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Lifeness signatures and the roots of the tree of life

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Abstract Do trees of life have roots? What do these roots look like? In this contribution, I argue that research on the origins of life might offer glimpses on the topology of these very roots. More specifically, I argue (1) that the roots of the tree of life go well below the level of the commonly mentioned 'ancestral organisms' down into the level of much simpler, minimally living entities that might be referred to as 'protoliving systems', and (2) that further below, a system of roots gradually dissolves into non-living matter along several functional dimensions. In between non-living and living matter, one finds physico-chemical systems that I propose to characterize by a 'lifeness signature'. In turn, this 'lifeness signature' might also account for a diverse range of biochemical entities that are found to be 'less-than-living' yet 'more-than-non-living'.

Keywords Tree of life · Origin of life · Definition of life · Protoliving systems · Protocells · LUCA · Lifeness signature

Introduction

At the very start of the nineteenth century, the French botanist Augustin Augier produced one of the earliest known published trees of life showing relationships of organisms (see Fig. 1, Augier 1801; see Stevens 1983). This tree-like diagram is replete with leaves and branches that represent members of the plant kingdom and their relationships with one another. Interestingly, this diagram also displays a system of roots that spread downward from the base of the trunk. Augier unfortunately did not give to these roots any role but graphical: they stand in the

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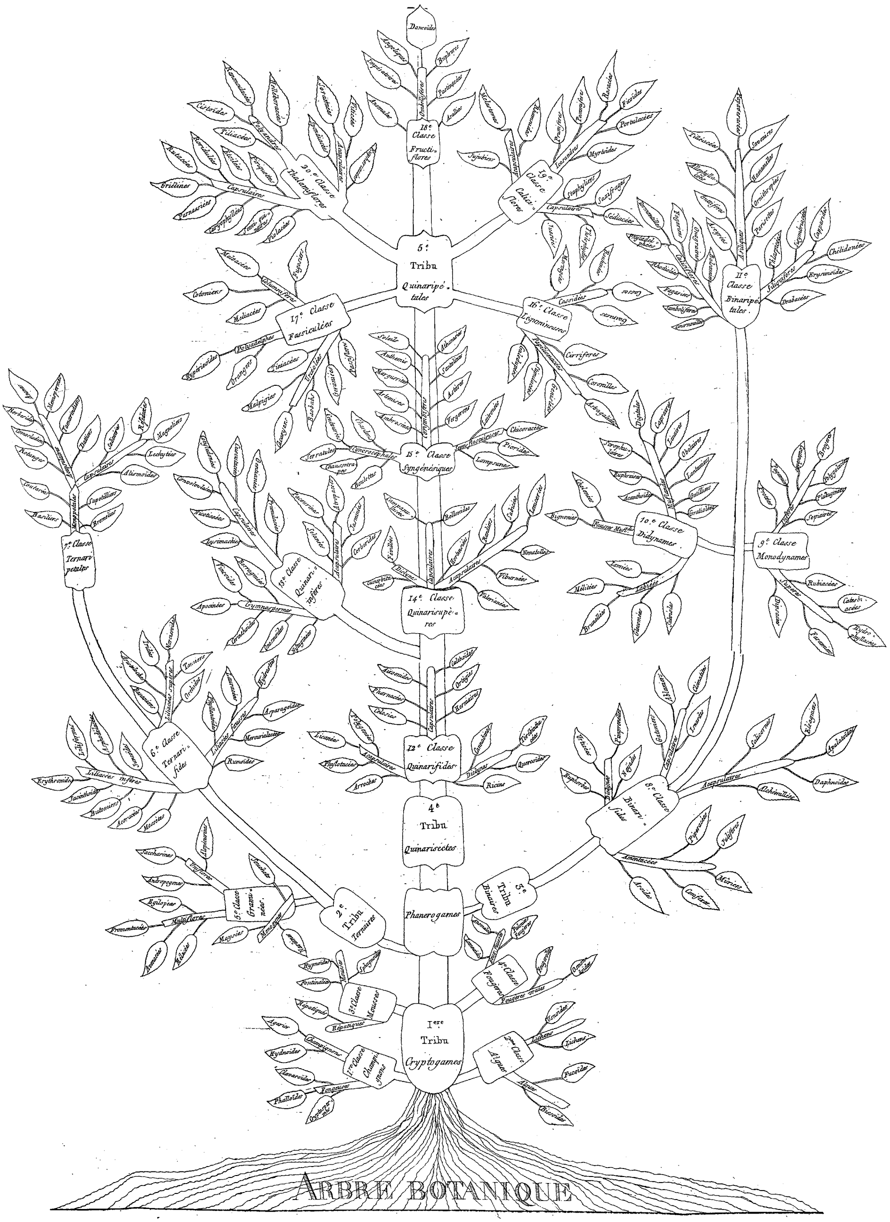

Fig. 1 Augier's "arbre botanique" (Augier 1801; see Stevens 1983)

diagram without label and without explanatory force. And such was the state of scientific knowledge at that time. Major subsequent representations of the tree of life stick to explanatory relevant elements and do not have roots. This is so of Darwin's famous branching diagram included in *The Origin of Species* (1859), of Haeckel's realistic tree-like picture featured in the *General Morphology* (1866), even of Doolittle's recent representation of the 'bush' of life (1999). What can recent scientific research tell us about these very roots? How far down can we go? In this contribution, I argue that origins of life research offers some glimpses about these roots. More specifically, I formulate two major claims. The first one is that trees of life ought to go deeper than the level of the 'ancestral organisms' where they generally stop: this deeper level is the level of much simpler and minimally alive entities that might be referred to as 'protoliving systems'.[1] My second claim is that below protoliving systems, the tree of life dissolves gradually into non-living matter along a multidimensional system of roots. This second claim follows from a gradual and multidimensional definition of life according to which the transition from non-living to living matter is a gray-zone populated by physico-chemical systems characterized by particular 'lifeness signatures'.

The 'ancestral organisms' and the tree of life before them

When looking at a tree of life, one often sees small branches receding into larger ones, and large branches that in turn merge into even larger ones until one reaches the bottom of a trunk. Run backwards, the story of evolution would appear to converge onto some sort of common ancestor of all living forms. As Darwin put it, a logical outcome of descent with modification and natural selection is that "probably all the organic beings which have ever lived on this earth have descended from some one primordial form, into which life was first breathed" (1859: 484). Most, if not all, trees of life illustrate this thought by displaying a trunk, or a system of trunks, that start at some ancestral state. Of course, molecular biology has given empirical support to such a claim, in particular by showing that specific biochemical themes, be they chemical constituents of the cells or metabolic pathways, appear to be common to all known terrestrial life forms (Pace 2001).

This hypothetical ancestral state is often taken to be the point in time when the three domains of life, Bacteria, Eucarya and Archae (Woese and Fox 1977), were not yet distinct and were somehow still merged into a single domain, probably a few billion years ago. What constitutes this single domain remains a matter of controversy. For some, it is a unique organism or a cloned population of similar organisms; in this case, the ancestral state may be referred to as the "cenancestor" (Fitch and Upper 1987), or as the "last universal common ancestor", or in short, as "LUCA" (Forterre and Philippe 1999). For others, this single domain would consist in a population of several types of organisms that, as a whole but not individually, would possess most of the basic features common to contemporary life forms; this

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[1] The term "protoliving system" has been used in origins of life studies with the meaning of rudimentary, primordial -and potentially minimal- living systems (Fox 1991) It is with this meaning that I use the term here. This meaning might be found to be synonymous to that of "protocell" even though protoliving systems do not require, strictly speaking, to be cell-like. Also, the term "protocell" is now often used in synthetic biology with a broader meaning, for instance including systems made out of non-prebiotically compatible compounds or resulting from artificial life research (e.g. Rasmussen et al. 2009) than the initial meaning it had in origins of life studies (e.g. Krampitz and Fox 1969; Stillwell 1980; de Duve 1991).

population would also be subject to so much lateral gene transfer that lineages would be impossible to establish; this ancestral population has been referred to, for instance, as a “communal ancestor” (Woese 1998), or as a “mutiphenotypical population of pre-cells” (Kandler 1994).

Independently of the ‘single-cell’ versus ‘population’ controversy, the ancestral state, when taken as the last point in time when the three different domains of life were only one, remains commonly understood as the locus of organisms that bear strong functional resemblance to the simplest life forms of each of these three domains. As a consequence such ancestral organisms are already fairly complex entities. This complexity can be inferred from ‘minimal genome’ studies. In a given environment, a minimal genome would be a repertoire of genes that would be necessary and sufficient to support cellular life. It was found in the 1990s that the number of truly essential genes of any organism might be quite small compared to its complete genome. For instance, some gene knock-out experiments have shown that the bacterium *Bacillus subtilis* might still do well with only ~300 genes left out of its original pool of ~4,100 genes (Itaya 1995). Cross-species comparison of genomes via computational comparative genomics indicate that certain genes tend to be quite closely related across species. For instance, the comparison of the genomes of *Haemophilus influenzae* and *Mycoplasma genitalium* shows that a couple hundred genes, called orthologous genes, had sequences that were more similar to each other than they were to other sequences from the two genomes (Mushegian and Koonin 1996). It was found that only ~15 genes had to be added to this set of orthologous genes in order to cover the full spectrum of essential cellular functions, including translation, transcription, replication, membrane transport, and energy conversion. In total therefore, a minimal genome might consist of only ~200–300 genes (Mushegian and Koonin 1996).

Other studies based on phyletic patterns of orthologous gene sets, on phylogenetic species trees, and phylogenetic gene trees, show that essential genes tend to be highly evolutionarily conserved, both in terms of sequence evolution and in terms of wide phyletic spread (Jordan et al. 2002). Of course, such results might strongly be affected by gene loss and horizontal gene transfer (Gogarten et al. 2002), and it is not at all certain that an ancestral genetic signal might still persist within the genomes of extant organisms. Nevertheless, there appears to be a rather strong confidence that organisms of the ancestral state possessed already several hundreds of genes, potentially even ~ 600 genes (Koonin 2003). For some, these genes would be made out of DNA (Forterre 2002; Dworkin et al. 2003); others favor a mixed RNA-DNA genome and replication system (Leipe et al. 1999).

In any case, the conclusion that can be drawn is that these organisms were already rather complex biochemical systems whose spontaneous appearance from inanimate matter appears quite unlikely (Moreira and Lopez-Garcia 2006). First, they would be endowed with a modern genetic polymer, DNA or RNA, whose prebiotic synthesis is far from obvious; they would also possess several hundred genes whose simultaneous random abiotic synthesis is questionable. Second, this genetic system would be capable of replication, hence the presence of a replication machinery, potentially coupled to a repair system that would ensure a high fidelity persistence of the genome. Third, these organisms would possess a translation machinery that would carry out the synthesis of proteins in a comparable fashion to that of modern organisms in the sense that it would be based on the activity of some sort of ribosome. Fourth, they would be characterized by a metabolic activity that would very likely produce energy in the form of ATP by generating a proton gradient across the cell. Finally, they would also be encapsulated within a membrane that would also be capable of growth and division.

The simultaneous presence of all these properties and chemical subsystems make it unlikely for such ancestral organisms to have appeared spontaneously on the primitive Earth. Rather, they must have been preceded by simpler biochemical systems endowed with fewer and/or less sophisticated functionalities. I propose to refer to such simpler and more primitive entities as ‘protoliving systems’ (as in Fox 1991). Such protoliving systems would be minimal living systems in the sense that they would be physico-chemical systems endowed with a self-sustaining metabolism and capable of evolution. Compared to the ancestral organisms, they would be based on simpler molecular components, potentially the simplest components one could devise to make the systems function in a life-like manner. These protoliving systems might, for instance, possess simpler and more prebiotically compatible genetic polymers than DNA or even RNA, and rely on peptide nucleic acids (PNA) or others (e.g. Eschenmoser 2007). They might also use simpler catalysts than proteins even if much less efficient, for instance oligopeptides and minerals (e.g. Rode 1999; Commeyras et al. 2004). They might also have a much simpler and rudimentary lipidic membrane that would split without any molecular regulatory system but simply thanks to physical pinching forces (e.g. Monnard and Deamer 2002), or even no lipid-based membrane at all but mineral compartments in a hydrothermal chimney (e.g. Martin and Russell 2003). Whatever these protoliving systems might have actually been, my point is that other living systems must have preceded the ancestral organisms that are referred to as “LUCA” or as the “communal ancestor”, respectively in the single-cell and population hypotheses. In other words, the tree of life goes deeper than the stage that is most of the time referred to as its roots, and protoliving systems represent such an earlier stage.

Now, if protoliving systems are taken to be ‘minimal’ living systems given the environment of the primitive Earth, it then seems that anything simpler would not qualify as alive or, maybe more exactly, as ‘truly alive’. As a consequence, the deeper shape of the tree of life at its roots very much hinges on the question of defining life. If life is a yes/no property of any given biochemical system, and if protoliving systems are the simplest of such systems, then the tree of life starts with protoliving systems and does not go deeper. If, on the other hand, life is not such a clear-cut property but appears gradually through many biochemical cumulative stages, then the tree of life has roots that go deeper than protoliving systems and reach into non-living matter.

When defining life matters for the tree of life

The question of defining life has been, and still is, a matter of great controversy that has spurted numerous proposals and counter-proposals. Already in the 1970s and according to Carl Sagan himself, there was no broadly accepted definition of life: rather, it seemed that each biological discipline had a particular tendency to define life in its own terms, hence a plurality of definitions of life, be they anchored to physiological, metabolic, biochemical, genetic or thermodynamic preferences (Sagan 1970, 985). More recently, several dozens of definitions of life have been referenced (see for instance Palyi et al. 2002, 15–56; Popa 2004, 197–205). I would argue that these definitions tend to fall into two broad categories: on the one hand, 'list-based definitions', and on the other, 'model-based definitions'. In list-based definitions, living systems are defined with a list of necessary and sufficient properties that typically include growth, reproduction, self-repair, energy-harnessing capability, matter-harnessing capability, variation capability, information capability and so forth (see for instance Oparin 1961; Bernal 1967; Monod 1970; Crick 1981; Mayr 1982; de Duve 1991; Farmer and Belin 1992; Koshland 2002; Morange 2003). Any physico-chemical system is said to be alive, as per a given definition, only if it possesses all the properties mentioned in this definition. Model-based definitions, on the other hand, define living systems not on the basis of a list of necessary and sufficient properties, but on the basis of a model that describes the very functioning of such living systems (see for instance Maturana and Varela 1973; Gánti [1971] 2003; Ruiz-Mirazo et al. 2004). For instance, Gánti's model consists in three functionally dependent cross-catalytic subsystems—a metabolic network, a template information system, and a membrane that encloses everything—whose correct coupling ensures the growth and subsequent replication of the chemoton. In the case of such model-based definitions, a physico-chemical system is said to be alive only if its functioning can be represented by a model that is similar to the model proposed in the definition, or that includes such a model.

Despite the many attempts to define life, be they 'list-based' or 'model-based', there still appears to be much disagreement on any of them: counter-examples are put forward that either might fulfill the definition conditions despite not being intuitively alive, or might be excluded by the definition contrary to our intuitions. For instance, some argue that viruses should not be included within the circle of living systems in so far as they lack metabolic activity (e.g. Luisi 1998; Ruiz-Mirazo et al. 2004); others argue the contrary, all the more now that it has been discovered that viruses form 'viral factories' that seem to possess the required properties of some of the list-based definitions of life (e.g. Raoult and Forterre 2008). Some also argue that self-replicating strands of RNA, like those that might have played a crucial role in the 'RNA world' scenario of the origin of life (e.g. Gilbert 1986), might count as being alive since they are capable of replication and variation (e.g. Luisi 1998); for others on the contrary, such RNA strands lack metabolic activity and membrane enclosure, and therefore cannot count as living systems (e.g. Shapiro 1986; Segré et al. 2001). Some might argue that oligo-peptidic autocatalytic networks might rightly qualify as being alive (e.g. Kauffman 1993); others would reject this claim on the grounds that such autocatalytic networks might not be capable of evolution (e.g. Luisi 1998; Ruiz-Mirazo et al. 2004). In a similar fashion, one could question whether minimal protoliving systems as those proposed by Szostak et al. (2001) or Libchaber and his team (Noireaux et al. 2005), might more properly qualify as alive than, for instance, those pursued by Rasmussen and colleagues (Rasmussen et al. 2003). And so forth.

What this debates clearly illustrates is that there are indeed a fairly wide range of properties and models that can be called upon to delineate living systems from non-living ones in many different ways. Incidentally, this also shows that there seems to be a 'gray-zone' in between what one would intuitively qualify as living and what one would definitely count as non-living. This gray zone might also very well encompass the range of physico-chemical systems that are likely to have represented key milestones along the transition from non-living to living matter. Indeed, a much favored hypothesis among scientists about the origin of life is that of a very gradual process, involving the appearance, fine-tuning and coupling of several properties, and spanning many millions of years (e.g. de Duve 1991; Popa 2004). As a consequence, there would not be any sharp delineation between non-living and living matter: 'life' would be the outcome of a succession of 'more-or-less alive' systems. Stated differently, the property of 'being alive' or of 'belonging to the class of living systems' would be better captured as a matter of degrees rather than as a matter of a dichotomous 'yes/no' delineation. This has led some to argue that a framework based on classical logic and its law of bivalence is not appropriate to define life, and ought to be replaced by a framework resting on fuzzy- or multi-valued logic (see Bruylants et al. 2010). In such a case, a zero-to-one scale would be defined by attributing a zero-value to a set of physico-chemical systems that would definitely count as non-living, for instance simple chemical compounds of the primitive Earth such as methane, ammonia or carbon dioxide, and a one-value to systems that would definitely qualify as living, for instance protoliving systems of the type mentioned earlier. Within such a scale then, any physico-chemical system might receive a score between zero and one that would represent its 'lifeness' or degree of being alive: it would be more alive than a molecule of methane, yet less alive than a protoliving system.

Such an approach not only makes it possible to reconcile differing viewpoints about dichotomous definitions of life and border-line cases, but also takes into account some of the latest scientific knowledge about the origin of life and the very likelihood of a gradual and multi-step transition from non-living to living matter. Viewing life as a matter of degrees entails a tree of life that would have roots going deeper than protoliving systems (value of one on the scale of lifeness) and somehow dissolving into non-living matter (value of zero). These roots would represent biochemical systems that would be more alive than simple chemical compounds yet less alive than protoliving systems, and that would also be historical ancestors to these protoliving systems. These roots, however, do not form a solid line, but rather fade away as they move from fully living systems down to non-living matter.

'Lifeness signatures'

If physico-chemical systems may receive a score of lifeness between zero and one, and as a result qualify as 'more or less' alive, one may ask on which grounds such a score might be attributed. An answer might be that the lifeness score should be a good measure of how far such a system is from non-living matter (score zero) and how close it is to living matter (score one). Since score one has been defined as that of a protoliving system, a minimal system endowed with a self-sustaining metabolism and capable of evolution, any system with a score of less than one would not be able to display this full set of properties. But it would seem that there could be several ways to do so: a system might be capable of metabolic activity without being able to reproduce itself, hence to evolve by natural selection; another one might be able to self-replicate without any metabolic activity; yet another one might be capable of metabolism and of reproduction, yet not of variation, hence not of Darwinian evolution; and so forth. The degree of lifeness of any system can therefore be interpreted as a weighted average of the degrees of performance of this given system along a set of functional dimensions, including for instance: replication, variation, metabolism, individuation etc.[2] And indeed, any given system might be more-or-less successful at making copies of itself, more-or-less successful at generating variants of itself, more-or-less successful at metabolizing components or energy tokens from given sets of available nutrients and energy sources, more-or-less successful at individuating itself with a sophisticated membrane, or even more-or-less successful at co-organizing or coupling any two of the above properties.

Each degree of performance along any of these functional dimensions can also receive a more precise definition. For instance, individuation might be realized in different ways and with different performance scores. Starting simple, it appears very likely that membranes evolved much in complexity over time. As illustration, one can tentatively propose, at least, five different stages of individuation performance. Stage I might correspond to enclosure within mineral compartments, for instance within growing hydrothermal chimneys with circulating flows of sulphide-rich hydrothermal fluid and iron-containing water (e.g. Martin and Russell 2003); such compartments would have provided individuation means, yet would be fixed, with little possibility of molecular exchange with the environment. Stage II might correspond to rudimentary bi-layer vesicles that are likely to have resulted from the self-assembly of simple amphiphile molecules (e.g. fatty acids) following their abiotic synthesis and their concentration in pools or droplets of water; such vesicles might have indeed been the first types of membranes encountered on the primitive Earth; yet they remain very fragile, sensitive to concentration, temperature and pH (e.g. Monnard and Deamer 2002). In stage III, the addition of other molecules such as sterols or amphiphilic polypeptides could have resulted in slightly more robust vesicles that would be stable across a broader set of chemical conditions, and that would be larger as well (e.g. Rohmer et al. 1979; Luisi 2002). Stage IV might then correspond to the insertion of specialized transporters and active catalysts; these new compounds might have enabled vesicles to create and maintain chemical disequilibria with their environment, thereby opening up more sustainable metabolic activities (e.g. Ourisson and Nakatani 1994). In stage V, the subsequent additions of other more complex organic compounds such as

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[2] Such dimensions might correspond to properties of “list-based” definitions or relate to the functioning characteristics of the models involved in “model-based” definitions of life.

polysaccharides, energy-transduction components or surface-layers components would have resulted in the appearance of the extremely sophisticated and multi-functional membranes like those of current organisms.

Similarly, one might conceive of ways of defining stages for each one of the remaining functional dimensions mentioned above (replication, variation, metabolism and coupling), or for any other one that might be found relevant. One would then end up with a compound number of functional scales along which the performance of any given physico-chemical system might be evaluated. And, in order to fit the zero-to-one overall scale of lifeness, all stages of all functional dimensions would be renormalized to a zero-to-one scale. For any given system, the weighted average of all the scores along the functional dimensions would then result in its overall lifeness score.

Yet, and most importantly, the pattern of functional scores that each system would get along the different functional dimensions would then represent its particular 'lifeness signature'. Such a lifeness signature makes it possible to understand where any given system lays on the multi-dimensional pathways leading from non-living to living matter. For instance, a system might be good at replicating itself, yet might do poorly in the other functional dimensions (example: an autocatalyst). Another system might do well in terms of individuating and replicating itself, yet poorly elsewhere (example: a lipid vesicle). Yet another might have an excellent metabolic activity while scoring low in terms of variation or individuation (example: an autocatalytic set). And so forth. Overall, 'lifeness signatures' might lead us to identify types of systems that may represent key evolutionary milestones from non-living to living matter (see Fig. 2). Let us note, however, that such milestones need not be arranged in a successive cumulative manner, but might end up showing parallel pathways towards a minimal fully functional and alive protoliving system.

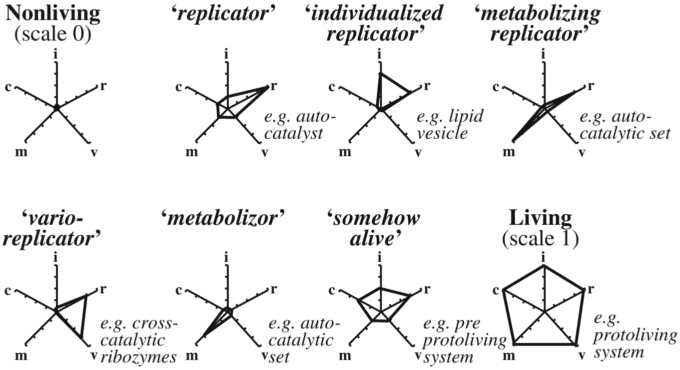

Fig. 2 Lifeness signatures for systems in between non-living and living matter. The 'lifeness signature' of a given system is represented by a polygon joining the performance scores of this system along each one of the relevant functional dimensions. In the cases depicted here, '*i*' represents individuation, '*r*' replication, '*v*' variation, '*m*' metabolism and '*c*' coupling. Each scale goes from the center (value zero) to the periphery (value one)

Such a multidimensional way of representing border-line systems in between inanimate matter and life helps reconcile differing viewpoints about the lifeness of some of the most controversial systems. Indeed, each of the controversial examples might very well represent particular types of such 'more-or-less alive systems', each with its own particular lifeness signature. For instance, auto-catalytic RNAs might be accounted for by a lifeness signature that would be strong on replication, average on variation, poor on individuation, metabolism and coupling. On the other hand, oligo-peptidic autocatalytic networks might have a lifeness signature that would be strong on replication and metabolism, yet weak everywhere else. In other words, the multidimensional scale make it possible to reconcile different definitions of life by equating them to different 'lifeness signatures'. Also, qualifying particular physico-chemical systems via the multidimensional scale of their 'lifeness signature' shifts the focus away from trying to find the 'ideal' clear-cut dichotomous delineation of living systems: rather, this highlights the importance of identifying particular types of 'more-or-less alive systems' and of providing means of understanding their key features and their inter-relatedness.

A consequence of this view is that, at its very bottom, going back in time, the tree of life not only dissolves gradually into non-living matter, but does so in a plurality of ways, following the evolutionary pathways of the most successful 'lifeness signatures'. In other words, the tree of life would not have a single root that would start below the level of the ancestral organisms and below the level of protoliving systems, but a system of several intertwined roots (see Fig. 3): some of them might correspond to systems that might have been good replicators but with a poor metabolism; others to systems with a good metabolic activity yet little success in generating variants; and so forth. One might imagine ways for systems to partly evolve along one or several of the functional dimensions that correspond to their

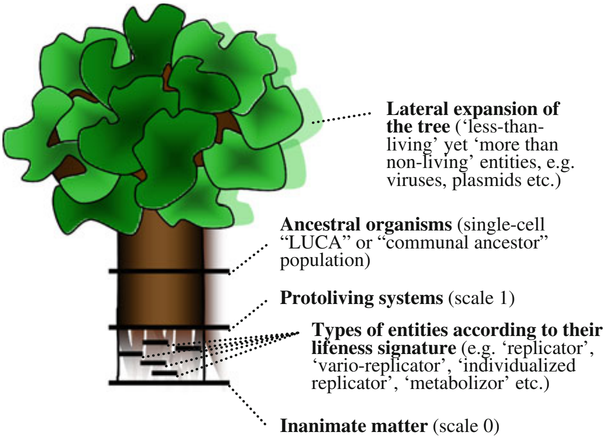

Fig. 3 The roots of the tree of life. Whereas most trees of life tend to start at the level of the 'ancestral organisms', two deeper stages have been added: the stage of the protoliving systems and the stage of the borderline more-or-less alive systems whose lifeness signatures determine their positioning on specific early roots of the tree of life that gradually emerge from non-living matter. The lateral shading of the tree would correspond to 'more-or-less alive systems' concomitant with fully living ones

lifeness signature. One can also imagine ways for such systems to develop new functionalities or gain them by lateral transfer from entities with a different signature, thereby pursuing a slightly different evolutionary path. In any case, the existence of multiple types of ‘more-or-less alive systems’ to which would correspond different ‘lifeness signatures’ would entail a rather ‘bushy’ system of multiple roots that gradually fade away into non-living matter as one tracks the multiple origins of life backward in time.

Three more points

Identifying real-case examples of ‘lifeness signatures’

One of the key scientific challenges associated with this multidimensional view of the transition from non-living to living matter is to pin down actual examples of different lifeness signatures that would also be prebiotically relevant. Unlike in some of its upper branches, populating the tree of the life at its base remains highly speculative: the fossil record is scarce and spurs lasting controversies when it comes to interpreting minute structures or molecular compounds that might, or not, be remains of primitive forms of life (e.g. Schopf 2006; Brasier et al. 2006). It is even possible that no fossil older than 3.7 billion years will ever be found. As a result, the best one might likely be required to settle for is possible scenarios populated by experimentally reconstructed entities that are compatible with the environmental conditions supposed to be those of the primitive Earth (e.g. Kasting 2005), and that are built from chemical compounds that are also shown to be prebiotically relevant.

There have already been several experiments showcasing specific chemical-systems that display, to a certain extent, some of the functional dimensions I mentioned above. Unfortunately, such systems are limited and often remain far from appearing compatible with the abiotic conditions of the primitive Earth: in particular, they may rely on sophisticated chemical compounds whose prebiotic synthesis is unknown or judged unlikely, or they may unfold in very particular chemical conditions that might appear unlikely to be found in the natural world. Such systems include sets of chemical compounds that can cross-catalyze each others’ synthesis, and that can therefore display properties of replication and quantitative growth; examples include autocatalytic sets of nucleic acids (Yjivikua et al. 1990), of RNA strands (Sievers and Von Kiedrowski 1994; Kim and Joyce 2004), or of polypeptides (Lee et al. 1996; Yao et al. 1998; Ashkenasy et al. 2004). Other systems may include sets of chemical compounds that can spatially self-organize into physically bound systems like micelles or vesicles, and that can therefore exhibit some form of individuation property. This is the case of certain organic compounds found in meteorites (Deamer 1985), of some amphiphile molecules that can self-organize into autocatalytic self-replicating micelles (Bachmann et al. 1992), and more generally of a broad range of lipids, be they phospholipids or fatty acids, for which some prebiotic chemical pathways might have been proposed (e.g. Hargreaves et al. 1977; Nooner and Oro 1979) and that can display surprising properties of growth, budding, fission or fusion (Hanczyc and

Szostak 2004), of surface catalysis (Rajamani et al. 2008), in addition to being good cases of individuation and of selective molecular exchange with the environment (Sacerdote and Szostak 2005).

Nevertheless, the multidimensional root system I have tentatively proposed, remains, in large parts, to be populated with prebiotically compatible systems. This is crucial if one is to identify the key milestones on the way to the appearance of fully-living systems on Earth. Yet, I would argue that, by being continuously revisited and adapted to fit the facts, such a tentative map has great value and can serve as a means of highlighting the different functional dimensions that matter on the way to life, and of identifying the inter-relatedness of systems that are more than non-living yet still somehow less than alive.

'Lifeness signatures': compatibility with other hypotheses on the roots of tree of life

In this contribution, my major claims with regards to the tree of life are that (1) the tree ought to be represented as going deeper than the 'ancestral organism level' where most trees tend to stop, and as having a 'protoliving system level' that would correspond to a much simpler, and minimal, form of living systems endowed with a self-sustaining metabolism and capable of evolution by natural selection, (2) the tree ought to be represented as going even deeper than protoliving systems with a system of several distinct yet intertwined roots that gradually fade away into non-living matter as the physico-chemical systems that are on these roots perform less and less well along the functional dimensions that characterize their particular lifeness signature. These two claims are compatible with different other claims about the roots of the tree of life, and upon which I wished to remain neutral. They are, for instance, compatible with different scenarios at the level of the ancestral organism, be they population- or single-cell-oriented, and also with scenarios that would include ancestral organisms without descent. They are also compatible with multiple scenarios at the protoliving system level: single-protoliving system scenarios, population-based scenarios, as well as scenarios that would branch out very early without long-term descent. And finally, my two claims are also compatible with different scenarios that would propose multiple origins of life.

Laterally expanding the tree

I have argued that the transition from non-living to living matter would be better represented by a multidimensional map that would capture key lifeness signatures and their inter-relatedness, and that would do so to account for the historically bound event which is the appearance of life on Earth some 3.8 billion years (e.g. Despois and Gargaud 2006). Yet, a related argument could be formulated that would have direct consequences on our way of delineating *current* living organisms from non-living matter. Similarly to the historical gradation and multidimensionality of 'more-or-less alive systems' on their way to life, one could picture the delineation of current forms of life as a gray zone that might be populated by 'less-than-living' yet 'more than non-living' physico-chemical systems. Such systems might have no relevance at all when it comes to explaining the origins of life, yet might indeed exist today, play a crucial roles in the biomass, and display particular lifeness signatures.

For instance, one could argue that entities such as viruses might belong to this gray zone: they are capable of individuation and of coupling the reproduction of their most visible entities, virions, to the metabolic and replication processes of their cellular hosts, and they might well represent also one of the most abundant biological entities on Earth (e.g. Edwards and Rohwer 2005). Viruses that infect other viruses might also be included (La Scola et al. 2008). The list of such borderline biochemical systems might also include those infectious proteinic molecules called prions, as well as self-standing genetic material such as plasmids or, more generally speaking, entities part of the "communal gene pool" that surround other living systems (Norman et al. 2009). And this list is, for sure, open. In a sense therefore, not only would there be no clear-cut historical transition between non-living and living matter, but there would also be no clear-cut delineation between current non-living and living systems. Such a view would result in laterally expanding the tree of life by adding a shaded zone of 'more-or-less alive' systems to some of its existing solid branches (see Fig. 3). It would make, not only, its roots fuzzy, but even some of its most current branches.

Conclusion

Trees of life do not start with a solid trunk or system of trunks: they have roots, or so have I argued. My claims are that such roots expand well below the level of the 'ancestral organisms' where most trees tend to start, and which can refer to single-cell hypotheses as in the case of LUCA or to population hypotheses. I have made two claims. The first one is that, below 'ancestral organisms', the roots of the tree of life go down to a level of 'protoliving systems' that are much simpler living entities than the 'ancestral organisms' are usually portrayed to be, and that are also defined as minimally living in the fuller sense. My second claim is that below 'protoliving systems', the roots of the tree of life form a multidimensional system of roots that progressively dissolve into non-living matter, thereby corresponding to a gradual or multi-stage transition from non-living to living matter along several functional dimensions. In this system of fading roots, I have proposed that physico-chemical systems be characterized by their 'lifeness signature', that is to say their degree of performance along a set of relevant functional dimensions. Not only might such a 'lifeness signature' alter the way we think about the roots of the tree of life, it might also change the way we generally delineate life from non-life.

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