"Earth and Other Planets In View Of Biogenesis". Arnold Hanslmeier, Stephen Kempe and Joseph Seckbach (eds.). Cellular Origin and Life in Extreme Habitats and Astrobiology, Springer, Dordrecht, The Netherlands. Submitted by invitation.

CAN THE EVOLUTION OF MULTICELLULARITY BE ANTICIPATED IN THE EXPLORATION OF THE SOLAR SYSTEM?

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Summary. The problem of the origin of metazoa is becoming more urgent in the context of astrobiology. By now it is clear that clues to the understanding of this crucial transition in the evolution of life can arise in a fourth pathway besides the three possibilities in the quest for simplicity outlined by Bonner in his classical book. In other words, solar system exploration seems to be one way in the long-term to elucidate the simplicity of evolutionary development. We place these ideas in the context of different inheritance systems, namely the genotypic and phenotypic replicators with limited or unlimited heredity, and ask which of these can support multicellular development, and to which degree of complexity. However, the quest for evidence on the evolution of biotas from planets around other stars does not seem to be feasible with present technology with direct visualization of living organisms on exoplanets. But this may be attempted on the Galilean moons of Jupiter where there is a possibility of detecting reliable biomarkers in the next decade with the Europa Jupiter System Mission, in view of recent progress by landing micropenetrators on planetary, or satellite surfaces. Mars is a second possibility in the inner Solar System, in spite of the multiple difficulties faced by the fleet of past, present and future missions. We discuss a series of preliminary ideas for elucidating the origin of metazoan analogues with available instrumentation in potential payloads of feasible space missions to the Galilean moons.

1. Introduction.

Understanding the evolution of development in multicellular organisms is one of the most challenging problems in biology, along with the still-to-be understood origin of life on Earth (and in the universe), the central core of the modern science of astrobiology. One giant step in this direction has been taken by John Tyler Bonner (Bonner, 2001). He focused on a significant transition in the evolution of the Earth biota (Maynard Smith and Szathmáry, 2001, Ch. 12), when development was not complicated by the billion years that followed the late Proterozoic, at a time when the Earth had witnessed over two billion years of microorganism evolution. Some progress is possible with the

development of a model for the origin of multicellular organisms based on the idea that it had a selective advantage to be multicellular, as in poor environments microorganisms could use each other as nourishment to survive (Kerszberg and Wolpert, 1998).

The problem of the origin of metazoans is pivotal for astrobiological research. Besides the three possibilities in the quest for simplicity outlined by Bonner (cf., Section 2), in the long-term the exploration of the Solar System seems to be another way to elucidate the key factors of evolutionary development. This may be attempted on the Galilean moons of Jupiter (cf., Sections 3 and 4), or on Mars in spite of the difficulties faced by the fleet of past, present and future missions.

During the past decade, in the wake of the Mars Allan-Hills meteorite controversy (McKay et al, 1996), the discovery of extra-solar planets (Perryman, 2000; Santos, 2008), evidence of life in extreme environments (Pikuta et al, 2007; Morozkina et al., 2010) and complex organic molecules in outer space (Kwok, 2009), as well as new research on the origins of life (Chyba and Hand, 2005; Schuster, 2010), provided the scientific foundation for studying the origin and distribution of life in the universe. By 1998 a detailed astrobiology roadmap was in place to answer three questions:

(i) How does life begin and evolve?
(ii) Does life exist elsewhere in the universe?
(ii) What is the future of life on Earth and beyond?

NASA founded an Astrobiology Institute, and funded academic and research institutions around the USA to undertake research to answer these questions (Dick and Strick, 2004).

In principle, it would be desirable to obtain preserved samples from putative biospheres, in a returning probe to Earth, in order to perform exhaustive analyses in search for biomarkers, most desirably putative forms of life showing high complexity. From the engineering point of view there are many bioethical issues involved. Furthermore, the quest of information on the evolution of biotas from planets around other stars seem to be not feasible with present technology, particularly in two areas of research that employ direct, but remote observation:

• Direct visualization of living organisms (Schneider et al., 2010): the shape of an organism 10 meters in length and width, a spatial resolution of 1 meter would be required. Even on the putative closest exoplanet, Alpha Centauri, the required baseline B would be at 600 nm B = 600,000 km (almost the Sun's radius).

In reflected light, the required collecting area to obtain 1 photon per year in reflected light is equivalent to a single aperture of B = 100 km. In addition, if this organism were moving with a speed of 1 cm/s, it would have to be detected in less than 1000 s. All these numbers are unrealistic, unless laser-trapped mirrors, as proposed by Labeyrie et al. (2005), are realized. But as pointed out by Schneider and collaborators, in their present conception, laser-trapped mirrors are fragile in the solar wind.

• SETI: the existence of so many exoplanets discovered up to the present (Friedlund et al., 2010) increases our expectations to find intelligent life forms evolving elsewhere in the universe. In spite of uncertainty over the origin of life on Earth, a landmark paper in 1959 spurred on the search for intelligence elsewhere. Its authors, physicists Giuseppe Cocconi and Philip Morrison, argued that artificial radio signals at a 'magic frequency' would be evidence of intelligent life. In the early 1980s NASA began building a program to detect such signals.

This ultimately produced a customized 'multi-channel spectrum analyzer', which

became active in 1992 (Morrison et al., 1977). However, congressional politics wound up any SETI observations under the auspices of the US government only a year after they began. The remnants of the NASA SETI program were transferred to the non-profit SETI Institute in California. With private funding, the SETI Institute has continued to search with the multi-channel spectrum analyzer and also secured funding for the Allen Telescope Array – an array of some 350 radio dishes that are each six meters in diameter, which will operate as a dedicated SETI facility 24 hours a day. Smaller SETI endeavours are carried out around the world by a variety of institutions. So although no longer government-funded, the search for cognitive exobiology remains in place.

These examples, as well as further astrobiological research, rest under the implicit but simplifying assumption that exogenous life can achieve enough complexity to be detected. Altogether, this favours *in situ* analyses to be performed by probes in different places in the solar system, where conditions seem to be within the range to support life, at least the kind that is analogous to how we know it on Earth.

Even the simplest known unicellular organisms on Earth are capable of homeostatic adaptation, and are genetically and metabolically complex. The capabilities for homeostasis are, in a sense, mandatory evolutionary precursors for the ability for differentiation. Even in unicellular organisms, homeostasis is a precursor for the further increase in complexity, involving dramatic changes in the signalling, cell cycle regulation, etc. that probably allowed the emergence of multicellular lineages (Hoenisberg et al., 2008; Sebé-Pedrós et al., 2010). In this chapter we have a two-fold aim. First we want to recount and evaluate how "universal" are the conditions for the emergence of multicellularity. Arguably, multicellularity can be thought to be a prior step in the evolution of traits with high complexity, such as intelligence and language. From this remark it does not follow that we can expect the latter to be an inevitable consequence of the former. We know that the selective pressures leading to human intelligence and language capabilities must be quite specific, and that the physiological possibilities of organisms are not enough to promote them (Maynard-Smith and Szathmáry, 1997, Ch. 17; Fitch, 2010). Yet, multicellularity can be regarded as a prior step to achieve increased degrees of morphological complexity, range of sizes, and novel traits, that allow using empty niches. After all, it was not until nearly after 80% of the history of life on Earth when differentiated multicellularity appeared, and which resulted on the early Ediacarian fauna and the great Cambrian explosion (Glaessner, 1983; Cloud, 1986). The latter, ultimately led to traits unimaginable for an ecosystem composed of unicellular (or simple multicellular) organisms. We are aware that multicellularity might not be the only route towards complexity, but we consider that it is among the simplest strategies to achieve it.

A question that we would like to answer is, given the easiness with which multicellularity developed on Earth (about 25 times to the best of our knowledge; Buss, 1987; Bonner, 2000), can we expect that, if life in an unicellular form exists elsewhere in the Solar System, multicellular forms will also be present? We will address this question in terms of the possible hereditary mechanisms and constrains that we could expect in any replicating entity composed by material building blocks (as opposed to information based). This would support the large body of astrobiological research that works under the "complex life assumption", and which covers not only the theoretical background, but also the practical aspects. That is, the search for biosignals in the solar system.

This will in turn lead us to our next goal: can we devise a technique to identify and characterize simple multicellular organisms in the next generation of space missions, aimed specifically for the search of life in Mars and in the Jovian moons? We will try to base our proposals making the fewest biological assumptions, in order to avoid the inherent bias to search for life as we know it, and with the use of currently existing technology: spectroscopy, microscopy, and cytometry.

2. How can one simplify the mechanism of development?

2.1 BASIC PRINCIPLES THAT UNDERLIE DEVELOPMENT

One might argue about the details of development that those details *are* development, but we will take a different approach. It seems that there are some basic principles upon which all the details may rest. The present chapter focuses on how to find those principles and what they might be. We consider three ways along which we could pursue this quest for simplicity:

- The straightforward, descriptive biological approach where the mechanisms are at least superficially exposed.
- The beginnings of multicellular development yield another clue. One might assume that at first only the minimum steps necessary were present. What happens inside a cell is incredibly complex despite its small size, but with the evolutionary origin of multicellularity some minimal signals between cells must have taken place, giving rise to the origin of multicellular development. Those extracellular beginnings were simple and were only subsequently followed by an increase in complexity.
- Mathematical modelling. One can ask what is the simplest way to achieve some developmental change in form. This is an approach to which we shall return periodically to see how it fits in with our more central message of what might have been the nature of the first cell signalling when multicellular development arose in early evolution.

These are not the only ways of achieving simplicity, for there are others as well. To give a well-known example, the foundations of molecular genetics were achieved by Max Delbruck and Salvador Luria using viruses (bacteriophages), and by the very simplicity of these naked, parasitic genes (and their rapid rate of reproduction) it was possible to gain an extraordinarily deep understanding into the fundamental nature of the effects of mutations. The use of viruses to solve important biological problems remains important today, as shown recently by Burch and Chao (1999), who have elucidated some basic questions of population genetics, again by taking advantage of the molecular simplicity of viruses.

2.2 COMPROMISING THE DEVELOPMENTAL STAGES: THE UBIQUITY OF TRADE-OFFS IN THE TRANSITION TO MULTICELLULARITY

Among the major evolutionary transitions, multicellularity (and perhaps sexual recombination) enjoys the privilege to have evolved many independent times, at different taxa, and in different ways. This is a gift that allows us to understand the causes and benefits of this trait in some detail. The main steps in the evolution of

multicellularity are succinctly summarized (Bonner, 2001; Grosberg and Strathmann, 2007): what we have, then, is the evolutionary origin of multicellular development. First, there was a selection for an increase in size by becoming multicellular, and once achieved, there was a selection for a better integration, a better coordination of the adhering cells to compete effectively for energy and for a way to reproduce successfully. Then, with each successive step of size increase, propelled always by the fact that the uppermost size niche is never filled, there has been a further selection pressure for integration and coordination, often by new and innovative devices to accommodate the newly created larger organism.

In dissecting the evolutionary stages of multicellularity, we must discriminate between *colonies of cells*: the individual cells might be related or not, but the colony as a whole works for a common good. The *undifferentiated multicellulars* are composed by cells that are all related with each other. These essentially reproduce clonally (at least a few times) and remain together after the division. In the *differentiated multicellular* organisms, the cells can adopt distinct specialized function, which usually affects their morphology, patterns of gene expression, etc. while still being composed by related cells developing from a single mother cell according to a specific developmental plan. Colonial forms are found in archaeans, bacteriae, and eukaryotes; while true multicellular organisms primarily in eukaryotes. Differentiated multicellularity is the one that involves many complex traits involving signalling and cell cycle regulation, and which has allowed for highly complex organisms to develop.

The precise mechanisms that trigger and propel the passage to multicellular organization seem to lie on the trade-offs between the uses of resources or traits from which the cells can benefit (Michod, 2007). Ultimately, individual cells gain or loose reproductive success by allocating varying amounts of resources to distinct vital functions or traits. For example, in becoming colonial, the individual cells give up their free motility (dispersal), but gain survival by the advantages of size (Bonner, 2001), or by minimizing the interaction with non-cooperative individuals (Pfeiffer and Bonhoeffer, 2003). Also, cells might allocate their individual resources to either reproduction, or to a specialized a function (e.g. photosynthesizing), achieving in this way a differentiation between cell types (Michod et al, 2006). Even for simple organisms like cyanobacteria, the division of labour among cells is a stable solution for heterogeneous environments. However, each cell cannot individually perform the two ways of energetic harvesting: fermentation and photosynthesis (Pfeiffer, Schuster, and Bonhoeffer, 2001). In metazoans, a trade-off comes from the cytoskeleton, because cells employ its elements to either form the mitotic spindle and divide, or to assemble the cilia machinery and disperse (Buss, 1987; Solari, Kessler and Michod, 2006). From different points of view we confront the existence of trade-offs in the evolution of development. These trade-offs reflect the conflict that critically determine whether for a cell it results convenient to remain free, or to include itself as part of a multicellular individual. Ultimately, a more efficient signalling system reflects the evolutionary maturity where conflicts are avoided and development proceeds efficiently (Hoenigsberg, Tijaro and Sanabria, 2008).

These examples illustrate that the existence of trade-offs between functions or traits are imposed as constraint to what individual cells can achieve. A mixed strategy, to which these individual cells can opt, is their aggregation, and the subsequent division of labour. In this way, multicellularity confers the benefits of the different individual types

or advantages at once, plus the conveniences of the emergent aggregated multicellular individual.

Besides the existence of a trade-off, a second but equally critical factor is that of genetic conflict. Within a colony, a mutant may appear that confers such individual a clonal advantage over the other cells. This mutant will first parasite the colony, and eventually disrupts the multicellular development. Thus mechanisms have to evolve in order to minimize the risk of appearance of these genotypes. As suggested above, cooperative cells might have already solved this problem (Pfeiffer and Bonhoeffer, 2003), for example by some kind of cellular kin recognition, so when in passing to the multicellular stage, genetic conflict is a minimal disadvantage. Besides, because in multicellular organisms the cells are genetically related, genetic conflict is also kept to a minimum. But mechanisms to punish cheaters that only take advantage of the colony might be required, including cell death (Kerszberg and Wolpert, 1998).

Arguably, trade-offs can be considered a universal feature that is expected in virtually any type of reproducing entity evolving under ecological constraints. However, in the astrobiological context, assumptions on hereditary mechanisms are harder to argue. For instance, whether genetic conflicts can arise, depends on such mechanisms. Still, in our hereditary system, genetic conflict is more an antagonistic factor, a problem to solve, than a requirement for the emergence of multicellulars. We will retake this part of the discussion in section 2.3.

2.2 HANDICAP OF BIOLOGISTS IN RELATION TO DETECTION OF MULTICELLULARITY

We have been arguing that the potential discovery of exogenous life, e.g. in Europa or Mars is of immediate urgency. Under the assumption that life exists in any of these places, two possible scenarios unfold: the exogenous life forms are either unrelated to life on earth, or they are related (e.g. by panspermia). In either case, the search for multicellularity in the solar system becomes relevant because it might tell us about the "universality" of the traits that earth's living creatures have developed.

We have no idea whether what we have learned about evolution is of universal validity. What has been called the *fundamental handicap of biologists*: "our knowledge in biology is restricted to only one example" (Shklovskii and Sagan 1966, Ch. 14), raises the question whether in attempting to do astrobiology, we are instead just extrapolating biology to exoplanets, whose normal conditions are instead extreme on Earth. This line of research on extremophiles can rationalise such an extrapolation. This can be a trap, since we have been tempted to think that because life is able to exist on such exceptional habitats, then it could emerge in those environments. As a matter of fact, for long we thought that the archaean extremophiles lied at the root of the tree of life, but recently this assumption has been challenged: these extremophiles may be instead subsequent adaptations of organisms that lived in moderate conditions (Gribaldo and Brochier-Armanet, 2006). This observation has not been fully taken into account in astrobiology.

In addressing the likelihood of the existence of exogenous multicellulars, are we confronted with this fundamental handicap? There is a very good body of mathematical modelling that has studied the origins and evolution of multicellularity. But how much of this can be extrapolated is evidently not clear. However, because many of the

mathematical and computational models overlook many biological details (from signalling to inheritance), it suggests that some of the arguments are to some extent robust.

Yet, in practical terms the search for complex forms of life is very much handicapped. Even in our own biota, it is easy to think of counterexamples to the simple stacked-cell archetype: (1) intertwined tissues (e.g. neural and gangliar), (2) coenocytic organisms; they have pseudo continuous tissues where a compartmentalized, yet continuous cytoplasm hosts multiple nuclei (e.g. the Bryopsidale algae, *Rhabdocalyptus*, the Glass sponge), (3) progressively contained cells, like Russian dolls, where one cell encloses other cells, which in turn enclose other cells, and so on (e.g. *Volvox*). We can imagine many alternative ways of complex multicellular-like organizations, but it would be most exciting to discover those arrangements that we did not conceive even theoretically, and for which detection is even harder to anticipate.

2.3 DARWINIAN AND LAMARKIAN EVOLUTION: THE ROLE OF HEREDITY FOR THE EMERGENCE OF MULTICELLULARITY

We suggested above that the transition to a multicellular stage, is accompanied by trade-offs, and that in such transition genetic conflict is a major problem to solve by the emerging species. We also advanced the hypothesis that genetic conflicts actually arise as a consequence of the limited and digital inheritance system that we know and have. (To be clear, our inheritance system, DNA, is practically unlimited due to the enormous amount of possible states. However, it is typically the case that because of the low mutation rates, there are simultaneously only a few numbers of mutants appearing in a population in a short interval of time, between the appearance of the first mutant and fixation of any of the genotypes. Thus the available states over which selection can act, are limited). Other types of inheritance (Szathmáry, 1999) might not incur into genetic conflicts, but may have other kind of hindrances of their own. However, under these distinct forms of inheritance, individual cells may find it detrimental or beneficial to become part of multicellular individuals. In any case, *some kind* inheritance must exist, and trade-offs are expected. How much can we anticipate with these two elements? We have to consider the possible kinds of replicators, which roughly, can be classified into four types. Replicators can have limited or unlimited heredity, and can be genotypic or phenotypic.

If inheritance is *unlimited* (continuous traits with affected by polygenes, approximate this situation) then there is a huge possibility to fine-tune the factors that stabilize multicellular aggregations. Specifically, signalling and homeostasis can be nurtured through selection to be sensitive to differential changes, rather than to a discrete presence/absence set of signals. In turn, this fine regulation may allow for refined mechanisms to avoid conflict (e.g. Michod, 2007). We will not deepen into this type of inheritance, since this class is realized by life as we know it on earth, and most research in multicellularity comprises this class (see Grosberg and Strathmann, 2007 for a review).

Limited inheritance, occurs when the number of states (e.g. mutants) is far much smaller than the number of individuals in the population. Only a few genes may allow enough degree of differentiation to support colonial aggregations. However, the

potential for the evolution of novel complex traits might be strongly impaired by the few numbers of possible genotypes. Although this might be a reason to fix increases in the size in the genome in exotic life forms, there might well be constraints on the number of their analogous genetic units. Nevertheless, there is no reason to discard mechanisms that avoid genetic conflict and thus fix a stable multicellular level. As an obvious analogy, consider relatedness among the cells in a multicellular organism. Development from of a single cell might enforce relatedness, and this is not debauched by the limitedness of the inheritance system. The advantages of a multicellular might still apply over those of the unicellular alternative. As mentioned above, some traits with strict Mendelian inheritance could mimic this type of inheritance, and no wonder that many organisms have remained in evolutionary stasis, perhaps stuck in a local fitness optimum without apparent modifications for hundreds of millions of years.

Both types of genetic inheritance can support evolutionarily stable multicellular states, as long as mechanisms to avoid selfishness can exist. However, if life elsewhere emerges as a *phenotypic* replicator, there is little chance that differentiated multicellularity can arise. In this inheritance system, any phenotypic change is passed to the next generation in a Lamarckian fashion. Thus a *phenetic conflict* – the analogous to genetic conflict – is entangled with differentiation. Hence a multicellular organization would be much a more challenging evolutionary transition, compared to that of the genetic inheritance. Nevertheless, this does not exclude the possibility of cooperation and of strict regulation (essentially intra-colonial selection) of the potential defectors.

Differentiated multicellularity seems unlikely, for two reasons. First, because any phenotypic difference is heritable, the distribution of cell types (and their replication rates) within the organisms would be driven by, or at least correlated with, the environmental fluctuations. At best, this would be a way of senescence, but any strict control of cell types and proportions seems far-fetched. This immediately limits the possible degree of complexity (amount of differentiation into tissues, organs, etc.) that can evolve. The second reason is related to the effects of canalization. In our genetic system, mutations and environmental factors are often “canalized”, meaning that their effect is buffered by other genetic modifiers and epigenetic factors, so that the phenotype shows minimal variation resulting from such mutations or environmental effects. This has played a critical role in the evolution of development. But by definition, canalization is absent in phenotypic replicators.

In particular, if the phenotypic replicators have unlimited heredity, we face the worst-case scenario. This is allusive to the blending inheritance idea, where selection quickly consumes all variability, and evolution halts. Similarly, differentiation would simply come to a standstill by an “optimal” cell type that would propagate in the whole organism, leading to an evolutionary dead end. A possible way out would be a threshold mechanism in the replication, where each phenotype has metabolic basin of attraction that buffer slight phenotypic deviations, but if a threshold is crossed, the new phenotype progresses. In this way simple versions of differentiation are possible, but without a major increase in complexity. A clear-cut example of this type of systems, is the lipid world’s replicating vesicles (Segre et al, 2001). This type of organisms (so far only a theoretical construction), consist of vesicles that have certain proportions of different lipids. These proportions convey information transduction and are heritable, at the same time. However, the number of possible states is limited, and the modification of the lipid proportions by environmental addition of certain chemicals is possible. Yet, these

external modifications are inherited, thus development is simply unstable. Although this model has been proposed as an alternative for life's origin for its hereditary properties and physical-chemical plausibility (Shenhav et al, 2003), it is clear that it cannot account for increased levels of complexity.

Summarizing, a colonial organization and undifferentiated multicellularity are valid structures that could emerge in any kind of hereditary system (with different degrees of difficulty, though), which could confer the individuals with some additional advantage by size, cooperation, etc. Differentiated multicellularity would require hereditary mechanisms of a very specific kind: digital information transduction across generations. This narrows our expectations for detecting complex life form. However, we ultimately rely on evidence to proceed in our intellectual quest, but with these educated guesses we can begin our journey towards the search for complexity.

3. Detection of biomarkers in Solar System exploration: Possible single-cell evolution

The considerable evidence for the presence of a liquid ocean over a silicate core makes Europa a candidate for the emergence of a second evolutionary pathway of autochthonous life. A fundamental subject in astrobiology is the origin of habitable ecosystems—a question in geochemistry—rather than the alternative search for the origin of life itself—a question in chemical evolution (Chela-Flores, 2010). Since certain bodies may share a similar geophysical past with the Earth, a question suggests itself: *Can available instrumentation be the 'pioneer' in the discovery of habitable ecosystems in geophysical environments similar to the early Earth, where oceans were in contact with a silicate core?*

Evidence already exists for a positive answer. Although inconclusive, Dalton et al. (2003) compared the spectra of Europan surface elements with experiments where extremophile microorganisms were exposed to conditions that recreated the moon's surface radiation, pressure and temperature. The spectra showed good agreement with each other for certain bandwidths. Although encouraging, this is inconclusive. Spectrograms of small Europan ice regolith samples might give a more certain conclusion. In any case, we propose an alternative, based on a central piece of this dilemma: the element sulphur (S).

A reliable window on the nature of the early terrestrial habitable ecosystems is the Pilbara Craton (Australia), a rich fossiliferous archive of the early steps of evolution, having preserved details of ancient hydrothermal vents. It contains a ~3.47 Ga barite deposit with microfossils of a complex set of sulphate-reducing bacteria (Shen and Buick, 2004). The large spread in the $\delta^{34}S$ values provides the earliest reliable biomarker from the early Earth. Europa may represent the only other case in the Solar System in which liquid water has been in contact with a silicate core over geologic time in perfect analogy with the early Earth (Bland *et al.,* 2009). It is therefore reasonable to make the following hypothesis:

On Europa the presence of hydrothermal activity at the interface of the silicate core and the ocean can provide a variety of chemicals playing a role in sustaining microbial life at the ocean floor.

This hypothesis is subject to a feasible experimental test: Europa's non-ice surficial

elements were found to be widespread, patchy and, most likely, endogenous. We argue that penetrators should be inserted in orbital probes in the future exploration of Jupiter's System (Gowen *et al*., 2009). There are alternative views on the effect of space weather on the radiation-induced S-cycles produced on the surficial molecules; but S is common to both interpretations (Carlson *et al*., 1999; McCord *et al*., 1999). Fortunately, just because of this presence of sulfur on the icy surface of Europa we are presented a unique opportunity to identify reliable biomarkers, since there are ways of identifying biomarkers by simply looking at the isotope anomalies when we suspect that microbes may have been present in the environment. The redistribution of the primordial isotopic mixtures can be followed up in terms of the appropriate parameter, namely the "delta" parameter (Chela-Flores, 2006):

$$\delta\,^{34}S = [(^{34}S/^{32}S)_{sa} / (^{34}S/^{32}S)_{st} - 1] \times 10^3 \; [^{0}/_{00}, \text{CDM}]$$

For simplicity this function will be referred to as the delta-34 parameter, or simply as the delta parameter. Its value is close to zero when the sample coincides with the corresponding value of the Canyon Diablo meteorite (CDM), a triolite (FeS) that was found in a crater north of Phoenix, Arizona. This parameter allows a comparison of a sample (sa) with the standard (st) CDM. The relevant terms are the dominant sulphur isotope (^{32}S) and the next in abundance (^{34}S). In fact, $(^{34}S/^{32}S)_{st}$ coincides with the average terrestrial fraction of the two most abundant isotopes of sulphur. We obtain positive values of the delta parameter when by comparison we have a larger quantity of the less abundant isotope ^{34}S. Nevertheless, the advantage of having defined such a parameter is that negative values will indicate an abundance of the most abundant isotope ^{32}S. Besides, we remark that in non-terrestrial Solar System materials (such as lunar dust, or meteorites), the values of the delta parameter are close to the CDM average.

This, in turn, signifies that biological processes will be more easily recognizable when sulfur, rather than the other biogenic elements (hydrogen, carbon or nitrogen) will be considered. There is an overwhelming amount of data supporting the view that metabolic pathways of sulfur bacteria have enzymes that preferentially select the isotope ^{32}S over ^{34}S. As pointed out above, this will be reflected in habitats that are depleted of ^{34}S. In other words, in lakes, seas or oceans, where the sulfur microbes are present, the value of the $\delta^{34}S$ parameter will have characteristic large negative values.

This suggests that focusing on sulfur might be more reliable means for estimating biological effects (if any) on Europa. In contrast, to the isotopes of hydrogen, carbon or nitrogen, sulfur shows fractionation with a relatively narrow distribution range in meteorites, as well as the Moon fines, breccias and fine-grained basalts retrieved by the Apollo missions. In the case of meteorites these values are about $2\,^{o}/_{oo}$ relative to the standard CDM average (Kaplan, 1975, Farquhar and Wing, 2005). The measurements of isotopic ratios of the biogenic elements were not considered during the Galileo Mission. Fortunately, they are in principle measurable in future missions to Europa. A remarkable and most useful phenomenon is that the largest known S-fractionations are due to microbial reduction, and not to thermochemical processes.

Besides, sulfate abiotic reductions are generally not as large as the biogenic ones

(Kiyosu and Krouse, 1990). From experience with a natural population, this type of biota is able to fractionate efficiently S-isotopes up to $\delta^{34}S$ of -70‰ (Wortmann *et al.*, 2001). Dissimilatory sulphate reducers are ubiquitous on Earth, producing the largest fractionations in the sulphur stable isotopes. These microbes are widely distributed in terrestrial anoxic environments. Consequently, they are the most evident candidates for the microorganisms populating a habitable Europan ecosystem. Microbial fractionation of stable S-isotopes argue in favour of penetrators for surveying the surface of not only Europa, but also Ganymede, where surficial sulphur has been detected (McCord *et al.*, 1997). The Europa-Jupiter System Mission (EJSM) intends to explore in the 2020s both of these satellites.

According to our hypothesis we predict that penetrators (supplied with mass spectrometry) should yield different results for fractionated sulphur. The icy patches on Europa should give substantial depletions of ^{34}S, while measurements on Ganymede should give significantly lower values for the depletion of ^{34}S. (As the largest of the Galilean satellites lacks an ocean-core interface, according to our hypothesis it would not be able to support life.) These diverging results—large minus $\delta^{34}S$ for Europa, and small minus $\delta^{34}S$ for Ganymede—would provide a clear test for the hypothesis that a habitable ecosystem has emerged on Europa. The test is within reach of available technology for planning the eventual penetrator payload.

4. Detection of biomarkers in Solar System exploration: multicellular evolution on the Europan surface and the Martian poles

4.1 INSTRUMENTATION SUGGESTED FOR A FUTURE SPACE MISSION

As discussed above, reduction states of sulphur in different forms may provide indirect, but reliable evidence of life. Yet, this is far from any organismic characterization. Perhaps a following step would be to try to determine the range of traits of the putative organisms.

The most reasonable alternative is direct imagining. We are expecting to deal mainly with micro and mesoscopic organisms, which require powerful optical or electron beam microscopy. Both technologies have already been implemented in the Phoenix Mars Lander, a mission that successfully reported the apparent presence of water including ice-rich regolith (in the soil) as well as snow (Smith et al, 2009; Whiteway et al, 2009), but unfortunately there was no organic matter on the landing site. Not only organic matter was not detected by the wet experiments, but also the micrographs showed no signs of organisms. Although the mission was not aimed at detecting bio-signatures, the power of the microscope was enough to detect even small bacteria of 0.1 μm. This lack of evidence does not rule out the presence of life on Mars. A more probable place to detect life would be in the polar caps, where the dune spots have diffusion patterns that suggest biotic dispersion (Ganti et al, 2003; Horvath et al, 2009), and which most abiotic models fail to explain (Kereszturi, et al, 2009).

Another phase, which is at the core of our interest in this chapter, is to characterize some organismic properties. With a rather simple technology it may be possible to have a significant insight about the complexities that may be abundant in exogenous life. The favourite targets at the moments are Mars (in the poles) and Europa. In both worlds, life

specimens might be trapped in the surface ice, which is in principle accessible to the penetrators. A piece of ice containing some organismic tissue can be minced in such a way that the biological sample, if multicellular, is disrupted into smaller constituents, tentatively cell analogues. It would be hard at this point to perform any biochemical or calorimetric analyses, particularly in the Jovian system.

Since we expect that the hypothetical organisms in Europa have metabolism based in sulphur, we require techniques that allow monitoring of distinct chemical states of this element. Although sulphur compounds are very elusive, particularly *in vivo*, spectroscopy in the X-ray wavelengths (near 2.47 eV) shows a characteristic peak (termed *K-edge*) that allows discerning among distinct radical types involving this element (Rompel et al., 1999). The advantage of spectroscopic observation of sulphur is that it is non-invasive and therefore may allow coupling with a flow cytometer.

Cytometry is a technique commonly used in diagnosis of diseases and experimental biology. This technique passes a suspension of cells through a narrow channel that is monitored by an optical device that characterizes every single particle. Conventionally, cytometers employ optical emissions at clearly distinct wavelengths –usually those resulting from labels from specific antibodies (e.g. green fluorescent protein)– to categorize, accurately count and even sort the cells, organelles, chromosomes, etc. according to several physical, chemical, physiological and molecular properties that can be targeted using antibodies. However, as an astrobiological technique, immunologically based labelling seems to us a weak option, owing to the fact that the antibodies aim for specific macromolecules. With Europa in mind, we argue that X-ray absorption spectroscopy can be developed as an alternative to immunological labelling, and to wavelengths which are less ambiguous than the infra-red and optical spectra (Dalton et al., 2003), basing the categorization of the putative cells on the spectrum of absorption near sulphur's K-edge.

4.2 DESCRIPTION OF THE IN SITU PROPOSED EXPERIMENT

An *in situ* experiment demands that the "preparation" of the samples is simple, quick, and energetically efficient. In order to create a suspension from an ice sample directly taken from the regolith, a simple procedure is needed. We assume that the ice contains frozen tissues, which can be mechanically disrupted. The most compelling way for automatization and payload requirements in astrobiological research are tissue pressing (Singh, 1998), and a "fine mincing" using the "Medimachine®" (Novelli et al, 1996). In the first, a sample is pressed through a very fine mesh that disaggregates the tissue (this might requier prior melting and filtering of the ice sample). The second is a small apparatus that scraps the sample into tiny particles on the order of microns, the typical size of a mammalian cell. The disaggregated tissues can then be suspended into a solvent (water, alcohol), and circulated through the flow cytometer for precise scrutinity.

Thus the instrumentation required for cell-analyses and characterizations comply with the engineering constraints. The mass spectrometer has been already miniaturized to weight a few grams. A flow cytometer can weight on the order of half a kilogram, and occupy less than 100 cm^3. The tools for cell disaggregation occupy and weigh only a fraction of that. With astrobiological objectives in mind, it would be a worthwhile challenge to reduce these dimensions even more.

5. Discussion and conclusions

We have discussed two main arguments in this chapter: firstly, the likelihood that multicellular life can (or has) emerge(d) elsewhere (with views on Europa and Mars). The complexity level that we could expect would be, to be on a safe margin, colonial and undifferentiated multicellularity. These can be supported by any inheritance system that is based on chemical metabolisms. At the same time, ecological constraint might ease their existence.

Secondly, we have discussed ways to detect and analyze such life forms. We have in mind instruments that could be contained in small penetrators. These instruments could provide us with significant data on the physical and chemical properties of the icy surfaces of Europa or Mars. We aim to optimize our insights in two ways. We go beyond the now canonical goal of detecting life. This is certainly desirable. Our knowledge would advance upon characterization of such life forms. The twofold gain comes first from identifying exogenous life, and second from providing information about the evolutionary steps of our own biota.

Life on Europa might be found in the cracks, or we may find biosignatures of it on the icy surface. In either case the penetrators are valid preliminary suggestions for adequate instrumentation. Direct observation of Europa's ocean, perhaps photographic, must await for the hydrobot/cryobot missions, but not in the foreseeable future. Yet, the tectonics of Europa (Greenberg, 2005) stir and mix the ice, which is to our favour, because it can make these samples accessible at some point in the surface from which, in principle, ice samples can be extracted.

Initially, the sulphur isotopes may provide a first evidence for life. But samples can be analyzed using a combination of microscopy, spectroscopy, tissue disruption and flow cytometry. All together, these techniques can provide us with an enormous amount of information about (a) the existence of multicellular organisms, (b) their degree of differentiation ("level of complexity") and (c) characteristic of individual cells. Even if we would not find multicellular organisms, the last point entirely justifies the use of the cytometer, since it would allow our first insight into the cell biology of exogenous unicellulars, or even of molecular protobiotic replicators of high complexity.

We might find after all that life elsewhere in the solar system, if present, is not entirely unrelated to ours. If life exists on Mars, it is most likely struggling for survival under conditions that are at best extreme to our standards. On the other hand, if life exists on Europa, it is most likely close to its "initial". We may consider that the Earth, by comparison, to be at a most favorable stage between these other two, enjoying its golden age of a rich and vast biodiversity. Still, panspermic seeding, even if at a very low frequency raises the possibility that life-to-be-discovered may not belong to independent trees of life. Instead, our first contact with exolife may uncover new branches of the same phylogenetic tree, where the exo-organisms may successfully exploit niches unimaginable for us.

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