to be a blatant piece of obscurantism. 'What is *really* driving the system?' they will demand. The implication is that if, in an experiment, a factor was varied and produced an effect, then surely that was *the cause*.
> To assume that a given cell or a given condition is doing all the work may be good practice when setting up analytical experiments. However, any strong claim that one event is normally sufficient for the occurrence of another event will meet with frank incredulity [at least] from most ethologists."

When suggesting a group of molecular biologists to be open for a moment to consider a complementary way of thinking about causality when characterising a system, as for instance "triadic causality", which derives from semiotic logic, it was pointed out to me that maybe what I was actually attempting to do was to change the names of well established concepts and terms. I think this point is sufficiently important as to deserve a few lines. In that context, I emphasised that it is not the names or the customary terms that I am interested in, it is the logic that may lay behind such terms that one should consider important. For example, the feedback concept, introduced by the cyberneticians in the 1940s, brought to science not only new terminology but also a logic, or more precisely, a kind of causality, namely, circular causality. This notion became of central importance in the biologists' conceptual toolbox. This does not mean that the notion of feedback was not intuitively accessible to students and laymen before cybernetics. All we can say is that it was not previously explicitly conceptualised in the western scientific world of the post-Newtonian age of modernity. For instance, Gregory Bateson (1972, 1979) claims

that if the formulation of the “mechanism” for evolution proposed by Wallace would have gained better acceptance than the Darwinian formulation, cybernetics could have been invented 100 years earlier. We can also say that even much before the advent of Newtonianism that ”simple but complex” notion was surely accessible to intuitive minds. What happened with Newton and Hume was not that other kinds of causality (or rather, other ways of thinking about causality) disappeared. What simply happened is that they were not taken into consideration. Where was the feedback notion when ”mechanicism” was taking total predominance? One can be sure that there were a lot of feedback loops taking place in the living world during Newton’s time. Today it is not possible to construct a mechanicist explanation in biology without resorting to circular causality. Are there other notions of causality that may have been overshadowed for centuries and which, in combination with the less overshadowed ones, may be effectively active in the shaping of that reality which certainly is out there but that we anyway have to construct in our minds?

It is precisely the impressive results of molecular biology that have devaluated the role of linear causality in biological systems. The overwhelming omnipresence of “cross-talk”, “redundancy”, “pleitropy”, “epistasis”, “polygenes”, “cryptic variants” (e.g. the “jukebox” effect in development), for example, has posed serious challenges to the logical foundations of biology. Based on this experience, it is easy to foresee that a further challenge to those foundations will become evident when biologists learn to recognise (as it is happening already) the existence of informational processes (implicit in e.g. signal transduction, non-trophic interactions, etc.) and of emergent properties and processes in a historical and hierarchical perspective. The concatenation of emergent and hierarchical levels will require different logics to think about causality, not just renaming well-established terms and concepts. Strong “logical” candidates for formulating new empirical strategies are, in my view, “downward” and “triadic” causality, in addition to the already well-established linear (dyadic) and circular causality.

As the notion of “information” has become so important in all branches of biology (in many cases through the influence of molecular biology on these branches) it is important to clarify whether this term implies the necessity of considering a logic which may be different from the standard linear material cause and effect, by no means substituting it but rather overlapping with it, necessary precisely because it is not reducible to impacts of billiard balls. It is not the task of renaming molecular

biology's standard terms that it is interesting but enquiring into what kind of logic they really imply.

In discussions about the limits to reductionism in biology there are several ideas that come forward. The idea of levels of organisation and hierarchical levels, the idea of boundary conditions and constraints, the existence of emergent properties and processes, the tension between chance and determinism (necessity), the idea of selective and stochastic processes, the historicity of biological processes, the idea of developmental trajectories, the importance of the context, and teleology, goal oriented behaviour, teleonomy, functional considerations, direction, intentionality, choice, selection, gratuity and the likes.

This means that in spite of the proclaimed dominance of the reductionistic approach in the mainstream, there has been the necessity for the existence of a great plurality of thoughts in biology in order to address these only partially understood issues that keep reappearing in biology under new settings and contexts. Think of the rich "parallel" history of evolutionary and developmental thought represented by more than 120 years of debate and experimentation in symbiosis (see Sapp, 1994; Dubos and Kessler, 1963); the tradition and the growing field of epigenetics; the history of systems theory and organicism; the incorporation of cybernetics in biological explanations; the advent of complex systems paradigms, including the theoretical framework to which I will be referring in this work, biosemiotics, which derives from a theoretical tradition that throughout the 20th century increasingly considered the importance of communication processes in biology. This very rich epistemological landscape in biology is not the fruit of a speculative effort aimed to negate or downgrade the successes of reductionism. On the contrary they may be born with the intention of improving and taking the best advantage of these progresses when they have to be applied out of the lab, in the organism, in the field, in the ecosystem, in open systems characterised by multitrophic and multisemiotic continuous interactions.

Since it is claimed that there are two types (or stages) of reductionism - ontological (constitutive) and epistemological (explanatory) - it is normally assumed that accordingly there are two kinds of antireductionisms. "Epistemological antireductionism holds that even if *in reality* everything is explained by particle physics, we cannot, given our finite mental capacities, grasp the ultimate explanation of most complex phenomena, and would not be able to do so even if we knew the law or laws governing their ultimate constituents" (Nagel, 1998: 4). It can also be the case

that the ultimate explanation of the precise physical event in each case would not tell us what we want to know: “explanations at higher levels often remain practically preferable and for many purposes indispensable” (Nagel, 1998: 4). On the other hand ontological antireductionism would be “the position that some physical phenomena, even though they can be explained in terms of principles that fit their specific features, simply do not have an explanation at the ultimate level - that is, in terms of the universal laws governing their ultimate material constituents” (Nagel, 1998:5).

Usually the soft reductionists would admit some epistemological limits to reductionism although the ontological point would remain firm. The most accepted view is that it is all right to be epistemologically non-reductionist given the limits of our mind and our methods to grasp the totality of the ontologically accepted reducible reality, the Laplacean dream. But it is less common to see reductionism questioned at its ontological level, i.e.: the notion that at the very end absolutely everything is reducible to the evolution of material particles since the time in which a paradoxical "nothing" exploded (and thus the whole of causality is determined by the collisions of these particles). But the contrary can also result to be practical: reductionism is epistemologically a marvellous and exciting tool but ontologically it may have not much scientific value or use because 1) it is based on a dogmatic conviction and not on any sound scientific “rational” principle, there is no experiment to convince you, there is no quantity to be measured (and may be that is just a normal feature of any ontology) and 2) because it does not really add anything crucial to epistemological reductionism but on the contrary it may block the way to complementary kinds of reductionism.

Nagel (1998: 6) has claimed that “emergence” is the main alternative to reduction and that it relies on a supposition of indeterminism. According to him, indeterminism in basic physics leaves some things unexplained which are nevertheless explicable by principles that govern phenomena at higher levels of complexity. However, Nagel believes that the problem of emergence can be posed even about a physically deterministic world. This position is probably at the base of most complex dynamics programs which can be said to adhere to ontological reductionism although recognising its epistemological limits.

Rose (1998: 178) reasons that:

> "Each 'level' of complexity of nature involves new interactions and relationships between the component parts which cannot be inferred simply by taking the system to pieces. Yet philosophical [i.e.: ontological] reductionism implies that even if higher order properties are emergent they remain secondary to lower-order ones. The lower the order the greater the primacy ... it seems as if only lower order explanations can be 'truly' scientific."

One of the main components in von Bertalanffy's organicist system theory is the idea that there are laws appropriate for each level of organisation, from atoms to ecosystems (Gilbert and Sarkar, 2000: 3), i.e.: level-specific rules - which in the terms of this work will come to resemble "emergent codes" throughout the hierarchy. Gilbert and Sarkar (2000:3) claim that this does not mean that each level is independent of the lower one; on the contrary, laws at a level may be *almost deterministically* dependent on those at lower levels, but they may also be dependent on levels "above". The problem is of course what magnitude one gives to this kind of "quasi-determinism". The mere acceptance of the influence exerted by levels "above" puts serious questions to the qualification of *almost* determinism which still implies a "command chain" from below and which may block our understanding of the increasing semiotic freedom observed in living systems (Hoffmeyer, 1996).

The main critique raised against many theories considered vitalistic is the fact that they have claimed that living matter is greater than the sum of its parts because of some life "force" or principle ("entelechy," *"elan vital," "vis essentialis,"* etc.) which is "added to or infused into" the chemical parts (Gilbert and Sarkar, 2000:1). But the arguments of the materialist detractors of vitalism are also sometimes highly contradictory, or at least not resolute, in this sense. To start with, the vitalist argument could in principle turn out to be a materialist viewpoint since the "elan vital" *is added* (so one could understand a posteriori) to the physico-chemical substrate of life. It doesn't matter what process "adds" this new element, the starting point is material. The kind of organicism or "material holism" (i.e.: material anti-reductionism!) described for example by Gilbert and Sarkar (2000: 1) could be (unjustly) said to resemble vitalism in this sense. Since life is an emergent property, independently of how and what combination of bottom-up or top-down processes gives rise to such emergent property - i.e.: the "threshold" of life - in that moment the "*elan vital"*, the distinctive characteristic of life, is added, or in any case it first appears: first you don't

have it, now you do, and now you have it, now you don't - quite a qualitative jump. But organicism, if understood as materialist, continues to be reductionistic. Materialistic holism, i.e.: material anti-reductionism, is an oxymoron. Any stance that adheres to ontological materialism is by definition reductionist: it reduces mind to matter and its mechanics. This has been extremely useful and desirable at the epistemological level. But ontologically it blocks the way to accepting "the reality" of emergent properties. By trying to overcome the paradox of vitalism, the "holistic materialist" (the "material anti-reductionist") may find himself or herself in the awkward position in which Newton found himself when he was criticised by the ultra-mechanicists who, inspired by Descartes, considered that the Newtonian concept of "force of gravity" was equivalent to a subreticious reproposition of "hidden qualities".

The limits of our individuality impose reductionism upon us as a fundamental mode of being. Every act of perception is a reductionist operation by definition. By perceiving, thinking, explaining and talking we are forced to reduce. We continually digitalise the continuum as we speak or reason. So the question (to me) is not about being reductionist or holist, but about the possibility of complementing material reductionism with other kinds of reductionisms (instead of looking for paradoxical anti-reductionisms). As claimed by physiologist Denis Noble (Bock and Goode, 1998: 172), reductionism and integration are not incompatible. It is the integrationist agenda that has become important. In a similar mood Thomas Nagel (Bock and Goode, 1998: 175) has expressed his doubts about considering the impulse towards reduction as a cultural phase which might be reversed by a move towards the acceptance of certain higher-order explanations as fundamental. He claims that the reductionist impulse conforms to a deep need for understanding. In this sense he foresees that things which we now cannot reduce will lead us to search for new kinds of reduction. My question is, could we conceive a *complementary* non-materialist reductionism that contributes to the integrationist agenda? Mechanicists were dualists. Materialists were monists. Could we possibly think of "form and substance" not as a dualism but as a "duality"?

Gilbert and Sarkar (2000) explain the difference between organicism and reductionism and how they can be alternatives to each other, but it also needs to be explained how organicism, if understood as "materialist holism", can possibly be considered something different from reductionism and, paradoxically, in what way does it differ from vitalism. Pushing ontological reductionism up the ladder of the emerging hierarchies produces vitalism as a side effect. According to Gilbert and

Sarkar (2000: 3) in embryology organicism has tried to reconcile ontological materialism with "epistemological emergentism". But taking the alternative combination could also result as interesting. One could for example adopt an "ontological emergentism" - i.e.: the acceptance of the "reality" of emergent properties, with particles being the first emergent property if you wish - and settle for epistemological reductionism, preferably if of different overlapping kinds.

Moreno and Umerez (2000: 107) have argued that notwithstanding all the different views regarding the nature of physical causality (including the different shades of material, efficient or mechanical causes, and even a kind of platonic interpretation in terms of inherent formal cause), they all share the basic idea of its *universality* and *exclusivity* as causal principle. Moreno and Umerez claim that it is possible to admit the principle of the universality of physical causality (which they call the "materialist principle") and, simultaneously, reject its *exclusivity* as explanatory principle for every kind of system. They say further that the necessity to postulate the existence of another type of causal link in biological systems is not in contradiction with the principle of universality of physical causation, because anyway such a new type of causal link requires complex underlying levels of physical organisation (Moreno and Umerez, 2000: 107). The exclusivity of the "materialist principle", is based on faith on the Big Ban explanation and it is also limited by it, i.e. by the tacit agreement on omitting the pre-Big Bang era, and the impossibility of a rational explanation (in material terms) for the non-space in which the Universe has to expand itself. Only by a conventional and normative agreement can we implement such an omission, and only by intuition can we think about it.

I do not see what exactly is it that strict adherence to ontological materialism brings home to epistemological reductionism. May be the choice of ontological reductionism has served as a "vaccination" against the puerile ostracism that sometimes has sadly found place in life sciences by which alternative logical formulations of (biological) phenomena have been met with disqualifying labels that if attributed to a scientist, she or he has joined a sort of black list. The consideration of emergence and teleology, individually or in combination, has very often been met with such attacks that ascribe labels of different kinds of vitalisms, theisms or any other "illicit feature". "Vitalist" or "creationist" were probably not meant to be insults, but they function as such when a scientist is ostracised by being included in those labels. The cases are countless. For example, in spite of the fact that Wallace's

formulation on the theory of evolution was taking shape faster and also, in Charles Lyell's opinion, in a much clearer language than Darwin's formulation, Darwin initially did not consider Wallace a menace to his priority over the theory because he was ascribing to Wallace the label of "creationist" (Løvtrup, 1987: 209). But it turned out that Wallace was no creationist, the only difference between his and Darwin's formulation was that his was clearer in language, it was being completed faster, and may be, as Bateson hints, with a more sound and explicit cybernetic logic. Wallace could have very well called his natural selection "regulation". Social Darwinism was not yet in vogue so there were still some gentlemen around, and it was enough that Darwin told Wallace how he had been working on those ideas for 20 years (as opposed to Wallace that had spent only 10 years to arrive to the same conclusions) for Wallace to acknowledge Darwin's priority over the theory and even contribute to it. Be that as it may, I do not see why a sound and logical epistemological formulation that is not necessarily traceable to ontological reductionism had to be postponed until the ontological problem of the origin was solved, something that Darwin did not achieve anyway. Wallace, as a "cybernetician", was seriously considering mind as an emergent property. Darwin was not. He was just attempting to bring down the last reserves about the materialistic ontology. But the massive re-emergence of "emergence" in contemporary biology seem to contradict Darwin. Once more Wallace was ahead in complex paradigms. Mind and pattern as the explanatory principles which, above all, required investigation, were pushed out of biological thinking in the later evolutionary theories which were developed in the mid-nineteenth century by Darwin, Huxley and company (Bateson, 1972: 450).

So ontological reductionism has served as a "safe conduct" to avoid ending in what Gilbert and Sarkar (2000: 4) call "bad company". In spite of all the very fine contributions made by Hans Driesh in embryology, including his intelligent and honest initial reductionistic approach, he is "bad company". Today, the growing field of epigenetics would profit a whole lot by rediscovering some of Driesh's notions and observations. Even Lamarck, the father of modern evolutionary theory - and "probably the greatest biologist in history" (Bateson, 1972: 427) (in part thanks to him biologists have a name for their profession) - turns out to be a bad company, and many others whose contributions may have just been inspired by an idea similar to the one expressed by embryologist Dalcq (1951) when he was professing his faith to organicism as a way of "reconciling the struggle for objectivity with a full respect for

life" (quoted in Gilbert and Sarkar, 2000: 3). Many of the "blacklisted" were just exercising rationality freely in order to contribute to research, just like Darwin or any other "whitelisted". We learn by their successes as well as by their mistakes and their bold ideas which both, the white- and the blacklisted, usually have. Even Darwin. Gilbert and Sarkar (2000: 4) quote Maienschein when he asks why does "concern with the organism as a whole ... come in more recent decades to be associated with fuzzy thinking and sloppy vitalism?" and they report embryologist Lewis Wolpert as saying "the notion that so-called emergent properties are required for understanding living organisms is 'a bunch of yak, all talk and nothing more´"", and they ask "why should some scientists be so hostile to organicism and emergent properties?". They claim that such a question is not a "scientific" or a "historical" one, but a "sociological" question. "Indeed, scientists and philosophers have made a severe distinction. One was either a reductionist or a vitalist" (Gilbert and Sarkar, 2000: 4). The only thing that may save you from the razor, not Occam's but Robespierre's, is if you express your ontological doubts in terms of indeterminism, or better yet quasi-determinism, by doing whatever pirouette possible to overcome the schizophrenia to which Ulanowicks refers.

The most important consequence of assuming ontological reductionism that I can think of is that it sets the normative value for the Laplacean dream, so that the material causes at the lower level can be used as the main intervention level to solve any kind of problem in the "whole thing", even "emergent" ethical problems, which then theoretically could be solved with pills (may be by inhibiting their emergence?). In order to intervene in the "whole thing" from below, the "whole thing" must be reducible. For example, "A programme devoted to the detection of which levels of serotonin might predispose a person to an increased statistical possibility of engaging in one of a number of activities, from suicide through depression to murder, followed by the mass screening of individual children to identify at-risk individuals, their drugging throughout life, and/or raising in environments designed to alter their serotonin levels - which is after all the action programme that would result from an attempt to define the genetic/biochemical as the right level for intervention - only has to be enunciated to demonstrate its fatuity" (Rose, 1998: 185).

Adhering or not to ontological reductionism, a matter of personal belief, does not have such crucial bearings in the epistemological strategy with the exception of providing the impulse to the hubris that makes science to know no limits. I do not

consider the non-resolution of such ontological choices an obstacle to the epistemological advancement of an "integrationist agenda" useful to the empirical endeavours. The question is not whether to be reductionist or holistic. The question is whether we can conceive a superimposed and overlapping kind of reductionism, not to compete with but to complement material reductionism. This is basically an epistemological choice. The reluctance to consider a different kind of causality in emergent systems may come from the intensity with which an explanation of the problem of the origin and/or the start-up or build-up mechanism of the system is searched. I am afraid that by waiting for such an explanation we would unnecessarily delay the use of alternative logical ways of organizing different kinds of causal relations in empirical and practical applications.

Independently of whatever our theory of the origin is, we have to deal with the present stage of evolution. The fact is that today we deal with, and we are part of, coevolutionary systems which present emergent properties. This coevolutionary mode, that one can presume to be as old as the biosphere, could also have played a role when life was in the process of covering the entire surface of the planet as we know it today. Be that as it may, this is what we have today: a coevolutionary mode. This is what we have to deal with both evolutionarily and ecologically. What are evolving today (and since a long time ago now) are entire complex and sophisticated networks at all levels, from genomes to phenotypes, from prokaryotes to Internet. This does not mean at all that we do not need to understand the past, i.e.: natural history, including the human species, its "learning" process, its history and cultural traditions.

Teleology is scientifically a problematic concept, and therefore the proliferation of euphemisms to describe it. But the effect of euphemisms, (created to escape who knows from what), is that the concept loses clarity. In my opinion, the word teleology expresses well what it has to express. It sounds good, it sounds cult, even scientific, so why should we blur its meaning with idiosyncratic euphemisms? Although the semantic roots of the "teleology" concept goes back to classical Greece, the word as such was coined by Christian Wolff in 1728. The etymological roots on which Wolff was drawing suggest the idea of "*giving an account of something by reference to an end or goal*" (Lenox, 1992: 324).

According to Lenox (1992: 330), to neo-Darwinians, the concept of teleology carries with it unshakeable metaphysical commitments that are theistic, vitalistic, or both. But instead of doing away with it they have just resorted to euphemisms.

Discussions of teleology in the last decades have focused on providing an account of teleological concepts and explanations within biology (natural selection, function, goals, design) that does not commit the person using these concepts to any of the "illicit features" (backward causation, anthropomorphism, vitalism, etc.). "Indeed, avoiding the twin specters of 'natural theology' and 'vitalism'... was part of the motivation behind the attempt, by leading neo-Darwinians, to replace the word 'teleology' with 'teleonomy'" (Lenox, 1992: 331). Bringing up new euphemisms over and over again will not solve the problem. Pushing the ontologically reductionist neo-Darwinian mechanism up the ladder of biological hierarchies, brings along "teleonomy", or "anthropomorphised teleology" as a side effect. Teleology is an emergent property.

But historically the euphemism of euphemisms has been "natural selection", for it implies both downward causality and teleology. To start with, it is a mental concept. There is no physical correlate to it. The problem is that it is retained to be foundational of a purely material-mechanicist framework. That is why there may be a tendency to "materially" reify the concept but with the boomerang effect of consistently giving place to one of the most rejected "illicit features", (chauvinistic) anthropomorphisation of teleology in nature. This must be the reason why it is so hard to find a final definition of the concept. Natural selection appears to be nothing less than the whole context, i.e.: the total sum of factors and simultaneous circumstances that determine a particular event. This brings natural selection closer to cybernetics and information theory. All the *contextual* parameters that "enact" the "selection" (naturally, of course) - practically no less than all the historical and unique conditions of a given moment - can be compared to the totality of possible paths and constraints that create higher probabilities towards certain paths over many others, as advocated in information theory. The problem would be of course, how to model such paths and such constraints. In this sense I claim that the way in which "regulation" is being conceptualised in molecular and developmental biology has some close analogies to the concept of "natural selection". After all, "natural selection" is about regulation (as it is explicit in Wallace' language). There is an analogy and a continuity, between developmental and physiological regulation and natural selection. The genome contains architectures for patterns that have survived and have become established as "habits", or networks. These are architectures within architectures within architectures. Recipes of successful tools for establishing networks, systems of

correspondences, systems of ideas in circuit. These networks and patterns have become established in response to constant variation of conditions. The "redundancy" built in the surviving system is what confers the flexibility (resilience) necessary to establish a meta-pattern (habit or network) at a level above any of the single habits that belong to the redundant circuit, as a survival strategy to deal with constantly varying conditions, i.e.: the evolving context.

Another problem with natural selection is that it is taken for granted that the formula "survival of the fittest", understood in terms of quantitative reproductive rate, is a technical necessity of the concept. This may, and has led to many chauvinistic anthropomorphisations of nature while on the other hand it could also be true that "... it was not the crudest, the simplest, the most animalistic and primitive aspects of the human species that were reflected in the natural phenomena. It was rather, the more complex, the aesthetic, the intricate, and the elegant aspects of people that reflected nature." (Bateson, 1979: 5).

If life is life within life, as I believe it is right now, the question of the unit of selection is also a question of embedded hierarchical levels and emergent properties. As we move up the scale from genes to species, the "survival of the fittest" formula, loses its physicality, it appears weaker, diluted, because at certain levels entities begin to need the survival of other entities and communication becomes much more complex and less linear, creating complex trophic and semiotic networks that become units of selection themselves as well as "selection determinants", or what I will be later defining as "emergent interpretants". This means including also the niche, the habitat and the ecosystem as units of selection and as part of the constraints that determine "selection". Communication patterns are also units of selection as well as selection determinants.

As Gregory Bateson (1972, 1979) pointed out, it is not about the unit of selection that we should be worrying about, but the unit of survival. The minimal unit of survival is not the gene, nor the breeding individual, the family line, the population, the sub-species or some similar homogeneous set of conspecifics. The minimal unit of survival is the *organism plus its environment*. In what way can such a unit of selection relate to the quantitative version of the formula "survival of the fittest" remains an open question. If the unit of selection is the organism and its environment, who is it that has to be the fittest to survive? And what kind of fitness are we talking about? "Fitness" becomes harder to quantify, it becomes more of a qualitative variable, closer

to homeostasis, balance, health, ecosystem function, resilience and cycles of renovation. Potentiality and readiness for change is already built into the survival unit. The heterogeneity of the wild population is already one-half of that trial-and-error system which is necessary for dealing with the environment. The flexible environment must be included in the unit of survival along with the flexible organism (Bateson, 1972: 451).

One has to put aside for a moment the problem of the origin in order to realise that in practical terms at the present evolutionary stage we can only talk about coevolution. The sum of the contextual parameters that "act" the selection gets very complex and interconnected in a multiplicity of emergent trophic and semiotic networks, and the interrelation of trophic and semiotic causal links may not correspond to a combination of contextual parameters that we call "survival of the fittest" reduced to individual organisms or species. Natural selection is everything that lies outside of all the material components of the system, that is, the relations of all these beings and physical entities. Natural selection is a relational concept. It emphasises the importance of considering the relations of the components.

Aristotle appears to restrict full-blown natural teleology to the biological domain (Lenox, 1992: 326), an *epistemological* choice that I very much share. The naturalistic approach to teleology allows Aristotle to offer teleological explanations that sound remarkably like modern "adaptational explanations" (Lenox, 1992: 327). I believe this is so because the concept of "natural selection" is inherently teleological. Natural selection turns out to be a "natural disguise" for teleology. This is probably the reason for its success as a concept: it is a masterpiece of political correctness. Asa Gray, who together with Ernst Haeckel is considered one of the greatest supporters of Darwin during his time, noted in *Nature* (1874) that "Darwin's great service to Natural Science [is] in bringing back to it Teleology: so that instead of Morphology versus Teleology, we shall have Morphology wedded to Teleology." To this Darwin quickly responded: "What you say about Teleology pleases me especially and I do not think anyone else has ever noticed the point" (Lenox, 1992: 329). On the other hand, Haeckel sent his book to Darwin in which he was defining teleology as a metaphysical doctrine and therefore concluding that Darwin had finally done away with teleology. Darwin's response in a letter to Haeckel stated that "... the manner in which you often refer to me in your text, and your dedication and the title, I shall always look at as one of the greatest honours conferred on me during my life"

(Løvtrup, 1987: 233). "... it appears that gradually Darwin '...began to see that what he considered to be misinterpretations were, *in effect*, softening the blow of evolutionism and, paradoxically, gaining him adherents'." (Løvtrup, 1987: 232). Based on this, one has to admit that Darwin was a marvellous public relations man. Be that as it may, Darwin's ambiguity still represents the mood towards the concept and the word in contemporary biology.

One of the most articulate spokesman for the "teleo-mechanical" point of view in the study of development and physiology, Karl Ernst von Baer, summarised the debate as follows:

> "Nearly a century ago Kant taught that in an organism all the parts must be viewed as both ends and means at the same time. We would rather say goals and means. Now it is announced loudly and confidently: Ends do not exist in nature, there are in it only necessities; and it is not even recognized that precisely these necessities are the means for reaching certain goals. Becoming without a goal is simply unintelligible" (Lenox, 1992: 330).

Lenox points out that this remark was made as part of a critique of Darwinism - seen as a theory that reduced the explanation of living phenomena to the interaction of chance and necessity.

If one considers both kinds of "determinants" of natural selection, biotic and abiotic, one could agree, with Aristotle, that the teleological part lies in the combined teleology of all the biotic determinants.

Natural selection, being the context, has causal "agency" in the shaping of the substrate. Not all variations are the result of point-mutation, nor is all the re-shuffling of the generated variation at different levels of domains in the hierarchical architecture of the genome exclusively achieved by vertical inheritance. This applies to different genetic determinants, regulatory elements, proteins domains, whole proteins and genes. The fact that *C. elegans* can contain a protein which is structurally highly similar to a human protein, and which may even be functionally interchangeable in both systems - meaning only that the macromolecule of one system is capable of being incorporated in the "mechanism" of the other system, i.e.: it could "fool" the system, but not necessarily meaning that both mechanisms, "the function", are the same, and not meaning at all that the context is the same - would be

considered as a case of convergent evolution if a genealogical vertical connection could not be traced in the best established evolutionary tree. The occurrence of convergence, together with the capacity of genomes to be used as codes for the production and exchange of variation, and the capacity of physiomes for modulating such variations in their environments, hint to the plausibility of horizontal communicability in evolutionary time. This would be an instance of downward causality. However, the issue of convergent evolution is much more complex than what I have implied here. Molecules with as little as 10% of structural homology may present a great degree of functional homology, so the convergence would be placed at a specific domain. Also, some cases of convergence are purely functional and not at all structural as in the case of bioluminescence where different biochemical substrates and enzymes have produced the same innovative "idea". Convergence can also be seen as the modular use of existing "ideas" or "solutions", which are always "systems of ideas in circuits" i.e.: a protein will always have a correspondence with the circuit to which it belongs.

Lamarckians and neo-Lamarckians have been rather criticised for not finding a plausible "mechanism" to account for environmental influences in evolution, but the Lamarckian issue remains open in biology because at its essence what it really considers is the actual fact of the irreducible complexity between the genotype, the phenotype and the environment. The continuous re-appearance of this issue is just an indication of the coevolutionary and symbiotic nature of living systems under present conditions.

Evolution through natural selection has also given rise to a species of organisms, *Homo sapiens*, which has the functional capacity for reshuffling, synthecising *ex novo* and introducing variation in the evolutionary dynamics of living matter. Breeding and biotechnology can exert a causal "agency" in the shaping of the substrate. That is also downward causation. It should be reminded here that biotechnology, in the form of gene technology, is only the domestication of "natural genetic engineering" (Shapiro, 1992, 1997, 1999), a sub-form of downward causality - part of "natural selection" if you wish. The knowledge about biotechnology is an emergent property which is not codified anywhere in the genome space. Such knowledge, which in business is called "immaterial property", lies between the networks of neurons of many scientists, the written protocols to carried out the processes and the thousands of computers that

maintain bioinformatic databases. These are all super-emergent things whose causal links are increasingly freer from the molecular dynamics on which they are based on.

The fact that an emergent property is emergent means that the logic of the material linear causality of "cause and effect" is left behind as a substrate for the emergent level, and a new set of rules form a new (meta) code appropriate to the level that the emergent property represents. When we talk about emergent properties we usually take for granted that emergence occurs from a complex combination of material-mechanic (dyadic) causal events giving rise to the emergent level. Very seldom do we consider the case of a "second order" emergent process, which originates from a complex combination of causal factors which are themselves emergent properties of a first order. Logically, the higher the emergence order of the factors involved, the more divorced these causal links are from the kind of causality (material-mechanic) which determined the first order emergent property at the molecular level. (I think this is what Hoffmeyer (1996) has referred to as "semiotic freedom"). This has enormous empirical implications because, considering only the first order emergence process and the dyadic material-mechanic causality that gives rise to it, we are mislead to think that the modelling of emergent properties is just a matter of more carefully and extensively quantifying the material-mechanical components.

The kind of counter-illumination camouflage created by a species of bacteria (*Vibrio fischeri*) which helps a squid (*Euprymna scalopes*) "escape" the eye of his predator, represents, in terms of the present work, an example of an emergent code. The interbacterial codes that are well exemplified by "quorum sensing" (part of the bacteria-squid system) are another instance of emergent codes at a different level of the same system. These examples, to which I will return later, share the triadic logic implicit in a code (see section 2.2), but the resulting codes may be materially, organisationally and functionally very different. When we are tracing non-trophic interactions in multitrophic systems, or when we are tracing the signal networks in a physiological system, what we are actually elucidating is across which hierarchies can we identify emergent codes in a given system, and the causal links of these codes and patterns. In the bacteria-squid-predator system, one could accept that each species has done a long way of evolution "on its own" before becoming acquainted in the system. But the important point is that once the circuit is connected coevolution and symbiosis take the lead, i.e.: the evolutionary dynamics becomes coevolutionary. The

interaction gives rise to the evolution of specific phenotypes in each organism which blend together in the system creating a larger "common phenotype" in the form of a specific semiotic network, which implies a system of correspondences.

> "According to the principle which Uexküll once phrased 'Where there is a foot, there is a path, where there is a weapon, there is an enemy', it should be possible to point out correspondences easily and unambiguously" (Plesner, 1976; quoted in T. von Uexküll et.al., 1993: 12).

The principle of correspondences basically says that "in the sphere of living things each affordance presupposes a counteraffordance - that is, it can be realised only through an interaction" (von Uexküll et.al., 1993: 12).

The emergence of codes has a relation to the emergence of new causality, a different kind of logic, dependable on but different from the logic of mechanical causality. Everywhere and every time a code emerges, there is already an *emergent interpretant* which is logically "above" the formality of the code itself.

Of the limits that material-mechanical reductionism may present, two of the most important are:

1) the impossibility of considering causal links determined by embedded hierarchical emergent properties, systems, agents or processes, and

2) the tendency to exclude the context. Both points will be the concern of the present work. In relation to this, we will bring to the attention that two very evident and actual trends are operating in empirical biology at all levels: the fact that biology is becoming the "science of sensing" and, not by chance concomitant to that, the fact that there is a very generalized call in biology to give the highest consideration to the context in many empirical fields.

1.5 Biology becomes the science of sensing

In the last two decades there has been in biology a major shift from a focus on information as a material agent of causality towards a focus on information as context-dependent, i.e.: a focus on signification processes (Hoffmeyer, 1996).

I take as a given the powerful trend that sees biology as the "science of sensing", e.g.: from the central role of signal transduction in all cellular and inter-cellular

activities to phenomena such as quorum sensing; from semio-chemicals and info-molecules between conspecifics to non-trophic interactions in multitrophic systems; from gene technology to biosensing and biocontrol.

We can thus notice the unconscious emergence of a concept of "natural regulation". By that I mean that "regulation", as the mechanism that orchestrates and directs (i.e. interprets) the signals represented by molecules that bind to each other in specific ways when their concentrations are statistically relevant, starts to look as something that exists, whereas nobody knows *where* it exists. Nevertheless, regulation at all levels has become essential.

A cross-sectorial look of current biological scientific literature reveals that at all hierarchical levels there is:

- increasing importance being ascribed to the "context"
- consideration of communication systems and information
- a generalised call for the integration of molecular biology with developmental, physiological and ecological approaches.

1.5.1 The importance of the context

Lack of proper consideration of the context is one of the main limits of reductionism and is systematically becoming a main concern in all subdisciplines of biology. The importance of the context is a recognised challenge to all empirical endeavours, and multiple knock-out strategies will have to be rethought accordingly.

The recognition of the importance of the context is another clear sign of the semiotisation of biology, for the context is a defining element of semiosis. The context, as a meta-code, provides the key for the interpretation of codes, which are the result of habits, "crystallised" patterns of behaviour, codified actions. A code is something to be interpreted.

As we go up in the scale of emergent processes, empirically, the context acquires further importance and complexity, being its spatial arrangement larger and comprehending "down-stream" processes in the hierarchy (see Salthe, 1993).

The importance of the context is beginning to be acknowledged at all levels. For example, at the level of cell biology, Nurse (1998: 94-95) claims that knowledge about the different macromolecules making up macromolecular assemblies and "machines" such as the ribosome, the replisome and signal transduction pathways "...

yields useful information on the types of reaction that can occur and on their kinetics, but this information can be misleading because the conditions found within the macromolecular assembly may not be the same as those which apply to the operation of a few components in dilute solution, which are the conditions used in classical biochemical *in vitro* analysis". He gives the following example:

> "Firstly, the assembly of macromolecules into complexes allows the channelling of intermediates to occur. This has been described in the context of intermediate metabolism when a low molecular weight component undergoes several sequential chemical changes within a complex ... Channelling allows intermediates to be passed on from one component to another without free exchange with the region surrounding the complex, and this allows reactions to occur which would not be energetically favourable in free solution. Secondly, the conditions present within a macromolecular complex may differ substantially from those generally found *in vitro*. The concentration of protein may be up to a hundred times greater leading to a significant exclusion of water, and ionic conditions may also differ. Such differences will certainly influence the kinetics of reactions and possibly also the nature of reactions that can take place" (Nurse, 1998: 94-95).

In few words, macromolecular reactions are context-dependent.

Contextual problems increase as we move up to higher hierarchical levels as when considering the level of an organelle or a whole cell where scale extends far beyond that observed during molecular interactions. For example the organisation of the entire cell in space implies the existence of morphogenetic mechanisms such as spatial fields and diffusion gradients which can impart positional information to the cell. In general greater extension in space-time scales will give rise to new phenomena (Nurse, 1998: 95).

1.5.2 Integration of molecular and ecological approaches

We have accumulated vast quantities of empirical data that characterise the material-energetic exchanges in living matter at all hierarchical levels of biology. As we will see, a semiotic characterisation will be based on, but not limited to, this material characterisation. It will be rather superimposed on it. The fact is that now

biologists have arrived at the point in which they need to characterise semiotic processes in all branches and hierarchical levels of empirical biology. A central claim of this work is that the semiotic characterisation will be central in the progress of the integrative agenda that passes through molecular-genetic, epigenetic, physiological, organismic and ecological levels.

With the rapid development of molecular techniques, there is in all branches of biology a sort of interaction with molecular biology. While on the one hand the integration of molecular, physiological and ecological approaches is more than necessary, there is yet the risk of importing some of the ambiguities of the "informational talk" from molecular biology to other hierarchical levels. In relation to this we may also find a tendency to reduce physiological and ecological complexity to its molecular "components": the massive characterisation of signal-transduction networks and the elicitors and components of the cascades that determine complex genetic reactions in response to variable environmental cues (what could be called "the signalome", see section 3.2). The understated goal would be that of mapping an ecosystem in terms of molecular kinetics. One should of course consider also the epigenetic continuum in between.

As early as 1975, shortly before his death, biochemist and the biophysicist Gordon M. Tomkins - a major figure in the development of molecular biology (Kay, 2000) - sketched a model for the evolution of biological regulation and the origin of hormone-mediated intercellular communication. He claimed that

> "Since a particular environmental condition is correlated with a corresponding intracellular symbol, the relationship between the extra- and intracellular events may be considered as a 'metabolic code' in which a specific symbol represents a unique state of the environment." (Tomkins, 1975: 761).

He further argued for an apparent generality of such a code. More recently biochemist and molecular biologist Mogens Kilstrup (1998) has reframed Tomkins' contribution in a semiotic perspective. The recent discoveries in the field of signal transduction have confirmed how right Tomkins had got it.

Around the same period, biochemist Marcel Florkin in his treatise from 1974 "Concepts of molecular biosemiotics and molecular evolution" - one of the most ambitious attempts to integrate a view of nature as a sign-producing system into an

elaborated theoretical frame (Emmeche and Hoffmeyer, 1991) - recognises the signified (that to which the sign refers, its "meaning") of biomolecules as being involved at levels of integration higher than the molecular one, for instance at the level of self-assembly in supramolecular structures, and the physiological and ecological levels. He designates all the signal-molecules that today are known as info-chemicals, semiochemicals, pheromones and info-molecules as "ecomones", the non-trophic molecules contributing to insure, in an ecosystem, a "flux of information" within and between organisms. These two contributions put together create a link between endosemiosis (communication processes within organisms) and exosemiosis (communication processes between organisms). (see section 2.1).

In this direction, the field of signal transduction networks constitutes one of the first conceptual links between hierarchical levels. Signal transduction has opened the doors to the integration of molecular techniques with embryologic, developmental, physiological and ecological approaches. It has also re-dimensioned the centrality of DNA as the sole source of biological information. Whereas DNA was the dominant and central element in the conceptual and experimental framework of biology, today its place is being taken by signal transduction. Signal transduction research has remarkably contributed to a major paradigm shift in biology in which now the discipline is seen as a "science of sensing". Once we recognise that sensing is one of the necessary properties of life, we can not do without considering triadic logic in order to construct our understanding of living phenomena.

Therefore, the central part of this work will be dedicated to outlining some general semiotic principles of signal transduction networks and what is their role and position in relation to the larger semiotic networks in which they function, i.e.: in the hierarchical formal processes of mapping, translation, transformation and transmission of information.

The integrative agenda thus depends on the consideration of the flow of information within organisms (the genetic, epigenetic and physiological levels) and between organisms of the same or different species, i.e.: the ecological level of functionally integrated multitrophic systems.

Chapter 2
Signals that build signs

2.1 The biosemiotic paradigm

Besides molecular reductionism and spontaneous semiotisation there has been since the beginning of the 20th century a less noticed although equally important trend being developed in biology. Based on early contributions by biologists and scientists like Jakob von Uexküll, Konrad Lorens, Gregory Bateson and Thomas Sebeok among many others, a new paradigm took shape to make explicit the relation between biology, communication and semiotic processes. Today under the general name of biosemiotics, this theoretical frame deals with communication processes in living systems, from molecules to ecosystems. In the last two decades biologists like Thure von Uexküll, Jesper Hoffmeyer, Alexi Sharov, Kalevi Kull and Claus Emmeche (among others) have consistently contributed to the linking and developing of a variety of related sources into a coherent paradigm. In a sense they have continued and consolidated a "German-Baltic-Scandinavian connection" which pervades the history of biosemiotics since the times of Karl Ernst von Baer (for a thorough review on the history of biosemiotics see Kull, 1999).

If one thinks about it, the sentence "communication processes in living systems" may sound redundant since there cannot be communication processes outside living systems. That is precisely the point: "communication", or in semiotic terms "semiosis", is a defining property of all life manifestations (Sebeok, 1985/1976). This is one of the premises of the biosemiotic framework which sees biological processes from a sign-theoretic perspective. It allows an alternative way to study living systems not only as based on the organisation of molecules, but also as based on the production and communication of signs in nature (Hoffmeyer, 1996). The focus of attention is not so much on the natural selection of replicating molecules or genotypes (as in the traditional neo-Darwinian paradigm), but on the signs-links and interpretation systems of various semiotic agents on all biological scales: from molecular recognition to cellular self/non-self distinction, from the molecular semantics of gene expression and regulation to the semantics of inter-organism

communication (from bacteria to elephants), from individual cognition to the swarm intelligence of ants and bees (Emmeche, 1998a: 11).

Biosemiotics sees organic evolution as a gradual build-up of semiotic networks of organisms covering the totality of the surface of the Earth and thus giving rise to an autonomous sphere of communication: the semiosphere (Hoffmeyer, 1996). The term semiosphere was originally suggested by Yuri Lotman (1990) in his very sound semiotic theory of culture. Independently, Hoffmeyer (1996) developed a wider and biological conception of the concept[1]. In this latter perspective the semiosphere is a sphere like the atmosphere, the hydrosphere or the biosphere. It pervades the biosphere and consists in communication: sounds, odours, movements, colours, electric fields, waves of any kind, chemical signals, touch etc. (Hoffmeyer, 1997a).

Organisms not only belong to ecological niches, they are always also bound to a semiotic niche, i.e. they will have to master a set of signs of visual, acoustic, olfactory, tactile and chemical origin in order to survive (in the semiosphere). If on one side we have the semiotic niche on the other we have what Jacob von Uexküll in 1940 defined as "Umwelt": the phenomenal worlds of organisms, the world around them as they themselves perceive it, i.e.: what they actually "construct" out of the semiotic niche (Uexküll, 1982 [1940]; Hoffmeyer, 1997b). For example, the Umwelt-space of a moth will contain no sounds emitted by birds or other vertebrate animals with the single exception of the 20.000 hertz frequency used by hunting bats. The Umwelt-space maps onto a "response space" defined by the set of possible activities of the organism. (Hoffmeyer, 1998a: 41). The semiosphere poses constraints or boundary conditions to the Umwelts of populations since these are forced to occupy specific semiotic niches (Hoffmeyer 1997b).

Semiotic networks in ecosystems and physiological systems, mass-energy transfers, trophic chains and biomass growth and decay are not mutually exclusive explanatory tools:

> "… to the extent evolution favours the establishment of refined semiotic interaction patterns between species, it will also tend to open the way for a multitude of physical interactions between species ... In this perspective symbiotic relations are not to be considered just funny accidents, rather they

[1] In this regard, John Deely (2001: 629) has suggested to use the term "signosphere" for the cultural setting and to adopt the term semiosphere in the biological conception proposed by Hoffmeyer.

> constitute a systematically occurring phenomenon in the semiosphere" (Hoffmeyer, 1997b: 367).

Any elemental biological organism already interacts semiotically with its invironment when it selects or avoids energetic or material objects in it (Nöth, 1999: 78). But the semiotic interactions of organisms are by no means limited to physical dependence modes. There are other possibilities for semiotic mutualism in which one organism uses regularities exhibited by other organisms as cues, just in the same way it may use perceived regularities from the abiotic world for similar purposes (Hoffmeyer, 1997b: 367-368). Semiotic interactions will tend to combine different species into integrated functional networks which cannot be analysed in terms of two-species interaction models (Hoffmeyer, 1995: 377).

Semiosis, the processes of production, communication and interpretation of signs - i.e.: coding and de-coding - takes place within and between organisms. The term "endosemiosis" refers to the processes of interpretation and sign transmission inside an organism. On the other hand, "exosemiosis" refers to the processes of sign interpretation and transmission between organisms of the same or different species and in general the interpretation of environmental cues. All endosemiotic sign processes are (directly or indirectly) linked to phenomena in the organism's environment. Organisms are wrapped in semiotic networks in which specific circulating signs are accessible only to complementary systems of interpretation. The exosemiotic sign processes, which transform the objective environment into subjective universes, are intrinsically related to the endosemiotic sign processes in a continuous basis (von Uexküll et. al., 1993).

By recognising the cell as the most elementary integration unit, T. von Uexküll et. al. (1993) differentiate four endosemiotic integration levels:

1) The microsemiotic level - sign processes occurring within the cell and between its organelles, which take place through relations between networks of genes, enzymes, signals and second-messengers.

2) Cell-to-cell communication by cytosemiotic processes in neighbouring cells (direct metabolic and electrical contact at 'gap-junctions'), including coordinated responses of group of cells that share a regulating signal.

3) Endosemiotic networks that link the most diverse cells into functional units, including systems for short-distance sign vehicles (e.g. transmitters) and systems for long-distance sign vehicles (e.g. hormones, antibodies).

4) The combination of cells into organs and/or systems [as well as the emergence of higher order physiological codes] (von Uexküll et. al., 1993).

Von Uexküll et. al. (1993: 9) also stated that a linear hierarchical scale cannot account for the complexity of semiotic processes. Therefore, biosemiotics searches for multidimensional and ramified models as well as for circular models joining together different integration levels (von Uexküll et. al., 1993: 9). So these integration levels should not to be considered as sharp frontiers given their coextensive nature. More subtle integration levels can be identified in between these levels and these are not necessarily manifested as emerging physical structures but sometimes can also be manifested as a new complex logical product based on already existing structure. Later I will be revisiting this relation between endosemiosis and exosemiosis when considering signal transduction networks. There I will define "the signalome" as the substrate through which emergent codes constitute levels of integration at different physical and logical levels of the hierarchy.

By considering processes of communication (semiosis) as a central characteristic of living systems from the lowest to the highest aggregation levels, biosemiotics seeks to develop a notion of "biological information" that is relevant to the different hierarchical levels of the living world and to the multiple biological disciplines that study them. Biological information functions at and between different levels of complexity that go from the molecular-genetic level to the epigenetic (whole-cell) level up to more systemic levels which include various types of communication systems such as nervous, immunologic, endocrine and ethological systems, up to ecosystems. At all these levels and systems "biological information" as the vehicle for communication must present common features and causal relations (Emmeche, 1998a). The emphasis is not merely on the "transfer of information" *per se*, as if it was a material thing that can be physically moved from one place to another (whether in genetic or in ecological systems) but on the emergence of communication networks and interpretation contexts and systems. Above all, the emphasis has to be put on the "continuous chain of information" from the lower to the higher hierarchical integration levels and vice versa.

Hoffmeyer (1995) has claimed that the reification of communication to 'nothing but' transmission of molecular signals - which yields the idea that information is (exclusively and simply) something that can be moved or transported as a physical object - has favoured quantitative characterisations of communication and signalling networks within living systems but at the cost of a grave underestimation of the interpretative or semiotic competence of such systems. In a biosemiotic understanding biological information is inseparable from its context; it has to be interpreted in order to work. In this regard, Gregory Bateson's approach to information, hierarchical contexts and analog/digital communication has been recognized as highly relevant to a more fully developed semiotic approach to biology (Hoffmeyer and Emmeche, 1991).

There is also a need for considering synchronicity and diachronicity together. Hoffmeyer and Emmeche (1991) have distinguished between the vertical semiotic system - consisting in genetic communication down through generations - and the horizontal semiotic system - which allows for communication throughout the ecological space (Hoffmeyer and Emmeche 1991, Hoffmeyer 1997a). One evident trend in evolution has been the development of organisms with ever more complex Umwelts i.e. organisms with a subjective experience of the world supported by increasingly sophisticated sensory apparatus (and corresponding nervous system in higher animals) which would enable organisms to form fine-tuned internal impressions of what lay round about them (Hoffmeyer, 1996: 33). Due to this trend the horizontal or ecological semiotic network has gained increasing autonomy relative to the semiotic genetic system (Hoffmeyer 1997a).

The synchronic-diachronic interplay of these two systems can be grasped through the notion of "code-duality" (Hoffmeyer and Emmeche, 1991) which advocates for the consideration of historical and evolutionary aspects in the semiotic networks which are "horizontally" operative in organisms and ecosystems. This notion has drawn inspiration from the works of Bateson (1972, 1979) and Wilden (1980) (Emmeche et.al. 2002). Basically code-duality is definable as the ability of a system to represent itself in two different codes, one digital and one analog (Hoffmeyer and Emmeche 1991: 126). Life exhibits a semiotic interaction between the two states, the analog coded state of the organism itself and its redescription in the digital code of DNA. As analog codes the organisms recognise and interact with each other in the ecological space giving rise to the horizontal semiotic system (or ecological

hierarchy), while as digital codes (after eventual recombination through meiosis and fertilisation in sexually reproducing species) they are passively carried forward in time between generations through the vertical semiotic system (or genealogical hierarchy) (Hoffmeyer, 1997a).

> "... the memorized description in the digital code must be translated to the physical 'reality' of the actual living system. For this translation (the developmental process) to take place, the fertilized egg cell, or some equivalent cell, must be able to decipher the DNA-code ... This need for the participation of cellular structure shows us that a sort of 'tacit knowledge' is present in the egg cell. And the existence of this tacit knowledge hidden in the cellular organisation must be presupposed in the DNA-description." (Hoffmeyer and Emmeche, 1991: 127).

In other words, the "tacit knowledge" must stand in a system of correspondences with the genome architecture. The extent of this tacit knowledge is still vague and hard to evaluate, but at least it has to include positional information, embryonic "structural templates" that may set the orientation of certain epigenetic trajectories and above all it must include a sort of essential or embryonic signalome (see section 3.1.2) that is already functional in interpreting the configuration of signals that start fertilisation and which selectively operate on DNA to start differentiation and development of the (inherited) embryonic signalome itself and consequently of differential use of DNA by the organism. So it is much more than amino acid sequences what organisms inherit[2].

Hoffmeyer and Emmeche set the boundaries for the code-duality between the DNA-digital code and the phenotypic analog. I will go further to claim that the mutual determination of digital and analogical information can also be observed (i.e.: can be useful for organising data) at different integration levels in the endo- and exosemiotic

[2] Jablonka (1998) mentions four "inheritance systems": 1) the epigenetic inheritance system i.e.: regeneration of cell structure and metabolic circuits - the transmission of cellular morphology 2) the genetic inheritance system i.e.: DNA replication - the transmission of DNA base sequences 3) the behavioral inheritance system i.e.: social learning - the transmission of patterns of behaviour, and 4) the linguistic inheritance system i.e.: the socio-cultural learning - transmission of language structures. In addition, Kull (2000) emphasises the role of the environment. A particular manifestation of phenotype or behaviour are connected (or limited) to a particular environment. The stability of environmental conditions is a necessary part of the inheritance systems, being itself a carrier of a part of information from generation to generation (Kull, 2000). In a sense the environment is also an inheritance system. This systems conform, of course, a continuum.

processes through out the continuum of development. In fact, the dynamic up-and-down causality mediated by signs is an ontogenetic historical continuum that oscillates within these boundaries of the code-dual nature of organisms and ecosystems. Now we need to "unpack" the semiotic process within these boundaries. How does the digital become analogical and the analogical goes back to digital? Is the code-dual nature of digital-analogical information also present at different integration levels of the continuum that ranges from DNA to organisms? In the light of current knowledge about genome architectures, signalling networks and regulatory systems we have to consider communication within and between genomes, i.e.: the genome space, and within and between phenotypes i.e.: the global phenotype, that is, aggregates of analogs in horizontal communication. In other words, we have to consider the mutual constitutivity of these two "spaces" as well as the semiotic process "in-between".

The distinction between analog and digital codes is not a simple one (Hoffmeyer and Emmeche 1991: 130; for a wider treatment of this distinction see also Bateson 1972, 1979; Wilden 1980; Heims, 1991, Hoffmeyer, 1996). As we will see, this distinction depends on the hierarchical nature of contexts (and thus the existence of meta-contexts) in the multidimensionality of semiotic processes. Heims (1991: 92) tells about how Bateson kept insisting on the clarification of the way the terms digital and analogical were constantly being used at the Macy Conferences on Cybernetics (in which cybernetics as a dicipline was being defined) during discussions that focused primarily on detailed neurophysiological data. "Bateson recalled the historic conflict in Great Britain between the geneticists, especially his father [William Bateson], and the staticians, especially Karl Pearson, concerning whether variation of biological species formed a continuum or is discontinuous".

According to Bateson (1979: 249)

> "A signal is *digital* if there is discontinuity between it and alternative signals from which it must be distinguished. *Yes* and *no* are examples of digital signals. In contrast, when a magnitude or quantity in the signal is used to represent a continuously variable quantity in the referent, the signal is said to be *analogic*".

In digital systems response is a matter of "on-off thresholds". In analogic systems response is *graded* (i.e.: varies continuously) according to some variable in the trigger event (Bateson, 1979: 122-123).

In digital communication a number of purely conventional signs combine according to rules that can be said to be similar to algorithms. The signs themselves have no simple connection (e.g., correspondence of magnitude) with what they stand for. In analogic communication real magnitudes are used, and they correspond to real magnitudes in the subject of discourse (Bateson, 1972: 373).

> "In the natural world, communication is rarely either purely digital or purely analogic. Often discrete digital pips are combined together to make analogic pictures ... and sometimes ... there is a continuous gradation from the ostensive through the iconic to the purely digital. At the digital end of this scale all the theorems of information theory have their full force, but at the ostensive and analogic end they are meaningless" (Bateson, 1972: 291).

Wilden (1980) pointed out that "The analog is pregnant with *meaning* whereas the digital domain of *signification* is, relatively speaking, somewhat barren ... what the analog gains in semantics it loses in syntactics, and what the digital gains in syntactics it loses in semantics" (Wilden 1980: 163).

According to Hoffmeyer and Emmeche (1991: 131)

> "The trick of digitalisation consists in introducing gaps into continuums, thereby creating boundaries. These boundaries, however, do not themselves belong to the continuum, neither are they part of the gap. The boundary is the locus of an external intervention and thus necessarily defines a goalseeking system that drew that boundary. Therefore a system of a higher logical type - defining the goal - is necessarily established in the process of digitalisation".

I will refer to such a system of a higher logical type as the "emergent interpretant" (see section 2.5.4) which sometimes can be equivalent to some of the integration levels in endo- and exosemiosis.

2.2 Triadic causality

As stated before, the concatenation of emergent and hierarchical levels in biology will require a different logic to think about causality. Strong "logical" candidates for this task are "downward causality" (to which we have already referred in previous sections) and "triadic causality" - in addition to the already well-established linear (material) and circular (feedback) causality.

Linear causality, as exemplified by material cause and effect, can be seen as a mix of the Aristotelian categories of material and efficient (or mechanical) causality. This kind of causality is characterised by "dyadic" action and therefore I will refer to it as dyadic causality in accordance with Charles Sanders Peirce's distinction between dyadic and triadic action (see below). For practical reasons it is useful to make such a distinction between "dyadic causality" and "triadic causality", which derive from Peirce's semiotics (although it has to be said that in Peirce's writings the distinction is made between dyadic and triadic "action"; but we could without major problems substitute the word "action" by "causality" in order to be consistent with the other conceptions of causality that may be integrated into a system of explanations, as e.g. downward causality and circular causality.)

As we have seen, circular causality corresponds to negative or positive feedback. Although it may be based on a dyadic (material-mechanical) action, the fact that it is embedded in a self-corrective system may also give to it a triadic nature, depending on whether the direction "impinged" upon, or taken by, the system (which is being informed by the feedback) is more or less independent of the nature of the material-mechanical links that communicate the difference which guides the system, i.e.: whether the cybernetic system subject to feedback is a physical system or whether it is a living, sensing and interpreting system.

According to Lucia Santaella (1999: 501) Charles Sanders Peirce - the founder of modern semiotics - was led to reinterpret Aristotle's doctrine of causation because of historical inconsistencies and the narrow view of causality adopted by his contemporaries, namely, that a cause is an event of such a kind as to be necessarily followed by another event which is the effect (the main notion of causality accepted since David Hume). As mentioned above, in Peirce's semiotics there are two general kinds of actions in the universe: "dyadic" and "triadic". In this view, the Aristotelian categories of material and efficient causalities are considered to correspond to "dyadic

action" while the categories of formal and final causalities are reduced to "triadic action". I will assume this practical modern reframing of the Aristotelian notion of causality as a useful epistemological tool for biology, and I will leave the Aristotelian formulation open to the more ontological kind of arguments.

According to Peirce, dyadic action is "brute, unintelligent, and unconcerned with the result" that may come from it. "A compulsion determined by the particular condition of things", which acts as "to make that situation begin to change in a perfectly determine way; ... what the general character of the result may be in no way concerns the efficient causation". Dyadic action is cruder and simpler than formal and final causation (which are of triadic nature). A dyad is a discontinuous fact occurring "here and now", it is brute force, compulsion, an effective action, blind, nonrational, singular in occasion. This is the world of forces and impacts (Santaella, 1999: 500-501). Dyadic action is mechanical or dynamic, and is concerned with efficient causation as described for example in connection with material-energetic transfers and biomass (Emmeche, 1998b).

On the other hand, triadic action (formal and final causalities) is governed by law, it deals with the ideal or final. In Peirce's description final causation is:

> "that mode of bringing facts about according to which a general description of result is made to come about, quite irrespective of any compulsion for it to come about in this or that particular way; although the means may be adapted to the end. The general result may be brought about at one time in one way, and at another in another way. Final causation does not determine in what particular way it is to be brought about, but only that the result shall have a certain general character" (Santaella, 1999: 500).

The triadic action type is semiotic, or intelligent; it concerns formal and final causation as described in biosemiotics and evolutionary theory and in general whenever genealogical or ecological communication and "information transfer" from cells to ecosystems is considered. The two kinds of action are irreducible, but inseparable and superimposed. In biology there has been a tendency to consider exclusively the dyadic type of causality. But as the biosemiotic approach has repeatedly emphasised, in the last two decades there has been in biology a shift from a focus on information (as a material agent of causality) towards a focus on

signification processes i.e.: information as context-dependent. The fact that metabolic processes, food chains or non-trophic interactions are not just simple and dyadic actions but complex relations dependent on constant communication within and among organisms (of different or the same species) represents the semiotic dimension (Emmeche, 1998: 75).

According to Santaella (1999: 514), semiosis, or the action of signs, can be seen as a technical version of final causality. The Percian definition of the sign is a logical description of the way triadic causality functions in nature. A sign is an irreducible triadic relation. It represents a relation between three factors: 1) the primary sign - the sign vehicle - i.e.: the bearer or manifestation of the sign regardless of its significance (that which stands for something else) 2) the object (physical or non-physical) to which the sign vehicle refers, and 3) "the interpretant" i.e.: the system, or the interpretation key, which construes the sign vehicle's relationship to its object (Hoffmeyer, 1996: 19). This relation is customarily represented as a sign triad (Figure No. 1)

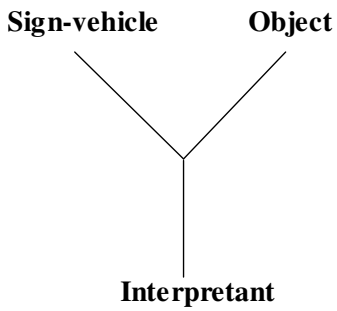


Figure No. 1. The sign triadic relation.

The most general character of this action is in its destination. It is an action directed toward a goal. This action can be intentional or not, it can be conscious or not, purposeful or not. As pointed out by Emmeche (1998) and Santaella (1999), Peirce's semiotics [just like Bateson's biological cybernetics] makes us aware of the purposeful dynamism of life. The semiotic definition of life does not reject the idea of teleology being inherent to the living world. "There is mind wherever there is triadicity; wherever there is a tendency toward learning, growing, or evolving, there is

mind, no matter how rudimentary its action may be" (Santaella, 1999: 503). In order to avoid anthropomorphic confusion it is important to make a clear distinction between finality and intentionality. Final causality is of course involved in intentionality, which is the psychological self-conscious version of a final cause, but is not limited to it: "It is a widespread error to think that a final cause is necessarily a purpose. A purpose is merely that form of final cause which is most familiar to our experience" (*CP* 1.211), that is, purpose is just one type of final causality, it is a conscious version of it, but purposes can also be unconscious, and in the biological world, Peirce held that purpose can be merely an "action *virtually* directed toward the removal of stimulation" (*CP* 5.563) (Santaella, 1999: 503).

2.3 An integrative concept of "biological information"

As we will see, Peirce's distinction between dyadic and triadic action, and his logical description of the way signs function in nature, correspond very closely to the concept of context-dependent-information in biological systems developed by Gregory Bateson (1972, 1979).

This concept of "biological information" departs from any paradoxical physicalist account of information (i.e.: it considers as true that information is information not matter nor energy, and thus that certain materials such as DNA or any of the so-called "informational" molecules are not *per se* information). It also departs from the purely probabilistic accounts of the mathematical theory of information formulated by the cyberneticians, although instead of excluding these accounts it rather overlaps with them.

In Bateson's definition the smallest unit of information is a difference or distinction, or news of a difference. So information means a difference that makes a difference to a system capable of picking it up and reacting to it, i.e.: for there to be a "difference" - news of a distinction - there has to be a biological system that senses it. Otherwise they would not be differences, they would be just impacts. So information means a difference that makes a difference to some system with interpretative capacity (Bateson, 1972, 1979; Emmeche, 1994).

A sign, or in Bateson's terminology, an idea, can be a complex aggregate of differences or distinctions. It can be formed by the smallest units of informational processes, i.e.: news of a single difference (Bateson, 1979: 250), as e.g.: the binding

of a single signal-molecule to its membrane-receptor. More elaborate signs can be formed by complex aggregates of elementary differences (which constitute more complex differences). This implies the emergence of codes: “Every effective difference denotes a demarcation, a line of classification, and all classification is hierarchic ... differences are themselves to be differentiated and classified” (Bateson, 1972: 457). That is, they have to be recognised as *patterns*.

“The number of potential differences in our surroundings ... is infinite. Therefore, for differences to become information they must first be *selected* ...” and *categorised* by an interpretative system with such capability of pattern recognition (Hoffmeyer and Emmeche, 1991: 122).

As it can be noticed, in this perspective biological information is not restricted only to DNA and amino acid sequences. It is an emergent property based on sensed differences and complex aggregates of differences. Biological information functions like signs in the sense that it is context dependant and requires interpretation processes. There is no information without interpretation (i.e.: pattern recognition), and herein the importance of the context.

This way of understanding information and sign-function gives place to the following distinction between causal links:

1) On the one hand we have the world of non-living billiard balls and galaxies - the material world - characterised by the kinds of regularities described in the physical sciences, where forces and impacts are the “causes” of events (Bateson, 1979, Bateson and Bateson, 1989: 211). This is what Bateson identified as the “pleroma” which corresponds to the Percian description of “dyadic action”.

2) On the other hand we have the world of the living - where *distinctions* are drawn and a *difference* can be a cause - all processes in which the analog of cause is information or a difference, i.e.: the entire biological and social realm, the world of communication, necessarily embodied in material forms subject to physical laws of causation as well as the distinctive processes of life (Bateson, 1979, Bateson and Bateson, 1989: 207). This is what Bateson identified as the “creatura” which corresponds to the Percian description of “triadic action”.

The two kinds of action are irreducible, but inseparable and superimposed. “... information does not belong to the sphere of matter and energy, but to the subjective and non-dimensional sphere of structure, pattern and form ... At the most fundamental level the distinction between life and non-life is dependent on this ability: the

response to differences ... Nothing in the world of living systems makes sense unless we include in our explanations this peculiar ability to respond to selected differences in the surroundings" (Hoffmeyer and Emmeche, 1991: 123). And " ... differences are not intelligible in the absence of a purpose" (Hoffmeyer and Emmeche, 1991: 126).

Hoffmeyer has claimed that the necessary but sufficient condition for a system to have the ability to transform the differences in its environment into distinctions is that it has developed self-reference based on code-duality, i.e. the continued chain of digital-analogue (i.e. DNA-cell) re-interpretations guiding the genealogical descent (Hoffmeyer 1993b, 1997a, 1995, 1996; Hoffmeyer and Emmeche, 1991).

As mentioned before, in biology there has been a tendency to consider exclusively the dyadic type of causality, i.e.: linear cause and effect, the material-mechanical logic of physics. In the last century, with the advent of cybernetics, we have witnessed the full incorporation of "circular causality" in biology, i.e.: feedback. Emergent properties are unwillingly accepted by the reductionist ontology, and thus downward causality is not easily recognised in life sciences yet. The same can be said about triadic causality.

Being both a biologist and a co-founder of cybernetics, Bateson made very particular contributions to both disciplines. He is considered a pioneer in the study of communication in living systems and evolution. In this sense, his contribution to cybernetics was very special among "the Cybernetic Group" (Heims, 1991) because for him communication was a characteristic property of the living world, one of the premises of current biosemiotics. His use of cybernetic concepts created a link to semiotic logic thanks to his innovative notion of information, which departed from that of his cybernetic colleagues. But his formulation of information as differences sensed by living systems - which is perfectly equivalent to the triadic logic of sign-function in semiotics as postulated by Peirce - did not hinder him from using the rest of the conceptual tool-box from cybernetics like e.g.; the notions of feedback, digital and analogical codes, and even information as improbability or restraints, which in his view emphasised the importance of the context in a developmental pathway. Thus by having introduced triadic logic into the study of communication in biological systems (implicit in his notion of information), Bateson was truly what today we could call a biosemiotician. He would be amazed to see how the general ideas he was postulating for the study of communication systems in biology fit so well with the astonishing findings of molecular biology today, for example in the field of signal transduction

networks. Maybe this is the case because Bateson was very well informed about the neurophysiological research being conducted in his time.

2.4 The genome space and the global phenotype

2.4.1 Genome architecture

At the turn of the 20th century there has been a great re-conceptualisation of the nature and organisation of genome architectures. The discoveries of the middle of the century lead to portray biological organisation as encoded in an apparently static structure of nucleotide sequences. At the end of the century the picture was that of a highly dynamic genome structure. Whereas genomes were portrayed as passive templates for recording and transmitting random variations that would be preserved long enough in order for natural selection to transform such meaningless gibberish into informed instructions, now the picture is of a genome which has an active role in its own evolution (Fox, 1999: 290; Caporale, 1999).

In this new portrait some important evolutionary issues have emerged: the mosaic structures (or architectures) of genomes, their dynamics, their plasticity and their stability as systems. In this dynamics it turns out that the ability of genomes to play an active role in their own evolution is also a part of the developmental processes of the organisms that carry them.

Four decades of dissecting genome function at the molecular level have shown that genomes are organized as hierarchies of composite systems (Figure No. 2).

At higher levels whole genomes, plasmids, viruses and multilocus regulons display characteristic architectures. In turn each smaller subsystem displays its own characteristic mosaic organisation integrating smaller and smaller subsystems into the larger system. A chromosome or a plasmid may contain several operons. Operons are organised in protein coding regions (genes) and regulatory sites. Each component of an operon has in turn its own internal organisation: a single gene contains sequences encoding modular protein domains that carry specific functions (DNA binding, substrate binding, etc.). These open reading frames are systems rather than elementary units. The distinct domains are capable of encoding genetically separable functions and each can be used many times in various combinations with other domains (Shapiro, 1992, 1997, 1999).

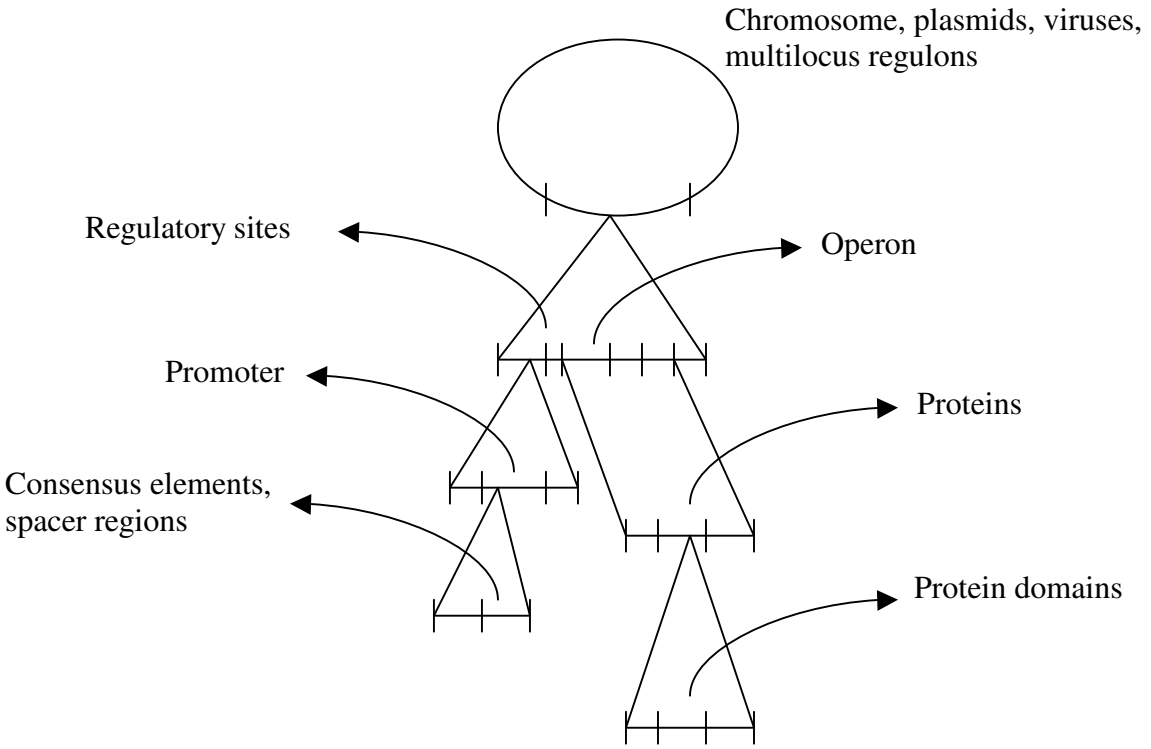


Figure No. 2. Genome architecture: "hierarchies of composite systems"

In the same manner the regulatory elements and binding sites are organized hierarchically. For example at a lower level the regulatory region can be broken into promoter, operator binding site and so on. In turn a promoter can be broken down into -10 and - 35 consensus elements and spacer regions of defined length. But at higher levels, the promoter can integrate the whole operon into a transcriptional regime of coordinated functions determined by a group of operons which have to be simultaneously activated during some particular conditions. That is, the repetition of the same (or similar) promoter in different operons allows for the regulation of several different operons located at separate sites on the chromosome by the same repressor protein. Such coordinately regulated operons constitute a regulon (Shapiro, 1992, 1997, 1999). The complexity of genomic integration into multilocus systems becomes even greater if we consider the biogenesis and functioning of cellular organelles and

the processes of multicellular development, where suites of loci throughout the genome respond to intra- and intercellular signals (Shapiro, 1999: 25).

The taxonomy of genetic determinants has grown widely. In Figure No. 3 an incomplete list of generic genetic motifs contained in genome architectures is shown. In recent years there has been increasing attention to the repetitive genetic elements and to the mobile genetic elements (Fedoroff, 1999). All these determinants and higher order systems are said to be interconnected and organized into specific "systems architectures" by dispersed repetitive DNA elements (i.e.: promoters and regulatory binding sites) which, according to Shapiro (1999: 26), constitute the physical basis for integrating distributive genomic networks. But binding sites in regulatory regions are only one class of repetitive DNA elements. According to Shapiro other aspects of genetic function (e.g. replication, proof-reading, recombination, chromosome mechanics) are also controlled by particular genetic codes, and the motifs, which constitute the appropriate codons, are inscribed in DNA molecules (as repetitive elements). Other essential processes also depend on dispersed repetitive elements that do not encode proteins e.g.: codons that make up telomeres, centromeres, and replication origins. Repetitive DNA elements set the "system architecture" of each species because of their fundamental roles in genome transmission and in determining patterns of gene expression. From this perspective what makes each species unique is not the nature of its proteins but a distinct "specific" organisation of the repetitive DNA elements that must be recognized by nuclear replication, segregation, and transcription functions. It is significant that these repetitive sequences show a high degree of taxonomic specificity. Many organisms within a phylum have functionally interchangeable proteins but differ critically in how protein synthesis is regulated during development (e.g., mice and elephants). "Sibling species" pairs have no significant phenotypic differences (or even changes in the order of genetic loci on the chromosomes) yet the two species can be distinguished by their repetitive DNAs (Shapiro, 1999: 25-27).

Some genetic determinants and motifs

- whole genomes /chromosomes

- plasmids, viruses, multilocus regulons, operons

- polypepttide coding sequences / multidomain protein-coding sequences
  - DNA binding sites
  - inducer binding sites, etc.

- regulatory sites / transcriptional determinants/ binding sites
  - consensus sequences or "boxes"
  - operators
  - DNA binding sites for transcription factors and RNA polymerases
  - promoters
    - -10 and - 35 consensus elements
    - spacer regions of defined length
  - multilocus regulons and replicons

- replication origens, partitioning sites

- repetitive genetic elements
  - heterochromatin (simple sequence repetitive DNA)
  - small repetitive arrays
    - triplet repeats in coding sequences
    - introns

- mobile genetic elements
  - transposons
    - transposable binding sites at the termini of transposable elements
    - temperate bacteriophages
    - retrotransposons
  - gene cassettes/integrons

- codons that make up telomeres, centromeres, and replication origens

Figure No.3. An incomplete taxonomy of generic genetic determinants

2.4.2 Evolution is a balancing act

In order to maintain their presence in ecosystems, living organisms must have considerable genome stability as genomes are transmitted from generation to generation. Alterations in the genetic message by any of the different mechanisms of spontaneous mutagenesis set some limits on genetic stability but at the same time this constitutes one of the basic means for evolutionary development. In other words, the capability of variation is what confers genetic plasticity. So there is a trade-off between stability and variability of genomes. There are multiple mechanisms that generate genetic diversity and which may contribute to genome plasticity: from local sequence changes (replication infidelity, environmental mutagens) to more elaborate DNA rearrangements (recombinational reshuffling), to DNA transmission and acquisition between organisms (horizontal gene transfer) (Arber 1991, 1999). Many of these mechanisms (especially those regarding recombination processes and DNA acquisition) depend on enzymes which often do not act fully at random on the DNA. The involvement of specific gene products in these processes and the important role that is attributed to the generation of genetic diversity for the evolutionary expansion of life leads Arber to call these genes ”evolutionary genes”, i.e., genes (and gene products) that modulate the frequency of genetic variation (Arber, 1999: 36). Genes for repair processes and genome maintenance can also be considered as evolutionary genes (together with those that produce variation) since one may assume that the efficiency of DNA repair relates both to a moderate genetic stability and to the evolutionary need for a certain proportion of genetic variants in populations of cells. These include recombination complexes, transposition processes, repair and proof-reading systems as well as systems for uptake and transfer of DNA. Arber has postulated that evolutionary genes must themselves undergo biological evolution and he calls this sort of meta-evolution ”second order selection”.

2.4.3 Communication in the genome space

Of the highest importance has become the communication potential between genomes. Besides the strategy of development of gene functions in the vertical transmission of genomes from generation to generation there is also the possibility for the acquisition of short DNA segments from other organisms. This is what is

commonly referred to as horizontal gene transfer (Syvanen and Kado, 1998). Due to acquisition barriers the horizontal strategy works best for relatively small DNA segments such as domains, single genes and operons. Whereas random encounter plays an obvious role in horizontal gene transfer, it always also requires the activities of many enzymes and functional systems (Arber, 1991). Here, mobile genetic elements play a decisive role in the mobilisation of chromosomal genes onto natural gene vectors (Heinemann, 1998; Hall, 1998; Arber, 1999; Shapiro 1999).

There are of course many limiting factors that act as modulators of the balance between stability and plasticity in the process of acquisition of genetic material. These factors include surface incompatibilities, restriction-modification systems and functional compatibilities between exchanging entities (Arber, 1991:9; Lorenz et al., 1998). Because of both the (quasi) universality of the genetic code and the functional similarities of different living organisms, a gene or the functional domain of a gene that had a function in a donor organism may also be able to function in a potential recipient organism (Arber, 1991:10). Thus, according to Arber, the classical evolutionary tree should be drawn with horizontal shunts that represent the possibility of horizontal flux of genes from one strain of microorganism to another. In this way, the evolutionary tree actually becomes a "multidimensional evolutionary network".

What all this means is that genome evolution is beginning to be seen as a dynamic process that includes not only gene shuffling within the genome, but also gene transfer across genomes (Sonea 1990; Syvanen and Kado, 1998; Caporale, 1999). Heineman (1991) and Jørgensen (2000) picture bacteria as the hub in a model describing the dissemination of genetic material among the four other kingdoms (plants, animals, fungi, archae). Both gram-positive and gram-negative bacteria exchange genetic material among them and examples of gene transfer from bacteria to organisms that belong to one of the other four other kingdoms have also been documented. In this way bacteria become the center of DNA flow, and the uptake of naked DNA by natural transformation becomes a very important process in dispersal and in evolution (Heineman, 1991, 1998; Salyers et al., 1998; Jørgensen, 2000).

Bellgard et al. (2000: 298) propose the term "genome space" as the entire set of genomes of all living organisms. The genomes of bacteria, archaea, and eukarya may be considered as three genome subspaces within this genome space. During evolution, genes have been transferred between species within a subspace as well as between species in different subspaces. As suggestive as the notion of a "genome space" may

be, this is practically all they say about it in their paper entitled “Dynamic Evolution of Genomes and the Concept of Genome Space”.

Now we start to see the genome space as a plastic entity that is continuously producing and incorporating mutations events; intra- and inter-organisms shuffling of DNA mobile elements and genes; and intra- and inter-species gene transfer. It is now being observed that many of these events - including mutations - are not purely random but may be "induced" by a given context (not only by chemical insults, but also by other kinds of environmental cues).

Cells possess numerous biochemical systems capable of changing and reorganising DNA sequences and genetic determinants: “tools” cells can use to modify their genomes. As pointed out before, cells also posses a wide range of repair and proof-reading functions to remove accidental (stochastic) changes in DNA sequence resulting from replication errors and physicochemical insults. This suggests that bacteria have little tolerance for purely random variability (Shapiro, 1997: 99). There are also well-documented studies of *in vivo* cellular DNA manipulations in eukaryotes (Shapiro, 1999: 27). Shapiro refers to these systems as “natural genetic engineering systems” (Shapiro, 1992, 1997, 1999), what Arber (1991) calls “variety generators”. In fact, genetic engineering in labs, cutting and splicing DNA, depends upon reagents developed by several decades of research on DNA biochemistry: enzymes and systems extracted from living cells such as nucleases, ligases, polymerases, vectors packaging extracts, etc. (Shapiro, 1992: 102). *What distinguishes cellular biochemistry from chemistry outside the living cell is that cellular events are subject to biological regulation by signal transduction networks*. Shapiro (1999: 28) presents evidence that DNA biochemistry is no exception to this rule and that natural genetic engineering is also subject to biological regulation mediated by signals that control both the timing and the localisation of changes, and he claims that the depth of these regulatory interactions (between cellular signal transduction networks and natural genetic engineering systems) is likely to prove typical rather than exceptional (Shapiro, 1997: 103). There is a fundamental difference between thinking about genetic change as a biological function regulated through signal transduction or thinking about genetic change as the stochastic, accidental result of replication errors and physicochemical insults. The point is that the ability to trigger mutagenic events could be extremely advantageous to organisms

under stress conditions if on the other hand context interpretation induces the system to guarantee genome stability under ordinary circumstances.

We can now observe how contemporary cell biology increasingly focuses on the operation of signal transduction networks and how these networks, in the reductionist strategy, are becoming the conceptual tool that is attempting to link the different sub-branches of biology from the molecular level up to ecosystems (Bruni, 2002). Shapiro compares these cellular networks to molecular parallel computers of considerable sophistication and with an incredible capacity for information processing that allows each cell to interpret its own status and adjust gene expression accordingly. In the light of this, he concludes, we should get ready for some surprises as scientists explore more deeply the notion that signal transduction networks can guide the abilities of cells to engineer their own DNA (Shapiro, 1997: 103).

Incidentally, as we will see in some of our examples, genome communication is not limited to the evolutionary process. It is becoming increasingly evident how inter-species genome communication influences development in the ecological space. Signals from organisms of one species determine genetic responses in organisms of different species.

2.4.4 The global phenotype

Just as we are bound to bear in mind that all living manifestations are historical entities we have to remember also that all living entities are alive at the same moment. So the global genome space is a dynamic space that contains and continuously renovates the potentiality of the global phenotype (the totality of living specimens at a given moment). But unless we want to remain stuck to a geno-centric view, we have to consider *the mutual semiotic constitutivity* of the global genome and its phenotypical counterpart. It may be hard to imagine the continuous nature of this genome space and of the global phenotype since genotypes are carried around by organisms that by their individuality seem to be separated from each other. But what determines this continuity is precisely a communication process, or more generally, semiosis. For example current concepts about biodiversity advocate for protection of genetic diversity within a species since it is claimed that in the process of variation in time (evolution) each individual is a carrier of diversity. If evolution is to be

considered a gradual process depending on point-mutations this would mean, paradoxically, that in a sense bio-diversity is a continuum in space and time.

Horizontal communication of fragments of the digital redescription of organisms complicates our view of the semiotic interaction between the analog coded state (the global phenotype) and its digital redescription (the global genome). It turns out that the digital code is not only part of the vertical semiotic system but it is also part of the dynamic horizontal process in the ecological space. (For the concept of code-duality - the unending chain of coding, recording and translation, between the analog and the digital states of organisms, i.e.: the organisms themselves and their DNA redescriptions - and for the interplay between vertical and horizontal semiosis, see Hoffmeyer and Emmeche, 1991).

The potentiality for "change" and for the production of novelty implicit in the genome space is regulated, or better yet, mutually determined by the actual manifestation of such potentiality (the global phenotype, i. e., the analogical counterpart) in relation to the existing environmental conditions. That is, we can consider the interplay of code-duality, the constant digital-analogical-digital translation, from global genome to global phenotype and so on, as a sort of homeostatic system of mutual determination. What connects all this is the process of semiosis: the indeterminate chain of triadic relations that "make sense" out of the material-energetic exchanges present in the bio-mass, and which give rise to the distinctive characteristics of lively dissipative structures and their goal-oriented behaviour.

No matter what our theory of the origin of living matter is, as long as there has been living matter on this planet, there has been a global phenotype, with its global genome space included, a biosphere, and more importantly, what makes it "global", a semiosphere. No matter how long did it take to arrive to its "spherical" shape, to its "all around the sphere" globality, semiosis has always been a feature of it. Just as we can talk about a "threshold of life" and accordingly a "(bio)semiotic threshold" we have to acknowledge the emergence at a certain point of an "irreducible complexity" in the evolving genotype-phenotype-environment.

How can genomes constitute a single space if they are separated from each other by their respective phenotypes? But we seem to have no problem in homogenising diversity in terms of biomass. Does "biomass" unite what is separate while "biodiversity" separates what is united? In fact the genotype-phenotype dichotomy is

a very strange one because it is obvious that the genotype is part of the phenotype that carries it around. Genomes don't walk around by themselves! Isn't the genome just one more organ, an information *depository* organ, but please, not "the" information depository organ? The synchronicity of the global phenotype, its irreducible complexity, points to co-evolution and symbiosis as the main evolutionary modes. Its counterpart (individual) evolution reduces life under the threshold of its actual manifestation.

The reason why in biology it has been impossible to separate development from evolution (actually an absurdity) is because semiosis (communication) between genotypes together with their phenotypic carriers, be that between individuals of a species or between individuals of different species, is inevitably (as intrinsic to the idea of time) simultaneously a synchronic and diachronic process, and therefore semiosis has to be always considered simultaneously in the vertical semiotic system (the genealogical hierarchy) and the horizontal semiotic system (the ecological hierarchy). Through this semiotic process the global phenotype and its genome space coevolve responding to changes and creating changes as well in the compound biotic and abiotic elements of the biosphere.

2.5 Tools for mapping semiotic networks

2.5.1 The continuous "chain of information" between levels of complexity

Another important assumption of this work is that instead of originating exclusively at the molecular-genetic level, "biological information", as the vehicle for communication, must present common features and causal relations at all different levels and systems (Emmeche, 1998a), and, therefore, information transfer must occur also from one emergent or hierarchical level to another. Such a claim will allow us to consider a less genocentric view of living systems.

In figure No. 4 I show a hypothetical hierarchy of levels for information transfer within and between living systems. In this regard let me remind once more the remark made by Chargaff in 1962 about the implausibility of DNA as the repository of biological information if it was not possible to identify a "continuous chain of information" from the lowest level to the highest (Sarkar, 1999: 199). As mentioned before, this argument poses a very interesting and central question to contemporary

biology: how can we conceive "the continuous chain of information from the lowest level to the highest" and perhaps from the highest to the lowest? This is the field in which a sign-theoretic approach would be pertinent to biology in general and to biotechnology in particular.

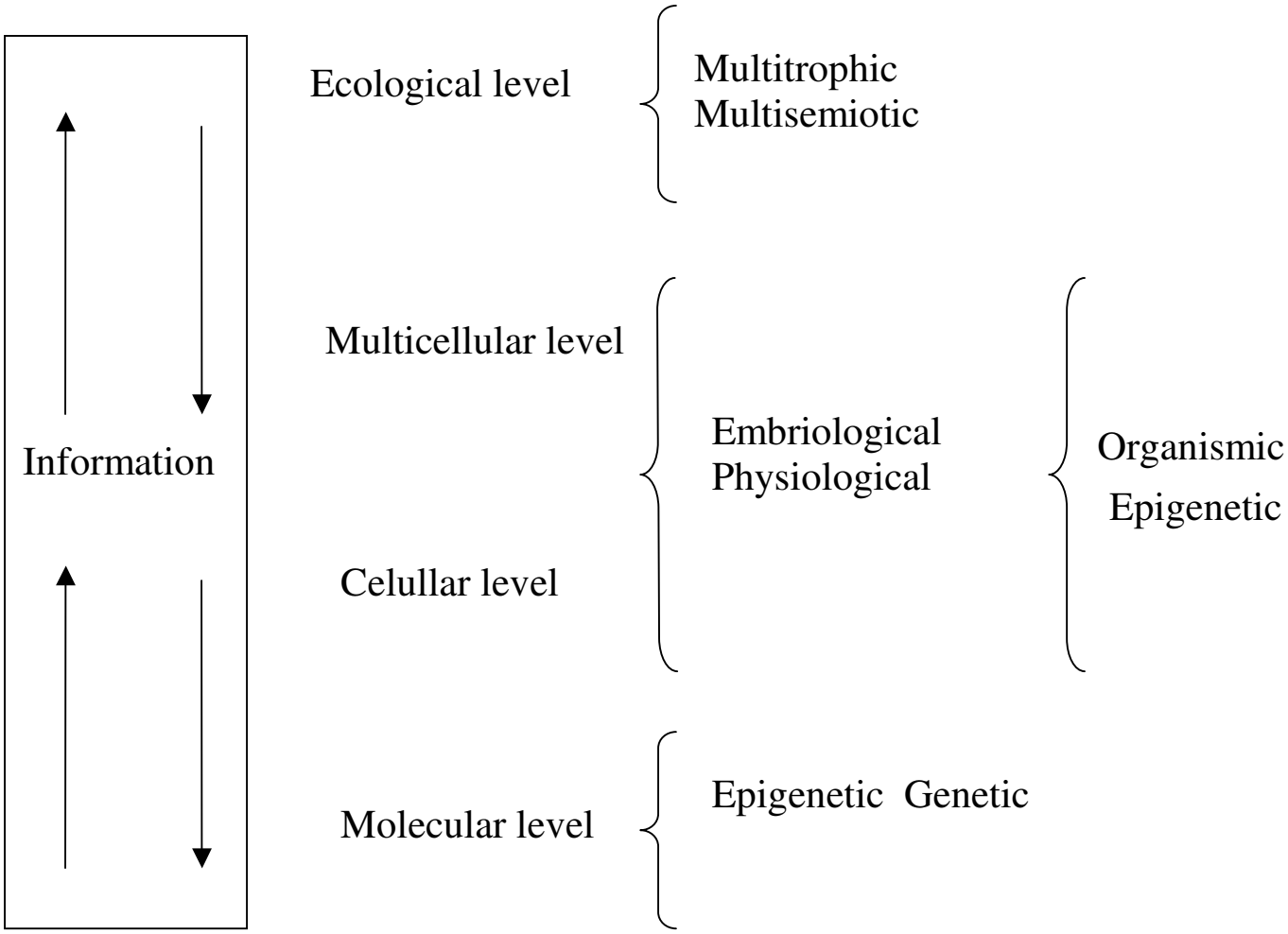


Figure No. 4. Biological information between levels of complexity

What is at stake here is the problem of "localisation" of information which poses also the problem of localisation of related "things" such as regulation. Bateson

reminds us how this matter has bedevilled communication theory and especially neurophysiology for many years:

> "It is flatly obvious that no variable of zero dimensions can truly be located. 'Information' and 'form' resemble contrast, frequency, symmetry, correspondence, congruence, conformity, and the like [including also "specificity"] in being of zero dimensions and, therefore, are not to be located" (Bateson, 1972: 408).

What Chargaff called the "chain of information" could not work in a dyadic mechanical frame of causality, but would have to be redefined as the emergence of integration levels, and, while at a given level there may be a myriad of dyadic (mechanical) causal relations, the emergence process is mediated by triadic (semiotic) causality. The details of the complicated dyadic "mechanisms" of the myriad signal-molecule cocktails that constantly and dynamically poke into, or bind to, receptors are not sufficient to explain the emergence of novel semiotic contexts by the addition of such mechanisms. A mechanical dyadic explanation of signalling molecules suffices only at a given hierarchical level. But the subsequent relevance of these isolated events (up or down scale) in a hierarchical and evolutionary perspective make better sense when seen within a triadic logic (Bruni, 2002).

It is this "chain of information", with its upward and downward causality - the emergence of new semiotic contexts at different hierarchical levels and their reciprocal influences - that I think is central for the integrative agenda in biology.

In the spontaneous inductive strategy different types of "biological information" (and codes) are constantly being invoked without any clear conceptual link with previous or new concepts of biological information across hierarchies. For example, does quorum sensing (see section 2.5) imply a new kind of biological information? (Bruni, 2002). What about the information implied by the recognition specificity provided by a particular protein domain in the relation *R*-gene *Avr*-gene (Jones, 2001), or for instance the information provided by the specific profile of a blend of volatiles emitted from a cabbage plant? (Shiojiri et. al., 2001). Current theories on the evolution of induced defence are based on the concept that current herbivory or disease is correlated with the risk of future attack. In this sense it is claimed that current or past attack can be seen as having the potential to provide information about

the future environment. How do these types of biological information relate to each other? Or to genetic information? Or to the information that is said to be transferred by phosphorylation? Or by bioluminescence? In other words, what common principles and causal relations can be identified in all these different types of "information"?

Biology, lacking a unified paradigm to deal with all these communication codes, languages and sensing, and being so committed to physical reductionism, has not been able to come up with a coherent picture of all these semiotic processes across the different emergent levels of organisation.

We say we have found regulation in "signal-transduction". But have we found it? What regulates what?

How can we relate the different emergent levels of informational processes and semiotic contexts in developmental trajectories? How are interpreting systems formed at these levels? How do codes and semiotic networks emerge in this picture?

In the emergence of these semiotic networks "information as specificity" plays a central role together with the interplay between digital and analogical codes in nature.

How is "information" conveyed through the continuum?

I suggest that this process is mediated by what I will now define as *digital-analogical consensus.*

2.5.2 Digital-analogical consensus

Digital-analogical consensus can be defined as the mediatory action of codes which are formed at different hierarchical levels out of an indefinite number of dyadic causal relations, specific "lock and key" interactions, that by their simultaneous occurrence give rise to emergent and "de-emergent" specificities and triadic relations.

This process can be described as the formation of an interpretant by the simultaneous (synchronic) occurrence of a combination of factors, determinants or circumstances.

In other words, the continuous code-dual process that through an indefinite set of digital messages in simultaneous occurrence forms an analogical message that is interpreted (sensed/formed) as the context by the emergent interpretant.

In a reducible perspective, this could be viewed as the emergence of new analogical signs (properties, contexts, pieces of information) by the aggregation of digital signs.

By the same token, the analogical mode (the bulk of information) influences the circulation of digital information (downward causation). These influences from above can contribute to determine new configurations at lower levels.

But the emerging analogical compound effect may also constitute a "quasi-digital" piece of information to a higher level of aggregation ("to be or not to be"). In this way the new analogical sign can be a digital contribution to a still larger or more complex analogical sign (Bruni, 2002).

We encounter emergent processes in which new levels and kinds of signification in biological processes appear. And these new levels of signification are not always specified by the precedent lower hierarchy process. As with many emergent properties, one can not exclude downward causation.

The process has a hierarchical and emergent nature and it implies levels of integration in which a myriad of dyadic causal relations at a given level contribute to the emergence of the interpretant in a process mediated by triadic causal relations. Here, hierarchical levels do not imply a limited number of levels. It refers rather to the emergence of interpretants in a continuous process.

Through this constant hierarchical digital-analogical conversion, “information” is “conveyed” from lower to higher systems and vice versa, information and contexts emerge and semiotic processes have a causal link to higher, parallel or lower levels in the hierarchy.

An emerging "state" constitutes a difference that can be sensed by some system with interpretative capacity. Every effective difference denotes a demarcation, a line of classification, and all classification is hierarchic. In other words, differences are themselves to be differentiated and classified (Bateson, 1972: 457). But also complex aggregates of differences are to be differentiated and classified. That is why semiotic description is always hierarchic.

> “... there is that hierarchy of differences which biologists call ‘levels.’ I mean such differences as that between a cell and a tissue, between tissue and organ, organ and organism and society.

> These are the hierarchies of units or *Gestalten*, in which each subunit is a part of the unit of the next larger scope. And always in biology, this difference or relationship ... is such that certain differences in that part have informational effect upon the larger unit, and vice versa" (Bateson, 1972: 458).

In 1950 geneticist Hans Kalmus claimed that since the action of a particular gene was sometimes felt in a distant cell, genes acted more like a "broadcasting system" than "wired telecommunication" (Sarkar, 1999: 203). DNA digitally encodes an analog, i.e.: a protein. This analog by binding or not binding a correspondent protein (or nucleic acid), that is, by being or not being (there), may also become a digital message. But the simultaneous expression of a set of genes may constitute itself an analogical message (with its respective context). This type of message is not itself specified by digital DNA. In this sense Kalmus' "broadcasting system" "irradiates" an analogical multidimensional wave rather than the linear digital impulses of wired telecommunication.

Just as in human language larger narratives represent a kind of analogical information that emerges from the underlying digital code (natural language), larger aggregates of digital information become analogical when its complex interactive dynamics become explicit. This dynamic up-and-down causality mediated by signs is an ontogenetic historical continuum that oscillates within the boundaries of the code-dual nature of organisms and ecosystems.

In chapter 3 I will elaborate some detailed examples of this principle in relation to signal transduction and metabolic regulation. For the moment let me furnish some short illustrative examples from different hierarchical levels hoping to facilitate its understanding:

1) The transformation pathway in bacteria (as well as conjugation and transduction) works through a set of emergent "lock and key" binding mechanisms (which may be more or less specific). That means that "competence" is the aggregation of different specificities.

2) The relative composition of a blend of volatiles emitted by a particular plant infested by a specific herbivore (Takabayashi and Dicke, 1996; Shiojiri et. al., 2001) is the real message and not the compounds themselves, i.e.: the blend functions as an analogical message formed from a complex mix of digital information, the presence or absence of a certain threshold concentration of each compound. However each

compound's concentration is in turn an analogue that is formed out of the digital presence or absence of each molecule, which in turn are tridimensional analogues, an so on.

3) The complex specificity of the host-symbiont relation is determined by an aggregate set of specificity determinants which in turn are conformed by lower level specificities. In section 2.6 I give a more detailed treatment of this example by describing the *Vibrio fischeri-Euprymna scolopes* symbiotic relationship as a semiotic network built from emergent specificities that range from the workings of the Lux operon in the genetic organisation of the bacterium, to the different levels of specificity determinants in the symbiotic relation, up to the emergence of the particular context that gives the squid the chance to avoid being predated at higher trophic levels (with the help of the bacterium).

4) The fact that in some cases the recognition of a given avirulence factor may require not one but several host genes, which in turn may need several other biotic or abiotic (contextual) cues, offers the possibility of a creative response to a very specific challenge, i.e.: the formation of a very sophisticated habit, the emergence of a resistance response. This sophisticated habit based on the interpretation of a complex cocktail of dyadic relations could be compared to our capability of producing new thoughts by "synthesising" a whole new idea from subordinate thoughts.

5) The configurational changes experimented by the ribosome (an analog) during protein synthesis result from specific combinations of proteins bound to it in complex interactions. Here each protein acts as an individual digital message (by binding or not binding), but at a lower level each protein is an analog that results from complex interactions of digital messages.

6) In neurons, inositol triphosphate receptors (InsP3Rs) are sensitive to both inositol triphosphate and calcium ions. They are thought to act as "coincidence detectors" to correlate the activity of pre- and postsynaptic inputs, which is central to memory formation (Berridge et. al., 2000: 13). This is hardly just some idiosyncratic detail in a particular "mechanism", this is a general rule in biological processes. Almost all genetic circuits obey to suites of signals and regulators that have to act simultaneously and which result in the emergence of a complex phenotype.

7) In the phenomenon of "synaptic summation", when two neurons, A and B, have synaptic connection to a third neuron, C, the firing of neither neuron by itself is sufficient to fire C; but when both, A and B, fire simultaneously (or nearly so), their

combined action will cause C to fire. From the physical point of view, this combining of events to surmount a threshold is called a "summation". But from the semiotic point of view this synergy would not be a summation. The system operates to create differences. There are two differentiated *classes* of firing by A: those firings which are accompanied by B and those which are not. Similarly there are two classes of firings by B. The so-called "summation", when both fire, is not an additive process from this point of view. It is the formation of a logical product - a process of fractionation rather than summation (Bateson, 1972: 457).

We can consider the interplay of code-duality (Hoffmeyer and Emmeche, 1991) - the constant digital-analogical-digital translation, from the genome space to the global phenotype, and vice versa - as a sort of homeostatic system of mutual determination. What connects all this is the process of semiosis: the indeterminate chain of triadic relations that "make sense" out of the material-energetic exchanges present in the bio-mass, and which give rise to the distinctive characteristics of living dissipative structures and their goal-oriented behaviour.

It should not be hard to acknowledge the context-dependent nature of any kind of information. A context can be a larger aggregate of information, a set of simultaneous occurrences that conform a sign that is received in its complexity by the interpreting system. The context could be characterised in terms of digital-analogical consensus, but evidently it is not fully quantifiable.

The important thing is that at a certain point, at a certain time and place, a quality is formed and/or sensed, and that perception generates causality or "agency", that is, it presupposes a next step. For this reason, when dealing with semiotic processes qualities become important. So in order to characterise a semiotic network it is necessary to characterise the context, which by being an analogical complex combination of factors - and by having an effect upon different logical levels of causality in the hierarchical process under study - has a qualitative nature hard to quantify. This is the level at which we have to rely on monitoring patterns to aid our characterisation of the context. This characterisation can be achieved not so much by pinpointing every quantitative detail of the picture but by relying on the cyclic appearance of patterns, in our effort to get, as much as possible, a less static picture of the context in evolution.

The next problem would be how to explore and how to chose which patterns can be useful in the characterisation of the context of the semiotic network under study. This is related to the problem of how to take the best advantage of the enormous quantity of empirical data that is being produced, in other words, how to organise the data into an integrating description that relies on patterns.

Digital-analogical consensus provides complex possibilities for fine-tuning responses to variable contexts in an incredible creative combinatorial manner. Visualising biosemiotic processes in this way can be useful to organise hierarchically the suits of factors that determine or influence emergent properties in a given causal network.

In summary, one has a configuration of many digital instances, presences, combinations of locks and keys, threshold concentrations, that create an analogical state, a compound effect, a quality which (triadically) finds its relevance, its significance, at an emergent level. Signal transduction networks are an instance of this kind of relations. Combinations of many signal transduction networks compound an analogical message that (homeostatically) regulates a higher order process, property, state or phenotype.

2.5.3 Complex specificities

Digital-analogical consensus is closely related to the very common notion of biological specificity. However, there are new kinds of specificities at much higher levels than the basic stereochemical specificities. Actually, these basic stereochemical specificities combine to give rise to more complex specificities. This emerging process is related to, and is probably at the base of, the increasing semiotic freedom exhibited by complex organisms, i.e.: the extent of logical (or causal) independence that some processes can acquire with respect to the physical dynamics of the substrate that underlies such processes. The most extreme example of this would be the path through different levels of ascending complex “lock-and-key” mechanisms that goes from stereochemical specificities up to free will or natural language (a sophisticated emerging “system of correspondences”).

There are basic types of specificities which give rise to new and more complex types of specificities:

- the specificity of each DNA sequence for its complementary strand, as modulated through the specificity of DNA base pairs.

- the specificity of the relation between DNA and protein, modulated by “genetic information”, understood as the specification of a protein sequence, i.e. the linear amino acid residue sequence of a protein from a DNA sequence as a process of “translation”, i.e. the triplet-amino acid specificity.

There are more complex types of specificities:

- gene-enzyme specificity
- enzyme-substrate
- antibody-antigen
- signal molecule-receptor
- activation complex-DNA, and so on....

The simultaneous and complex "activation" of an indeterminate number of these "lock and key" mechanisms mediate the emergence of new informational-semiotic contexts and new and more complex "lock and key" mechanisms and specificities like for example:

- “cocktail” of signals-cellular response
- host-symbiont
- organism-niche.

Specificities at different levels become an analogical message out of the complex interaction of many lower-level specificities. These complex specificities establish “systems of correspondences”, “systems of ideas in circuit” (see below). The importance of hierarchising semiotic contexts is that sometimes at a given level what may look as an “either-or” choice of function or manifestation, may be determined by the compound effect of a larger analogical message, a bulk of information, that has a causal link to the lower level. For example, whether a pathogen protein acts as a virulence or as an avirulence factor is determined by a larger gestalt at a level above the dyadic resistance-(a)virulence protein relation.

Digital-analogical consensus emerges as a general pattern for sign construction, i.e.: for generating complex specificities and lock and key mechanisms, creating immense combinatorial semiotic possibilities for regulating and fine-tuning complex,

detailed and decentralised responses to equally complex, detailed and decentralised stimuli.

2.5.4 The emerging interpretant

I stated before that an indefinite set of digital messages in a simultaneous occurrence may form an analogical message that is interpreted (or sensed) as the most comprehensive assessment of the context by, or at, an *emerging interpretant*.

It is worth to spend a few lines to make clear in what sense this notion is being understood here. Let's start from the Percian definition of the sign. The three factors of a triadic relation i.e.: the sign-vehicle, the object to which it refers and the interpretant may belong to various orders of reality as single objects, general classes, physical impulses, organic activities, mental representations, natural laws, etc. What constitutes the sign relation is the particular way in which this triad is bound together (Santaella, 1999: 515).

"The interpretant" is the regularity by which the sign-vehicle links a particular object (e.g.: a particular contextual demand, a necessity, a stress, a state of affairs) to a specific effect or response.

The sign-vehicle (e.g.: a signal-molecule, a blend of signals, etc.) acts as a mediator between the object to which it refers (a particular aggregate of contextual parameters) and the effect that such a sign-vehicle (and indirectly the object) produces on a system (or on a stage in the process of development of that system).

The mediation of the sign in relation to the object leads to the production of the interpretant which, however long the chain of interpretants may grow, will always be due to the logical action of the object, that is the action mediated by the sign. "Furthermore, this triad implies a constant expansion of the process of semiosis since the interpretant, in turn, determines a further sign, becoming thereby a sign to that further interpretant. Semiosis is, thus, an infinite process or an endless series in a process that operates in two directions, 'back toward the object' and 'forward toward the interpretant'." (Santaella, 1999: 515). An interpretant can be understood as the synchronicity of circumstances and factors that determine that in a certain process there is sign-function.

The sense in which I will be using the term does not imply an autonomous entity of any kind. It can be rather viewed as a level of integration. The easiest way to grasp

the meaning of the concept is to think about it as *the level* at which a complex configuration of signals or signs makes a difference to some living process or entity. But the emerging interpretant is not the entity or the process itself. In practical terms, from the observer's point of view, it is a focal level that for the purpose of our analysis we can identify as the point or the moment of convergence for different kinds of factors that acting together "select" a direction for the whole system. It will be an emerging interpretant if the resulting action is a pattern or a habit that can be observed regularly in relation to a particular state of the context.

I put the emphasis on the emergent characteristic of the interpretant because it has to be considered as something that is generated at every second in a continuous basis. Actually the formation of an interpretant is the creation of meaning (and/or function) itself.

> "We cannot directly observe the interpretant according to which a living system codes effects of the environment on its receptors into signs. We have to infer it from the system's behavior; we have to interpret endosemiosis ... by reconstructing their 'history': we take the last act or behavior of the living system as an indexical sign pointing to the interpretant which, as the coding instance, has assigned to the sign the meaning it has with regard to the system" (von Uexküll et. al., 1993: 15).

The emerging interpretant is not to be understood in any anthropomorphic sense and neither as an autonomous entity of any kind but as a stage in the process of emergence in the semiotic network under study.

It could be argue that the consideration of emerging interpretants at the sub-cellular level would be a reification of those sub-cellular levels and complex biochemical reactions as if they were living entities below the cellular level. The answer to this is that we may consider these sub-cellular levels from a biosemiotic point of view, as embedded in a hierarchical system of emerging interpretants at higher levels. The problem of reification, or entification of the emerging interpretant at levels below the cell (as the level at which the sign-vehicle makes a difference) arises only when we want to consider the problem of the origin and the build-up of the first system. In order to avoid confusion with the "attractors" of complex dynamics, a distinction must be made between the "attractors" in complex physical

systems and the "emerging interpretants" in complex living systems. The latter present a higher potentiality for freedom with respect to the constraints and boundary conditions that surround it. Information implies causality beyond dynamics.

Obviously, our identification of the emerging interpretant depends on the phenomena that we wish to examine. The emerging interpretant can be seen as the locus at which a goalseeking system (which can be contained in a larger system) defines its goal. It is that part of the emerging system that achieves a higher logical type of manifestation with respect to the dynamics that underpins it.

2.5.5 Systems of correspondences - Systems of ideas in circuit

At the present stage of evolution we have to deal with coevolutionary systems which present emergent properties. What is evolving today (and since a long time ago now) are not single entities but entire complexes of sophisticated networks at all levels, from genomes to phenotypes, from prokaryotes to Internet.

What is informed by the genome are integrated systems of functional domains, which constitute elemental units for a great diversity of emergent codes. All these codes share the principles of specificity modulated by digital-analogical consensus working within a triadic logic in the process of emergence of complex phenotypes. The different functional domains in a single protein allow its interaction in and with different directions of the network and with different actors of the system. Each functional domain represents a correspondence with other domains distributed in the products that are coded in the genome, as well as correspondences with products coded in or by the environment, including organisms of the same or different species[3].

For example, a homologous phosphorylisable sequence (i.e.: a sequence which is susceptible to being phosphorilated in a particular residue) can be encountered in different proteins combined with a variety of other domains that give its particularity to the protein. In this way, the code of phosphorylation is distributed in the whole system. These correspondences at the level of functional domains is what is actually coded in the genome i.e.: networks of correspondences which are used to constitute metabolic codes; not complete complex phenotypes. What is coded in the genome are

[3]This is related to the "principle of correspondences" as discussed in Uexküll et. al. (1993: 12) which states that "in the sphere of living things each affordance presupposes a counteraffordance - that is, it can be realized only through an interaction".

the elemental units of specificity which are used and arranged modularly in the distributed network, as well as the "recipes" for successful structural elements. Part of the arrangement is implicit in the complex architecture of the genome. But the model for integrating circuits is an analogue implicit in the *embryonic signalome* (see section 3.1.2). The analogical "know-how" to ensemble and differentiate systems of correspondences must be inhereted in the embryonic signalome. Once cells start dividing, the new cells get the library and the whole system of interpretation. During differentiation the library remains the same, therefore differentiation starts by changes in the *signalome* (see section 3.1.1).

The combinatorial possibilities of domains constitute complex codes with different infrastructural organisation and mechanisms but which share common logical principles. In this view, DNA is a library of distributed architectures of integrated systems of corresponding (specific) sequences: the emergent digital units of the DNA code. The sequences or domains - be that binding sites, integrating repetitive motifs, protein domains, regulatory sequences, etc. - are used modularly within systems of correspondences and specificities that reach beyond the organism into its niche.

Let's take for example the following evolutionary consideration about a family of signal-transduction components:

> "Both Gsα and Ras are members of a family of intracellular GTP-binding switch proteins collectively referred to as the *GTPase superfamily* ... The many similarities between the structure and function of Ras and alpha Gs-alfa and the identification of both proteins in all eukaryotic cells, indicate that a single type of signal-transducing GTPase originated very early in evolution. The gene encoding this protein subsequently duplicated and evolved to the extent that cells today contain a superfamily of such GTPases, comprising perhaps a hundred different intracellular switch proteins. These related proteins control many aspects of cellular growth and metabolisms" (Lodish et. al., 2000: 905).

It is not that these proteins control these many aspects by themselves. They are a part of a sophisticated network that in its complex relations regulates the coordination of many simultaneous processes. Whereas it is said that a single type of protein

originated very early in evolution and from there came all its variants, it has to be emphasised that the function of the protein has to be seen in coevolution with the integrated network in which it functions. The protein cannot pop up being already potentially "useful" (functional) to something (within a system) and then "jump" into a circuit where that "usefulness" has a value. It is not plausible, to me, that a functional signalling molecule emerges, then it diversifies into 100 different variants and then each variant jumps into a slightly different but homologous mechanism within 100 different networks. Somehow the components and/or the networks must coevolve.

Be that as it may, convergence should not be considered as something uncommon. The same idea can be achieved with similar, or even with totally different, infrastructure. Convergence may occur at different levels. It can be that two different signals act through totally different infrastructural arrangements in eliciting the same genetic response. It can also be the case that the pathway is very similar but some steps are in some ways different. There can also be an instance in which some identical steps or mechanisms are used in pathways that lead to different "final" responses. In other words, there is convergence in the use of modular components. Pathways can be modularly arranged (by using existing ideas) to produce answers to similar and new kinds of challenges. The evolution of hierarchical specificities requires a different evolutionary mechanism, not so much based on single genes, but on modular components. Certain forms in the context pose a question, so to speak, of which the emergent component is an answer, a "functional" idea or a sign. This can be appreciated in the evolution and development of many specificities, like for example antibody and antigen in mammalians, or avirulence factors and response-determinants in plants.

At ecosystem level, biodiversity is the library for the ecological systems of correspondences which are involved in the development and organisation of ecosystems. If we destroy the information, i.e.: if we interrupt networks, we destroy the regeneration capacity of the ecosystem. Conversely, if we disable ecosystem-function, the information loses its sense, there will be no context for its interpretation. These two metasystems of correspondences, the genome and biodiversity, are in correspondence with each other. So besides a taxonomy of species, we are now developing a taxonomy of circuits.

What evolves and develops are systems of correspondences.
What survives are "systems of ideas in circuit".

2.5.6 Delimiting semiotic networks

Now we have a concept of biological information relevant to different hierarchical levels. What do we have to keep in mind in order to delimit a semiotic network?

Semiosis is multidimensional, i.e.: innumerable semiotic processes occur at the same time in multiple directions and emergent levels. Some of them may intersect, others may not (Santaella, 1999: 516). Semiotic networks can be temporally and spatially separated and still be in communication. Or they can be causally linked although they belong to (or can be identified at) different levels of the biological hierarchy. Therefore it is not an easy task to delimit a semiotic network. However, in Bateson's work there is already some criteria that differentiate semiotic kinds of networks from more physical kinds of chain-reactions. Such criteria need to be developed further.

As Bateson pointed out, in the hard sciences effects are in general caused by rather concrete conditions or events - impacts, forces, and so forth. But once you enter the world of communication, organisation, regulation, controls, etc., you leave behind that whole world in which effects are brought about by forces, impacts and energy exchanges. You enter a world in which "effects" are brought about by *differences.* As he shows, the whole energy relation is different (Bateson, 1972: 452).

First, we have an economics of energy and materials (bioenergetics) within a single cell, an organ, a coral reef or a tropical rainforest. Second, we have an "economics of information" (semiosis) within these entities. According to Bateson (1972: 460) these two pictures do not fit together very well precisely because the units are differently bounded in the two sorts of ecologies. In bioenergetics it is natural and appropriate to think of units bounded at the cell membrane, or at the skin; or of units composed of sets of conspecific individuals. These boundaries are then the frontiers at which measurements can be made to determine the additive-subtractive budget of matter-energy for the given unit. In contrast, in informational physiology and ecology, the semiotic aspects deal with the budgeting of pathways, codes and of probability. The resulting budgets are fractionating (not subtractive).

The world of information and differences is not limited to the imaginary "Gauss' surface" that we may draw in order to enclose our selected emergent interpretant, not even when we are talking about a higher organism, whose informational pathways extend much beyond its skin.

"*The boundaries must enclose, not cut, the relevant pathways"*, which protrude with messages and other pathways beyond the boundaries in which we have enclosed our relative unit (Bateson, 1972: 460).

Bateson further claims that in light of this, the very meaning of "survival" becomes different when we stop talking about the survival of something bounded by the skin and start to think of the survival of "the system of ideas in circuit"(Bateson, 1972: 461), i.e.: the survival of semiotic networks. This points to the difficulties that may arise when we attempt to separate endosemiosis from exosemiosis or when we fail to pay attention to the diachronic-synchronic continuity of the system. Maybe such separation is theoretically possible when dealing with bioenergetics (although I doubt it), but it is certainly impossible when dealing with semiotic networks.

The survival of "systems of ideas in circuits", i.e.: the workings of semiotic networks, is a function of the context, which is the more complex level of description of a system given that it comprehends all the pathways and restraints which in one way or another condition the totality of the system. Therefore, the characterisation of the context is of crucial importance when setting the boundaries of a semiotic network. Because of the richness of details that may constitute the context of a given system, its characterisation may appear sometimes as an impossible task. The answer to this is that instead of concentrating on the totality of pathways and restraints at a certain level we can concentrate on the hierarchical nature of pattern-formation and contexts when choosing the imaginary borders of the network under study.

Linear "cause and effect" explanations can be said to be "positive": "We say that billiard ball B moved in such and such direction because billiard ball A hit it at such and such an angle". On the other hand cybernetic explanation is always "negative", i. e.: "it considers what alternative possibilities could conceivably have occurred and then ask why many of the alternatives were not followed" (Bateson, 1972: 399). In other words, the course of events is said to be subject to *restraints*: factors which determine inequality of probability. Without restraints, the pathways of change would be governed only by equality of probability (Bateson, 1972: 399). Restraints of many

different kinds may combine to determine uniquely a given pathway or sequence of events.

In biological systems these restraints, or determinants, include cues, i.e.: sources of information which will guide the system in its "selection" or in its development. From the point of view of the cybernetic observer, these pieces of information are restraints in the sense that they increase the probability of a given manifestation or event to happen, or a given pathway to take course (Bateson, 1972: 400). From the semiotic point of view these pieces of information are differences that make a difference to an emergent interpretant within a hierarchical structural-functional system. Cybernetics deals with the probabilities of pathways while semiotics deals with the *choices* of pathways that the system makes based on the global interpretation of such restraints and probabilities in relation to its internal coherence. In this sense living systems are said to be stochastic. The restraints - including sources of information - lay out the probabilities of the pathways among which the informed system, based on its global interpretation will tend. So in biological systems restraints do not fully determine the outcomes of events; they increase the probabilities of certain pathways over others. A specific complex configuration of cues guides the system in its development at every instant, in a continuous way.

The role of a protein in a pathway, the physiology and the anatomy of some part within the organism, or the function of a species in a ecosystem are all things that require a "negative" explanation by an analysis of restraints (Bateson, 1972: 400). When we assume such an analysis from a semiotic point of view what we are considering is the configuration and evolution of the context.

In semiotic networks (and also in cybernetic circuits, as originally stated by Bateson), formal processes of mapping, translation, or transformation can be imputed to every step of a given sequence of phenomena. These *mappings* or transformations may be very complex, e.g., where the output of some system is regarded as a total transform of the input; or they may be very simple, e.g., where the output is an analogical transform of the input (Bateson, 1972: 401).

For example, as we will see later, signal transduction does not function with a single signal. The process consists in translating the analogical concentration of signals (sensed by the compound effect of a number of "digital" signal-receptor bindings) into an analogue concentration of single transforms that reflect the analogical information of the concentration present at the input.

The hierarchical nature of contexts (contexts within contexts) is universal for the semiotic aspects of phenomena. Therefore we tend to seek for explanation in the ever larger units. Without context there is no communication (Bateson, 1972: 402). It turns out that in biological systems, regulation is nearly always linked to semiotic controls and for this reason, regulation will tend to be the compound effect of many limiting factors at different levels of the hierarchy, but regulation, which is close to homeostatic balance, will always be integrated at higher levels of the system.

The elementary unit of information, *a difference that makes a difference*, is able to make a difference because the pathways along which it travels and is continually transformed are themselves provided with energy. The pathways are ready to be triggered. We may even say that the question is already implicit in them (Bateson, 1972: 453).

At a selected or identified emergent interpretant there is a contrast between most of the pathways of information inside the "system" in which is manifested and most of the pathways outside of it. For example lets take a mammalian cell's signal transduction pathway of the kind that uses relay systems to transduce the signal from the cell-surface receptors to the nucleus, with the resulting alteration of transcriptional activity. The first part of the journey, the arrival of the signal molecule to the vicinity of the receptor, that which will produce the first difference, is energised from "behind", by some source outside the system, and, if it comes from the environment like e.g. an odorant, it can be said to be energised in the ordinary hard-science way. If instead the signal is generated by another living system the network could then be extended in that direction. But once the difference is transduced inside the system, this type of travel is replaced by travel which is energised at every step by the metabolic energy latent in the protoplasm which *receives* the difference, recreates or transforms it, and passes it on (Bateson 1972: 453). (However, exceptions may occur on both sides of the line, i. e.: some external chains of events may be energised by relays, and some chains of events internal to the system may be energised from "behind", depending on our delimitation of the network. However, as a general rule we can say that the coding and transmission of differences outside the system is somehow different from the coding and transmission inside) (Bateson, 1972: 454).

This is exactly the case of signal-transduction networks. If one strikes the head of a nail with a hammer, an impulse is transmitted to its point. But it would be incorrect to say that what travels from the receptor to the nucleus in a cellular process is an

"impulse". It would be more correctly called "news of a difference" (Bateson, 1972: 454).

> "... at every step, as a difference is transformed and propagated along its pathway, the embodiment of the difference before the step is a 'territory' of which the embodiment after the step is a 'map'. The map-territory relation obtains at every step" (Bateson, 1972: 455).

2.6 Emergence of semiotic networks: from molecules to ecologies

As an example of an emergent (coevolutionary) semiotic network I will start with a well characterised aquatic multitrophic system, bacteria-squid-predator, that at the bacterial level involves what has been called "quorum sensing", one of the many transcription regulation systems in prokaryotes, one which is coupled to intercellular communication mediated by signal molecules that are thought to constitute inter-bacterial communication codes. From there, I will proceed to the realm of terrestrial multitrophic systems - that share the "logic" of quorum sensing - up to higher emergent levels of semiosis like for instance plant to plant communication. The dynamics involved in the evolution of these phenomena represent interesting instances of emergence of informational contexts along the biological hierarchy from molecules to ecologies, evidencing that a linear mechanistic causality does not suffice to couple the different emergent levels. To overcome the ambiguous "spontaneous teleology"[4] so frequent in biology, a semiotically informed approach will be needed.

The model organism from which the "quorum sensing" concept derived was the bacterium *Vibrio fischeri* (sometimes *Photobacterium fischeri* in the literature). This bacterium came to light (literally!) by studying a species of squid, *Euprymna scolopes*, which swims around the ocean's surface by night, searching for food. To any predator below, the squid appears as a very dark object moving against the very bright background of the moon. Quite a dangerous situation for the squid who "to solve this problem", is said to "have evolved" a light organ in which it cultures a very pure, very dense population of *V. fischeri*

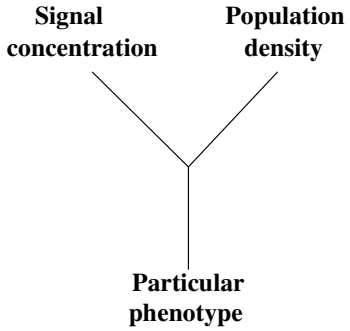
[4] I use here "spontaneous teleology" in analogy to "spontaneous semiotics", in the sense that although the word teleology seems to be anathema in life sciences, in their everyday language scientists customarily endorse organisms and evolution with teleological characteristics, which are often also

This bacterium produces a substance called luciferase, which glows with the same intensity as the moon (blue-green light, 495 nm)[5] rendering the squid invisible to predators below by erasing the shadow that would normally be cast as the moon rays struck the squid from above. A sort of camouflage known as counterillumination. The mutualistic advantage is that by glowing, the squid escapes getting eaten and in turn it provides food and shelter to the bacterial colony, which will be kept away from other competing bacteria (Ruby and Lee, 1998; McFall-Ngai, 1999; Visick and McFall-Ngai, 2000).

When *V. fischeri* is inside the squid's light organ the cells reach a critical concentration at which it starts producing luciferase. When free living in the "outer" environment and at low cell density, bioluminescence becomes an expensive luxury for the bacteria and light production is quickly minimised (Greenberg, 1997: 371).

The question here is, how can the bacterium (or its metabolism) know, or better yet, *sense* that it is inside a light organ and therefore it is time to activate the genes that produce luciferase?

The concentration of a small diffusible signal molecule produced by the individuals of the colony provide the crucial element for which the concentration is reflective of population size, modulating accordingly a given phenotype (Swift et al., 1999: 291). This is what has been called "Quorum Sensing"[6]. The word "quorum" is a legal term that refers to the number of members of a group required to be present at a meeting in order to legitimize a given decision. Quorum sensing can be represented as a triadic sign process as shown in Figure No. 5.



anthropomorphic. So it is very common to find descriptions like: "to solve this problem, the squid has evolved a light organ".

[5]Actually it is not luciferase that glows. Luciferase is the enzyme that catalyses the oxidation of some organic compounds (luciferins) in a reaction that emits visible light.

[6] The term first appeared in the *Journal of Bacteriology* in a minireview written by Clay Fuqua, Steve Winans and E. Peter Greenberg in 1995. It originated with Winan's brother in law, a lawyer who was trying to understand what the researchers were talking about (Greenberg, 1997: 371). Ever since it rapidly became a standard term in the scientific literature.

Figure No. 5. The quorum sensing sign triad. The concentration of a small diffusible signal molecule inside the bacteria reflects population density and may eventually trigger a modulation of the phenotype. Or, in other words, the concentration of the signal molecule acts as a sign in that it provokes the formation of a changed phenotype of the population, i.e. an interpretant, which relates to population density in a way echoing the way the concentration of the signal molecule itself relates to population density.

Although there are many examples of environmental cues (including the concentration of different extracellular substances) that can be transduced as a signal that triggers a metabolic response, quorum sensing refers specifically to those cues that build up as the consequence of cell density.

Let's now take a quick overview of the molecular model through which the bacterial colony produces light in the squid's organ. In *V. fischeri*, the genes that encode for the ingredients of luciferase and other substances necessary for the bioluminescence reaction are contained in the lux operon, consisting of the following genes:

- *luxA* and *luxB* which encode the alfa and beta subunits of the enzyme luciferase
- *luxC*, *luxD* and *luxE* which encode components of the fatty acid reductase complex, i.e. the enzyme which synthesises the necessary aldehyde substrate for the luciferase.
- *luxG* which is a gene with unknown function and who's presence does not seem necessary for bioluminescence.

The products of theses genes constitute the phenotype that is to be regulated by quorum sensing (bioluminescence). In addition, the operon contains two other genes necessary for quorum sensing itself: *luxI* and *luxR*. (Salmond et al., 1995; Sitnikov et al., 1995; Greenberg, 1997; Swift et al., 1999). The three main components of the quorum sensing system are produced by the very same operon that they have to regulate (a phenomenon originally known as "autoinduction"):

1) The signal-molecule: a low molecular weight molecule of the acyl-homoserine lactone (AHL) family, and specifically in the case of *V. fischeri*, *N* - (3-oxohexanoyl)-

L-homoserine lactone, or OHHL for short. Notice that this signal-molecule is not itself directly encoded by the operon but it is the "product" of a process catalysed by the direct "gene product".

2) The signal-generator: an enzyme encoded in the *luxI* gene (and thus called LuxI protein) which in turn synthetases the signal-molecule from different precursors that come from other biosynthetic pathways (a huge source for further regulation tollbooths and consensus requirements, and thus of coevolutionary pathways).

3) The response-regulator: encoded in the *luxR* gene (and thus called LuxR protein) which binds the signal-molecule to form a complex that acts as the transcription activator that in turn binds DNA near the Lux promoter, and so doing paves the way for the RNA polymerase, i.e., the enzyme which is actually producing the RNAtranscript of the whole operon.

When the local concentration of signal-molecule (OHHL) is low the majority of binding sites at the response-regulator (LuxR) molecules are left open, and the luxR protein will then take on a conformation that cannot bind to the regulatory site in DNA. As a result very little luciferase can be made. When the local concentration of signal-molecule is very high the response-regulator binds the signal in such a way that a conformation change is induced in the regulator which in turn enables it to bind to the specific site in the DNA and turn on the transcription of the whole operon at a higher or more efficient rate (by enhancing the RNA polymerase binding). We see here an instance of digital-analogical consensus at the lower levels of the semiotic network in the bacteria-squid-predator system. Each individual signal molecule is an analogue (a shape, which was digitally encoded in DNA). Its specific attachment to a single regulator (another analogue) functions as a digital message, by being or not being there. But the real message to the emergent interpretant is the concentration of signals which is received by the system as an analogue message that triggers the operon collectively in the colony.

But before the operon is turned on, how can LuxI (the signal-generator) and LuxR (the response regulator) be made so that the operon can be turned on? Apparently the operon is never completely "shut off". LuxR is consistently transcribed at a low level so that there is always some molecules around to affect regulation, and there is always a basal level of LuxI being made that guarantees low concentrations of signal-molecule. When these low concentrations add up as the

consequence of many cells getting close together (as when inside the squid's light organ) the binding of the two molecules increases, establishing a positive feedback loop that amplifies the signal and results in full production of the bioluminescence ingredients (Salmond et al., 1995; Sitnikov et al., 1995; Greenberg, 1997; Swift et al., 1999).

It seems as if every time that a signal-regulatory network is elucidated it is always discovered that there is further regulatory complexity. There is always integration of different regulatory mechanisms and signals depending upon many different cues like for example nutritional status, environmental stress, surface viscosity, cell density and many others, in order to elicit a complex phenotype. Not to mention the regulation of interconnected pathways like for instance those that originate the precursors from which the signal-generator produces the signal-molecule. Therefore it is customary to speak about global and primary regulatory controls. In this case the quorum sensing system would be considered as primary, i.e.: "specialised" in its particular "local" function (bioluminescence), while additional controls participating in the network, as for instance the common cAMP-CAP complex (which is also necessary for completing consensus in the quorum sensing system), would be considered as global regulatory controls, i.e.: active in many other circuits (posing the interesting problem of "categorial perception", i.e.: interpretation of meaningful patterns out of ubiquitous signals, a phenomenon to which we will return later). In addition, other common agents and factors of regulation are implicated in the bioluminescence reaction such as heat shock, oxygen or nutrient starvation, and SOS DNA-repair-inducing agents (Sitnikov et. al. 1995). Furthermore, there is mounting experimental evidence for "multilayered hierarchical quorum sensing cascades", for example in the production of many virulence factors by the bacterium *Pseudomonas aeruginosa* (Latifi et al. 1996: 1144).

The participation of cAMP (cyclic adenosine monophosphate) in quorum sensing is very interesting, although not surprising, because of the universality of this signal also in eukaryotic systems. In the bacterial Lux operon and its relatives, cAMP works as a further regulator of the network by binding a specific protein, the catabolite activator protein (CAP), pretty much like in the lac operon. By itself RNA polymerase would bind fairly weakly to the *Lux* promoter. Similarly, the cAMP-CAP complex by itself (and much less CAP alone) does not have high affinity for the CAP-site in the *Lux* control region compared to what would be its optimal affinity for that DNA

binding sequence. However, when cAMP-CAP and RNA polymerase bind to the*Lux* control simultaneously, they stimulate each other's binding, forming the cAMP-CAP/RNA polymerase complex, which makes more contacts with the DNA than either cAMP-CAP or RNA polymerase alone). This phenomenon which is usually referred to as "cooperativity", occurs, for the same reason that a repressor dimer has higher DNA-binding affinity than a repressor monomer, for example (Lodish et.al., 2000: 354). Cooperativity of this kind (which is the rule and not the exception) could be thought of as a minimal unit of digital- analogical consensus. But the cooperativity required in the system is nearly always larger than such a single unit. It is the consensus analogical mode of a particular context that determines how partial bits of information are to be interpreted in the system. It is worthwhile to keep in mind that digital-analogical consensus can be achieved at higher logical levels beyond the contact of three or four molecules

2.6.1 Microbiologists turned their attention to the "context"

In 1992 it was found that the same signal molecule (OHHL) that was responsible for the regulation of synthesis of luciferase in *Vibrio fischeri*, was also responsible for the regulation of synthesis of the carbapenem antibiotic in the terrestrial plant pathogenic bacterium *Erwinia carotovora*. The significance of this discovery layed in the fact that up to that moment OHHL-mediated autoinduction was considered to be uniquely connected with bioluminescence in the marine bacterium and its close phylogenetic neighbours. The fact that two such different organisms share a common signalling molecule (and mechanisms) led researchers to believe that they had stumbled upon a bacterial language of communication mediated by OHHL, and/or structurally similar molecules, which might be far more widespread than originally supposed (Salmond et al., 1995: 615; Swift et al., 1999: 291).

But that was not all. In experimental settings it was found that mutants of *Erwinia carotovora* that were unable to make carbapenem antibiotics on their own could do so when cross-fed with a second strain of mutants. The second strain of *E. carotovora* was supplying a signalling molecule which triggered antibiotic synthesis in the first group. This discovery hinted at the possibility that there could also be "cross-talk", i.e.: that signal-molecules produced by one species could be detected by the metabolic machinery of a different species. In fact, similar cross-talk was later

observed in relation to the swarming motility behaviour of mixed colonies of *Pseudomonas putida* and *Serratia liquifaciens*. Swarming is one of six described forms of bacterial surface translocation and it has been characterised in detail in *Serratia liquifaciens* (Eberl et al. 1996; Eberl et al. 1999). Contrary to swimming, that can be achieved by individual cells, swarming colonies can be seen as specialised cells organized in subpopulations communicating through quorum sensing signal molecules. It is considered an important social phenomenon since cultures of different species in certain conditions might be able to collaborate in the process of surface colonisation. Such collaboration of two or more species of bacteria for the achievement of swarming has been observed in experimental settings in which one species differentiates into swarming mode (long hyperflagellated cells organized in an outer, motile layer), while the other(s) produce a surfactant to condition the surface for better motility. This seems to involve a species that emits a signal which triggers a response in another species in order to create a "community phenotype" (Eberl et al. 1996; Eberl et al. 1999:1708).

During the 1990s the list of Gram-negative bacteria that possessed quorum sensing systems expanded and so did the list of phenotypes regulated in this manner and the family of homoserine lactones that serve as signal molecules (Salmond et al., 1995; Swift et al., 1999). Although presenting some differences, Gram-positive bacteria are also known to possess quorum sensing regulation systems, i.e. cell-density dependent phenotypes (Kleerebezem et al. 1997). Some phenotypes include a range of virulence factors and multiple exoenzymes, antibiotic production, conjugation, biofilm formation, and swarming motility.

Researchers now wonder how did these signals evade detection for so long admitting that such exchange of external signalling molecules between single-celled organisms was unexpected and therefore nobody was looking for them. For decades, microbiologists had been isolating cells out of the culture medium in which they had grown and throwing that medium away together with the signals. That is why some bacteria would loose their pathogenicity in the experimental settings. It was the *context* that was being thrown away!

A neodarwinistic point of view may lead us to think that every time we encounter a so-called antibiotic in nature we have before us a case of biochemical warfare. Perhaps this is not something we should take for granted. For example, it has been demonstrated that one of the *Pseudomonas aeruginosa*'s quorum sensing signals (3-

oxo-C12-HSL) could also be part of the set of virulence phenotypes exhibited by this opportunistic human pathogen, in the sense that it has been proven to have a direct effect upon the immune system, impairing the host's response to bacterial infection (Swift et. el., 1999: 306; Pesci and Iglewski, 1999: 152; Wu et. al., 2000: 2482). If this molecule was not known to be also part of a signalling system, we could easily conclude that it was exclusively a virulence factor, a weapon. The same can be valid about many antibiotics that may turn out to be not just weapons, but also, or mainly, communication devices (Cundliffe, 2000: 410-413).

In a narrow "struggle for life" view, it may also be tempting to think exclusively in terms of semiotic warfare, like for example when *Vibrio anguillarum*, a fish pathogen that inhabits the same ecological niche as some *Aeromonas* species, produces an AHL (3-oxo-c10-AHL) presumably to outcompete the *Aeromonas* species by blocking its quorum sensing systems (Swift et. al., 1999: 307). The signal-molecule of the *V. angillarum* competes for the binding sites in the *Aeromonas* species' receptors, i.e.: as an antagonist of the *Aeromonas* signal-molecule, thereby inhibiting the physiological activity of its quorum sensing circuit. Perhaps more illustrative would be a case of "inter-kingdom semiotic warfare". The red macroalga *Delisea pulchra* produces a range of 14 different halogenated furanone compounds that are structurally similar to the acyl homoserine lactone molecule family. These furanones specifically inhibit the quorum sensing-dependant swarming motility of *Serratia liquefaciens*, which is a deleterious bacterial trait for the alga since it is related to biofilm formation and colonisation (Givskov et. al., 1996; Rice et. al., 1999). In other words the alga reduces the levels of bacteria on its surface through molecular mimicry, i.e. by producing signal analogues, icons, which interfere with the bacterial endogenous signals (in fact molecular mimicry - structural and/or functional - has become a popular entry in biology journals given its potentiality for biotech solutions).

But there is not only semiotic warfare. As in symbiosis in general, there are plenty of examples of mutualistic interactions via quorum sensing, not only in the symbiosis bacteria-higher organism, but also in bacterial interspecies communication, or cross-talk, as the example previously mentioned in relation to swarming motility behaviour in mixed colonies. There is also evidence that some bacteria may become virulent in response to cell signals from quite unrelated bacteria in the environment and different species have been reported to team up and communicate in order to

coordinate their pathogenic response (Eberl, 1999: 1708-1710). This simply means that any assessment of an organism's virulence must take into account the context and the likelihood of signalling molecules being present, i.e.: assessment of the semiotic niche.

AHLs are not the only signalling molecules for bacterial cell-cell communication. There are many other peptide pheromones and also other bacterial signal systems the cross-talks of which are very commonly being reported. Certain cross-talking signals have also been identified in biological systems as different as bacteria and mammals (e.g. cyclic dipeptides found in marine bacteria have been found in mammalian systems as neurotransmitters) (Rice et. al., 1999: 28).

It is becoming apparent that quorum sensing is just part of a complex regulatory network, where additional environmental information is transduced through other pleiotropic regulators of gene expression. Some systems are very specific while others are more promiscuous in their interactions with different types of signals. But it is now commonly accepted that the many cell to cell communication and environmental sensing systems in bacteria constitute a complexity of codes and languages. And it has been suggested that these are new codes to be cracked. The title of the review article by Salmond and his collaborators (1995) may be representative for the mood: “The bacterial ‘enigma’: cracking the code of cell-cell communication”.

To visualise how semiotic networks convey information triadically through digital-analogical consensus - creating higher order specificities which are not fully directly determined by the lower order (physical) stereochemical specificities, i.e.: there is plenty of room for semiotic freedom - and how these networks are necessarily constituted by systems of correspondences that presuppose the existence of complementary elements distributed in the system, let us go back to the 3 main molecular actors in the quorum sensing circuit of *V. fischeri*:

1) The signal-generator (the LuxI protein) possesses specific functional domains (or active sites) that serve to synthesise the signal-molecule starting from two specific substrates that must be selected and recruited from those existing in the cellular pool. It is believed that a region (in the C-terminal domain) is involved in the selection of the right acyl chain that will give its specificity to the signal- molecule, while another region (in the N-terminal domain) contains the active site where the precursors are joined together (Sitnikov, 1995: 809; Greenberg, 1997: 374).

2) The response regulator, the LuxR protein, to which the signal-molecule binds in order to form the complex that activates transcription of the operon, is a modular protein with individual functions carried in specific regions. The C-terminal domain contains both the DNA binding and transcriptional activation functions. The N-terminal domain carries several functional sites, and this is the binding zone for the signal-molecule. In the absence of the signal-molecule, it appears that the N-terminal blocks the ability of the C-terminal to bind the specific site on DNA and activate transcription. Binding of the signal-molecule to the N-terminal releases the inhibitory effect by unmasking the DNA-binding and transcriptional activation functions of the C-terminal domain (Salmond et. al., 1995: 617; Sitnikov, 1995: 806; Greenberg, 1997: 373).

3) The specificities of the acyl-homoserine lactone signal-molecules can be better appreciated if we see them as a family of molecules. The several molecules identified so far in Gram-negative quorum sensing systems share a common structure. They are small molecules that have a fatty acyl group (an acyl chain) linked to a modified amino acid (homoserine lactone). The chain lengths vary in different signalling molecules and it is this feature that gives its specificity to the signal-molecule. They all appear to be able to diffuse through the membranes of bacteria. Some signals appear to be unique to one species while others are shared by several. Some species produce a single or a few signalling molecules, others produce a range. Different signal-molecules, differing only in the length of their acyl side-chains, may be synthesised by a single *luxI* homologue. And more interesting, the structures of the signal-molecules from two different bacterial species can be identical but the corresponding LuxI synthetases that produced them may exhibit only 21% of identity. It is therefore not possible to predict the identity of the AHL signal molecule(s) from the sequence data of a given LuxI homologue suggesting that the “shape” in the lower level process is not always the only important factor for the new emergent level (in this case the signal-molecule) (Salmond et. al., 1995; Sitnikov, 1995; Greenberg, 1997).

The relative concentrations of the signals and their activities may vary according to the context, so that the right cocktail of signals triggers the right response. The threshold concentration of signal-molecules necessary for transcription of a specific set of genes also varies with the species. This means that the specific threshold concentration is a significant aspect of the sign. Or, in other words, it is the

simultaneous and complex "activation" of an indeterminate number of "lock and key" mechanisms that determines the emergence of new informational contexts and new and more complex "lock and key" mechanisms. Every new emergent "state" constitutes a difference that can be sensed by a system with interpretative capacity, i.e.: an emergent interpretant.

Let us briefly continue the road "up-scale" in the ontogenesis of the squid-bacterium-association. It has been suggested that the population-dependent regulation of gene expression can be viewed as an example of multicellularity in prokaryotic populations. Quorum sensing is nearly always symbiosic since in most known cases the colony that coordinates the simultaneous expression of a given phenotype is a symbiont of a higher organism and very often the cell-density-dependent phenotype is related to the colonisation and/or the interaction with the host. This makes this phenomenon quite an interesting case for exploring the emergence of semiotic networks and the interrelation of informational contexts at different levels of complexity. It also raises interesting questions about the coevolution of the host-symbiont specificity.

> "Specificity in this association [squid-bacterium] is achieved through a reciprocal dialogue between the host and symbiont in a series of stages that ultimately result in the establishment of a stable relationship that endures throughout the lifetime of the host" (Visick and McFall-Ngai, 2000: 1779).

Escaping the egg-hen paradox, the first two signs of this dialogue are the reciprocal presence of two "analogs": the squid and the bacterium (or rather a small colony of it). Against all odds this encounter ineluctably takes place. Of the estimated 1 million bacteria present in 1 ml of seawater in the squid's environment, only 0.1% are *V. fischeri*. It has been calculated that as a result of seawater flushing into and out of the squid during its ventilation process, only an average of 1 *V. fischeri* cell would enter and exit the body cavity every 0.3 second. However not a single aposymbiotic specimen (squid without light organ symbionts) has ever been detected (Visick and McFall-Ngai, 2000: 1779-1780). This record of success in colonisation against all odds means that the "reciprocal dialogue" is a very precise and concrete one. The fact that when *V. fischeri* is absent, or too low in number, the light organ remains uncolonised even with high numbers of nonspecific bacteria in the environment,

indicates that there is a "host-imposed" positive selection for *V. fischeri* (MacFall-Ngai, 1999: 242).

When a juvenile squid hatches from the egg, it does not contain any symbionts. It needs to acquire the symbionts from the sea water. By cultivating and expelling symbionts into the environment, the squids is said to "horizontally" transmit the symbiont from one generation to the next (Ruby and Lee, 1998: 807). A few hours after the squid is hatched, symbiotic colonisation rapidly begins. After the contact, both organisms induce each other into a series of morphological and developmental changes which result in the enhancement of the association (Visick and McFall-Ngai, 2000: 1779).

Before undergoing the developmental changes that take place exclusively in the presence of the bacteria, and which lead to the mature functional organ, the juvenile squid is able to develop its (still virtual) light organ all "by itself", but only to a point in which it is primed for the interaction. In order to develop the particular features that allow the squid to use and "manipulate" the light, it needs the presence of the bacteria. The underdeveloped organ constitutively "comes" with some features to make sure it collects the needed bacteria. It has two ciliated epithelial fields each consisting of a layer of cells on the surface of the organ that extends into two long appendages. It is believed that the function of these ciliated fields is to harvest and recruit the *V. fischeri* to initiate the symbiosis. After colonisation (and following specific signals) the ciliated fields are lost through a process of apoptosis (cell death and tissue collapse). The bacterium is also thought to play an active role in its own "recruitment" process since it has been demonstrated that nonmotile *V. fischeri* (either nonflagellated or flagellated but defective in motility) cannot initiate colonisation (Visick and McFall-Ngai, 2000: 1780).

There are many different factors that determine and assure the symbiont-host specificity. Each of these "specificity determinants", which give each organism its "symbiotic competence", may belong either to the symbiont or to the host. Each determinant works through a particular specificity but it is the collective and mutual interaction of all of them that determines the compound symbiont-host specificity.

Some of these determinants include physical and chemical barriers in the path that leads to the organ and inside the organ itself, which only *V. fischeri* can overcome (Visick and McFall-Ngai, 2000: 1781). The host "creates" a habitat in which only *V. fischeri* is able to initiate and maintain a stable association. Other determinants

include adaptations of the host immune system to recognise the bacteria as "self". Upon entering the light organ the symbionts interact with a population of macrophage-like cells (which are part of the squid's immune surveillance system). It has not been clearly established whether the macrophage-like cells engulf nonspecific bacteria (thus helping *V. fischeri*) or whether they instead provide a mechanism to control symbiont number (and thus symbiosis health), or both (MacFall-Ngai, 1999: 242; Visick and McFall-Ngai, 2000: 1782).

While some *V. fischeri* cells may have contact with host macrophage cells, the majority of the symbionts in the population are eventually found in intimate association with the epithelial cells lining the crypts inside the organ. This association between the bacteria and the squid's tissue is mediated by a specific receptor-ligand "lock and key" that assures that the right symbiont binds to the epithelial cells (MacFall-Ngai, 1999: 246; Visick and McFall-Ngai, 2000: 1782).

Several hours after the bacteria have entered the light organ, the symbionts are induced to change; they loose their flagella and decrease their individual size while the population increases rapidly resulting in a high cell density. This is how 12 hours after the hatching of the juvenile squid, what is apparently the most relevant product of the association emerges: light.

Although dark bacterial mutants (defective in structural *luxA* or in quorum sensing regulatory *luxI* and *luxR* genes) commonly arise spontaneously in lab-culture, of the hundreds of analysed bacterial isolates from the light organs of *E. scolopes* of all ages, no nonluminescent strains have been found! (Visick and McFall-Ngai, 2000: 1783). Since luminescence requires an alleged 20% of a cell's metabolic capacity, neodarwinian mechanisms demand that a strong selective pressure must be present to maintain this trait.

If bioluminescence is the *raison d'être* of the symbiosis from the squid's point of view, there must be a sophisticated and stringent mechanism to ensure that only luminescent *V. fischeri* can establish or continue the symbiotic relationship. It is believed that one possible mechanism may involve direct sensing of light by the squid (Visick and McFall-Ngai, 2000: 1783). The light sensing capability of the squid points also to other directions in the semiotic network. With the first daylight each morning, the squid expels 90% of its organ's bacteria into the sea in a delicate balance that avoids unhealthy overgrowth without completely eliminating the symbiont population. By doing so, the squid gets rid of the unnecessary cell-density-dependant

bioluminescence during the day, and it “horizontally” provides symbionts to future generations. This pattern of behaviour is not a “programmed” circadian rhythm but depends on the animal response to the cue constituted by increasing daylight.

As mentioned before, a mechanical dyadic explanation of signalling molecules suffices only at a given hierarchical level. But the subsequent relevance of these events (up or down scale) cannot be coupled or grasped through that kind of explanation. The significance of a biosemiotic kind of explanation is to put these isolated events into a hierarchical, developmental and evolutionary perspective which may make better sense when seen within a triadic logic. Evolution of light production cannot be accounted for by the working of the Lux operon and its evolution through a neodarwinistic mechanism. When seen as the aggregation and emergence of new specificities that constitute new semiotic networks, the coevolutionary nature of the association and thus of the Lux operon becomes evident.

The specific advantage to *V. fischeri* occurs only in its mutualistic relation to the squid. The squid not only utilises the bacteria's light emission as a source of camouflage, but it has itself evolved to take full advantage of such light source. The squid's light organ develops only in the presence of its specific luminescent partner; it is in an immature state until the bacteria have successfully colonised it. Nevertheless the immature organ and its predisposition to follow the developmental path induced exclusively by that specific symbiont must be somehow inherent in the squid’s genome and in the fertilized egg as “tacit knowledge” (Hoffmeyer and Emmeche, 1991: 137). This developmental path makes sense only in relation to the light produced by the symbiont. Within a few weeks after the bacteria colonise the squid, the fully developed light organ is present. The mature organ possesses four structures specifically to manipulate the use of the light source provided. It has a reflector tissue to direct the light emission, a transparent lens type structure, a shutter mechanism (constituted by a black ink sack) to control the intensity of emission and it has yellow filters to shift the wavelength of luminescence closer to that of the moonlight and starlight (MacFall-Ngai, 1999: 247).

It is generally supposed that bioluminescence has evolved independently many times in some thirteen different phyla (ranging from bacteria to unicellular algae, coelenterates, beetles and fish). This is reflected not only in the gene and protein structures, but also in its biological, biochemical and functional diversity, as well as its sporadic phylogenetic distribution (Hastings, 1998). It is usually inferred that the

functional importance of bioluminescence is the fact that another organism detects and responds to the light. It has also been suggested that bioluminescence did not originate until organisms came to possess photoreceptors, given the fact that in a Darwinistic or a neoDarwinistic context there would be no selective advantage to produce light if nothing was able to detect it. So the evolution and development of the lux operon quorum-sensing semiotic network does not involve only bacterial cell-to-cell communication, or the bacteria-squid dialogue that induces each other into a series of morphological and developmental changes, or the evolution of the squid's own photoreceptor to control its light organ, but of course it involves also the predator whose photoreceptor does not perceive the "difference" because of the camouflage. Therefore, knowledge about the squid's predator is necessary in order to understand the development of the semiotic network constituted by (among other things) the Lux operon.

2.6.2 The multitrophic plant-herbivore-parasitoid-pathogen system

While we can consider genetic heredity as a vertical (diachronic) communication system between generations of organisms of a same species, genomes-phenotypes are also engaged in developmental and ecological communication, as can be appreciated from the semiotic network described above. The genetic match (specificity) between participant species of a given network is more evident in cases of symbiosis where genetic determinants in one organism have a functional role in the regulation of specific developmental pathways in an organism of a different species. But the channels of communication, both in evolution and development (there is no way we can separate these two processes), are ramified into pathways that would not be traditionally considered symbiotic in nature although the principles of mutual determination, specificity and functional integrity may be there.

The integrative agenda thus depends on the consideration of the flow of information within organisms (the genetic, epigenetic and physiological levels, i.e.: endosemiosis) and between organisms of the same and different species, i.e.: the ecological level of functionally integrated multitrophic systems (exosemiosis). According to Haber (1999:179) when studying multitrophic relations it is preferable to concentrate on "small" ecological systems or subsystems, like plant-insect complexes, or communities in the smallest aquatical or terrestrial habitats, and he

asserts that what assures that biodiversity is adequately taken into account in this type of research is a combination of the concept of functional groups/ecological guilds and the study of food chains and food webs. In fact, a variety of empirical studies have yielded a general model that could be called the "multitrophic plant-herbivore-parasitoid-pathogen system". These studies, which increasingly consider above and below ground multitrophic interactions, so far have furnished a good picture based on material exchanges, i. e.: trophic webs, between the participating taxa.

Haber (1999: 179) suggests that future investigations must focus on interactions of species, in two different ways: 1) on the interactions between the species themselves (which would be the task of biocoenotic research); and 2) on the interactions or connections of the species with their abiotic environment (which is "classical" ecology). In the framework advanced here it would be a great mistake to keep these two aspects separate. Both kinds of interactions determine the *context* in which organisms are immersed and synergically constitute the "semiotic niche" of the species.

According to Kratochwil (1999), there is no doubt that many organism species are constantly linked by certain interactions, and that these interactions may be obligatory. But then he adds that such an interaction structure has systemic character only when it can be differentiated from other systems and when an independent matter flow is ascertainable. In other words, it is normally claimed that interactions amount to matter (nutrient) and energy flow. In fact, Haber (1999: 176) points out that the great biological and ecological research programs of the last decades mainly focused on the discovery or confirmation of universal natural laws, predominantly on absorption, transformation, and processing of energy and matter. Kratochwil (1999: 14) quotes Günther's definition of niche according to which the niche is a dynamic relation system of a species with its environment. It is composed of an autophytic/autozooic and an environmental dimension. The autophytic/autozoic dimension comprises the phylogenetically acquired morphological and physiological characteristics of the species while the environmental dimension is the sum of all effective ecological factors. However Günther's definition (dating from 1950) states that the autozooic dimension (in the case of animals) comprises also ethological characteristics. This consideration makes the issue of interactions much more complex than just matter and energy exchanges and it does in fact introduce the semiotic dimension. To this we can add that today it is very common among botanists

to assume an "ethological dimension" for plants as for example when they talk about behavioral traits, not to mention communication between plants.

Haber (1999:179) claims that biological diversity is often understood exclusively - or mainly - as diversity of tangible structural entities. This state of affairs gives rise to a huge knowledge gap about the diversity of "ecological niches", i.e. of relations between organisms and the environment. This means that there is very little consideration of the surprising variety of communication systems within species and populations, as well as of the diversity of behaviour expressions and learning processes. Clearly what is lacking is the semiotic dimension.

There is in the literature an increasing interest in "complex interactions", "multitrophic links", "connectivity", responses to "multiple enemies", "cross-talk" between multiple pathways, "non-trophic interaction" relations between biotic and abiotic factors, between above and below ground, between ecological and historical developments. What does this mean epistemologically? It looks as if after making inventories of components and dissecting individual pathways, the reductionist strategy is in need for a complementary perspective to "connect" all the reduced parameters which in the "field" influence each other creating cocktails of non-linear causal links.

The reductionist strategy has concentrated on the accountability of material stocks (whether in terms of matter or energy). But we see now in these ecological studies a renewed interest in the "flow of information" within these complex processes. We find terms such as "semiochemicals", "chemical information", "signals", "sensing", "recognition" and "perception".

Having research been dominated by the reductionist approach it is no surprise that with the rapid development of molecular techniques there is in act a sort of interaction of all branches of biology with molecular biology, including ecology. In this regard there is also in the literature a call for integration of molecular and ecological perspectives (Baldwin et al. , 2001; Paul et al., 2000). How are these approaches being integrated? Are they really being integrated? Or is it just a reduction of the latter to the former? With the importing of the concern for "information" from molecular biology to ecology, we may also be importing the ambiguity that has characterised the "informational talk" in fifty years of molecular biology. And yet it may very well be that the element which may bring about the longed integration

across hierarchical levels and subdisciplines is precisely the "information" notion, or better yet, the semiotic dimension.

The inter-bacterial code active in quorum sensing is also part of the semiotic network operative in the "above- belowground multitrophic plant-herbivore-parasitoid-pathogen system" since almost all cases of quorum sensing are related to symbiosis and symbiosis is always present in these systems. Nearly always the bacterial colony that coordinates the simultaneous expression of a given phenotype is a symbiont of a higher organism and very often the cell-density-dependent phenotype is related to the colonisation and/or the interaction with the host. Besides this fact, there are several lessons that quorum sensing research can teach us when considering networks of info-molecules in multitrophic systems, but the most important might be the necessity of a careful consideration of the context.

In a "multitrophic" system, which is simply what reality offers (since the bitrophic and even the tritrophic systems are artificial realities), there are multiple interrelations that create many possibilities for combinations of mutualisms, amensalisms, parasitisms, predation, comensalisms and antagonisms. For example, in a terrestrial multitrophic system bacteria can antagonise with a fungal "pathogen" (isn't the bacteria pathogenic to the fungus?) which in turn can antagonise with the host-plant, being the bacteria a mutualistic symbiont of the plant (Seddon et. al., 1997; Keel and Défago, 1997). Soil microbiota determine many types of interactions in the rhizosphere including the so-called plant growth promoting rhizobacteria, symbiotic nitrogen-fixing bacteria and mycorrhizal fungi that constitute a mycelium "bridge" connecting the plant's roots with the microhabitats of the surrounding soil and to other plants of the same or different species (Barea *et al.*, 1997). We encounter fungal-fungal interactions (those considered pathogenic and those considered beneficial interacting between them) (Whipps, 1997); interactions between mycorrhizal fungi and foliar fungal pathogens in the phyllosphere of host plants (West, 1997); host-plant mediated interactions between arbuscular mycorrhizas and plant parasitic nematodes (Roncadori, 1997), between arbuscular mycorrhizas and subterranean and foliar-feeding insects (Gange and Bower, 1997); between insect herbivores and pathogenic fungi on the phyllosphere (Hatcher and Ayres, 1997); between micro-herbivores (such as bacteria, fungi and viruses) and macro-herbivores (invertebrate and vertebrate) (Faeth and Wilson, 1997); just to name a few cases recently under investigation.

These systemic interactions include also temporally and spatially separated species interactions (Faeth and Wilson, 1997: 202). The experimental approaches consider both, top-down effects (control exerted by predators on lower trophic levels) and bottom-up effects (control exerted by resources available to each trophic level). The consideration of several processes acting simultaneously (predation, disease, competition for resources, competition for enemy free space, limitations imposed by abiotic conditions, etc.) is now being recommended (Karban, 1997: 199).

The material exchanges of these complex interactions (nutritional quality of tissues, nutrients and metabolites concentrations, etc.) have been the subject of many studies and are well documented. On the other hand the "non-trophic" relations, which may not be as easily discernible as trophic interactions, are just recently being characterised. There is now no doubt that non-trophic interactions play an equally important role in ecosystem functioning as trophic interactions do. The nature of these interactions hints to semiotic processes. As pointed out by Van der Putten et. al. (2001: 548) "The information used by aboveground invertebrate herbivores and carnivores overlays the food web and includes cues to factors that mediate indirect interactions between species". They further add that "Food-web models could be useful for evaluating the consequences of above-belowground links of multitrophic interactions. Although these models are based mainly on flows of energy and nutrients, they could be extended to account for spatial and temporal heterogeneity. Some organisms, such as parasitoids and pathogenic fungi, might strongly affect the stability of food chains, whereas they make very little direct contribution to flows of energy or nutrients" (Van der Putten et. al., 2001: 553).

Baldwing et. al. (2001) encourage the merging of molecular and ecological approaches. However they do not advance a theoretical frame that could integrate these levels. They review the molecular details of the signalling processes and point at some of their ecological consequences. Instead of merging molecular and ecological approaches (and one should also consider the epigenetic continuum in between), there may be a tendency of reducing the latter to the former, that is, decompose (reduce) the ecological complexity into its molecular "components", with the understated goal of mapping an ecosystem in terms of molecular kinetics. While this approach is indispensable as a starting point, given that semiotic processes operate through such a complex substrate of interacting molecules, it can not be expected to exhaustively

account for the emergence of novel semiotic hierarchical networks that give rise to systems of semiotic control over the flux of metabolites.

So if we have on the one hand the complexity implied by the consideration of multitrophic interactions and the reciprocal influences of what happens, not only above and below ground, but also laterally in the whole periphery of the niche or even in the whole ecosystem, on the other hand we have that this complexity increases further when we consider the plethora of non-trophic interactions. Here the elemental "mechanism" or "action" is the ability of organisms to perceive, integrate and exchange molecular signals with a myriad of beneficial and harmful organisms, i. e.: sophisticated molecular mechanisms to respond to and to communicate with, for example, hosts, pathogens and symbionts (Staskawicz and Parniske, 2001: 279).

Commenting upon a study that hints to the involvement of the bacteria within a herbivores' gut in the production of the elicitors that plants use to recognise the herbivores themselves and consequently "call for help" from the third trophic level (the herbivores' predator), implying actually four levels of interaction (bacteria-herbivore-plant-herbivore's predator), Baldwin et. al. (2001:355) point out that this complexity is likely to increase when the influence of mycorrhizae and endophytes will be considered. To approach this complexity it will be necessary to consider the intrinsic relation between endo- and exosemiotic codes. They claim that an intimate understanding of the ecology of the plant system (in their case *N. attenuata*) involved in defence against insects is necessary to decipher the transcriptional 'Rossetta stone'. "Plant-insect interactions are played out in an ecological arena that is larger than the plant itself and incorporates many community-level components, as indirect defences so clearly illustrate. These higher order interactions can reverse the fitness outcome of a trait ..." (Baldwin et. al. 2001:353).

Dicke and Bruin (2001: 988) argue that in the study of chemical information transfer between plants (the last frontier in inter-organism communication being explored), much can be learned from research on chemical information in interactions between animals. In this regard they point to the growing body of evidence that animals exploit many sources of information (on resources, on competitors, on natural enemies) to adjust their behavioural decisions. In this context it is very tempting to reduce information to "chemical information" as it happened before with "genetic information" where information became DNA, i.e.: matter. Maybe our tendency to give priority to "chemical information" over other kinds of cues and regularities

comes fom the fact that it can give us the illusion of a material exchange, and thus it simplifies the ambiguities of a not well explained information notion, if it does not eliminate it altogether.

Dicke and Bruin (2001: 988) also remark that although interesting in itself, the medium of communication is of course not the main topic if one asks whether communication between damaged and undamaged plants occurs at all and how this affects the ecology of plant-attacker interactions. In fact, they notice, the underground transfer of information may be facilitated by root networks and by mycorrhizal connections that may transport nutrients and potentially also elicitors of defence over considerable distances. As I interpret this remark, the main topic is the emergence of the network and the interpretation context. In other words, the "medium of communication" is only one of the 3 aspects that constitute something that we may call "information". Besides the "medium" (the sign), for information in order to be information there must also simultaneously exist that to which the sign refers (its "meaning") and the interpretation key which completes the triadic relation giving rise to the interpretation system to which the difference (created by the "medium") makes a difference.

Usually in biology, when evolutionary considerations are invoked what we really are interested in is what is the relevance of a particular phenomenon, function, trait, behaviour, etc. Dicke and Bruin (2001: 988) assert that apart from mechanistic questions, evolutionary questions should be addressed asking why plants do (or do not) exploit their neighbour's information and whether their strategy is affected by e. g. environmental conditions or previous experience. This is equivalent to saying that the relevance and consequences of dyadic (mechanical) relations, i. e. their "significance", has to be seen within a triadic logic. As much as plant-to-plant communication is the last frontier being explored in interorganisms' communication, this is often investigated for interactions between conspecifics. Dicke and Bruin (2001: 988) see no good argument why plants would not be able to exploit chemical information from heterospecific damaged plants.

These mechanisms are based on a series of "recognition specificities" that apparently begin at the genomic levels of the interacting organisms (Jones, 2001: 281). By analysing the evolutionary aspects of these interactions it is hard to avoid noticing the co-evolutionary nature of such communication "mechanisms". A clear example comes from the existence of multiple resistance specificities (determined by

recognition specificities) that can occur as different haplotypes (set of alleles) in a resistance (*R)* gene loci in a plant. A single gene in a given host-plant can alternatively encode different "recognition specificities" that match different avirulence genes in a respective parasite (it is also hard to avoid noticing the parallelism with mammalians' immune systems). In other words, the plant's genome can recognise (through different binding specificities) sorts of "warning" signals encoded in the parasite's so-called avirulence genes, and thus initiating the resistance response.

There has been a lot of interest about the molecular and evolutionary mechanisms that create and sustain such diversity of recognition specificities (Jones, 2001: 281). Two orthologous resistance genes encoding proteins with up to 90% amino-acid sequence identity can have distinct recognition capacities binding to two different corresponding avirulence proteins. This means that just a few amino-acids may be enough to confer the required specificity (Jones, 2001: 282). In other words there are particular domains that make the major contribution to the unique recognition capacity of individual *R* genes. However in some cases it may not be clear cut when a given protein should be considered as an avirulence or as a virulence factor, that is, as a communication device (a sign for the potential victim) or as a weapon, and in some cases the same protein may play both roles (Nimchuk *et. al.*, 2001: 288). What determines the difference then must be the context, i.e.: the presence of further simultaneous information that conveys a larger consensus analogical message. This is what is implied when we repeatedly read about the influence of biotic and abiotic environmental conditions and of previous experience. This is also probably the main source of discrepancy between experimental and natural systems: the context.

Once more we find ourselves surrounded by words like communication, sensing, recognition and perception. The word "information" usually implies more or less similar albeit not well defined meanings. But in this new context, what is it that an organism can communicate? What is it that can be recognized or perceived? What can be sensed from the environment? And more importantly, who is the subject of these actions? For example, Paul et. al. (2000: 221) point out that current theories on the evolution of induced defence are based on the concept that current herbivore or disease is correlated with the risk of future attack. In this sense, they claim, current or past attack can be seen as having the potential to provide information about the future

environment. On this basis, induced resistances will be selected for only if (i) current attack is a reliable predictor of future attack, and (ii) if attack reduces plant fitness.

Here, "information" is the correlation of past or current attack with future attack. Aren't we talking about learning and memory? What is the information "conveyed" by current attack? But more importantly, information to whom? How and who knows that current attack "predicts" (it would be more precise to say indicates, or signs) the future attack? There is clearly an interpretation context involved here and current attack *per se* constitutes no information. It becomes information only in relation to the emergent interpretant. Contrary to mechanical actions and reactions, information requires a triadic logic. It involves not two but three elements in simultaneity constituting what in semiotics is referred to as the triadic sign-function. Information (current attack) is something that stands for something else (future attack) to some system with interpretation capacity, a subject (the lineage through the aggregate of individual plants) capable of perceiving (recording, recognising) the difference created by the event of the current attack, i.e. the reduced plant fitness. Current attack is information only if seen in this triadic relation.

There is also reference to the relationships between plant responses to biotic and abiotic stress. The abiotic environment also provides "information" about the risk of future herbivore or disease. Responses to biotic and abiotic stress are linked because optimising induced responses to minimise the physiological effects of attack is highly dependent on the abiotic environment (Paul et. al., 2000: 224). We can clearly see here the context-dependent nature of any kind of information. A context can be a larger aggregate of information, a set of simultaneous occurrences that conform a sign that is received in its complexity by the interpreting system.

One of the major critiques raised against plant-to-plant communication research is that ubiquitous cues cannot be meaningful information in interactions between damaged and undamaged plant (Dicke and Bruin, 2001: 982). This critic gives overly importance to the material medium of information and ignores the triadic nature of information and the specificities created by the context. This is the same problem that arises when dealing with any apparently ubiquitous signals in inter- and intra-cellular communication, like e.g. Ca^{2+} and cAMP second messengers. And yet, nobody would deny that they convey "meaningful information". What is at stake here is how the emergent interpretant can enact a sort of "categorial perception" to recognise the

information from the contextual background, a problem we will address later when referring to "signal transduction networks".

Knocking-out components to evaluate their importance in the working of the system has of course been an extremely successful strategy. This has been widely used from genetic systems (knocking genes) to multitrophic systems (incapacitating or removing one of the participating species). This is of course in the tradition of the old principle of treating one variable at a time, the rest remaining equal. But one of the problems with this strategy is that most knock-outs (genes or species) can be only achieved under highly controlled conditions. This means that all the contextual parameters in the field can not be correlated with the knocked component. There can be many factors under natural conditions that affect or are affected by the knocked components. This is a common source of discrepancy between experimental settings and natural conditions. A myriad of simultaneous consensus factors and cues determine the semiotic background in which the component acts. For example, Baldwin et. al (2001: 353) report a study on the transcriptional reorganisation induced in the plant *N. attenuata* when it is attacked by its specialist herbivore *M. sexta*. The study estimated that more than 500 genes respond to herbivore attack. These coordinated changes parallel the metabolic reconfiguration following pathogen attack and according to the study point to the existence of central herbivore-activated regulators of metabolism. This is one way of relying on patterns in order to "ratchet the system up to a higher informational level". In this case the procedure is not knocking out a gene but a putative inducing factor of a huge set of genes. Even that pleiotropic inducer may be only one detail of a whole context that may potentially change the whole array profile.

Many authors report their worries about discrepancies and inconsistencies between experiments under controlled conditions and field experiments. This recurrent theme is a hint of the importance of the context in communication processes as it was learned from the quorum sensing experience. Seddon et. al. (1997) argue that much early *in vitro* work was done with the hope that antagonism would be equally effective in the plant environment. Such expectations were naive and unrealistic and many factors other than the direct interaction between antagonist and pathogen play a role *in vivo*. The host plant, the microclimate of the infection court, other microflora and inhabitants of the phyllosphere, environmental parameters and insults (solar radiation, fungicides, etc.) - all contribute and modify this interaction. It is little

wonder that many of these earlier biocontrol attempts failed or were invariably inconsistent (Seddon et. al, 1997: 8). Hatcher and Ayres (1997) ask whether it is possible that mechanisms effective in the laboratory are not effective in the field, and point out that disagreement between laboratory and field results is all too familiar and there may be many reasons for this. Often the growth conditions of the plant are critical. Thus, for example, amounts of pathogenesis-related (PR) proteins produced by plants are strongly dependent upon growth conditions (Hatcher and Ayres, 1997: 141).

Fokkema (1997) laments that exploitation of the beneficial effects of mycorrhizal fungi and antagonistic microorganisms is handicapped by the often observed inconsistency of the beneficial effects under field conditions. He suggests that for future research it is important to identify the major cause of failures. Adequate representation of beneficial organisms at the proper place and time seems the most crucial factor. He advocates for simulation models that consider the responses of introduced populations to a variety of environmental conditions and this will be helpful in selecting more ecologically competent strains. Moreover, the reliability will be improved when we know the conditions under which our introduced micro-organism will work or not (Fokkema, 1997: 94). According to Clay (1997), endophyte infections of grasses resemble other mutualistic plant-microbe symbioses, such as mycorrhizas and root nodules, that enhance plant growth. Like those symbioses, the benefit of endophyte infection may vary with environmental conditions in conjunction with the genetic background of host plants (Clay, 1997: 157), and of the interacting organisms i.e. interactions in the "genome space". This evidences the importance of the context when understood as the complex interplay of both the ecological and genealogical hierarchies.

Chapter 3
A sign-theoretic approach to biotechnology

3.1 The integrating role of signal transduction

Given the central integrating role of signal transduction in physiological and ecological studies, I shall now outline some of its semiotic implications.

First let me define what I will refer to as *the signalome:* a "frozen" picture of all the known (and yet to be known) basic physical support molecules of all the known (and possible) signal-transduction networks active or ready to be triggered in a given moment.

Before going into the semiotic aspects of signaling networks, let me sketch what could be the ideal characterisation of the signalome if we follow the "orthodox" reductionist strategy.

3.1.1 Reducing the signalome

The ultimate goal would be the massive characterisation of signal-transduction networks and the elicitors of the cascades that determine complex genetic reactions in response to variable environmental cues. In fact there is a tendency to extrapolate the methods of the "genomic era" to all levels of the biological hierarchy, including ecosystems. As pointed out before, in this bottom-to-top research strategy "biological information" is allegedly called to play an important role. The received view is that this knowledge will lead us to the understanding of the regulation and behaviour of complex microbial communities and multicellular systems and the responses of these biological systems to environmental cues. The strategy seeks the acquisition of greater knowledge of the way in which cells, tissues, organs, and whole organisms interact with, and respond to, environmental signals.

Besides the massive identification of genes and their functions we will have an equally massive characterisation and classification of regulatory elements of genes, protein regulators and signal-transduction components. With the help of global-array technologies it is possible to model and correlate gene expression networks and

protein profiles. The passage from genome sequences to higher hierarchical levels requires the generation and correlation of data about:

- the regulation and interactions of genes and gene products within cells,
- the interactions and communications between cells, and
- the biological responses and susceptibilities of cells and organisms to biotic and abiotic environmental cues.

What will lead us to the understanding of living systems is a passage from a focus on one pathway at a time to the integration of multiple pathways.

To additively reintegrate all the reduced parameters we need computer power and more sophisticated algorithms capable of correlating the multidimensional data pouring from expression arrays that may include up to 20,000 genes assayed in:

- different cell or tissue types,
- different genotypic states,
- different physiological states,
- different developmental states (considered at different times), after different sets of cues, perturbations or stimuli.

The central assumption is that biology "happens" from the DNA sequence, through the structure and function of proteins, through the interactions of DNA and proteins in simple pairs and as parts of complex networks involving the hundreds or thousands of genes and proteins that control complex biological responses.

There is acknowledgement of the complications of conceiving networks strictly in terms of biochemical mechanisms but it is said that things could be ratcheted up to a higher "informational" level by looking at how quantitative expressions of proteins or RNA change as you perturb systems.

In any case, the algorithms that should integrate such biochemical networks have to deal exclusively with the dynamics of molecular interactions which are reduced and deconstructed in the lab and then dyadically reassembled in the computer. The understated goal would be to map an ecosystem in terms of molecular kinetics. There is no room for "information" or communication within and among living systems in

this procedure. There is only molecular kinetics and reaction rates. There is only molecules poking into each other when their concentrations are statistically relevant.

3.1.2 The embryonic signalome

Signalling processes start already at fertilisation. This implies an *embryonic signalome* which is already a functional system. The genome without the embryonic signalome would have no meaning. The fertilised egg, at the moment of fertilisation, inherits also such a functional embryonic signalome.

The embryonic signalome must contain all the minimal organisation required to start the interpretation of the whole inherited system, including a minimal set of integrated signalling systems, DNA, cytoplasmic materials and the proximal environment where it is immersed i.e.: the fertilised egg does not only inherit a (digital) genetic code but it also inherits an (analogical) embryonic metabolic code. For example, the code implicit in calcium signalling plays a major role in the beginning of fertilisation. "Some of the earliest interest in Ca^{2+} signalling during embryonic development can be traced back to investigations of the role of Ca^{2+} in egg activation" (Webb and Miller, 2003: 539).

The diversification of the signalome is what permits differential and selective interpretation of DNA and of the new emergent metabolic codes that result from the previous steps in the process of development. This signalome, which includes DNA and its metabolic code, is part of the epigenetic inheritance system. What kind of homologies in different species can be expected in such embryonic metabolic codes, which are in part responsible for, or are cofactors in, the *differential* use of the information circulating in the system? The inherited embryonic epigenetic code implicit in the signalome is what starts the mediation process that brings about the developmental pathway of the fertilized egg. It is therefore of great interest to understand the inheritance pathways of the embryonic epigenetic code and what are the minimal requirements and the nature of such embryonic signalling processes.

For example, according to Berridge et. al. (2000: 17-18)

> "One of the fascinating aspects of Ca^{2+} is that it plays a direct role in controlling the transcriptional events that select out the types of Ca^{2+} signalling systems that are expressed in specific cell types. Such a role for Ca^{2+} in

> differential gene transcription is still in its infancy but is rapidly developing into an active area of research ... Differentiation culminates with the emergence of different cell types specialised for specific functions ... A key element of the differentiation process, therefore, is to install those components of the Ca^{2+} signalling toolkit that each specialised cell needs to fulfil its particular function".

3.1.3 The ecological signalome

Let us leave behind the minimal requirements for the embryonic system and consider the extension of the signalome at its most general level.

We have pheromones, signals released by one organism that can be picked up by the signal transduction networks of other organisms of the same species, thus informing behaviour or gene expression in the latter. This kind of communication can be encountered from bacteria (e.g.: quorum sensing) to eukaryotes.

Then we have inter-species and inter-kingdom signals, like for example when a species of bacteria in the guts of a herbivore emits an elicitor that plants' cells are able to recognise prompting the plant to respond by emitting another signal-molecule that attracts the herbivores' predators (Baldwin et. al. 2001) . Would these be pheromones? In general these are being called info- or semio-chemicals.

Lastly we have intracellular and, in the case of multicellular organisms, intercellular signals, i.e.: signals within organisms. These last networks are mainly the subject matter of signal transduction.

All these signals enter into the general category of ecomones defined by Florking (1974). All ecomones are in interaction within Tompkin's metabolic codes (Tompkin, 1975), which, as stated before, extend their networks beyond the boundaries of the organisms. The endo- and exosemiotic codes are intrinsically linked by systems of correspondences.

The interbacterial "pheromone" systems (e.g.: quorum sensing) have sometimes been thought as a form of multicellularity. Analogously, the hormone-based intercellular codes - within differentiating multicellular systems - have some formal correspondences and relations with inter-organisms and inter-species signalling networks. Besides bacteria and the rest of the unicellular organisms, signal transduction is concerned mainly with the extracellular signalling molecules that

function *within* an organism to control metabolic processes within cells and in development.

In all these codes we can trace semiotic regularities and relations. I believe we have to, if we are to contribute to the integrative agenda.

Having delineated the signalome very generally, let us now narrow it down to some specific examples in order to consider some of the semiotic regularities that can be observed within the functional codes involved in signal transduction. The generalisations proposed here can be extended to other parts of the signalome.

What I would like to show is how the process that I previously defined as *digital-analogical consensus* emerges as a general pattern for sign construction, creating immense combinatorial semiotic possibilities for regulating and fine-tuning complex, detailed and decentralised responses to equally complex, detailed and decentralised stimulus.

Within animals, intercellular signalling involving extracellular secreted molecules have been classified into four types:

1) The endocrine signalling system which uses hormone-signals for remote communication between cells. In these systems, hormones travel, usually through the blood vessels, and communicate a single difference by binding to a cell's membrane-receptor, or by diffusing into the cytoplasm and finding its receptor inside the cell.
2) The paracrine signalling system, in which signalling molecules released by a cell only affect target cells in close proximity, like for example neurotransmitters.
3) The autocrine signalling system, by which cells respond to signals that they themselves emit, as in the case of many growth factors.
4) An additional way of signalling involves signals which are attached to the plasma membrane of a cell and which can directly enter into contact with a membrane receptor attached to the adjacent cell, what could be called "fixed" signals.

Let us take for example the most widely characterised endocrine systems which involve water soluble hormones with cell-surface receptors. Usually, what is used as classification criteria for the different types of pathways is the typology of the cell-

surface receptor. Cell-surface receptors responsive to water soluble hormones (in endocrine systems) have been classified into families that give rise to different types of pathways (Lodish et. al., 2000).

In my examples I will consider some of the structural differences and the formal similarities of two types of signal transduction pathways that have been characterised in detail, those that involve G protein-couple receptors (GPCRs) and those that involve receptors with intrinsic enzymatic activity, in particular receptors with tyrosine kinase activity (RTKs).

Normally these systems work as follows: a signal (a hormone) emitted by a remote cell makes contact with the surface receptor of the "target" cell. This produces a conformational change (and dimerisation or oligomerisation of the receptor) that permits the activation of a cascade of events and components in which the "difference" created by the binding of the hormone to the surface receptor is "transduced" through different possible mechanisms.

The different *intermediate* steps may include a modular arrangement of ready-to-be-activated components that give rise to identifiable codes which are implemented through different infrastructure but which share some logical principles, interfaces and cross-talking pathways. For example, it is possible to trace some analogies and relations between the following two types of codes:

- There is on the one hand a type of code implemented by the production or release of any of several second messengers, from ions to lipids, as e.g.: Ca^{2+} or cAMP.

- On the other hand there is a type of code implemented through the use of post-translational modifications of cytoplasmic proteins, for example phosphorylation or proteolysis.

Both types of codes share the logic of digital-analogical consensus for fine-tuning specific responses to a given contextual state, and both are cofactors in a larger emergent code, i.e.: these two types of codes combine - "cross-talk" - in an emergent code resulting in more complex logics and patterns because of both, larger combinatorial possibilities and larger contact with cross-talking pathways.

The cascades of patterns of second messengers and of protein interactions and modifications is what then relays the signal - sometimes amplifying or diversifying it - to the nucleus where it is finally “translated” into a cellular response.

The interpretation key for each signal is embedded in the larger message that its concentration conveys. One single molecule will not be enough to transduce the necessary concentration threshold for the “last” signalling event of the cascade to happen, i.e.: the transcription of particular mRNAs that will work as signs in further semiotic networks, from translation and so on. Actually, what is conveyed is news of differences in concentrations.

The whole code of signal transduction is based on signs consisting in complex patterns of concentrations of different signal types and the subsequent modulations of concentrations in all the intermediary steps.

In Bateson’s terminology, the transform of a difference (caused for example by the binding of a single signal-molecule) travelling in a circuit is an elementary idea. The concentration of transforms is a less elementary idea, and still less elementary is the difference created by cocktails of concentrations of transforms of diverse signals acting simultaneously.

The distinction between components in the system has to do more with the physical modality of the mechanisms involved in the formation of a new sign - which will transduce the information further in the pathway - than with the formal logic with which such components operate.

The signalomes of these systems are generally constituted by:

1) signals
2) receptors
3) effectors
4) molecular switches
5) second messengers
6) adapter proteins
7) sensor molecules
8) channels, pumps and exchangers
9) buffers

Almost all the actors of signal transduction networks can be placed in one of the categories listed above. But it is worthwhile observing that some components could be placed in more than one category or they may play a role in one moment and play a different one immediately after. Being the transductional process based on "cascades", some molecules can change their role from one step to the next, being sometimes a signal, i.e.: requiring a specific concentration threshold to be recognized, and/or by being a necessary cofactor for creating some analogical consensus (as e.g.: when interacting with an adapter protein), or it can be an effector by (in)forming the next signal-effector, i.e.: by participating in the relay chain (as e.g.: with protein kinases).

The consideration of the relativity of roles may on the surface appear as unnecessary or trivial, but it helps to decentre our attention from any single component as the sole "regulator", as it is usually claimed when we say that such and such signal or second messenger "regulates" a given process. There is a natural tendency for the specialist to see the subject of his or her study as the main regulator. Thus for the researcher studying Ca^{2+} second messengers, calcium "regulates" many processes, while those who concentrate on membrane-receptors will individuate specific ligands as the "regulators". In a signal-cascade some of the "actors" may be in chronological order receptors, signals or effectors. This is the nature of semiotic processes: signs produce other signs in continuous and multidimensional processes. For example, specific threshold concentrations of inositol second messengers translate into specific threshold concentrations of Ca^{2+} second messengers which in turn participates in a given "consensus" logical product to establish an even higher analogical message.

But it does not seem correct to claim that a given ion, molecule or protein complex is "the" regulatory element of a given process. There is no signal transduction network which is regulated by such a single element, and there is no signal transduction pathway that stands on its own. Any "second messenger" is no less a regulator than the primary or the final signal. Primary signals come from and go to different directions and networks, all of which offer further possibilities for regulation. There is no final signal either because the process is continuous and signal pathways do not end. Signals are only transformed within larger circuits of branching and interconnecting chains of causation. These chains of causation are "closed" or integrated in the sense that causal interconnection can be traced around the circuit and

back through whatever position was arbitrarily chosen as the starting point of the description (Bateson, 1972: 404).

We tend to see the process of signal transduction as beginning with the extracellular signal and ending with the transcription of a gene, but the hormone is not the beginning and mRNA is not the end of the semiotic network, they are just transient signs that take the process into new developments which will produce new sets of interconnected informational pathways in an endless progression until the system ceases to be a living-semiotic system within a network, that is, when its whereabouts will be determined exclusively by physical dynamics and there will be no more room for sensing, constructing and interpreting signs out of concentrations of signal-molecules.

This is why at the level of signal transduction it would be more appropriate to talk about mediation (as some authors do) rather than regulation, being the regulatory properties always found at higher hierarchical levels of integration.

3.1.4 Where is regulation?

Because such complex networks are not exclusively determined by mass-energy restrictions, a random event, such as the building up of a given extracellular signal's concentration in the periphery of a cell, will produce a non-random response to such an event. This non-random response is not deterministic in the physical sense because the system that reacts to the random event has a repertoire of responses of which it will select the optimal one based on a global interpretation of the context. Selection of responses, "choices", can be achieved at different levels. If a response can be selected at a rather higher level of integration, the alternative responses must exist as possible and "distinguishable" coded patterns in the system (Bateson, 1972: 405).

Contrary to our genetic determinism (and now our signal determinism), choices at higher hierarchical levels, determined by sensing larger aggregates of differences, will have larger restrictive or regulating effects upon the whole hierarchy by influencing a larger set of circuits and networks as opposed to a single signal-pathway mediating or contributing to the expression of a single gene, which in turn contributes to a phenotype.

If alternatives do exist and can be selected at higher levels of integration than, for example, the single signal network, it may not be necessary to face selection at lower

levels which are already included in the selection of the higher level. This is why regulation should not be considered exclusively a local event, but also a compound effect more properly characterisable at a level closer to the context of the system under consideration.

There are many sources for complexity in integrated signal networks. It is the interactions of different signalling pathways that permits the fine-tuning of cellular activities required to carry out complex developmental and physiological processes (Lodish et. al., 2000: 894).

A hormone or a neurotransmitter does not control anything, it rather cooperates to something. We can say that it is a limiting or a cooperative factor, but not properly a regulator. Regulation is a continuous process and anywhere you enter the circuit you will find a sort of "local regulator" or a check-point which in turn is regulated and controlled by further ramifications of the semiotic network. In other words, all the pathways involved in such "control" or "regulation" processes are themselves opportunities for further regulation and control. Let us mention just a few examples:

- We can start from the "primary signals", e.g.: hormones. Because of their potent effects, hormones (just as neurotransmitters) must be carefully regulated. In some cases, complex regulatory networks coordinate the levels of hormones whose effects are interconnected (Lodish et. al., 2000: 856).The synthesis and/or release of many hormones is subject to positive or negative feedback controls. This type of regulation is particularly important in coordinating the action of multiple hormones on various cell types during growth and differentiation. Often, the levels of several hormones are interconnected by feedback circuits, in which changes in the level of one hormone affect the levels of several other hormones (Lodish et. al., 2000: 857). Actually, hormone secretion is regulated by a combination of neural and hormone signals (Lodish et. al., 2000: 898).

- Another "mechanism" for regulating cell-to-cell signalling is modulation of the number and/or activity of functional receptors on the surface of cells. For instance, the sensitivity of a cell to a particular hormone can be down-regulated by endocytosis of its receptors (i.e.: invagination of the extracellular domain of the receptors), thus decreasing their number on the cell surface, or by modifying their activity so that the receptors either cannot bind ligand or form a receptor-ligand complex that does not induce the normal cellular response (Lodish et. al., 2000: 894-895).

- More generally, “the ability of cells to respond appropriately to extracellular signals also depends on regulation of signalling pathways themselves” (Lodish et. al., 2000: 894).

One can be sure that the process that leads to endocytosis of receptors in order to decrease the sensitivity of the cell is also “regulated”. So every single component of the “regulating system” opens a further pathway for regulating, controlling or limiting possibilities.

Are we hopelessly caught up in a process of infinite regress?

It is often assumed that receptor tyrosine kinases’ (RTKs) signalling pathways have a wide spectrum of functions including regulation of cell proliferation and differentiation, promotion of cell survival, and modulation of cellular metabolism (Lodish et. al., 2000: 871). Is there anything else to be regulated? This practically means that RTKs regulate nothing less than life. The same could be said about other regulating mechanisms. How could this possibly be? Because it does not matter where you enter the circuit, that portion “regulates life” in some sense. The problem is that regulation has no dimensions or units, it is not *physically* localizable.

Lodish et. al. (2000: 886) assert that “The coordinate regulation of stimulatory and inhibitory pathways provides an efficient mechanism for operating switches and is a common phenomenon in regulatory biology”. From a mechanicist point of view this leads to a paradox: does the coordinate regulation provide an efficient mechanism or does an efficient mechanism provide coordinate regulation? Where is this thing? What is it?

Let me furnish another example. Different G protein-couple receptors are sensible to many extracellular signals including numerous hormones and neurotransmitters (such as epinephrine, glucagon and serotonin), thousands of odorants (that bind receptors in the mammalian nose) and even light (like in the case of rhodopsins receptors in the eye). Although these receptors are activated by different ligands and may mediate different cellular responses, they all mediate a similar signalling pathway (Lodish et. al., 2000: 862).

The ligand-receptor complex activates a G protein, which activates an effector (e.g.: adenylyl cyclase), which in turn generates a “second messanger” such as cyclic adenosin monophosphate (cAMP). cAMP is a very common and important second messenger. At specific concentration thresholds it mediates in many cellular

responses, mainly by informing some cAMP-dependent protein kinases which will in turn take the message further by "sculpting" specific (digital) "differences" on its substrate, providing it with a specific (analogical) recognition pattern, i.e.: by phosphorylating specific residues in other components downstream the cascade. The concentration of cAMP is thereby de-coded and the message is transformed into another code, that of phosphorylation.

Now, the level of cAMP is said to be controlled by the hormone induced activation of adenylyl cyclase (that will release cAMP). But another point of regulation is the hydrolysis of cAMP itself to 5'-AMP by cAMP phosphodiesterase. This hydrolysis terminates the effect of hormone stimulation. In turn, the activity of many cAMP phosphodiesterases is stimulated by an increase in cytosolic Ca^{2+} (which is induced by neuron or hormone stimulation). In addition, some cells also modulate the level of cAMP by secreting it into the extracellular medium (Lodish et. al., 2000: 871). We see here that in reality cAMP is not controlled by the induction of adenylyl cyclase. That is just a cooperative element in a wider interconnected network for modulating cAMP fluctuations and which entails feedback loops, cross-talks and hierarchical synergies and controls, like the pathways that control Ca^{2+} concentration which stimulate cAMP phosphodiesterase (remarkably it just so happens that in turn cAMP also collaborates in controlling Ca^{2+}!), not to mention the possibilities of control of a myriad of co-factors and their respective pathways. So it becomes hard to say who controls whom. That is why very commonly our description of such a process may end with something like:

> "The synthesis and degradation of cAMP are both subject to *complex regulation* by multiple hormones, which allows the cell to integrate responses to many types of changes in its internal and external environment" (Lodish et. al., 2000: 871, my italics).

What is this "complex regulation"? Where is it? It doesn't seem to be in the genome architecture. How can we begin to notice how "decisions" are being integrated hierarchically from higher levels?

3.1.5 Modularity

When we are exploring how differences are sensed, transformed and conveyed across hierarchical levels, forming therefore higher order differences, we are mostly concerned about regularities in the formal and logical aspects of such processes rather than in the regularity of the physical structures that underpin them. The material means implicated in the formal process can be bewilderingly diverse. Since biology has focused mostly on the diversity of structures, rather than on the formal logics behind biological-semiotic processes, induction has necessarily been the norm, presenting biology as a science with very few deductive principles, generalisations or rules, and focused rather on specific, local and apparently idiosyncratic cases, putting us in front of a jungle of proteins where it is sometimes difficult to see the forest from the trees.

Let us say that the difference created by cocktails of concentrations of transforms of diverse signals acting simultaneously conform an idea. The functionality of such an idea is somehow "shaped", or informed, by the context. The context poses the question and the system comes up with the idea.

A very similar "idea", the result of a complex aggregate of differences, can emerge (developmentally and evolutionarily) through different infrastructural configurations and local solutions.

Many examples can be found within signal transduction in developing systems where activation of different modular arrangements of components can give rise to the same intermediate or final responses. This is obvious at lower hierarchical levels, like for example when a particular cellular response can be modulated by different kinds of signal transduction networks which exhibit different signal-receptor complexes, although in some cases the two different pathways may share a common "idea", such as a Ca^{2+} second messenger, at some step of the particular cascade. i.e.: the same component can be used modularly for different purposes at different hierarchical levels, like for example when some receptors that are activated by different ligands and mediate different cellular responses, nevertheless mediate a similar signalling pathway by using components and steps common to both pathways (Lodish et. al., 2000: 862).

There are also documented examples at the evolutionary level where very similar creatures have arisen through different embryological pathways. For example - in spite of the historical rejection of the different variants of the theory of recapitulation in evolution - it is normally accepted as common sense that the larvae or embryos of a given species commonly resemble the *larvae* of a related species more closely than the adults resemble the adults of the related species. But it is possible to find examples of exceptions such as "among the marine worm like creatures of the older Enteropneusta, different species, of what used to be regarded as a single genus *Balanoglossus*, have totally different embryology" (Bateson, 1979: 186). A very similar "idea" has taken shape through different pathways.

We can say that such *modularity* is a central feature in signal transduction. The same result can be achieved through different "infrastructure" by combining common components and principles, or, conversely, different arrangements of the same, or very similar, components can result in very different responses (see figure No. 6). Thus we have that:

1) The same signal and receptor in different cells can promote very different responses (as diverse as proliferation, differentiation, and death).
2) Activation of the same signal-transduction component in the same cell through different receptors often elicits different cellular responses.
3) Different cell types may have different sets of receptors for the same ligand, each of which induces a different response. Some signalling-molecules can function in more than one modality (e.g.: epinephrine can function as both neurotransmitter and hormone).
4) Different receptor-ligand complexes can induce the same cellular response in some cell types.

However, there is always receptor-signal *binding specificity*, and the resulting receptor-signal complex exhibits *effector specificity*, i.e.: it mediates a specific cellular response.

How this specificity is determined is considered "an outstanding question in signal transduction" (Lodish et. al., 2000: 905).

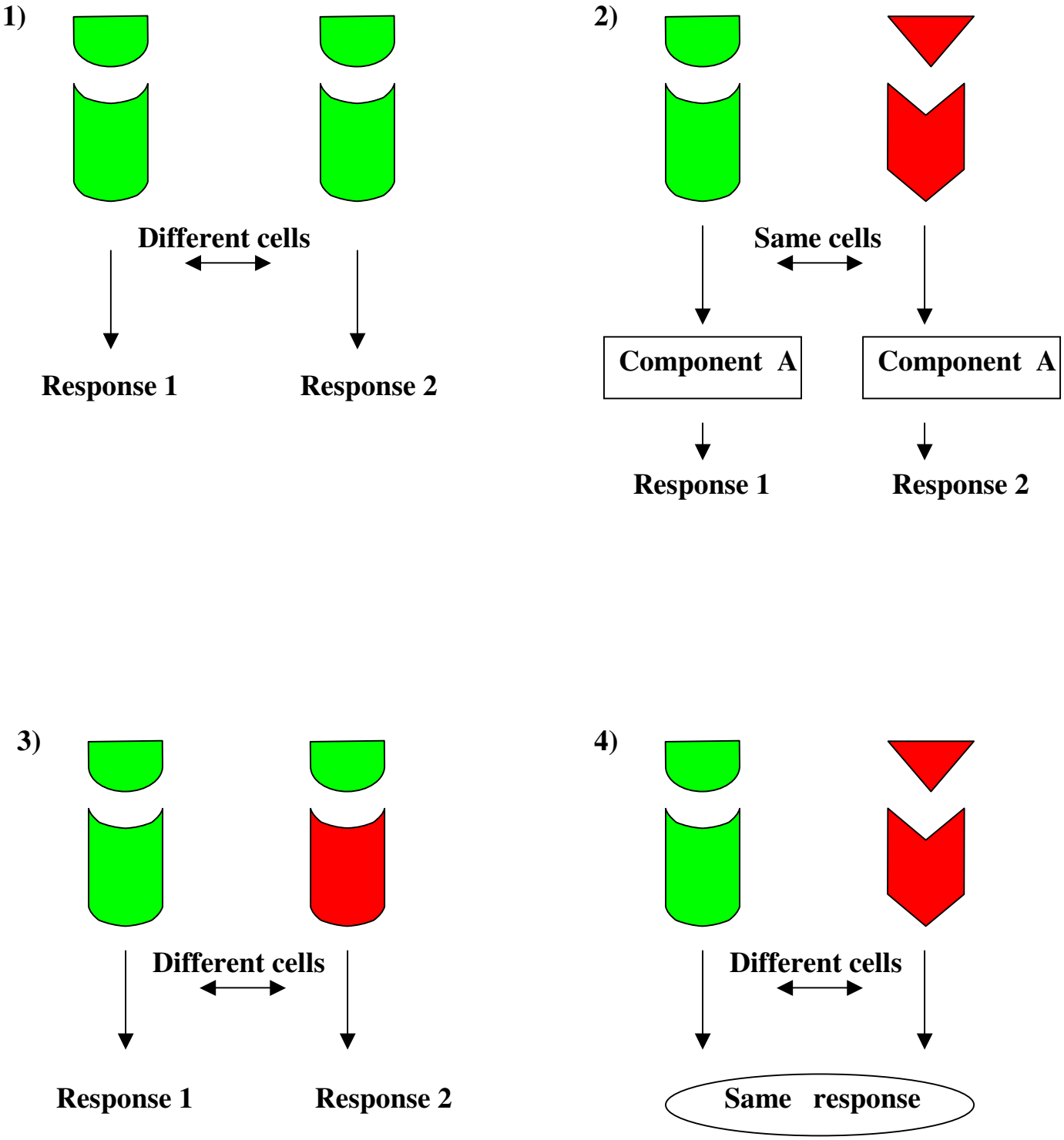


Figure No. 6. Modularity in signal transduction (see text)

3.1.6 Categorial perception

The multi-modality and modularity of signal molecules and relative "infrastructure" components, poses one of the central problems for understanding metabolic codes: the occurrence of different types of "cross-talk", "redundancy" and "categorial perception" at different hierarchical levels. In signalling systems and semiotic networks, these phenomena are intrinsically related to each other. Cross-talk and redundancy are already customary notions in biology and, like information, they are being defined inductively in different empirical approaches at different hierarchical levels.

On the other hand the term "categorial perception", which has been used in biosemiotics for a while (Stjernfelt, 1992; Hoffmeyer, 1996), captures very well the essence of the "outstanding question(s)" in signal transduction. i. e.: how specificity is determined, how ubiquitous signals or messengers convey specific information, how undesired cross-talk is avoided, how redundancy integrates the system. In other words how *categorial perception* is achieved.

Categorial perception as a prerequisite for the proper functioning of semiotic networks has been discussed in Stjernfelt (1992) and Hoffmeyer (1996). By categorial perception we can understand "the ability to slot a bewildering number of impressions into categories" (Hoffmeyer, 1996: 77). Let me restate it in terms of signalling systems by saying that categorial perception can be seen as the capacity for identifying and hierarchising patterns out of a contextual matrix, i.e.: the recognition of meaningful patterns out of ubiquitous signals.

In signal transduction, what determines the possibility for categorial perception is the convergence of complex arrangements of digital-analogical consensus which elaborate complex analogical signs that bind the specific context to the specific response. This is then what gives specificity to what otherwise could be ubiquitous "universal signals".

So if we are to understand the complexity of these codes we have to be able to identify the crucial digital-analogical-consensus instances by which complex signal configurations conform complex analogical signs.

It may be appropriate to make clear here what, in the language of this work, could be the difference between a signal and a sign. A signal by itself can be a sign, but this is probably the most elemental level of a sign. Simplifying we could say that a signal,

creating an elementary difference, is a digital message while a sign could be considered as an analogical message, although digital signals can work as signs as well, and complex signs may have a digital effect. But in any case what differentiates a signal from an "impact" is its triadic nature i.e.: the fact that rather than an "impulse", a signal transmits "news of a difference".

More generally we see that complex cocktails of signals is what really constitute signs, i.e. patterns that by the establishment of a habit or regularity become codified as signs that make a difference at the level of the emergent interpretant. A sign or an idea can be formed by the smallest units of informational processes, i.e.: news of a single difference (e.g.: the binding of a single signal-molecule to its membrane-receptor). More elaborate signs can be formed by complex aggregates of differences. This implies the emergence of complex codes which acquire logical independence and diversity with respect to the lower level code from which they have emerged.

The entity and the implications of "cross-talk" and "categorial perception" have only recently been recognised. A specific signal-receptor event may be transduced into a ubiquitous second messenger which then regains the specificity of the pathway somewhere downstream, where it directly or indirectly informs a specific effector that finally canalises the specificity of the "original" signal to the specific response. How exactly the specificity at the intermediate steps is achieved is not totally clear. At such points of the cascade, the system is vulnerable to undesirable cross-talk with other pathways. The point is that there is no simple linear cause and effect between the signal and the response.

3.1.7 Cross-talk

The term "cross-talk" needs a little qualification since it is being used in slightly different senses at different hierarchical levels. Thus we have for example that different species of bacteria can cross-talk to different degrees through homologous signals of the acyl homoserine lactone molecule family; the red macroalga *Delisea pulchra* cross-talks (interfering) with the quorum sensing system of the bacteria *Serratia liquefaciens* through halogenated furanone compounds that are structurally similar (but antagonists) to the acyl homoserine lactone molecule family; in eukaryotes, different signal transduction pathways are said to cross-talk; and within single pathways the Ca^{2+} and the phosphorylation codes cross-talk.

When you have “universal” signals that work specifically in specific pathways which communicate, sometimes it may not be wholly correct to speak about cross-talk between signals, for if the pathways are linked through a second messenger, then it would only be “normal” talk (in which case we could use the verb “interface”), whereas, cross-talk proper is what occurs between semiotically compatible systems, but which are not set up or prompt to communicate under “normal” circumstances, (e.g. mimicry, agonism, antagonism).

But mimicry, agonism and antagonism could also enter into the category of “normal” talk at higher hierarchical levels, being enough to enlarge our functional semiotic network to include the collateral sources of cross-talk.

Cross-talk can be better understood by considering the cases of homology in signalling systems, i.e. when molecules, components or modules of very different systems, which normally are not in communication, present functional compatibilities that would allow for a component of one system to interfere, deviate or work properly in another system.

In the case of integrated signal networks, where a common signal, e.g.: Ca^{2+}, is used at different levels and in different ways, what is necessary is precisely the avoidance of cross-talk, for otherwise the semiotic system could be ruined by uniformity. The avoidance of cross-talk in such systems is assured at the level of the emergent interpretant, which is the level that integrates the “key” for relating the most global assessment of the context to a specific response. In other words, at this level, complex patterns of signals, immersed in a given context, are distinguished and correlated.

This endows the system with capacity for categorial perception, i.e.: capacity for pattern recognition, which is the action of extracting contextual meaning from what would otherwise be ubiquitous signals, avoiding therefore anarchic cross-talk which would be deleterious to any “self-organised” system.

The nature of “cross-talk” has many different implications that will need to be considered carefully in future research. It suffices here to say that sometimes the system needs, and regularly uses, cross-talk, and sometimes it needs to avoid it, and it regularly does. This means that cross-talk needs to be understood as a complex combination of signals, pathways and therefore of regulatory agents. It is very important to stress that cross-talk has to be studied and identified at different hierarchical levels.

3.1.8 The Ca^{2+} code

One of the modular components of many signal pathways is what deserves to be called the Ca^{2+} code.

> "Of the approximately 1,400 grams of calcium that are in the human body, less than 10 grams manage to escape being trapped in the skeleton and teeth. These few grams might be an insignificant quantity, but they are extraordinarily *significant qualitatively*. They circulate in the blood and extracellular spaces, and penetrate cells to regulate their most important activities" (Carafoli, 2003: 326, my italics).

The versatility of calcium as an intracellular "second messenger" has led some authors to talk about its "universality" as a signal. This ubiquitous intracellular signal is held to be responsible for controlling multiple cellular processes throughout the life of eukaryotic cells from fertilisation to apoptosis, including embryonic pattern formation, cell differentiation and cell proliferation (Berridge et. al., 2000). "... the Ca^{2+} signal is important in cells from their origin to their death. It controls the creation of cells at fertilisation, masterfully guides them from infancy through adulthood to old age, and finally assists them at the time of their demise" (Carafoli, 2003: 331).

Cells at rest have a low concentration of calcium ions. But when the concentration rises to specific threshold levels many different functions and cellular responses can be activated.

One of the main questions calcium researchers are asking themselves is: how can these elevations of Ca^{2+} concentration regulate so many processes? Part of the answer lies in the versatility of the Ca^{2+} signalling system in terms of speed, amplitude, and spatio-temporal *patterning* (Berridge et. al., 2000: 11). But another part of the answer lies in what we have already said, that no single component of a signal-transduction network is by itself the regulator of a cellular response, it is rather one of many mediators.

Actually, it is not the simple linear rise in ion concentration that informs the system and triggers a response. It is rather the fluctuation of complex concentration thresholds.

For this purpose cells employ a sophisticated and extensive repertoire of signalling components, which comprises a "Ca^{2+} signalling toolkit" that can be assembled in combinations to create signals with widely different spatial and temporal profiles (Berridge et. al., 2000; Carafoli, 2003).

Ca^{2+} signals are generated by using both internal and external sources of Ca^{2+}. The internal stores are held within the membrane systems of the endoplasmic reticulum (or the equivalent organelle in muscle cells, the sarcoplasmic reticulum) and within the mitochondrion. The external sources come of course from the extracellular environment. Release from these internal stores and recruitment from the environment is achieved through various channels that respond to signals (see figure No. 6). There seems to be reciprocal interactions and cooperation between the different organelles and channels in modulating specific patterns of Ca^{2+} concentrations. For example, the endo(sarco)plasmic reticulum provides the Ca^{2+} that enters the mitochondria, which in turn modifies the Ca^{2+} feedback mechanisms that regulate Ca^{2+} from the endo-sarcoplasmic reticulum (Berridge et. al., 2000: 14). "Environmental" signals indirectly induce some of the channels that let Ca^{2+} in and out of the cytosol, contributing in this way to configuring specific patterns of concentrations of free ions.

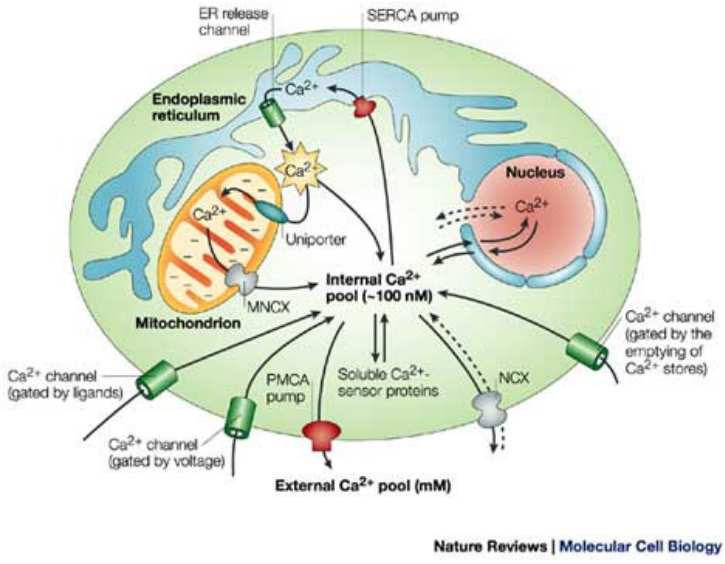


Figure No.6. Internal and external Ca^{2+} sources. Taken from Carafoli (2003).

It is important to stress here that when we are talking about a Ca^{2+} signal we are not talking about a Ca^{2+} ion per se but about a "spatio-temporal pattern". The digital signals represented by single Ca^{2+} ion constitute an analogical sign represented by spatio-temporal patterns of specific threshold concentrations.

Excellent and detailed reviews on the many families and isoforms of components involved in the Ca^{2+} code, and on the subtle complexities of these signalling systems can be found in Berridge et. al. (2000), Weeb and Miller (2003) and Carafoli (2003).

In summary, the Ca^{2+} signalling components include:

1) The sources and stores of Ca^{2+} ions, i.e.: the extracellular space, the endoplasmic reticulum (or sarcoplasmic reticulum), the mitochondria, buffer molecules, the nuclear envelope, and the cytosol, where the signals are configured.

2) Channels, pumps and exchangers (e.g.: Na+/ Ca^{2+}), i.e.: membrane-intrinsic proteins that transport Ca^{2+} ions across membranes. The channels are activated or disactivated (directly or indirectly) by extracellular signals (e.g.: neurotransmitters), other second messengers, voltage differences and by Ca^{2+} itself. Channels possess receptor domains being actually receptor-channels. Usually a consensus of different second messengers and other components, plus Ca^{2+} itself, is required for such an activation. There is a continuous fluctuation of Ca^{2+} concentrations created through the many different in-and/or-out-channels that operate at the different sources of Ca^{2+}.

3) Ca^{2+} buffers, i.e.: molecules that intercept free Ca^{2+} ions in the cytosol (or in organelles) and maintain them unavailable until they are required as free ions again, constituting an additional mechanism to give specificity to a given needed pattern.

4) Second messengers, i.e.: different Ca^{2+} mobilising messengers (generated when stimuli bind to cell surface receptors) that cooperate in different specific analogical consensus that activate or inhibit different mechanisms (e.g.: channels) for modulating influx and outflux of Ca^{2+} in the cytosol. The different Ca^{2+} mobilising messengers can coexist in cells where they seem to be controlled by different receptors that respond to specific signals (Berridge et. al., 2000: 12).

5) Sensor or decoding molecules, i.e.: proteins which respond to a given Ca^{2+} concentration pattern. By binding Ca^{2+} ions, sensor-molecules undergo a pronounced conformational change that allows them to continue the cascade towards specific effectors, usually protein kinases which alter other proteins, translating in fact the calcium message into the phosphorylation code and thereby directing the cascade

towards a specific response. A major family of these molecules is the family of EF-hand proteins which include hundreds of members, of which calmodulin is the most thoroughly investigated. There is a group of EF-hand proteins which are collectively called "neuronal Ca^{2+} sensors" which mediate neuronal functions such as the release of neurotransmitters (Carafoli, 2003: 330).

3.1.9 Creating patterns of patterns

The different degrees of excitability and concentration of different kinds of channels, depending on the levels of the appropriate Ca^{2+} mobilising messengers, modulate different kinds of spatio-temporal *patterns* of Ca^{2+} signals (Berridge et. al., 2000: 15). Variability of patterns is further enhanced by the existence of isoforms in the components of the toolkit.

The "autocatalytic" process of Ca^{2+} induced Ca^{2+} release enables different kinds of receptor-channels to communicate with each other to establish coordinated Ca^{2+} signals, often organized into propagating waves (Berridge et. al., 2000: 12).

Let us take for example two of the most common and well studied channels:

1) the inositol-1,4,5-trisphosphate receptor-channel (InsP3R) and

2) the ryanodine receptor-channel (RYR)

(Berridge et. al., 2000; Lodish et. al., 2000; Carafoli, 2003).

These channels coexist in cells as "clusters of channels", where they seem to be controlled by different receptors that respond to specific signals and where they are used cooperatively to fine-tune the formation of complex patterns of Ca^{2+} signals (see figure No. 7).

At low levels of stimulation, the degree of excitability is such that individual InsP3Rs or RYRs channels open. These elemental single-channel signals have been recorded as "blips"[7] when they result from the opening of an individual InsP3R channel, and as "quarks" when they result from the opening of an individual RYR channel. These are considered the fundamental events that are the building blocks

[7] From the *Webster's Encyclopedic Unabridged Dictionary*:
"Blip": a spot of light on a radar screen indicating the position of a plane, submarine or other object.
"Quark": one of the elementary particles believed to form the basis of all matter.
"Puff": an abrupt emission of air, vapor, etc. A short, quick blast, as of wind or breath.
"Spark": ignited or fiery particles thrown of by burning wood. The light produced by a sudden discontinuous discharge off electricity.

from which *more complex Ca^{2+} signals* (signs) are constructed (Berridge et. al., 2000: 15).

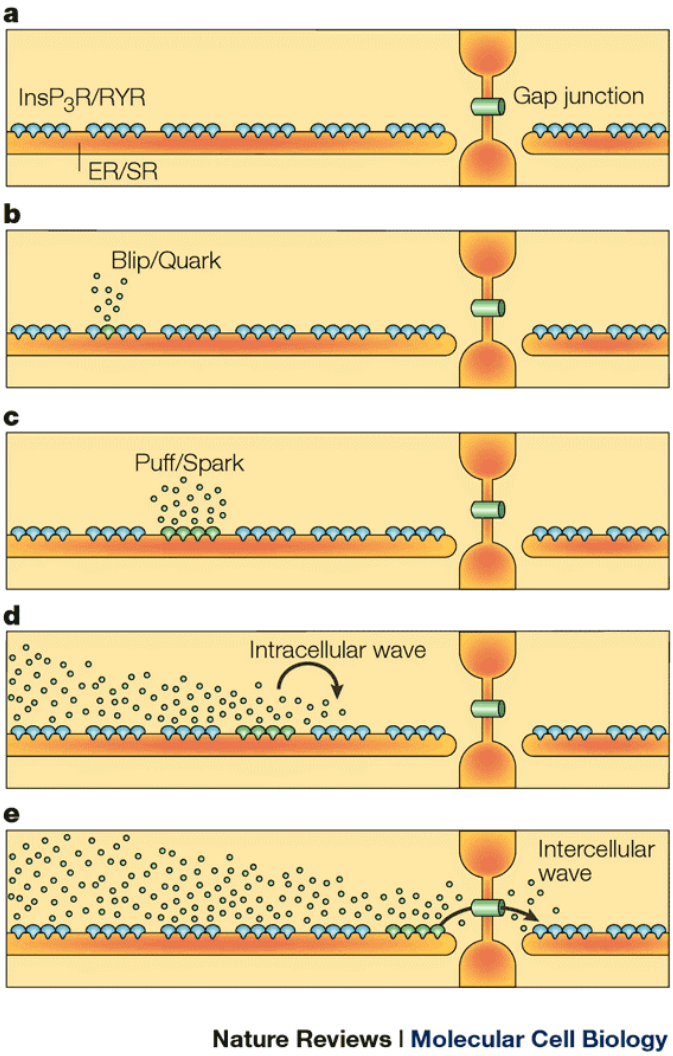


Figure No. 7. Clusters of channels create different patterns of Ca^{2+} signals. Taken from Berridge et. al. (2000)

These single channel emissions are rare events. More usual is the coordinated opening of clusters of channels. Such clusters of InsP3R channels generate compound signals known as “puffs” while clusters of RYR channels generate compound signals known as “sparks”. These signals may show different amplitudes suggesting that there are either variable numbers of channels within each cluster or variable numbers of channels open within an individual cluster (Berridge et. al., 2000: 15).

In addition to creating global responses, these elementary events have signalling functions within highly localized cellular domains (Berridge et. al., 2000: 16).

Sparks and puffs combine to form a more extensive kind of signal by constituting intracellular Ca^{2+} waves that sweep through the cell. For waves to occur, most of the InsP3R and RYR channels in the clusters must be sufficiently sensitive to Ca^{2+} to respond to each other through the process of Ca^{2+} induced Ca^{2+} release, therefore setting a positive feedback that amplifies the wave (Berridge et. al., 2000: 15).

Intra-cellular waves can become inter-cellular waves by propagating through gap junctions. Such intercellular communication contributes to the coordination of many cells. However it is not yet clear how the waves traverse the gap junction or whether before being transduced to the adjacent cell, the message is translated into Ins(1,4,5)P3 second messenger or even to some other extracellular mediator such as ATP (Berridge et. al., 2000: 16).

Finally, we have also frequency and temporal aspects of the signals. Ca^{2+} signals are usually presented as brief spikes. In some cases, individual spikes are sufficient to trigger a cellular response. When longer periods of signalling are necessary, spikes are repeated to give waves with different frequencies - ranging from a few seconds to 24 hours. Cells respond to changes in stimulus intensity by varying the frequency of Ca^{2+} waves.

In some cases, spikes can initiate gene expression more effectively than a steadily maintained level of the same average concentration (Berridge et. al., 2000: 17).

According to Berridge et. al. (2000: 17) “To use such a frequency-modulated signalling system, cells have evolved sophisticated ‘molecular machines’ for decoding frequency-encoded Ca^{2+} signals”. They point out two Ca^{2+} sensitive proteins that seem to decode wave frequency (Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C).

> "Now that the molecular and physiological mechanisms have been identified, the new challenge is to determine how this versatile Ca^{2+} signalling system functions in specific cellular processes. The Universality of this signalling system is evident in its *emerging function* during various developmental processes ..." (Berridge et. al., 2000: 20, my italics).

As I will try show, these patterns of signals function as signs which are usable in more complex combinations of patterns which entail an emerging code at a higher logical level.

3.1.10 The antidote to anarchic cross-talk

Stimulation of each class of receptor (GPCRs or RTKs) generally leads to the production of multiple second messengers, and both kinds of receptors promote or inhibit production of many of the same second messengers, including Ca^{2+} (Lodish et. al., 2000: 905).

As mentioned before, many of the components of the Ca^{2+} toolkit have several isoforms with subtly different properties (e.g.: binding domains, affinity, etc.) which add further versatility, specificity and calibration possibilities to the creation of spatio-temporal patterns of signals.

Calcium channels are informed by other intermediary second messengers, such as inositol 1.4.5-trisphosphate, or Ins(1,4,5)P3 for short, which specifically activate or deactivate them. In other words second messengers are mediated by second messengers (just like kinases phosphorylate kinases). " ... the versatility of the signalling network is enhanced by having different Ca^{2+} mobilising messengers linked to separate input signals."(Berridge et. al., 2000: 12). Downstream in the network, the same cell can use different Ca^{2+} sensors to regulate separate processes.

The circuit has further complexity because Ca^{2+} itself is involved synergically with Ins(1,4,5)P3 (and other consensus factors) to increase or decrease its own concentration.

Different channels coexist and are used cooperatively and modularly to fine-tune patterns. But they all create patterns of concentrations of the same ion which constitutes specific signs that inform specific responses.

So even if the isoforms of InsP3R-channels are highly specific, their mediatory role leads anyway to "universal" signals. Being Ca^{2+} a signal to many pathways this specificity could anyway remain vulnerable to the possibility of cross-talk. How is categorial perception achieved in the system?

Before going into the calcium case let me mention some possible explanations presently being considered to elucidate how categorial perception is achieved in signal transduction networks, i.e.: how a given concentration of a versatile second messenger informs the specific response and not other components of the network sensible to the same messenger:

1) One aspect that has been considered to explain this phenomenon is that specificity may be achieved thanks to the specificity that the downstream component - which in the previous step was informed by the second messenger - has for the subsequent substrate. For example, cellular responses to hormone-GPCR-induced-rise in cAMP vary among different cell types and tissues. "In virtually all eukaryotic cells studied, the action of cAMP appears to be mediated by one or more cAPKs [cAMP-dependent protein kinases], but the nature of the metabolic response varies widely among different cells. The effects of cAMP on a given cell type depend, in part, on the specificity of the particular cAPK and on the cAPK substrates that it expresses" (Lodish et. al., 2000: 887).

That is, the effects of a second-messenger on a given cell type depend, *in part*, on the specificity of the particular components that it informs and, of course, on the specificity of the components that follow after. But this is not enough to explain the crucial problem of categorial perception. When in one cell type there is more than one signal network that leads to increases in cAMP (like e.g.: in liver cells), and also leads to different responses, the hormone inducing the largest concentration of the common second messenger theoretically would also influence the activity of the networks that require a lower threshold concentration of the same second messenger.

2) Another argument to explain the process of "categorial perception" of these common signals is the consideration of the role that they may play as a "regulon" to coordinate the combined action of different responses by a hierarchical variation of thresholds in the concentration of the messenger that influences different components during the development of its concentration, in which case the "right" sequence of

thresholds would be achieved by the changes of concentration derived by a combination of hormones acting simultaneously to fine tune the response. For the same reasons as in the previous explanation, this by itself is not enough to explain how categorial perception is achieved. Rather, this fine tuning mechanisms could explain why then it would not be enough to have only the pathway with the hormone that expresses the highest concentration, which would overlap the other ones. The action of timely coordinated emission of differential concentrations makes a richer and much more differentiated concentration-development curve.

3) A third possible explanation for "categorial perception" in the case of a rise in cAMP that may produce a response that is required in one part of the cell but is unwanted, perhaps deleterious, in another part is by the discovery of anchoring proteins. Recent biochemical and cell biological experiments have identified a family of anchoring proteins that localise inactive cAPKs (the kinasis effector that is informed by cAMP) to specific subcellular locations, thereby restricting cAMP-dependent responses to these locations. This family of cAMP kinase-associated proteins (AKAPs) posses one domain conferring a specific subcellular location and another that binds to the regulatory subunit of the specific cAPKs (which is to be informed by the second messenger) (Lodish et. al., 2000: 888). In other words, anchoring proteins function as an efficient recruiting net for ready-to-be-activated cAPKs, but which could any way be susceptible of activation by cAMP of different origens.

This last possibility is very interesting because it may easily be related to the role that other types of molecules may have in the process of categorial perception like for example adapter proteins, molecular scaffolds and buffers. "Specific anchoring proteins may also function to localise other signalling proteins including other kinases and phosphatases, and thus may play an important role in integrating information from multiple signalling pathways to provide local control of specific cellular processes" (Lodish et. al., 2000: 888).

These three explanations do not exclude each other but they do not suffice to explain how complex specificities are achieved.

Let me now go back to the calcium code and suggest a possible explanation for categorial perception from the sign-theoretic perspective advanced in this work.

Since Ca^{2+} patterns have to be de-coded, their specificity as signals is related to a pattern of concentration thresholds of *different* elements that conform the vehicle of a more complex sign. It is because of this variability that different types of cells may exhibit very different responses to the same extracellular signal.

Let us continue with our example.

The inositol second messenger is generated when stimuli bind to cell surface receptors - and its concentration contribute to determine whether Ca^{2+} (present in low quantities) can activate the channels or not.

Ins(1,4,5)P3 diffuses into the cell to engage its receptor which is in a domain of the channel-protein. Together with some already present Ca^{2+}, it creates an analogical consensus for the release of more Ca^{2+} from the endoplasmic reticulum. Different isoforms of the Ins(1,4,5)P3-sensitive channel allow for differential sensitivity at the receptor domain of the channel adding more subtle combinatorial possibilities to the system. Different kinds of channels have different degrees of excitability depending on the levels of the appropriate Ca^{2+} mobilising messenger (Berridge et. al., 2000: 12). Increasing the level of Ca^{2+} enhances the sensitivity of the channels to the other consensus signals. This is the key to the "autocatalytic" process of Ca^{2+} induced Ca^{2+} release.

But the cytosolic autocatalytic action of Ca^{2+} seems to be more complex: it can be both stimulatory and inhibitory and can vary between the different receptor-channel isoforms. This gives place to some very interesting relationships between the level of activity of the channel (stimulation/inhibition) and Ca^{2+} cytosolic concentration (Berridge et. al., 2000: 12).

At low concentrations of the Ins(1,4,5)P3 second messenger, Ca^{2+} has a stimulatory effect on the channel but it inhibits it once a certain Ca^{2+} concentration threshold is reached, giving place to a bell-shape function. But it has been observed that sometimes the channels are not inhibited by high Ca^{2+} cytosolic concentrations - particularly when Ins(1,4,5)P3 is present, also at high concentrations. In this case, instead of a bell-shape, the relationship between channel activity and Ca^{2+} level is sigmoidal, with the peaks of the curve depending on the presence or absence of Ins(1,4,5)P3 which when present at certain threshold concentration collaborate in the digital-analogical consensus that enhances the stimulatory action of Ca^{2+}.

These instances of digital-analogical consensus shape the fluctuation curve and provide the system with the capacity for categorial perception, which is what gives

specificity to the analogical sign in order to avoid what would otherwise be anarchic cross-talk of ubiquitous signals.

Besides a given concentration threshold of channels, this particular case of digital-analogical consensus requires the simultaneous occurrence of certain specific threshold concentrations of for example Ins(1,4,5)P3 second messenger, Ca^{2+} ions, other Ca^{2+} binding proteins, adapter and scaffolding proteins, etc. These parameters must all have their own fluctuation curves, and the moments in which they intercept in complex combinations are the moments of digital-analogical consensus that will link a particular emission of Ca^{2+} patterns to a particular context in which the sign will be interpreted properly, i.e.: categorially and hierarchically.

Cytosolic buffers also play a major role in categorial perception. They are involved "...in shaping both the amplitude and duration of Ca^{2+} signals. During each spike, they act as a halfway house for Ca^{2+} by loading it up during the ON mechanisms and then unloading it during the OFF mechanisms". They also "... limit the spatial spreading of local Ca^{2+} signals. This is particularly important in neurons that contain high concentrations of buffers which are believed to ensure that Ca^{2+} signals are largely confined to synapses" (Berridge et. al., 2000: 14).

Adapter proteins also have to be mentioned in this regard. Many signal-transduction pathways contain large multiprotein signalling complexes, which often are held together by *adapter proteins*. Adapter proteins do not have catalytic activity, nor do they directly activate effector proteins. Rather, they contain different combinations of domains, which function as docking sites for other proteins. In some cases adapter proteins contain arrays of a single binding domain or different combinations of domains. In addition, these binding domains can be found alone or in various combinations in proteins containing catalytic domains. These combinations provide enormous potential for complex interplay and cross-talk between different signalling pathways (Lodish et. al., 2000: 856).

These are higher order complex specificities. A particular complex "lock-and-key" is created by which a complex configuration of concentration thresholds of signals and transforms of signals links a specific contextual demand to a specific cellular response, giving rise to the interpretant.

These complex lock-and-keys are higher order specificities. They are built on, but not limited to, the more basic individual stereochemical specificities between two molecules.

This principle may be behind many "auto-induction" processes in living systems which are never really "auto". They always depend on other consensus factors that modulate the "auto" effect. Otherwise auto-induction or auto-catalytic processes in living systems would be a once-in-a-life-time experience, disrupting the steady-state of the system by sending it into a positive feedback loop that could make the system collapse in the absence of any negative feedback control. This may as well hold a key to the understanding of many degenerative processes, from abnormal cell proliferation to the eutrophication of a lake, from apoptosis and ageing to pathogenic invasion.

The significance of a certain specificity, or of a more complex "lock-and-key", lies in the triadic relation between the match of the lock, the key and the door that it opens.

3.1.11 Fluctuations versus sustained rise

The purpose of the fluctuations of Ca^{2+}, rather than a sustained rise in cytosolic Ca^{2+}, has not been well understood only until recently. It has been suggested that one possibility is that a sustained rise in Ca^{2+} might be toxic to cells (Lodish et. al., 2000: 891). On the other hand, semiotically it makes perfect sense. Fluctuations of patterns (of Ca^{2+} concentrations) are pertinent when transducing a message into a higher order code. We see here how the logical product is not necessarily quantitatively proportional to the mass that expresses it. I.e.: logical products may not necessarily be formed incrementally in proportion to an increment in mass. In semiotic processes the variation of patterns is not always proportional to mass quantity. If this was the case, there would not be possibility for any kind of digitality or codification, i.e.: we would be back to the world of dynamics, forces and impacts.

The fluctuations of Ca^{2+} are related to the conformation of specific signs composed by specific threshold concentrations which cooperate in co-determining, informing and interpreting specific contexts. This fluctuation is part of a sign-network that works through a process of digital-analogical consensus. So the fluctuation is indispensable for the semiotic system. The sophistication of the code that is supported by such fluctuations and its embeddedness in a larger code gives the system remarkable semiotic plasticity for very sensible and complex fine-tuning and calibration functions.

The fluctuations are important because the different threshold concentrations at different times constitute part of a map of an equivalent territory in that precise moment. Fluctuations in thresholds are sensed as differences in the fluctuation curve. Some thresholds are important, i.e.: are part of signs, and some are not, depending on the rest of the context, including other signals and other signs, i.e.: consensus factors.

3.1.12 Semiotic toxicity

If the function of Ca^{2+} is a semiotic function, it makes absolute sense that there is a fluctuation rather than a sustained rise in cytosolic Ca^{2+}.

If we understand this phenomenon linearly, in dyadic terms, as just a simple increase of a needed metabolite whose presence and quantity at a given moment is determined by the amount of mass that needs to be metabolised, - contrary to Ca^{2+} that forms part of a configuration that will convey an analogical message - it is hard to understand the sophisticated fluctuations of concentrations, and why would that be the case. This is so because we are talking about information, not about mass.

A sustained increase in Ca^{2+} probably would not be toxic in the strict chemical sense. More likely a sustained increase in Ca^{2+} would be toxic primarily because it would disrupt the communication and regulation by zeroing the possibility of categorial perception of the different thresholds that are specifically composed by cooperative components in response to a particular constellation of signals and cues. In other words, the semiotic system would be ruined by uniformity. For example, an abnormal sustained increase of Ca^{2+} can permanently activate hydrolytic activities, mainly proteases, which is an event that is incompatible with cell life (Carafoli, 2003: 331).

3.1.13 The phosphorylation code

I can not go here into the details of the phosphorylation code but let me only enunciate its nature as a digital-analogical code for the creation of complex systems of specificities, comparable in this sense to the Ca^{2+} code with which it ”cross-talks” and co-operates in a modular fashion to participate in higher emerging codes.

In this case, some effector proteins - kinases and phosphorilases - create *patterns* of phosphorylation by cyclically phosphorylating and de-phosphorylating specific

residues in substrate proteins leading to sensitisation or desensitisation of cells to various stimuli. The phosphorylated form of some proteins is active, whereas the dephosphorylated form of other proteins is active.

Protein kinases modulate the activity or the binding properties of one or more substrate proteins by phosphorylating serine, threonine, or tyrosine residues. On the other hand, protein phosphatases remove phosphate groups from specific substrate proteins, i.e.: they de-phosphorylate them. The combined action of kinases and protein phosphatases can cycle proteins between active and inactive states.

In other words kinases and phosphorilases "sculpt" specific (digital) "differences" on their substrates, providing them with a specific (analogical) recognition pattern, i.e.: phosphorylation and/or desphophorylation of specific substrates produce meaningful patterns, a compound analogical message out of different single digital phosphorilated sites. So what may change, i.e.: what becomes relevant, is not the concentration of the substrate itself but the concentration of those with a specific phosphorylation pattern.

There are many possibilities for second messenger codes and the phosphorylation code to interface with each other, before, during and after the production of the second messenger and conversely before, during and after the production of phosphorylation patterns. When the concentration of the second messenger is de-coded, the message is transformed into the phosphorylation code.

3.1.14 Amplification

The term "amplification" also deserves to be further specified in this context. According to Lodish et. al. (2000: 887) the "overcomplication" of cascades - a series of reactions in which the enzyme catalysing one step is activated, or inhibited, by the product of the previous step - has at least two advantages:

1) A cascade allows an entire group of enzyme-catalysed reactions to be "regulated" by a single type of molecule. Some metabolic pathways are regulated by hormone-induced cascades, some mediated by cAMP and some by other second messengers. Such a single type of "regulating molecule" would then be viewed as the coordinator of an integrated set of enzymes, something analogous to the role of a

regulon in genome architecture, i. e.: a repetitive element that insures the simultaneous activation of cooperative elements in the required concentrations.

2) It is said that a cascade provides a huge amplification of an initially small signal. For example, blood levels of epinephrine as low as 10^{-10} M can stimulate liver glycogenolysis and release glucose, resulting in an increase of blood glucose levels by as much as 50 percent. An epinephrine stimulus of this magnitude generates an intracellular cAMP concentration of 10^{-6} M, an amplification of 10^4. Because three more catalytic steps precede the release of glucose, another 10^4 amplification can occur. In striated muscle, the concentrations of the three successive enzymes in the glycogenolytic cascade (i.e.: cAPK, GPK, and GP) are in a 1:10:240 ratio, which "dramatically illustrates the amplification of the effects of epinephrine and cAMP" (Lodish et. al., 2000: 887).

In a sense it would be wrong to talk here about amplification. The signal is not really amplified in spite of the fact that subsequent steps of the cascade build up higher concentrations of intermediaries. The signal may be said to be just "normally" transduced (i.e. not amplified) because those ratios of higher concentrations are implicit in the semantic value of the original signal. An amplification would rather imply concentration ratios much higher than what the "average" cascade would yield (granting that such increments could effect a proportional increment of response).

What is called amplification in this context is rather the transformation of a sign - constituted by a threshold concentration of a certain signal - into another sign - constituted by the threshold concentration of another molecule. In turn, these threshold concentrations combine to originate subsequent signs. An amplification would presuppose a meta-kind of sensibility in which by some kind of arrangement the original message would be sensed as being more drastic or urgent than "usual" and therefore a different arrangement of ratios could be useful for speeding or slowing the cascade, or for giving extra output (if possible) under special conditions. Amplification would be more similar to certain autoinductive processes with one or more positive feedback loops in the network. For example, one of the different effects of adrenaline is higher contraction rate of the heart, and thus higher blood pressure, which in turn enhances the circulation of adrenaline. This could more properly be considered an amplification.

When the system has a built-in potentiality to amplify a signal it means that it can be differentially more or less sensible to that signal, prompting in this way the necessary response before the concentration of what the signal refers to reaches intolerable levels, or escapes away, depending on the case. When on the other hand the system is capable of diversifying a signal, it means that the difference created by it is relevant to a related "cross-talking" pathway. A way for diversification may be the modulation of specific threshold concentration levels.

The amount of a particular receptor expressed in a cell at a given moment may be relatively low. Hormone receptors are present in minute amounts: the surface of a typical cell bears 10,000-20,000 receptors for a particular hormone representing only $\approx 10^{-6}$ of the total protein in the cell or $\approx 10^{-4}$ of the plasma membrane protein. The specificity of a receptor is a function of its *binding affinity* for the ligand. Changes in hormone concentration are reflected in proportional changes in the fraction of receptors occupied. At a rise of hormone concentration, the rise of receptor-hormone complex concentration will rise proportionally, according to its affinity constant. And usually the cellular responses will increase in the same proportion. But for many hormone receptors, the ligand concentration needed to induce maximal cellular response is lower than that needed to saturate all the receptor molecules in a cell i.e.: the maximal response of a cell to a ligand is generally achieved at concentrations at which most of its receptors are still not occupied (Lodish et. al., 2000: 859-860).

This discussion about amplification evidences the importance of considering the signal-sign-system in its triadic logic and not just exclusively in its dyadic workings. Dyadically, an increase in concentration levels of each subsequent enzyme may look as an amplification, a quantitative event, because we see how fewer molecules hierarchically mobilise higher quantities of other molecules. But triadically, it is the *regularity* of these ratios that gives coherence to the semiotic network in which the cascade is immersed. Triadic relations do not build up by an additive process of always increasing numbers of the next step molecule, as it is so clearly evident in the Ca^{2+} code. They result from the logical products produced by combinations of threshold concentrations of successive and/or simultaneous and adjacent steps. So the fact that there seems to be more materials downstream of the cascade does not necessarily mean an amplification of the logical result in the entire semiotic network. i.e.: the signal produces the "right" result, not more result. So it is the fractionating effect of the logical products that matters, not necessarily the additive effect of matter.

Given that in communicational systems we deal with sequences which resemble stimulus-and-response rather than cause-and-effect, much before we encounter energetic or material restraints we may encounter other types of limiting factors which are semiotic in nature. There is for example an economics of probability of the possible logical products that are present at a given moment and of the finite number of alternatives available to the system, which are context-dependent. This economics differs from the material budget in that probability - being a ratio - is not subject to addition or subtraction but only to multiplicative processes, such as fractionation (Bateson, 1972: 403). Let me try to provide an example of how such "fractionating effect" may work. When buffer molecules capture a necessary given number of free Ca^{2+} ions, keeping the cytosolic concentration under a certain threshold, the probabilities of activating the different pathways that are sensible to concentrations above such a threshold remain low. This can be compared to the example given by Bateson (1972: 403) in which a telephone exchange at a time of emergency may be "jammed" when a large fraction of its alternative pathways are busy. There is, then, a low probability of any given message getting through. Since the sign (or rather a part of it) that may prescribe a certain transcriptional response is a precise concentration of Ca^{2+} ions (plus of course a whole battery of other consensus parameters), all the individual Ca^{2+} dependent signal pathways of the network may contribute or not to the formation of the analogical sign. A Ca^{2+} ion bound to the buffer molecule is a busy line. The fractionating effect is not limited to that level. The threshold concentration of Ca^{2+} (as a compound analogue), by being or not being at a certain location and at a certain time is also a digital sign in a larger analogical message that leads for example to the transcription of a gene, whose product participates (by being or not being present) in other analogical products that give rise to complex emergent traits.

3.1.15 Transietivity, kinetics, isomorphisms, affinity, PH

The transietivity of the different concentration gradients of successive signals is of course related also to the specific biochemical rates of the different reactions that occur within the network. In this sense the biochemistry contributes to the "punctuation" necessary to convey an analogical message composed by configurations of concentrations of signals and other mediator-components. Kinetics helps to

determine the time-intensity nature of the signal. The rates of certain reactions act as a timer to control the length of time of an association, e.g.: molecular switches. The timing of the event, the duration of the signal, based on the kinetic rate is an analogical message, more complex than the mere digital presence or absence of the signal. To the analogical composition of the signal we have to add the specific concentration threshold necessary for the signal to be "meaningful" as well as the rest of the consensus cofactors that integrate a complex sign, which is what assures the proper "categorial perception" of what otherwise would be ubiquitous or meaningless signals.

The system is organised in such a way as to take advantage of these given physical restraints in order to incorporate them into a functional code. The same can be said about isomorphisms, affinity (specificity), modularity, PH tolerance and other structural restraints, that rather than determinants, are structural features that can be incorporated to articulate active codes through systems of correspondences. For example, there is a relation between affinity (Kd) and concentration in signalling systems. Concentration thresholds, which are part of complex cellular signs, are influenced by the signal-receptor affinity (which can also be an analogical variable), as well as by any habitudinal presence of signal-analogues (agonists and/or antagonists) in the system. Furthermore, different PH domains may modulate binding specificities in some molecules. These are some of the first rules implicit in metabolic codes that have been widely recognized by biologists.

3.2 Biosemiotic technology

3.2.1 Towards a definition of biosemiotic technology

The idea of a sign-theoretic approach to biotechnology implies the consideration of communication processes in living systems during technological applications of biology. If the characterisation, evaluation and assessment of informational processes (and thus the importance of the context) within and between organisms are recognised as important in biotechnology, a suitable logical framework for the organisation of this knowledge will be useful.

Biosemiotic technology would be that part of biotechnology which develops such epistemological tools. That is, the use of knowledge about "networks of biological

information" i.e. semiotic networks, and relative semiotic controls, whether for mapping and monitoring technics or for the subsequent steps of intervening, regulating or controlling the processes under consideration.

All biotechnological applications that are concerned with biological information pathways, not only from the cybernetic-probabilistic point of view but also from the functional or semantic point of view, constitute the field of biosemiotic technology.

One of the main contributions of biosemiotics to biotechnology is in the definition of the necessary integrative agenda of different subdisciplines and hierarchical levels within the life sciences - from molecules to physiologies to ecologies - in order to keep track of the relation of a given technological application with its particular context, be that a single cell, an organism, a crop, a patient, a niche, society or the environment.

From molecular biology to ecology, biosemiotic technology includes any technological application that relies on the understanding of sign systems and semiotic controls in organisms and ecosystems. A lot of modern biotechnology can already be considered as biosemiotic in the sense that the principles behind it imply transfer and interpretation of information in biosystems: from gene-expression, splicing and transfer to signal transduction; from quorum sensing to biosensors, biocontrol and ecological monitoring.

At the molecular level the principle behind natural or synthetic signal-analogues is at the center of many current and promising biotechnological applications. Signal-analogues can be agonists or antagonists. Agonists mimic the function of a signal by binding to its receptor and causing the normal response. On the other hand antagonists mimic the structure of the hormone but not its function by binding to the receptor but not activating the signal-induced effects, i.e., they work as inhibitors by blocking the physiological activity of the signal. What makes a good antagonist for a signal is a molecule that combines a domain with high affinity for the receptor with a domain that determines a specific configurational change in the receptor that decreases its affinity for the subsequent protein that has to be activated in the cascade. This leads to a whole field of signal-drugs, an area that will need delicate and sophisticated methods for mapping and understanding semiotic networks, i.e.: when intervening in the signalomes of complex organisms, including humans.

I shall now briefly present two examples from this technological landscape.

3.2.2 Quorum sensing and the post-antibiotic age

Let me return to quorum sensing as one of the interesting examples of incipient biosemiotic technology. It is not surprising that from the different applications of quorum sensing currently being explored, the most promising one has to do with its inhibition given that signalling-molecules in quorum sensing systems trigger the expression of a wide range of pathogenicity determinants in many organisms that infect plants, animals and humans. The alleged advantage of using quorum sensing for the bacterial colony is to avoid a premature detection by the host's immune system, which would give the host a chance to overcome the incipient colony. Instead the colony "quietly" grows until a sufficient number of cells have built up to release the pathogenic response when it is too late for the immune system to react. This is the point when the colony uses a signal to coordinate the attack. By studying molecular mimicry, like that developed by the alga *Delisea pulchra*, it might be possible to develop methods for blocking the signals so that organisms remain harmless and never express their pathogenic determinants. Some researchers see in this strategy the beginning of a post-antibiotic age in which we would attempt to "discipline" bacterial pathogens by understanding their "language". The great advantage over antibiotics is that quorum sensing inhibitors do not inhibit bacterial growth. They only interfere with the expression of virulence and colonisation and therefore there is no selective pressure to "evolve" resistance. Furthermore, since the molecules are diffusible, the signals are not stopped by physical barriers (they penetrate cells, organs and even biofilms) (Givskov, 1996; Rice, 1999).

There are other areas of potential applications for the manipulation of quorum sensing already identified. One is in the commercial scale fermenters for the production of antibiotics. In this case it could be advantageous to induce the colony to express its "protective" genes (which include antibiotics) earlier than it would normally do. Cultures of bacteria usually make antibiotics only as they finish the phase of rapid growth and enter the stationary phase. By adding specific cell signalling molecules at the start of the rapid growth phase they can be induced to make antibiotics throughout the period.

Another promising area seems to be food safety. At the stationary phase of the bacterial population, protective genes are switched on in response to environmental stress. Some of these genes code for virulence factors. This has implications for the

design of food safety strategies because many of the procedures used, such as manipulation of the pH and temperature and the use of preservatives, are environmental stresses that might be expected to trigger the release of signalling molecules that in turn switch on the protective genes increasing the potential virulence of surviving bacteria. Because the signalling molecules are highly diffusible, the nature of the medium around the bacterial cells is very important in determining whether or not the concentration of these molecules will increase to a point at which virulence is triggered. This means that the assessment of the semiotic niche includes the consideration of environmental stresses, the presence of signal-molecules emitted from harmless bacteria (which may trigger virulence factors in other species of bacteria) and the diffusion characteristics of the medium, among other things.

Another potential pharmaceutical application has been hypothesised in exploiting the immune response that animals exhibit to the bacterial signalling molecules themselves, although in this case one could expect a much more complicated map of the semiotic network. Finally, quorum sensing has been rapidly adopted for the design of bacterial biosensors. This has been done by using the regulatory part of the quorum sensing mechanism in conjunction with some common genetic marker, or by using the luciferase operon as a marker attached to a regulatory region which senses the desired parameter.

3.2.3 Biosensing: from cells to multitrophic systems

The very name of biosensing places this biotechnological application in the category of biosemiotic technology, or if you prefer, biosemiotic technology can be seen as an epistemological tool for devising complex biosensing. Even in the most mechanical conceptions of biosensing, where there is a physical mechanism using the specificities of organic compounds, and where the only biosensing entity is the human observer, there will be triadic logic involved. At its most simple technological level a biosensor is an analytical device incorporating a deliberate and intimate combination of a specific biological element (that creates a recognition event) and some kind of physical element that records and transduces the recognition event to the observer. According to Fraser (1997) most of the novelty of biosensors comes from the "bio"

side since the transducers of the recognition event are mostly physical instruments which have already been widely used in "physisensing".

Biosensors perform a diversity of sensing functions allowing the acquisition, capture, communication, processing, and distribution of information about the states of physical and biological systems. It is a characteristic feature of a biosensor that the device is tailored to the environment in which it is to operate. In the development and application of sensors to the field of process control, there is a trend that moves away from the use of measuring devices towards the use of sensing systems. This strategy seeks to decentralise measurements by focusing not only on product control but mainly on "on-line" process monitoring (Lading et. al. 2001:4). In other words, biosensors are involved in assessing qualities, not measuring quantities. That makes them semiotic devices. Quantities, i.e.: thresholds, can eventually be deduced from our knowledge about the metabolism of the biosensor.

When the biosensors that the human eye has to sense are organisms which in turn have sensed a difference (that makes a difference to the organism and then to the human observer) the web of semiotic networks to be analysed can increase considerably if we are to consider, for example, multiple biosensors for monitoring complex processes in an organism's physiology or in a multitrophic interactive system, whether at the level of a niche or ecosystem.

Biosensors, in their wider definition, have been used in many different modalities and applications, from the use, in the old days, of canaries to detect poisonous gases in coal mines, to the extensive use of antibody technology, up to the recent use of the most diverse and sophisticated genetic constructs. Today, biosensing represents a growing research area of its own and a complete biotechnology sector that develops tools and services for a great variety of applications. These devices may include nucleic acid sensors and DNA chips, immunosensors, enzyme-based biosensors, devices with natural and synthetic receptors, organism- and whole cell-based biosensors, etc. To this we can add the methodological tools for biodiversity and ecosystem monitoring that are based on sensible species, bioindicators or indicator species.

Since, at the physiological level, in vivo sensing is considered to be a priority for therapeutic purposes, biosensing research will meet increasingly complex requirements for the design of "on-line" biosensing devices or organisms. For example, the most quoted case of a widely used physiological biosensor is that of

glucose sensing for the control of diabetes. Continuous glucose monitoring in diabetics has been attempted through a variety of invasive or non-invasive methods. Despite much effort and some encouraging results, numerous obstacles remain, mainly due to poor sensor biocompatibility and fluctuating body chemistries. In vivo sensors are subjected to many obstacles once they are placed in their niches. But it is recognized that one of the hardest problems to overcome will be the recognition of the biosensor as foreign by the host, i.e., the cellular tissue responds, leading to membrane fouling and sensor encapsulation by fibrous tissue (Fraser, 1997). The cellular and humoral defence mechanisms will do their best to eliminate the spy. It turns out that biocompatibility is also a communication problem and careful attention to the semiotic niche could provide some hints as to how to camouflage the sensor while it does it job, and at the same time how to control its invasive presence. The fluctuating chemistry of the body is another important aspect to be considered from the semiotic point of view.

The possibility of fast, on-line, real-time sensing opens up new perspectives in a variety of applications in microbiology, medical diagnostics, biocontrol, biosafety, agriculture, ecological monitoring and in the pharmaceutical and food industries. In the pharmaceutical and biotechnological industries, the progress of microbial fermentation can be controlled and optimised through the use of biosensors. Biosensors measuring microbial growth and contamination of foodstuffs have been developed and are already in use (Lading et. al. 2001).

By relying on patterns, a web of biosensors could be conceived in the assessment of complex relations in a given hierarchical system. This could theoretically be achieved by combining different biosensing organisms or devices; by combining biomarkers within a single biosensor; or by linking one biomarker (e.g.: bioluminescence) to the simultaneous occurrence of a mix of analytes or environmental conditions (e.g.: a complex blend of volatiles). If the mixing of such regulatory elements is not feasible in the operon of the biomarker, then biosensors with different biomarkers would be required for such a job.

In physiological and ecological monitoring there are increasing expectations in connection with new broad-band array biosensors capable of classifying, assessing and gradient-tracking their dilute analyte targets in more or less complex and demanding physico-chemical and biological backgrounds. Because of their refined

capability for categorial perception, biosensors are capable of operating in “dirty” samples and complex mixtures.

3.2.4 Ecological monitoring

The general trend of globalisation has implied that also the spatio-temporal scope of environmental planning and technology design has expanded its range of action, setting up the creation of a massive techno-web to manage biodiversity resources and anthropogenic impacts on ecosystems. It is in this technological sphere - right at the interface between the natural system and our cultural “planning” - that we encounter monitoring technology.

But monitoring implies a previous step, which is sensing, and sensing in turn implies a range of semiotic processes of different sorts, as we have seen. The design of “sensing” has to precede the actual activity of “sensing”. In turn, our design will be influenced by the way we conceive the system that we wish to monitor. Sensing does not consist only in collecting data. Its efficient operation has a circular relation with the framework and the procedures that we have designed for structuring and codifying *the selected kind of data* in order to grasp their significance. Ecological monitoring, as technology applied to biosystems, has relied very little on the understanding of sign systems. However, biosensing is growing rapidly in environmental monitoring. The field of signalling in multitrophic systems is just at its beginnings but already at a “non-returning-point”. From that point of view, the whole idea of biocontrol is being reframed to include contextual considerations.

At the level of ecological monitoring, biosensors are being used to detect the presence of various chemical or biological materials in the environment, including other organisms or functional genes in populations, and to monitor continuous changes in environmental conditions. Some of the analytes to be monitored include metal ions, toxic materials, pollutants and different organic materials such as proteins, DNA, signal-molecules, viruses and bacteria, among others.

Again, what is needed is not measuring quantities but the rapid assessment of qualities, something that living organisms do much better than physical devices.

More sophisticated arrays of biosensing systems would be necessary when considering the complex semiotic networks that are currently being identified during biocontrol interventions in multitrophic systems. It is theoretically conceivable the

design of “webs” of biosensors which can be used to monitor health, biodiversity and ecosystem function in relation to human settlements, crops and wild ecosystems. Such a web could rely on the use of conceptual tools and empirical techniques that may range from single genetic constructs (organisms - from bacteria to plants - containing specific markers) to systems of “indicator” or “sensible” species that form functional semiotic networks which can be used as *indicator- patterns* of communication and relations in ecosystems.

3.2.5 The importance of sign-theoretic approach to biotechnology

Almost three decades ago Bateson gave us some hints as to how to proceed along a sustainable path (without at the time using the word “sustainability”):

> “...it would be convenient to have an abstract idea of what we might mean by ecological health. Such a general notion should both guide the collection of data and guide the evaluation of observed trends” and he goes on to define a healthy ecology of human civilisation as: “A single system of *environment combined with high human civilization* in which the flexibility of the civilization shall match that of the environment to create an ongoing complex system, open-ended for slow change of even basic (hard-programmed) characteristics” (Bateson, 1972: 494).

Among the different characteristics listed by Bateson in his attempt to work towards a definition of “high” we have: “A ‘high’ civilization should therefore be presumed to have, on the technological side, whatever gadgets are necessary to promote, maintain (and even increase) wisdom of this general sort. This may well include computers and complex communication devices”.

More recently, Hoffmeyer has added further hints in this direction:

> “...sustainable resource utilization presupposes that natural systems are allowed to follow their own complex and diverse regulatory mechanisms. And this is where information techniques enter the scene. So far we have simplified nature to match our heavy technical system. With the information techniques we would be able to fit our technical system to match the complexity and refinement of living nature ... Basically two kinds of information techniques should be

> distinguished. Techniques for manipulating, transferring and storing culturally derived informations, i.e. microelectronic techniques, and techniques for manipulating, transferring and storing biologically derived informations, i.e. bio-information techniques (e.g. gene splicing)" (Hoffmeyer, 1993a).

But even though new information techniques (both types) may constitute the technological basis for a production system which could better match the complexity of ecosystems we should also bear in mind that "... several of the premises which are deeply ingrained in our way of life are simply untrue and become pathogenic when implemented with modern technology" (Bateson, 1972: 502).

Hoffmeyer (2001) has summarised the importance of biosemiotic technology with the following simple and elegant formula:

$S/E \geq 1$

Here, S could be imagined as a "measure" of the semiotic fitness to control and optimise the flow of energy in living systems (cells, organisms, ecosystems) and societies, while E would represent the energy flow through these entities. When the flow of energy is much larger than what the semiotic controls can manage - in order to optimise such flow and minimise entropy - balances are pushed out of kilter.

In natural systems there is a near optimal fit of the means for semiotic control to the available actual flows of energy through bodies or ecosystems. Plants and animals have acquired exceedingly sophisticated endo- and exosemiotic means for controlling the flows of energy on which they depend. Through the evolutionary fine-tuning of semiotic controls in all processes of life, entropy is kept at a minimum or exported to the surroundings (Hoffmeyer 2001: 280-283).

As long as biological energy sources (i.e., photosynthesis and muscle power) furnished most of human needs for energy consumption these biosemiotic controls were largely undisturbed assuring that production systems were sustainable. But by mastering the use of non-biological energy sources, human societies have enormously increased the flow of energy that they can canalise into their production systems disturbing the relation between semiotic and energetic command in the larger system. We came to realise that "the flow of resources extractable from a given area can only be increased through operations which push natural systems farther away from their own balanced state" (Hoffmeyer 2001: 277-281).

> "The industrial revolution instantiated a rupture of this balanced situation, where the semiotic control function (S) would no longer match the size of the energy flow (E). In the industrial production system energy flows were dramatically increased, while the S component was not taken care of. This created a dangerously low S/E ratio, and it is suggested that this low S/E ratio constitutes a deep level explanation for the environmental crisis. In order to restore a sustainable production system, we will now have to develop technological means for a strong increase in the S factor of the production system, and it is suggested that this can be obtained through a development of considerate, gentle and clever forms of biosemiotic technology" (Hoffmeyer 2001: 277).

3.3 Biosafety and triadic causality: future perspectives

3.3.1 The biosafety map projected onto the semiosphere

At the level of conservation biology we will have the opportunity to blend our knowledge about genetic, metabolic, ethological and ecological codes in a non-deterministic way. In this view, ethology needs to be approached as the study of ethological codes and semiotic interactions within and between species in multitrophic and multisemiotic systems of correspondences, i.e.: in inseparable relation with phyto- and zoosemiotics. This may lead to *arrays* of indicator species that reflect patterns of crucial interactions, increasing therefore the resolution of our indicators.

The most important step for the conservation and sustainable use of biodiversity is often assumed to be the identification and elaboration of exhaustive taxonomic inventories. Many scientists are worried about the extinction of thousands of species yearly before anybody even had a chance to classify them. It has been estimated that about 1 million species have been taxonomically labelled and frequently it is repeated that there may exist five, thirty or even eighty millions of species yet to be discovered. But while taxonomy is of course necessary and useful, the understated goal of exhaustiveness and the lack of focus on relations between species seems a bit awkward in connection to monitoring. This is where semiotic networks enter into ecological monitoring. Measurements of biodiversity comprise the identification and quantification of species and the recording of population dynamics. In large, complex

and diverse ecosystems, as for instance a tropical rainforest, such measurements take on enormous proportions. Therefore modellers try to design monitoring systems that rely on what are considered "indicator species", a notion which obviously already has an explicit semiotic connotation.

We could pay attention to the sophisticated and vast systems of taxonomy developed by traditional autochthonous cultures in complex and diversified ecosystems. These taxonomies are not so much based on the demarcation between single species but on the multiple relations of a great diversity of species, i.e., the identification of meaningful networks and patterns. Based on this we could conceive sophisticated systems of bioindicators based on the patterns of semiotic interactions which may serve as signs of health and ecosystem function. This kind of mapping could also inspire screening procedures for extracting out of the library of biodiversity the "biological solutions" that could "mimic" the flexibility of the ecosystem as it is codified in its systems of correspondences. A strategy like this would more truly vindicate the role of the so-called "parataxonomist" in western globalised culture, that is, the role of the autochthonous people from traditional local cultures who in their everyday life are used to handle a huge amount of data relative to the species, their trails, their utterances, their habits and their relations with other habits, other utterances and other trails in the ecosystem.

As biotechnology advances, the genetic (and thus also the evolutionary) level will increasingly have to be included in ecological monitoring. Having to do with biological information at the different hierarchical levels of biology, a further development of the approach advanced in this work could provide a framework for charting a wider *biosafety map* in a hierarchical perspective.

We need to construct a sound and serene interface between the development of biotechnologies and the relation between health, biosafety, biodiversity and sustainability. This can be seen as the minimum common denominator of many bioethical discussions.

A biosafety map in a wider hierarchical perspective has to include considerations and relations between ecosystem health and function, food security and human sanitation and health. Monitoring of biosafety concerns occurs at many levels and sectors: in the labs, in agro-systems, in health care and disease prevention systems. These sectors are related and overlap with each other and therefore, based on our monitoring capabilities, we need to construct our maps in accordance to the

hierarchical nature of living systems. Some of the issues which deserve consideration in this context are:

- epidemiology
- bioinvasion
- genetically modified organisms
- gene transfer
- genetic erosion
- diversity erosion
- pollen and seed dispersal
- monocultures
- resistance
- biological risk assessment
- food and health security

I cannot go in detail here about how these related cross-sectorial issues of biosafety could be mapped onto such a semiotic model. I leave the issue open for future research. (For a suggestive preliminary ecosemiotic approach to bioinvasion see Emmeche, 2001).

Another open question that could be tackled from this perspective is the issue of the increasing expectations on the relation between bioprospecting and biotechnology, i.e., biodiversity as "raw materials", in the form of "information", for the different industrial sectors of biotechnology. As mentioned before, gene-technology (engineering and transfer of gene expression systems) is already based on semiotic processes. This opens the possibility for sophisticated and efficient breeding strategies. According to Hoffmeyer (2001: 287) a gene technologically based breeding strategy that takes into consideration the local conditions, instead of drastically changing (and homogenising) such local conditions, can be said to be a semiotic strategy. In this sense biosemiotic technology can be seen as a meta-technology to guide and control industrialised bio-production (Hoffmeyer, 2001: 289).

From the point of view of sustainability, these strategies must be embedded in the assessment and design of systems of correspondences that assure that our genetic construct matches the flexibility of the host ecosystem i.e.: it does not disrupt communication channels in the vertical and horizontal semiotic systems.

Sophisticated and flexible systems, not single ad-hoc constructs, is what we should hope from biotechnology.

In this sense and from this perspective the open question is: in what sustainable sense does the "library of biodiversity " represent an asset to the biotech sector?

Our maps will have to relate the semiotic networks involved in the production of organisation and health and, conversely, those involved in the production of entropy and pathogenicity. For such a biosafety map, a hierarchical understanding of health in living systems would be necessary. Pathogenicity is not exclusively a trophic problem. It is mainly a semiotic dysfunction. Such a map would be based on a projection of the semiosphere, which at different hierarchical levels relates physiology, diversity and function in aggregates of cells, organs, organisms, multitrophic systems, cities and whole ecosystems.

3.3.2 Towards a hierarchical understanding of health

A recurrent theme in ecological studies of biodiversity is the occurrence of resistance and pathogenicity. However these phenomena can not be fully grasped only in terms of trophic interactions because they imply a process of communication and context interpretation.

So far the term “non-trophic interactions” has been used to denote those kinds of interactions in (experimental) multi-trophic systems which “control”, “regulate” or simply “influence” the trophic exchanges that are being traced or monitored in the selected system but without being themselves trophic exchanges. It is no wonder that this kind of interactions had to become more evident when the systems for measuring biomass and fitness grew from one or two trophic levels to three to five trophic levels, i.e.: when trophic chains became trophic webs.

So in complex multitrophic systems we have to examine carefully what is it that differentiates these two types of interactions. Non-trophic interactions mean all the interactions that do not directly involve the transfer and metabolisation of organic matter. This means a lot of interactions. In the empirical field these interactions are being identified in the signalling systems that trigger specific responses between organisms of different species that are not directly involved in trophic exchanges. This is visible in the characterisation of infochemicals, semiochemicals and pheromones, especially in relation to resistance responses against predators. But there

are many other non-trophic interactions. Practically, the totality of signal-transduction networks operative in all the participating organisms and which inform the development and behaviour of a given organism in relation to many others; all the sexual and asexual exchange of DNA; all the regularities and behaviours of organisms that are sensed and interpreted by other organisms. In general, non-trophic interactions between living organisms refer exactly to semiotic interactions, where what is being "transferred" is information. The hierarchical nature of the interactions that lead to health, virulence and resistance in physiological and ecological systems is a very good example of non-trophic interactions.

Health, and the lack of it, has to be as old as life itself. This problematic concept, as it is intrinsically related to life, expands throughout the entire biological hierarchy from cells to ecosystems. At all these levels the notion presents numerous problems when defining a healthy system. There are different kinds of resistance constantly emerging at different hierarchical levels within biological complexity. There must be general and common principles that lie behind the different forms of resistance originating in Nature. Although it is quite different to speak about pest resistance, stress resistance, invasion resistance or to speak about antibiotic resistance, there might be some principles common to different mechanisms (if no other the production of a substance or a context that furnishes protection against something, and *communication* of these patterns to other organisms).

In the preface for the publication of the 5th Symposium of the Society for General Microbiology "Mechanisms of Microbial Pathogenicity" (1955), Howie and O'Hea (1955: X) begin by warning that discussions on how micro-organisms produce disease are very apt to follow a circular course to platitudinous conclusions. That is certainly not my purpose here. However I think it is useful to explore the analogies of these notions at the different hierarchical levels and their subjective, or relative, nature. In a hierarchical view, notions such as *resistance, virulence and health* should be seen as *subjective categories in ecosystems* which take their meaning depending on which side the observer decides to line up. These three terms can be generalised to include notions such as survival, predation, pathogenicity, invasiveness, function, balance, equilibrium, resilience and different kinds of symbiosis and interactions (mutualisms, amensalisms, parasitisms, comensalisms, antagonisms and protagonisms) etc. Kratochwil (1999) makes such a generalisation when he points out that "among themselves, species create bi- and polysystems and thus form so-called

bicoenotic links. These interactions between the organisms induce the emergence of characteristics which may contribute to stabilising the system (quasi-stability in the species composition). Such interaction patterns can be divided into probioses (mutualism, symbiosis, commensalism) and antibiosis (predation, parasitism etc.)" (Kratochwil, 1999: 13).

Let us consider the paradigmatic case of human bacterial pathogens. The host system senses the presence of the bacterium and resists it through the immune response. The host cell defences become virulent to the incoming bacterium which deploys resistance to that response through its colonisation traits that allow it to avoid, circumvent or subvert the "virulence" of phagocytic cells. Now the resisting bacterium deploys its virulence factors to which the human host resists by using antibiotics becoming again virulent to the bacterium, that at this point may in turn develop resistance to this new type of human virulence (antibiotics). I am well aware of the specific meaning of the word antibiotic and of the need to agree on that definition for practical reasons. However I will consider Kratochwil's enlarged notion in order to trace some analogies at the different hierarchical levels. The notion of antibiotic implies something that acts against life, something that kills. It is also agreed that it has "natural origin" i.e.: it is biosynthetic (a machine gun could also be considered an antibiotic but I will not go that far in my generalisation). The standard recognised antibiotics used in medicine are a sort of virulence factors, but they are also a resistance factor seen from the other side of the "fight". They can also be neither of these and simply be signals in a semiotic process. But taken in the most common meaning antibiotics are organisms' chemical weapons. In this sense all types of venoms and poisonous substances produced by organisms should enter into the antibiotic category. Where there is a venom there is also resistance, an antidote.

Our dualistic cultural tradition makes it difficult for us to abstract from the warfare vision of the "struggle for life" in order to see the - equally real - other side of the coin consisting of equilibrium, balance and mutualism. It has often been suggested that there has been a coevolutionary 'arms race' between, for example, plants and herbivores, as new chemicals are produced and subsequently overcome by insects (Gange and Bower, 1997: 116). It may be simplistic to picture the complexity of multitrophic dynamics exclusively with the warfare (arms race) metaphor since it is clear that not all, may be not even most, relations are antagonistic in Nature. Antagonism becomes a subjective category and what may seem a "pathologic" attack

at a certain level may turn out to be a healthy mechanism when seen in a larger gestalt. What is "resistance" for one individual or species may signify virulence for another and in turn, the resistance to virulence may be considered an emergent virulence. It may very well be that the escalation process of virulence being resisted and resistance being overcome is at the very base of an unhealthy system (the type of positive feedback that Bateson (1972) called "schismogenesis"). Moreover, virulence and pathogenicity are context dependent. A virulence factor and the pathogenic organism carrying it may not be such if not in a specific context. That context is a semiotic niche full of signs, some of which trigger virulence out of an otherwise "neutral" organism. For example, in order to invade host cells, salmonellae have to simultaneously sense proper levels of oxygen, pH, osmolarity, and an appropriate signal to the PhoP/Q regulon (most probably among other things). If even one of these conditions is unfavourable, the expression of the invasion genes is repressed and salmonellae do not invade the host (Falkow, 1997: 362). Each of these parameters becomes significant at a specific threshold value. In a different example, Alford and Richards' (1999) discuss the local causes for the global decline and losses of amphibian populations, which include ultraviolet radiation, predation, habitat modification, environmental acidity and toxicants, diseases, changes in climate or weather patterns, and interactions among theses factors. Many disease agents are present in healthy animals, and disease occurs when immune systems are compromised. They report that declines in the populations of *Bufo boreas* between 1974 and 1982 were associated with *Aeromonas hydrophila* infection, and it was suggested that environmental factor(s) (UV-B exposure, changes in pH, pesticides, pollutants etc.) cause sub-lethal stress in these populations, directly or indirectly suppressing their immune systems. Also a pathogenic fungus largely responsible for egg mortality in one population of *Bufo boreas* in Oregon may have been more virulent to embryos under environmental stress (Alford and Richards, 1999: 140).

We could say that there are no pathogens but pathogenic circumstances. In a sense it is the context that becomes pathogenic and at the same time it becomes ill. Since the context (the specific semiosphere) is constantly changing so is the semiotic niche of a particular system. Any assessment on the emergence of pathogenicity (or conversely the emergence of resistance) has to consider carefully the evolution of the context in relation to the semiotic niche of the potential pathogen, and in relation to its Umwelt. The material support for information (e.g. DNA, infomolecules,

semiochemicals, regulatory elements, etc.) has to be evaluated in the background in which it is inserted, be that genetic, metabolic or ecosystemic. The significance of non-trophic interactions in processes that lead to resistance, virulence and health, when seen as subjective categories in a wider gestalt of co-evolution and symbiosis, means that pathogenesis is basically a semiotic process. The wider gestalt may hint to the existence of a sort of “hierarchical health” in ecosystems.

Conclusions

What makes biotechnology different from natural biological production is the presence of cultural intentionality: the anthropic factor. Biological information is whatever makes sense, out of a difference, to the organism, whether at a physiological (endosemiotic) or at an ecological (exosemiotic) level. But information about biological information has to make sense to us.

The realisation of biology being a "science of sensing" in which being or not being makes a difference - a "being" that is susceptible of mimicry - supports without any doubts the claim that there is an ineluctable trend in biology that shifts the attention from information as a material agent of causality towards the world of signification. This could have profound pragmatic consequences in a time in which biotechnology is considered to be the industrial use of "biological information". A semiotic approach may turn out to be quite relevant when characterising the causal links that go from molecules to organisms, from labs to ecosystems.

Experiments that proof hypothesis are of course much easily carried out in laboratories and under controlled conditions. But as the importance of the "context" becomes explicit there is a stated need to go into more realistic field trials. So far the reductionist strategy being used to asses the causal links of a component in the larger system has been knocking out the component. This strategy, which is hard to act away from controlled conditions, is showing its limits as the contextual parameters and the complex "cross-talks" of components (or actors) create intricate webs with emergent properties whose changing qualities can hardly be assessed or measured by knocking (when possible) a single element.

Many biologists would agree that in the last decades there has been a big epistemological shift in biology. Whereas DNA was the dominant and central integrating element in the conceptual framework, today signal transduction is taking its place. In the mist of this complexity there is a very encouraging fact. A new phase of cooperation among empirical and theoretical scientists, from different disciplines and backgrounds, is beginning to emerge in order to face the borderless and complex nature of semiotic interactions in organisms and multitrophic systems. However it will be important for the modelling process to consider not only models related to the "arms race" metaphor, but to complement with models based on more systemic and

hierarchical properties such as health, resilience and ecosystem function, seen in the wider gestalt of co-evolution and symbiosis.

The important fact is that there is a new and exciting epistemological path opened in biology which is seriously considering the evolution of signals as one of the most important processes in living systems. These new efforts to tackle biological complexity will lead to mapping and monitoring systems based on the sign-networks operative at all hierarchical levels. These systems will have the opportunity to combine methods that range from biosensing techniques to methods for monitoring the traces and cues of indicator species in their specific contexts. The understanding of semiotic controls in the interactive behavior of cells, individuals, populations and species may prove useful or even necessary for the modelling process that in turn will lead to meaningful monitoring systems. This systems could then contribute to inform, and interact with, the policy-making processes for the regulation and management of health, biosafety, biodiversity conservation and sustainability.

We can of course aim to characterise and sequence exhaustively entire signalomes. For economical reasons we can also envision a strategy that relies on the identification and hierarchical organisation of crucial semiotic patterns which can then guide our quest for infrastructural details to fill in the relevant gaps in the sphere of the systems of interest. Conversely, what can guide our choices for the meaningful patterns at higher levels is our understanding about transduction of information across emergent levels and our knowledge about the rules of redundancy in the system, i.e.: coding instances.

We can aim to identify "overall structural motifs" of patterns and then concentrate on the local patterns that influence the metapatterns most critically. The contexts, the paths and the constraints are those of the whole developmental and evolutionary biosphere, a substantial global phenotype that serves as a substrate for the semiosphere, or conversely a semiosphere that serves as the "pattern which connects" the global phenotype, the sum of all living specimens in this precise moment.

I have proposed a framework based on triadic causality and on the interplay between digital and analogical codes in living systems which can be useful to organise hierarchically the suits of factors that determine or influence emergent properties in a given sign-network. I think this kind of mapping could be useful to (technologically) take better advantage of the intercommunication of sign systems at

and between hierarchical levels (therapeutically, for production and for ecological safety) and to integrate molecular, embryological, developmental, physiological and ecological approaches.

In order to correlate the emergent causality of information through out the hierarchical levels, a system of notation will be necessary to include a characterisation of codes and contexts at the different levels. This characterisations can not include the totality of the exhaustive mosaic of "all the actors" but will have to rely on the identification of "indicator patterns" that can guide the observer when characterising a semiotic network. A pattern, in fact, is definable as an aggregate of events or objects which will permit in such degree such guesses when the entire aggregate is not available for inspection (Bateson, 1972: 407). A system of notation based on the logic of digital-analogical-consensus can be useful to hierarchise the codes and the redundancy rules which are recognisable as patterns (i.e., which have statistical significance) within a finite aggregate of objects or events, so it becomes possible to delimit regions of the aggregate within which the observer can achieve better than random guessing by following a map of the formal relations within the system. Such a mapping of the distribution of patterns would be incomplete if we stay at a single level of the hierarchy.

As we have seen, the whole code of signal transduction is based on signs consisting in complex patterns of concentrations of different signal types and the subsequent modulations of concentrations in all the intermediary steps. Using Bateson's terminology, the transform of a difference (caused for example by the binding of a single signal-molecule) travelling in a circuit is an elementary idea. The concentration of transforms is a less elementary idea, and still less elementary is the difference created by cocktails of concentrations of transforms of diverse signals acting simultaneously. Complex aggregates of differences give place to emerging codes which acquire certain logical independence from the lower level codes from which they have emerged.

The emergence of these codes reflect the logic of digital-analogical consensus which provides great combinatorial possibilities for more complex logical products within the increasingly complex codes that are responsible for establishing higher order specificities and regulatory instances. The convergence of complex arrangements of digital-analogical consensus - the formation of complex "lock-and-key" mechanism - is what confers the possibility of categorial perception, which is

then what gives specificity to otherwise ubiquitous “universal signals”. If we are to understand the complexity of these codes we have to be able to identify the crucial digital-analogical-consensus instances by which complex signal configurations conform analogical signs that work in a network of triadic logic. In this way we can identify hierarchies of emergent patterns and search for the rules of specificity, cross-talk and categorial perception at the observed level, and at the same time we can maintain and eye on the causal links that point downwards, upwards and laterally in the multidimensionality of semiotic networks. Based on this hierarchy we could start to:

a) Define the nature and entity of different kinds of cross-talk at different hierarchical levels.

b) Define the nature and entity of different kinds of categorial perception at different hierarchical levels.

c) Establish criteria for delimiting the boundaries of overlapping systems.

d) Hierarchise the formation and organisation of patterns that are involved in coding.

e) Characterise and monitor the context at different levels - as the higher aggregate of cues, restraints and pathways which furnishes the key for local interpretations at lower levels.

My intention here was to suggest one possible key for characterising biological codes relying in the identification of some recurrent logical principles. I have proposed a minimal toolbox of concepts that I consider useful for this task. I have also used some examples from the best-characterised parts of the signalome to suggest how biosemiotics could make a contribution to the organisation and interpretation of the vast quantities of data being generated in this field. Once we do this, new interesting questions and alternatives hypothesis could emerge.

All codes by definition have logics. Unravelling a code means understanding a key for its logic. The code has its own logic, never truly accessible to us, but its regularities (which is a main characteristic of all codes) are “transduced” into equivalent regularities in our descriptive logic, our map.

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