Does "quorum sensing" imply a new type of biological information?

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Abstract. When dealing with biological communication and information, unifying concepts are necessary in order to couple the different "codes" that are being inductively "cracked" and defined at different emergent and "de-emergent" levels of the biological hierarchy. In this paper I compare the type of biological information implied by genetic information with that implied in the concept of "quorum sensing" (which refers to a prokaryotic cell-to-cell communication system) in order to explore if such integration is being achieved. I use the Lux operon paradigm and the *Vibrio fischeri – Euprymna scolopes* symbiotic partnership to exemplify the emergence of informational contexts along the biological hierarchy (from molecules to ecologies). I suggest that the biosemiotic epistemological framework can play an integrative role to overcome the limits of dyadic mechanistic descriptions when relating the different emergent levels. I also emphasise that the realisation of biology as being a "science of sensing" and the new importance that is being ascribed to the "context" in experimental biology corroborate past claims of biosemioticians about a shift from a focus on information (as a material agent of causality) towards a focus on the world of signification.

Introduction

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. One problem may be 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 have to interact with other emergent levels).

In a penetrating analysis by Sahotra Sarkar it was implied that after 50 years of debate on the "information" concept in molecular biology what in reality has survived is the stereochemical specificity suggested by Pauling and others at the end of the 1930s (though with many antecedents; Kay 2000: 43), according to which biological interactions are mediated by a precise "lock-and-key" mechanism between the shapes of the molecules (Sarkar 1996: 190).

But as mentioned above, problems arise with signal transduction networks and regulation, where we can see 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.

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 1999: 274), different types of "information" emerge 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. 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 (Hoffmeyer, pers. comm.).

As an example, I will consider the broad line of research that is currently being developed around the concept of "quorum sensing"

which refers to 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. The dynamics involved in the evolution of this phenomenon represents an intriguing instance 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. Insistence upon a reductionist explanation would require at least consideration of the code-dual nature of life (Hoffmeyer, Emmeche 1991). To overcome the ambiguous "spontaneous teleology"[1] so frequent in biology, a semiotically informed approach will be needed.

The *Vibrio fischeri* paradigm

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 in the surface of the ocean 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 which "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*.

This bacterium produces an enzyme called luciferase catalyzing a light producing reaction which makes the squid glow with an intensity and wavelenght reminiscent of moonlight (blue-green light, 495 nm). This renders the squid invisible to predators below by erasing the shadow that would normally be cast as the moon rays strike the squid from above — a sort of camouflage known as counterillumination. The mutualistic advantage is that by glowing, the squid escapes

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

getting eaten and in turn it provides food and shelter to the bacterial colony, which will be kept away from other competing bacteria (Ruby, Lee 1998; McFall-Ngai 1999; Visick, 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?

A small diffusible signal molecule produced by the individuals of the colony serves as the crucial element. The concentration of this molecule inside the bacteriae depends on population density and will eventually trigger a modulation of the phenotype (Swift *et al*. 1999: 291). This is what has been called "quorum sensing"[2]. 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 legitimise a given decision. Quorum sensing can be represented as a triadic sign process as shown in Fig. 1.

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.[3] Let's now take a quick overview of the molecular model for this process.

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[2] The term first appeared in a *Journal of Bacteriology* 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 standard in the scientific literature.

[3] As early as 1975, shortly before his death, biochemist and biophysicist Gordon M. Tomkins 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". He further argued for an apparent generality of such a code. (Tomkins 1975, Kilstrup 1998). In fact, quorum sensing seems to be just a specific case of Tomkins' metabolic code.

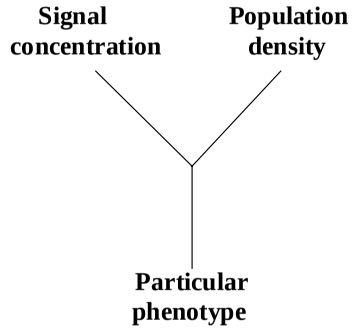

Figure 1. The quorum sensing sign triad. The concentration of a small diffusible signal molecule inside the bacteria reflects population density and may eventually trigger a modulation of the phenotype. Or, in other words, the concentration of the signal molecule acts as a sign in that it provokes the 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.

The Lux operon

In *V. fischeri*, the genes that encode the ingredients of luciferase and other substances necessary for the bioluminescence reaction are contained in the lux operon, consisting of (a) *luxA* and *luxB* which encode the alfa and beta subunits of the enzyme luciferase, (b) *luxC*, *luxD* and *luxE* which encode components of the fatty acid reductase complex, i.e. the enzyme which catalyzes the synthesis of the necessary aldehyde substrate for the luciferase, and (c) *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: *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 (in fact quorum sensing was 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 catalyzed by the direct "gene product".

(2) The signal-generator: an enzyme encoded in the *luxI* gene (and thus called LuxI protein) which in turn catalyzes the synthesis of the signal-molecule from different precursors that come from other biosynthetic 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 RNA transcript 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 transcription of the whole operon at a higher or more efficient rate (by enhancing the RNA polymerase binding).

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 a regulatory network is elucidated it is always discovered that there is further regulatory complexity. There is always integration of different regulatory mechanisms 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. In fact there is already mounting experimental evidence, for example in the production of many virulence factors by the bacterium *Pseudomonas aeruginosa*, for a "multilayered hierarchical quorum sensing cascade" (Latifi *et al*. 1996: 1144).

If we say that these signals are part of triadic sign-relations we can see here how the semiosphere unfolds itself in a myriad of interconnected signals (signs) and pathways of immense complexity (Hoffmeyer 1996). In the cellular processes with which we are concerned here, the cues involved in the regulation of the network are both endosemiotic and exosemiotic in nature.

Microbiologists learned to turn 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 lay 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). Needless to say the term swarming comes by analogy to the well known behaviour of bees. 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) produces a surfactant to condition the surface for better motility. This may very well involve a species that emits a signal that 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.

One may wonder how these signals could evade detection for so long. Researchers now admit that the exchange of external signalling molecules between single celled organisms was unexpected and that

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 neodarwinian 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* 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 al*. 1999: 306; Pesci, 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 communication devices (Cundliffe 2000: 410–413).

In a narrow "struggle for life" view, it may also be tempting to think of a sort 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 the latter's 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).

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., an assessment of the semiotic niche (Hoffmeyer 1996: 59).

Thus, 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 modes trigger the expression of a wide range of pathogenicity determinants in many organisms that infect plants and animals. 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. 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. In this strategy one might see 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).

AHLs are not the only signalling molecules for bacterial cell-cell communication. There are many other peptide pheromones and also other bacterial signal systems which cross-talk 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".

The emergence of semiotic networks

Once more we find ourselves surrounded by concepts that imply an unacknowledged semiotic understanding of nature. Regarding the processes described above, the literature is full of words like communication, sensing, code and language.

Strangely however, I have not found equally often the word "information", although it is implicit. Maybe the reason is that biological information is tacitly accepted to be exclusively genetic information, i.e. specification of amino acid sequence. But in this new context, what is it that one can communicate? what is it that a code hides? what can be conveyed through language? and what can be sensed from the environment?

One exception in the quorum sensing literature that tries somehow to define "information" is the paper by Kleerebezem *et al*. (1997) that concentrates on quorum sensing in Gram-positive bacteria, in which the phenomena presents some differences relative to its equivalent in Gram-negative bacteria. In Gram-positive bacteria the "mechanism" is more similar to the more common two-component signal-transduction

systems being routinely characterised in molecular biology. In this system the signal molecule does not bind the regulator directly in order to change its conformation so it can activate DNA transcription. Rather, in this case the signal molecule (a secreted peptide pheromone) "is recognized by the input domain of a typical sensor component of a two-component signal transduction system. Such two-component regulatory systems, consisting of a sensor and response-regulator protein which use *phosphorylation as a means to transfer information*, form a major mechanism of signal transduction in bacteria and play a key role in many of the changes in cellular physiology that result from changes in the environment" (Kleerebezem *et al.* 1997: 896, my italics, L. B.).

How does this type of information relate to other types of biological information like for instance the "genetic information" implied in the Lux operon, or the information that allows a predator to swallow a squid (the shadow), or that which allows the squid to avoid the former (counterillumination)?

Biology, lacking a unified paradigm to deal with all these communication codes, languages and sensing, and being so committed to physical reductionism, can hardly come up with a coherent picture of all these semiotic processes across the different emergent levels of organisation. The result is that as the details of the dyadic "mechanisms" of the myriad signal-molecule cocktails that constantly and dynamically poke into, or bind to, receptors are increasingly described and dissected, it becomes extremely complicated to explain the emergence of novel semiotic contexts by the addition of such mechanisms.

In 1962 the Austrian-American biochemist Erwin Chargaff noticed that although biological information might explain the highly specific relations between nucleic acid and protein, scepticism remained as to whether it would 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 not justification in claiming that 'DNA is the repository of biological information'" (Sarkar 1999: 199). Perhaps his intuition anticipated the kinds of problems such a limited concept of biological information would impose upon a science that could not refrain itself from talking about communication and sensing in virtually all of its subdisciplines and in all the hierarchical levels

under its lens. 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 causal relations, the emergence process is mediated by triadic causality (in this sense, should emergence and semiosis be considered the same thing in living systems? see Emmeche 2000).

As shown by Sarkar (1999), the genetic code cannot in itself account for the dynamics of gene expression, control and regulation. In this context, "information" simply means the specification of the amino acid sequence of the protein and the physics of the folding of a protein is also supposed to be taken care of by the amino acid sequence, i.e., folding is believed to be implicitly determined by the sequence (although recent findings seem to conflict with this universal hypothesis, see Eder, Fersht 1995).

In his notion of "information as specificity" Crick (1958) distinguishes only two types of specificity: (1) the specificity of each DNA sequence for its complementary strand, as modulated through base-pairing, and (2) 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. However, from this last specificity emerges a new one: the gene-enzyme specificity. Once we have proteins, new instances of "lock and key" mechanisms emerge: enzyme-substrate, antibody-antigen, signal molecule-receptor, activation complex-DNA, and so on. And the simultaneous and complex "activation" of an indeterminate number of these "lock and key" mechanisms determine the emergence of new informational-semiotic contexts and new and more complex "lock and key" mechanisms and specificities like for example host-symbiont and organism-niche.[4] 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,

[4] This relates 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".

one can not exclude the existence also of some kind of downward causation (Campbell 1974).

To visualise this process let us go back to the 3 main molecular actors in the quorum sensing system of *V. fischeri*. 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).

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).

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 length 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 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% 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 (see Fig. 1). 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 emerging "state" constitutes a difference that can be sensed by some system with interpretative capacity.

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 encode for 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 multi-dimensional wave rather than the linear digital impulses of wired telecommunication. In a reductive perspective, this could be viewed as the emergence of new analogical signs (properties, contexts, pieces of information) by the aggregation of digital symbolic signs. By the same token, the analogical mode (the bulk of information) influences the existence of digital information in a sort of downward causation. Also, such analogical compound effect may constitute a "quasi-digital" piece of information to a higher level of aggregation ("to be or not to be"). Just as in human language larger narratives represent a kind of

analogical information that emerges from the underlying digital code (written 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 onto-genetic historical continuum that oscillates within the boundaries of the code-dual nature of organisms and ecosystems (Hoffmeyer, Emmeche 1991).

Let's 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 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, 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, McFall-Ngai 2000: 1779f). 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, Lee 1998: 807). A few hours after the squid is hatched, symbiotic colonisation rapidly begins. After contact, both organisms induce each other into a series of morphological and developmental changes which result in the enhancement of the association (Visick, 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, 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, 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, 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, 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 non-luminescent strains have been found! (Visick, 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, McFall-Ngai 2000: 1783). The light sensing capability of the squid points also to other directions in the semiotic network. With the first day light 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 day-light.

Final remarks

The intention of this paper was to point out that 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 and evolutionary perspective which may make better sense when seen within a triadic logic (Salthe 1993). Evolution of light production cannot easily be accounted for by the working of the Lux operon and its evolution through a neodarwinian mechanism. When seen as the aggregation and emergence of new specificities that constitute new semiotic networks, the coevolutive 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, 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 to specifically 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 fishes). 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 after organisms possessed photoreceptors, given the fact that in a neodarwinian context there would be no selective advantage to producing light if nothing was able to detect it. So the evolution of the lux operon quorum-sensing semiotic network does not involve only cell-to-cell communication, or the evolution of the squid's own photoreceptor to control its light organ, but of course it involves also the predator whose photoreceptor do not perceive the "difference" because of the camouflage.

This brings me back to the question of my title: does quorum sensing imply a new kind of biological information? Maybe not. Biochemical specificities, whether nucleotide to nucleotide, triplet-amino acid or response regulator-signal molecule, when seen in a hierarchical and emergent triadic perspective are just differences that make a difference to a system with interpretative capacity. 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 ecosystems, from labs to the environment.

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Представляет ли “чувство сообщества” собой новый тип биологической информации?

При описании биологической коммуникации и информации необходимы унифицирующие понятия, чтоб соотносить разные “коды”, которые индуктивно “открывают” и определяют на разных уровнях биологической иерархии. В статье сравнивается биологическая информация генетического типа с информацией типа “чувство сообщества” (указывающее на прокариотную межклеточную коммуникацию). Использование в качестве примера парадигмы Lux-operon и симбиотической системы *Vibrio fischeri – Euprymna scolopes* позволяет описывать появление информационных контекстов на уровнях биологической иерархии (от молекул до экологии). Утверждается, что эпистемологическая сетка биосемиотики может обладать интегрирующей силой, способной преодолеть границы диадного механического описания при сопоставлении разных уровней организации. Подчеркивается, что реализация биологии в качестве “науки ощущений” и учитывание важности “контекста” в экспериментальной биологии подтверждают утверждения биосемиотиков о переключении внимания с информации на мир означивания.

Kas ‘kvoorumitaju’ kujutab endast uut tüüpi bioloogilist informatsiooni?

Bioloogilise kommunikatsiooni ja informatsiooni käsitlemiseks on vajalikud ühendavad mõisted, et seostada erinevaid “koode”, mida induktiivselt “avatakse” ja määratletakse bioloogilise hierarhia erinevail tasandeil. Artiklis võrreldakse geneetilise info tüüpi bioloogilist informatsiooni ‘kvooriumitaju’ (mis viitab prokarüootsele rakkudevahelisele kommunikatsioonile) tüüpi informatsiooniga. Kasutades näitena Lux-operoni mudelit ja *Vibrio fischeri – Euprymna scolopes* sümbiootilist süsteemi, kirjeldatakse informatsiooniliste kontekstide ilmumist läbi bioloogilise hierarhia tasandite (molekulidest ökoloogiani). Mööndakse, et biosemiootika epistemoloogiline raamistik võib omada integratiivset rolli ületamaks diaadilise mehhanistliku kirjelduse piirid erinevate organisatsioonitasemete seostamisel. Rõhutatakse, et bioloogia realiseerimisel “tajumisteadusena” ja “konteksti” tähtsuse arvestamine eksperimentaalses bioloogias kinnitavad biosemiootikute varasemaid väiteid tähelepanu nihkumisest informatsioonilt tähendusmaailmale.