

# Testing evolutionary convergence on Europa

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**Abstract:** A major objective in solar system exploration is the insertion of appropriate biology-oriented experiments in future missions. We discuss various reasons for suggesting that this type of research be considered a high priority for feasibility studies and, subsequently, for technological development of appropriate melters and submersibles. Based on numerous examples, we argue in favour of the assumption that Darwin's theory is valid for the evolution of life anywhere in the universe. We have suggested how to obtain preliminary insights into the question of the distribution of life in the universe. Universal evolution of intelligent behaviour is at the end of an evolutionary pathway, in which evolution of ion channels in the membrane of microorganisms occurs in its early stages. Further, we have argued that a preliminary test of this conjecture is feasible with experiments on the European surface or ocean, involving evolutionary biosignatures (ion channels). This aspect of the exploration for life in the solar system should be viewed as a complement to the astronomical approach for the search of evidence of the later stages of the evolutionary pathways towards intelligent behaviour.

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## Introduction

### *The universality of Darwinian evolution*

Although Darwin's theory of natural selection is recognised universally as the basis for discussion of any branch of biology, its relevance to what we now call *astrobiology* was only clearly pointed out during the commemoration of the centenary of Darwin's death (Dawkins 1983). The points made on that occasion are still relevant:

'[Darwinism] is probably the only theory that can adequately account for the phenomena that we associate with life'.

Dawkins' main concern, and ours in the present paper, is with the question of whether there are principles that are fundamental to all forms of life that may evolve in the universe. His main criticism, which we endorse, is that our writings have been rich in how extraterrestrial life might work, but poor in the discussion of how it might *evolve*.

Today, two decades later, when we are considering biology in general, and astrobiology in particular, we have the advantage that new and significant experiments are available; they give us some insight into the relative dominance of natural selection over historical contingency. We also have much clearer ideas regarding the distribution of the chemical components of life in interstellar space. Besides, there is growing evidence of the universality of the formation of solar systems, and in particular planetary hydrospheres and

atmospheres (Cosmovici *et al.* 2002): preliminary evidence suggests that there are water emission signals in planetary systems around Epsilon Eridani, Upsilon Andromedae and Lalande 21185.

### *What should we test during solar system exploration?*

Evolutionary responses to perturbations in a natural terrestrial environment can, in principle, lead to unpredictable evolutionary pathways. For instance, the large meteoritic impact in the Yucatan peninsula at the end of the Cretaceous perturbed the evolutionary pathway of large land animals. Notwithstanding the extinction of large predators (dinosaurs), it would have been possible to forecast, to a certain extent, an evolutionary response to such significant perturbation of the terrestrial environment. Indeed, independent evolutionary pathways triggered by perturbation to the environment may lead to features that tend to become similar even though they may be evolving in different lineages. This aspect of evolution can be illustrated with multiple examples, as we shall see below and later on in the Discussion section. We say that evolution in those cases is 'convergent'. A preliminary example is provided by the evolution of wings in birds and bats. For clarity, we should consider even at this stage a further example in detail: the New World cacti and the African spurge family are similar in appearance. These plants are succulent, spiny, water storing and adapted to desert conditions (Tudge 1991), yet they belong to separate

families. Their similar characteristics have evolved independently triggered by similar environmental challenges in completely different terrestrial habitats. This illustration is therefore considered a clear case of convergence, not a mere coincidence.

After such perturbations of the environment, the evolution of some features of the terrestrial biota will converge in other lines that have not been extinguished: large carnivorous predators, for instance, will inexorably arise. It should be underlined that at present astrobiology is in the process of identifying terrestrial-like environments that are significantly similar to those of the Earth (both in our solar system, either in planetary or in satellite environments, and also elsewhere in other solar systems). If we assume that evolutionary convergence takes place at the molecular, prebiotic and cellular stages in such environments, our working hypothesis, then the universality of Darwinian evolution in those new environments is the most outstanding question that we should endeavour to test as early as possible during the early stages of solar system exploration.

For these reasons astrobiology is truly beginning to force upon us the need to reconsider two fundamental questions:

- (A) How far can we attempt to forecast evolutionary responses to perturbations in a natural terrestrial environment? This question is analysed in the Discussion section.
- (B) How far can we attempt to forecast evolutionary responses to perturbations of environments that are analogous to those of the Earth? This question is one of the topics discussed in this paper.

We shall return to these questions in the remainder of this paper (with some further examples); they will be discussed more extensively elsewhere (Chela-Flores 2003). There is ample consensus that chemical precursors of terrestrial biomolecules may be, firstly, ubiquitous in the universe and, secondly, may also be precursors of life on planets (or satellites), whose biospheres are analogous to our own. During solar system exploration these considerations lead to the question of testing whether the evolution of life in the universe can be understood in terms of Darwin's seminal contribution, namely the evolution of life through natural selection.

## Testing convergent evolution in our solar system

### *How can we begin to test convergent evolution?*

In order to approach the empirical question of how far we can test the earliest stages of biological evolution in our own solar system, we should decide first whether we should expect any form of convergence in the exo-microorganisms that we might encounter, possibly close to the surface of icy worlds, such as Europa (cf. the section on 'Adequate responses to extant microorganisms on Europa' below and Greenberg *et al.* 2002).

In order to approach possible tests of the first stages of evolution, we may benefit from the results of the Galileo

Mission to the Jovian system: this mission has brought to our attention aspects of this Jovian satellite that are favourable to the origin of life. Europa rotates around Jupiter in an elliptical orbit, a motion that is constrained by the other three Galilean satellites (this is a classical result of celestial mechanics due to Laplace). As a result of this orbital resonance, the inner Galilean satellites have a significant tidal effect that heats and stresses their surfaces (an effect that is referred to as 'tidal heating').

Gravity measurements of the European moment of inertia from Galileo suggest that Europa has an inner core, a rocky mantle and a surface layer, mainly of liquid water. Impact craters also suggest that there is an ice-covered inner ocean, since they are shallower than would be expected on a solid (silicate) surface, such as that of the Moon. A robot especially built to penetrate ice overlying a mass of liquid water has been considered for some years – the so-called 'cryobot' (Horvath *et al.* 1997). Others, more recently, are approaching the question of melting probes with the advantage gained with previous experience with the development of a light-weight gas chromatograph-mass spectrometer developed for the Rosetta lander (Biele *et al.* 2002).

A somewhat more remote possibility is to build a corresponding submersible robot ('hydrobot') capable of bearing some experiments in its interior. With both of these robotic tools we would be in a position to make significant advances in planetary exploration: In the Europa ocean, or possibly on the iced surface itself, we are presented with the problem of deciding whether biology experiments should be planned in due course, and which tests should be taken to the stage of feasibility studies. One class of experiments shall be discussed in the last three subsections within the area of electrophysiology.

### *Prebiotic, chemical and biological experiments on Europa*

There are several classes of experiments to be discussed and clarifications to be considered in the future Europa campaign (Chyba & Phillips 2002).

- Firstly, at the prebiotic level, the content of the European ocean should be ascertained. A suite of measurements should be envisaged, in order to establish the subsurface context in which an autochthonous European ecosystem would presumably flourish. It would be advantageous to know the relative abundance of the main cations and anions present in the ocean, besides the volatiles such as oxygen, methane and carbon dioxide. (Certain knowledge of the ions present could be considered as a preparatory contribution for the electrophysiological test described in the last three subsections.)
- Secondly, the chemical evolution context should be probed. Indeed, searches for amino acids and nucleotides make sense, since such advanced stages of chemical evolution have been detected even in other Solar System bodies, such as meteorites.

However, it should be underlined that there are still gaps in our understanding of the likelihood of life emerging on Europa; for instance.

- The existence of sufficient free-energy sources is possibly the largest unknown factor (Chyba & Phillips 2002).
- If the ongoing debate on the origin of life deep underground (independent of sunlight) can be settled (Wächtershäuser 2000), then this discussion will have repercussions on whether Europa may have a resident autochthonous ecosystem.

#### *Adequate responses to extant microorganisms on Europa*

In spite of these uncertainties and the evident need for further theoretical and experimental work, we will inevitably have to consider simultaneously with the above-mentioned work how to respond adequately to the eventual encounter with extant or frozen extremophilic microorganisms. Such an event could indeed occur at an early stage of the European surface exploration.

From previous experience with European analogues on Earth (in the Dry Valley lakes of Antarctica), if a European biosphere has evolved in the ocean, we cannot exclude the fact that reprocessing of the ice-crust could bring microorganisms close to the surface from the body of water below (Parker *et al.* 1982). In addition, an independent argument supports the possibility that living organisms may survive in the crust, close to the surface (Greenberg *et al.* 2002): current geological appearance as well as tidally-driven tectonic theory argue in favour of recent resurfacing processes being capable of supporting a European biosphere. (The time scale for these events is established by the scarcity of craters on the icy surface.)

These two factors suggest, in turn, that biology-oriented experiments should be included at the earliest stage of exploration of Europa. Together with prebiotic and chemical evolution experiments, biological evolution experiments should also be included in the total payload available for the first landing missions.

In order to appreciate the significance of the class of experiments that will be discussed in the following section, we raise two related questions:

(1) *If convergent evolution has taken place elsewhere, have microorganisms gone beyond the stage of the simplest prokaryotes?*

One example of prokaryotic cells is cyanobacteria, such as *Oscillatoria*, which lack an internal nucleus. On Earth prokaryotic cells have evolved in what we may call a 'geologic instant'. It is sufficient to consider the Archean microfossils, which are older than two and a half billion years. Evidence for prokaryogenesis may even be one billion years older (Schopf 1993); but see the recent debate (Brasier *et al.* 2002) and also (Kerr 2002).

On these ancient rocks there are microfossils that have been interpreted as cyanobacteria. Therefore, even in the remote Archean eon, we already have unambiguous bio-signatures, which suggest the presence of simple prokaryotic life. Irrespective of the actual date of the Archean microfossils (2.5 to 3.5 Gyr BP), the thesis that prokaryogenesis occurred in a 'geologic instant' is persuasive. We should recall, firstly, that the Earth itself dates back to approximately 4.6 Gyr BP

Table 1. *Physiological responses in microorganisms and invertebrates (Shepherd 1988; Villegas et al. 2000; Chela-Flores 2001)*

Organism	Physiological responses
<i>Haloferax volcanii</i> (archaea)	Voltage-dependent ion channels and mechanosensitive ion
<i>Neurospora</i> (fungus)	Potassium channels have been characterized in the giant cells of this fungus; impulses last over one minute
<i>Paramecium</i> (protozoan)	Calcium channels involved in the protozoan movements; action potentials are typically of the order of 100 ms
<i>Rhabdocalyptus dawsoni</i> (sponge)	Ca- and Na-dependent channels
<i>Aglantha digitale</i> (jellyfish; cnidarian)	Action potentials have been characterized (nervous nets)
<i>Tunicata</i> (chordate)	Oocytes respond to an electric shock with a slow action potential

and, secondly, that our own star, the Sun, is expected to provide steady conditions for life for another 4 to 5 Gyr. Prokaryotes, therefore, seem to be a necessary consequence of planetary evolution of terrestrial-like environments (in view of the events that we have summarised above as a 'geologic instant').

(2) *Is it possible to recognize in a given prokaryote whether the first steps towards primitive systems for the conduction of ionic currents have already taken place?*

If simple responses to ionic environments are found to occur, for instance in microorganisms in the European ocean, then the first steps towards the generation of neurone-like cells would already have been taken. These evolutionary events are precursors of subsequent biological attributes of living organisms that have strong selective advantage, independent of any specific lineage, such as neurones, nervous systems, brains and eventually signs of intelligent behaviour (i.e. communication).

#### *A bridge between microbiology and astronomy*

The two related questions raised in the previous subsection would lead us to a bridge between microbiology and bio-astronomy. We can appreciate that in the simplest microorganisms, such as the archaean *Haloferax volcanii*, there is already a 'conduction system', namely a physiological response. We know that in this archaean there are calcium channels linked to movement (Besnard *et al.* 1997). In Table 1 we have gathered some physiological responses in microorganisms and invertebrates.

In the tree of life, already at the low evolutionary level of cnidarians, say in a medusa, or in a sea anemone, there are primitive nervous systems. These systems of neurones are referred as 'nervous nets' in the specialized literature in neurophysiology.

At low stages in the phylogenetic tree of life on Earth, we already find nervous systems made up of neurones in flatworms and nematodes, but it is remarkable that these

Table 2. *Some examples of cerebral ganglions in animals* (Sulston *et al.* 1983; Shepherd 1988; Villegas *et al.* 2000; Chela-Flores 2001)

Animal	Features of nervous systems and cerebral ganglions
<i>Notoplana acticola</i> (A marine species of flatworms; platyhelminths)	Receives inputs from sensory organs and delivers outputs to muscles, via nerve filaments
<i>Ascaris lumbricoides</i> (roundworm; nematode)	Receives signals from sensory organs and sends output signals to muscles; has peptidergic components
<i>Caenorhabditis elegans</i> (roundworm; nematode)	Small number of neurones (302); the entire nervous system has been studied by electron microscopy at every stage of development; the lineage of every neurone has been traced back to the zygote

organisms have also a primitive brain, more precisely a cerebral ganglion.

There are many examples of cerebral ganglions in animals. For instance, primitive nervous systems with developed peptidergic neurotransmitters and cerebral ganglions are known to have evolved in the roundworm (a nematode) *Ascaris lumbricoides* (Day & Maule 1999). Typically the ganglion receives inputs from sensory organs and delivers outputs to muscles, via nerve filaments. In Table 2 we have brought together examples of cerebral ganglions in animals.

From these examples we can infer that in multicellular organisms almost as soon as some coordinated electro-physiological responses are possible, they have been demonstrated to exist. (In addition to our examples we refer the reader to the detailed review of Hille 2001.)

We have assumed universal Darwinism (cf. the introduction). Consequently, these responses, which are observed at an early evolutionary stage in our phylogenetic tree, suggest the following (in principle testable) conjecture: nervous systems will also evolve at an early evolutionary stage elsewhere in the cosmos. If we find microorganisms in our solar system, we can begin to test this conjecture with the following question: *Have microorganisms elsewhere developed ionic channels or are the putative exo-microorganisms, at the level of mechanosensitive channels, responsible for simpler tasks, quite unrelated to nervous system activity, such as the control of osmotic pressure?*

All eukaryotes may have inherited potassium, calcium and later sodium channels (essential for the emergence of the first neurone) from an ancestral cation channel during the Archaeon, over 2400 million years ago (Shepherd 1988).

On the Earth biota these proteins are macromolecular pores in the cell membrane. In particular, in prokaryotes several channels have been identified by electrophysiology and genomic analysis, and their evolutionary links with eukaryotic channels have been discussed in detail (Derst & Karastchin 1998).

### Testing for differences in ionic channel responses

Ion channels of the cellular membrane represent a first step in the pathway toward the emergence of simple nerve nets and eventually nervous systems, which are highly dependent on these proteins. However, at early stages in cellular evolution we can already appreciate significant *differences in ionic channel responses*: in the giant cells of the fungus *Neurospora*, for instance, impulses are very slow – lasting over one minute. This is in sharp contrast with the more evolved responses of *Paramecium*, where calcium action potentials are involved in sensory responses and ciliary movement; these impulses, unlike the case of the fungus *Neurospora*, typically last for a period of the order of 100 ms (Shepherd 1988).

It would be a significant step forward in our understanding of life in the universe if, for instance, we were prepared to respond to a reprocessing of the European ice, which could be inducing microorganisms close to the surface, as already happens in the frozen surfaces of the Antarctic lakes (including Lake Vostok) or as is suggested by geophysical analysis of the Galileo images of the icy surface of Europa (Greenberg *et al.* 2002).

The physiological response (action potential) of the exo-microorganism could be compared with the extreme cases of action potentials typical of *Neurospora*, on longer time scales, or of *Paramecium*, on much shorter time scales (cf. the values of the action potentials in Table 1). The implementation of the electro-physiological aspects of this experiment, which would require only the assistance of remote control and the use of robotic arms, is a challenge that deserves attention during the planning of experiments (Chela-Flores 2002):

- (i) the introduction of a microorganism in a previously prepared solution of ions;
- (ii) the alteration of the concentration mechanically;
- (iii) inspection of the changes in possible cell polarization, in order to distinguish microorganisms from inorganic particles.

These three stages are based on standard electro-physiology. The implementation of such an experiment in a laboratory analogue of a melter (cryobot) seems to be feasible: Some filtering process for particles and microorganisms is required. Subsequently, standard voltage-clamp (VC) ion-channel analysis would follow. Since only simple electrical responses to the VC equipment is needed, remote control would not seem to be beyond technological feasibility, given the experience gathered with the previous missions to Mars and the planning of the Rosetta Mission for the landing on comets. The question of which ions to select has to be given some attention, but calcium, sodium and potassium ions are evident choices.

### What insights could we expect from these experiments?

We only need to recall, as we have done in the present paper (Table 1), that cells with different environmental requirements can generate different impulses.

The proposed *in situ* experiment can be carried out either in the ocean (inside a submersible) or even on the iced surface

(cf. the subsection ‘Adequate responses to extant microorganisms on Europa’).

New challenges arise from these unusual and novel settings.

- The question of miniaturization: this is a problem which does not seem to be beyond present day technology.
- In spite of the straightforwardness of the suggested test, difficulties typical of remote control have not been encountered previously in the area of electrophysiology. (We recall that signals would take about half an hour to reach the submersible in the European ocean.)

We should underline the relevance of testing the initial stages in the evolution of a neurone-like cell that has been presented in this section. For instance, a significant evolutionary stage would be exposed by the discovery of microorganisms with the capacity of propagating an electric impulse of short temporal duration (i.e. of the order of a millisecond).

The larger issue in astrobiology regarding the universal evolution of intelligent behaviour is clearly not restricted exclusively to the suggested search for ionic channels at the early stages of the evolution of nervous systems, which we have discussed in this section: The suggestion of searching for traces of extraterrestrial intelligence was made in the middle of the last century (the SETI project). Rephrasing the astronomer’s efforts, what is at stake is the search for evolution of intelligent behaviour.

### **Discussion: should we expect European biota to exhibit convergent evolution?**

The sharp dichotomy between chance (contingency) and necessity (natural selection as the main driving force in evolution) is relevant for the new science of astrobiology. Independent of historical contingency, natural selection is powerful enough for organisms living in similar environments to be shaped to similar ends.

The following examples suggest that, to a certain extent and in certain conditions, natural selection may be stronger than chance (further details are given in Chela-Flores 2003). These examples will suggest that the European search for life should focus on the early stages of the evolutionary pathway that would be analogous to our own.

Firstly, sticklebacks are small northern hemisphere fishes for which it has been demonstrated that natural selection has been the driving force in the evolution that has taken place in three Canadian lakes (Rundle *et al.* 2000). Secondly, Black European fruit flies that were transported to California offer a compelling case in favour of the key role played by natural selection in evolution (Huey *et al.* 2000). Finally, anole lizards from Caribbean islands offer some evidence for repeated evolution of similar groups of species, suggesting that adaptation is responsible for the predictable evolutionary responses of these lizards (Losos *et al.* 1998). We can speak in this case of evolutionary history repeating itself (Vogel 1998). In addition, further examples at the molecular level reinforce the hypothesis that evolutionary convergence is inexorably linked with evolution.

On the one hand, the northern sea cod is found on both sides of the North Atlantic. The distantly related order Perciformes contains the notothenioid fishes from the Antarctic. In spite of their distant relationship with cods, they have evolved the same type of antifreeze proteins, with repeats of the amino acids threonine, alanine and proline (Chen *et al.* 1997). The Arctic cod first appeared about 4 million years after the northern hemisphere sea cod. This episode in evolution represents a clear example of evolutionary convergence at the molecular level.

On the other hand, the mammalian lineage diverges from fishes about 400 Myr before the present. Nevertheless, in humans the long wavelength green and red visual pigments diverged about 30 Myr BP. However, a recent episode in evolution has granted the blind cave fish multiple wavelength-sensitive green and red pigments. Genetic analysis demonstrates that the red pigment in humans and fish evolved independently from the green pigment by a few identical amino acid substitutions (Yokoyama & Yokoyama 1990). Once again, this is another illustration of evolutionary convergence at the molecular level. Furthermore, evolutionary convergence of behaviour has been identified amongst animals with the largest brains in the oceans and on land, namely toothed whales (aquatic carnivores, for instance the sperm whale) and elephants (terrestrial herbivores). These animals, in spite of their radically different habitats, resemble each other more than they do other animals with which they share similar ancestries, diets or environments. One specific example of behavioural resemblance is social organization. Since these animals are not closely related through evolutionary history, their shared attributes constitute an example of *convergent behaviour*, not a coincidence (Weilgart *et al.* 1996).

Consequently, the numerous examples we have cited, which highlight the ubiquity of evolutionary convergence, strongly argue against biological diversity being unique to Earth and that within certain limits the outcome of evolutionary processes might be rather predictable (Conway Morris 1998, 2002). These Darwinian arguments provide a cornerstone to our thinking concerning the relevance of searching for evolutionary biosignatures from the early stages of the exploration of Europa, as we have maintained throughout this paper.

To sum up, we have suggested how to plan some experiments in order to test preliminary insights into the question of the distribution of life in the Universe. The main conjecture of this work is that universal evolution of intelligent behaviour is at the end of an evolutionary pathway, in which evolution of ion channels in the membrane of exo-microorganisms occurs in its early stages. Further, we have argued that a preliminary test of this conjecture is feasible with experiments on the European surface or ocean, involving evolutionary biosignatures (ion channels). This aspect of exploration for life in the solar system should be viewed as a complement to the astronomical approach for the search for evidence of the later stages of the evolutionary pathways towards intelligent behaviour.

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