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THINK AGAIN

Insights & Perspectives

The contours of evolution: In defence of Darwin's tree of life paradigm

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Motto "The answer Darwin conceived has proved correct (the man was annoyingly almost always correct)." The Origins of Creativity, Edward O Wilson.

Abstract

Both the concept of a Darwinian tree of life (TOL) and the possibility of its accurate reconstruction have been much criticized. Criticisms mostly revolve around the extensive occurrence of lateral gene transfer (LGT), instances of uptake of complete organisms to become organelles (with the associated subsequent gene transfer to the nucleus), as well as the implications of more subtle aspects of the biological species concept. Here we argue that none of these criticisms are sufficient to abandon the valuable TOL concept and the biological realities it captures. Especially important is the need to conceptually distinguish between organismal trees and gene trees, which necessitates incorporating insights of widely occurring LGT into modern evolutionary theory. We demonstrate that all criticisms, while based on important new findings, do not invalidate the TOL. After considering the implications of these new insights, we find that the contours of evolution are best represented by a TOL.

KEYWORDS
endosymbiosis, Eukaryogenesis, evolution, LGT, species concept, tree of life

INTRODUCTION

The concept of a tree of life (TOL) is an integral part of Charles Darwin's theory of evolution, and it has served as an invaluable guiding principle in organizing biological knowledge. A lot of research effort has been devoted to uncovering the many branches of the TOL and the branching pattern that best reflects evolutionary reality. However, a number of novel biological findings (discussed below) are claimed to invalidate this notion. These developments were coherently described in David Quammen's captivating and wide-ranging book "The Tangled Tree,"[1] where the TOL concept is criticized and implied to have been proven wrong by quite a few of the leading scientists cited. One of the first publications to worry about the challenges that lateral gene transfer (LGT) posed to (in this case bacterial) genome tree reconstruction was published in 1999,[2] while a 1993 publication already referred to a web of life.[3] Statements from more recent publications exemplify critiques of the TOL concept based on both LGT and endosymbiosis, for example, "Pattern pluralism (the recognition that different evolutionary models and representations of relationships will be appropriate, and true, for different taxa or at different scales or for different purposes) is an attractive alternative to the *quixotic* pursuit of a single true TOL." (Our italics)[4] and "Only simple metaphors (the 'Tree of Life') and dreams of hierarchical orderliness passed down to us from Linnaeus are at risk."[5] In this last article, entitled "How big is the iceberg of which organellar genes in nuclear genomes are but the tip?," the authors speculate: "If the iceberg of LGT does sink the ship of genome-based phylogeny, ..." Concomitantly, stressing the fact that some evolutionary processes, such as LGT, are not "tree-like," alternatives for phylogenetic trees, such as phylogenomic networks or

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Abbreviations: HR, homologous recombination; LGT, lateral gene transfer; LECA, last eukaryotic common ancestor; LUCA, last universal common ancestor; TOL, tree of life.

Box 1 – Defining and clarifying the Tree of Life (TOL) concept

Disregarding earlier conceptions of the TOL, instead starting with the vision of Darwin, the TOL is to be understood as a bifurcating diagram of evolving life forms by descent with modification (see "B" in Figure 1). Darwin focussed on eukaryotic macroscopic organisms and in our modern interpretation his species nodes would correspond with isolated organismal gene pools which can mix via sexual recombination. Cellular continuity in the eukaryotic part of the TOL is achieved by fertilization (fusion) and the resulting zygotes. In the prokaryotic domains, cellular continuity simply stems from binary cell division. In these ancient domains, the nodes of the bifurcating tree depict groups of cells which are defined as representing prokaryotic species (based upon core-genomes, operational taxonomic units -OTUs-,[16] and physiology); this species concept clearly differs from the eukaryotic one above. When we invoke an organismal/cell tree as the founding framework for the TOL, this is the bifurcating diagram of species we refer to. Our article confronts the difficulties of reconstructing the TOL (e.g., because of rampant LGT) but starts out by unequivocally stating the reality of this bifurcating diagram. Whether we are able to reconstruct it is irrelevant to its reality. The rare, but highly important, instances of "species fusions," such as the one at the basis of the eukaryotes, are all discussed in the main text. These can easily be accommodated in our TOL concept.

webs,[6] and the coral of life[7,8] have been proposed while dismissing the TOL as the "tree of one percent" (1% of the genome, that is).[9] We will present a detailed examination of these arguments for such positions in a later section: "An overview of critical considerations."

IN DEFENCE OF THE TOL PARADIGM

As described below, based on this examination of arguments critical of the TOL concept, we conclude that there is convincing evidence that the TOL, which we interpret as a "cell tree" (see Box 1), is an accurate and indispensable way of presenting biological evolution. Considering this evidence, we will show that, though the mechanisms at work and the evolutionary histories we are trying to reconstruct are much more complicated than was originally thought, this does not imply that the concept of an evolutionary tree is just a "simple metaphor" (see also below). To avoid any possible later confusion, when we talk about a "tree" in this context, we mean a linear diagram with diversifications (bifurcations), in the case of biology representing a steady flow of ancestors giving rise to (slightly) modified descendants, such as depicted in the single figure of Darwin's On the Origin of Species by Means of Natural Selection[10]; see Figure 1. Of note, the fact that we might not be able to convincingly reconstruct the temporal sequence of bifurcations in every instance of a set of diversifications from a common ancestor, does not in any way invalidate the concept of the tree. We also argue that discarding this fundamental explanatory concept would only harm basic biological insight. In further sections of this paper, we will describe the existence of a cell tree, the independent reality of a multitude of gene trees, and we trace proposed arguments against a TOL based on LGT and endosymbiosis to a mixing-up of the organismal (cell) tree with the multitude of gene trees. This confusion between a single, unique cell tree and many distinct gene trees is key to understanding much of the confusion in the field. Next, we bring up the existence of language trees to illustrate the power of using trees to describe/reconstruct processes of "descent with modifications."[10] We outline how evolutionary processes work by multiplication and diversification, and that this automatically leads to trees. As an illustration of the way of thinking that we will criticize here, we now cite the last paragraph of the first part of Quammen's book verbatim: "Darwin and Darwin's followers owned the tree image now. It would remain the best graphic representation of life's history, evolution through time, the origins of diversity and adaptation, until the late twentieth century. And then rather suddenly a small group of scientists would *discover*: oops, no, it's wrong." (Our italics). We will argue that, in fact, (i) there are no such discoveries fundamentally falsifying the TOL image, (ii) this erroneous conclusion is partly based on conceptual confusion, and (iii) discarding the TOL is not the proper way forward with regard to developing evolutionary theory and thinking. This does not mean that a critical look at the tree concept is not highly worthwhile, or that naïve conceptions do not have to be nuanced (this will only enrich our understanding). On the contrary, we argue that the monumental discoveries of endosymbiosis and LGT necessitate the intellectual effort of properly integrating these processes into a modernized theoretical framework for evolution. It does mean, however, that the TOL concept is *not* inherently flawed. The, often implicit, criticism, that the tree might be impossible to reconstruct (NB often confused with the prior claim that there is no tree!), is increasingly proving to be incorrect based on recent advances (see below) in reproducing ancient events in evolution's history.

As an aside, we should mention an earlier attempt to re-evaluate Darwin's TOL concept in the light of abundant LGT, by downplaying the importance of the former.[17] Basically, the article maintained that Darwin was mostly interested in evolutionary mechanism(s), and dismissed his use of the tree image as a "useful simile" or, even worse, "as an analogy for competition between species" only.[17] Careful reading of Darwin shows these claims regarding his position to be incorrect, and we consider getting rid of Darwin's TOL highly inadvisable.

EMERGENCE OF AN ENRICHED TOL CONCEPT

Remarkably, Darwin's theory has been able, to not only incorporate, but be highly enriched by subsequent findings, for example, Mendelian and, later, mathematical population genetics, resulting in what is now

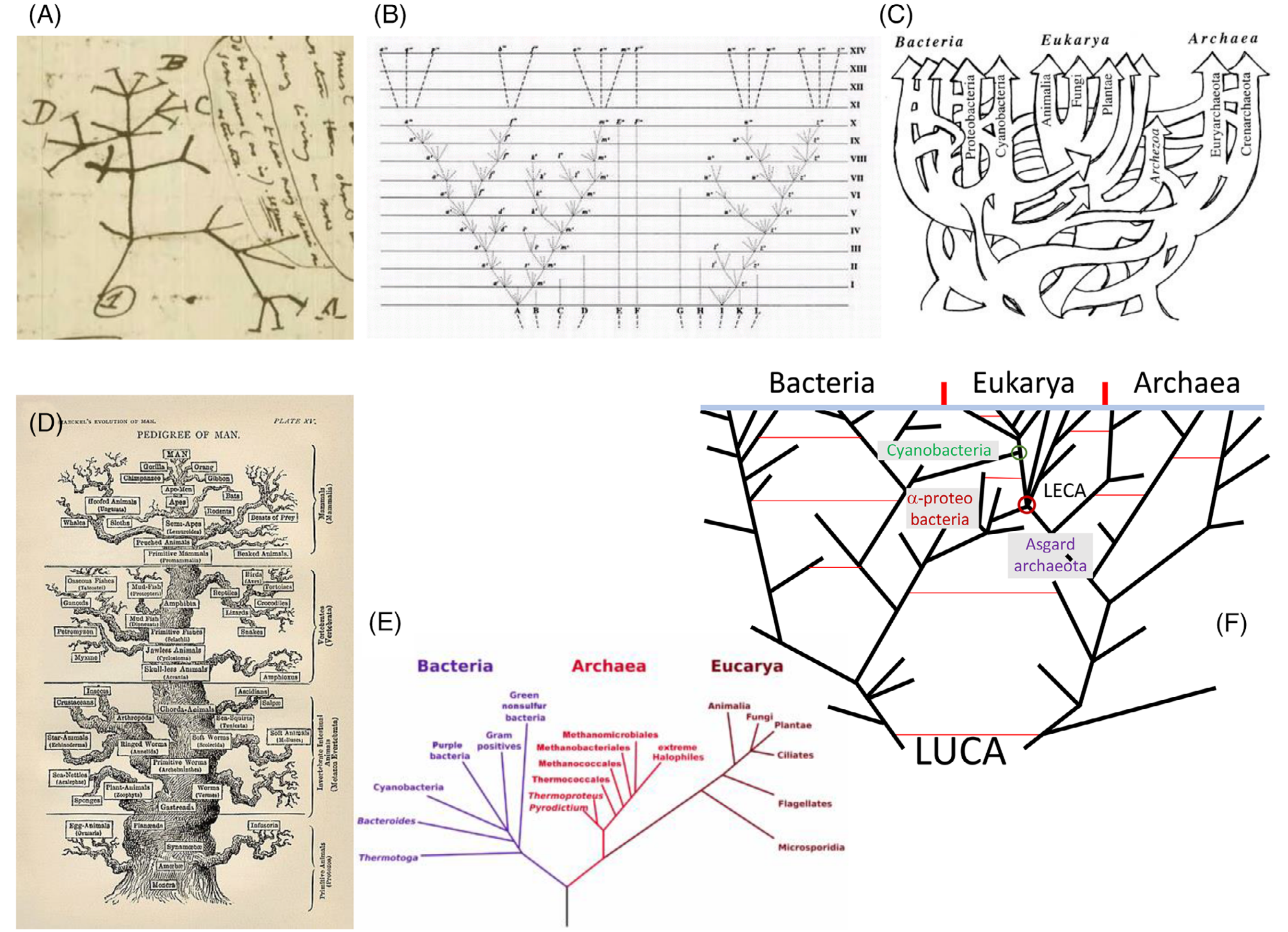

FIGURE 1 Depictions of the tree of life. (A) The first depiction in Darwin's Notebook "B." (B) The famous tree in.[10] (C) Doolittle's reticulated tree or net, appearing in.[11] (D) Haeckel's hierarchical tree. (E) The tree of Woese and co-workers, appearing in.[12] (F) The kind of modern depiction which is most scientifically accurate and fruitful, as we argue here. Broad black lines indicate highly schematized trajectories of cellular descent. Thin red lines specify LGT instances of specific genes (the small number of examples chosen to depict that LGT can occur both within and between the three domains), without an indication of direction. The merger between an Asgard archaeon and an alpha-proteo (like) bacterium at the basis of the eukarya, leading to the last eukaryotic common ancestor (LECA), is shown encircled in red.[13] The uptake of a cyanobacterium by an eukaryotic cell at the basis of the Archaeplastida is shown in green, while the multiple instances of secondary (e.g., euglenids; stramenopiles) or tertiary endosymbiosis (e.g., diatoms) in the eukaryotic domain[14] are not shown. "LUCA": The questions of whether archaea and bacteria arose independently from a precellular substrate, or both came from a simpler cellular precursor (LUCA), and if so, how LUCA came about, fall outside the scope of this article. The grey line on top indicates the present. Branch points in F are actually "fuzzy" in regions close to the branch point because of nuances in the biological species concept (see main text). When comparing Panel C and F, the mixing of a cell tree with gene trees (see main text) in C becomes obvious. Note the major differences in branching patterns for the three domains in E and F. In F, we have also emulated Darwin's tree (B) with respect to showing branches going extinct. With most modern tree depictions, it is easy to forget that the overwhelming majority of branches have gone extinct. Images A, B, C and E taken/adapted from ref. [15]; D Wikimedia Commons.

known as the modern synthesis of evolutionary theory. Here we will show how further revolutionary discoveries, namely endosymbiosis (and its indispensable role in eukaryogenesis; see below), subsequent acquisition of further organelles, and the widespread occurrence of LGT, mapped by ever-advancing DNA sequencing technology, enrich the Darwinian TOL concept. Instead of being based on directly observable characteristics of macroscopic organisms, modern TOLs are the result of integrating huge molecular data sets. One early major contribution, the work of Woese and colleagues, based on the remarkable divergence of 16S rRNA sequences, led to the three-domain TOL[12]; depicted in Figure 1E. Because of the centrality of 16S rRNA (see also below), trees based on these sequences seem to be impervious to LGT-based criticisms, and, indeed, Woese did not challenge the TOL concept as such, instead contributing to its further development. Considering all this progress, it is surprising that so many other researchers seem to want to discard it. Thus, we need a critical discussion of: (i) why this is so, (ii) the errors of the many alternative conceptualizations swirling around. That is what we will provide next.

AN OVERVIEW OF CRITICAL CONSIDERATIONS

Let us concisely discuss the arguments used to reject the evolutionary tree (i.e., the temporal sequence of bifurcations from common ancestors) as an accurate description of the history of life. Of note, most critics of the TOL depiction would say that the arguments are presented here in descending order of importance.

(i) LGT. Simply put, because part of the inherited traits of the organism is not of vertical descent, and such LGT can be rather extensive, the tree gives us such a misleading description that it should be discarded and replaced by a "web of life" concept.[1,4,9,11,18] Criticising the cell tree based on such gene characteristics does not occur in a cultural vacuum; the idea that genes constitute the "units of selection" (and that gene trees could thus be more important than organismal trees) was championed by Richard Dawkins' highly influential book "The selfish gene."[19]

(ii) Endosymbiosis. Leaving aside the well-known fusion of gametes during the eukaryotic process of meiotic sex,[20–22] which almost uniformly occurs within the species limits (for exceptions, see (iii), directly below), many instances of primary, secondary and even higher order uptakes of cells by other cells, followed by endosymbiotic gene transfer from the resulting organelles are extremely well documented; see[23] and references therein. For critics, this implies that the tree depiction is fundamentally wrong.[9,18]

(iii) The biological species concept. Here we will mostly focus on the nuanced comments made in the article by Mallet and coworkers: "How reticulated are species?"[22] The TOL depends on well-defined nodes: species. In this area, again, recent complicating insights and long-standing problems abound. A highly concise explanation of the term is needed first. Historically, we start out with the Linnaean concept (groups of closely resembling organisms); next Buffon introduced reproductive isolation (distinct species cannot sire reproductively competent offspring when allowed to breed). This concept was replaced by Mayr, who associated species with effective reproductive isolation under natural conditions. All of these definitions were effectively based on macroscopic eukaryotic organisms such as plants (i.e., members of the taxon embryophyta) and animals (i.e., metazoans). Applying the biological species concept to prokaryotes comes with difficulties, for example, when taking the realities of so-called core- and pangenomes into account.[24] This last aspect is of course intimately related to the LGT problem introduced above. However, also in the case of (both uni- and multicellular) eukaryotes, species concepts are more complex than was previously thought.[22] Where convergent evolution might hinder Linnaean sorting, many researchers thought that complete genome sequencing would overcome this challenge and allow easy reconstruction of the historical sequence of speciation events. Alas, also in eukaryotes, biological reality is more complicated. Convergent evolution also occurs on the molecular level, leading to technical challenges in reconstructing evolutionary history. LGT is much less extensive in eukaryotes, but still quite often detected in the unicellular "protists" and upon close contact with parasites, functioning as a reservoir of donor sequences, spoiling pure linear inheritance patterns. Correct temporal reconstruction of the TOL can also be complicated by the phenomenon of incomplete lineage sorting, where parts of the genome diverge at different rates in distinct species. Even worse: clear species boundaries themselves become difficult to define in eukaryotes as well (due to species hybridization and especially "introgression", which occurs upon backcrossing of a hybrid with a parental species). This has been found much more extensively than previously anticipated, for example, in African mosquitos,[25] Darwin's finches[26] and *Heliconius* butterflies.[27] It mainly occurs in groups blossoming upon rapid adaptive radiations, which thus often demonstrate increased phylogenetic incongruences. This would seem to make species themselves "reticulated", and thus not correctly represented by the point-like nodes of a classical TOL. All these different sources of phylogenetic/phylogenomic incongruences (plus methodological ones), as well as possible ways of dealing with them when attempting TOL reconstructions (e.g., algorithm improvements and incongruence search protocols), have, very recently, been reviewed in ref. [28]

So, do these three arguments spell the end of the highly fruitful TOL concept, forever to be replaced by a "bush or web of life"? We do not think so. In the next paragraphs, we will deal with each of them and show that the TOL concept (in the form of a "cell tree") remains the broadly correct and most fertile way of looking at evolution, which thus should be retained.

DISTINGUISHING BETWEEN THE CELL TREE AND GENE TREES

Before we begin discussing the arguments against the validity of the TOL introduced above, we would like to make a few important general remarks. Firstly, we would like to point out that phylogenetic practice seems not to have been influenced at all by these theoretical conclusions regarding the inadequacy of the TOL concept: instead, tree building has led to important improvements, ever finer details, and even much "deeper" trees (see Box 2). Secondly, all the criticism cited above uses the TOL as the "reference paradigm" and point out why and how a specific instance appears to deviate from it. As these deviations represent a range of initially unforeseen complications, their thorough analysis has led to the retention of a more nuanced tree concept. Thirdly, the field is plagued by two misconceptions already hinted at above: assuming that if we cannot reconstruct the tree, it does not exist, and more importantly, mixing up organismal (cell) and gene trees. This brings us to criticisms based on LGT.

To illustrate how a "net/web of life" concept was thought to replace the TOL, it might be useful to look at a highly interesting and valuable contribution to the phylogenetic reconstruction of early

Box 2 – Evolutionary contours: Theory versus practice

Has the phylogenetic practice of reconstructing bifurcating diagrams to represent the evolution of life been influenced by any of the theoretical considerations brought forward to discredit the concept of a TOL? The clear answer is: "not in the least". The last decade has seen the presentation of ever improved eukaryotic trees,[29,30] bacterial trees,[31,32] and archaeal trees.[33,34] However, in this context, an interesting attempt to reconstruct LUCA (see Figure 1F), published in PLOS genetics,[35] is informative. The authors grapple with the problem that extensive gene loss and LGT severely hamper their project. A tree based on genes that are universally present turns out to be a "tree of 1%", a number which does not seem able to inform phylogeny. Thus, the authors further worry: "Has lateral gene transfer obscured all records?" But using just a few quite rational assumptions they can conjure up an interesting description of LUCA. Using straightforward physiological constraints and assuming that bacteria and archaea are monophyletic taxa[36] they are left with ∼350 genes which fit very well with a physiological consortium near submarine, sulphur-rich, alkaline vents.[37] These genes turn out to be reciprocally rooted: that is, the bacterial and archaeal representatives constitute each other's outgroups, allowing the identification of strictly anaerobic deep branches compatible with the alkaline vent environment (for bacteria: clostridia; for archaea: methanogens). These articles[35,36] even make some further forays into possible connections between the reconstructed metabolism and emerging genetic (RNA) systems. These publications illustrate the power and fertility of assuming that successions of linear bifurcations took place, and aim to reconstruct them. In doing so, they belie all criticisms of the TOL concept.

events in evolution, entitled "Horizontal transfer of ATPase genes–the TOL becomes a net of life." by Hillario & Gogarten, published 30 years ago.[3] Analysing ancient metabolic proteins such as the proton-pumping ATPases and the TCA cycle-associated Glutamate dehydrogenase they demonstrate how extensive LGT can be and how this might lead to an incorrect reconstruction of the organism (cell) tree. However, their title illustrates the basic misconception we described. Darwin's way of looking at biological phenomena as evolving by massive multiplication and diversification as illustrated by tree diagrams, works for individual genes as well as for species of birds, or flowers, or barnacles. The resulting patterns should not be thought of as nets, but as trees in all instances. It is only when we *combine* organism (cell) trees with gene trees that a "net/web of life" depiction arises (see Figure 1). As an aside, both the "net" and the "web" metaphor no longer have the important arrow of time aspect of a "tree" (the also, often proposed, incorrect term "bush" would at least be better in that respect). Actually, even using the term "tree" has unwanted connotations and, thus, Haeckel's TOL,[38] shown in Figure 1D, is not an improvement on Darwin's diagram, depicted in Figure 1B. Of note, when comparing 1B and 1D, an unwelcome hierarchical aspect has also taken hold, which Darwin's conception happily lacks.

Because we currently understand much more with regard to LGT in prokaryotes (and, though more rare, in eukaryotes), we become increasingly able to accurately reconstruct individual gene trees, and their relationships with the separate cell tree.[39] However, upon finding evidence for quite a massive amount of LGT (especially in prokaryotes), partly based on the confusion described, the argument was often made that it invalidates the TOL as an accurate description of biological diversity, with the TOL being replaced by a web of life.[4,11,40] Our present considerations, as well as the progressing insights in the articles by Koonin and colleagues,[41–43] culminating in the concept of a "statistical" (consensus) TOL based on the set of genes most resistant to LGT, as also found in much more nuanced recent publications (e.g.,[44,45]) show this to be conceptually wrong. Also, while there is no *single* "resistant" gene whose phylogeny can be used to accurately reconstruct the TOL in its entirety, using this *set* of genes is another matter.

LGT COMPLICATES ACCURATE TOL RECONSTRUCTION BUT DOES *NOT* MAKE IT IMPOSSIBLE

Now that we have disposed of the "strong form" of criticism based on LGT, how about the "weak form," that is, that LGT makes accurate reconstruction of the TOL impossible? Has the degree of LGT been so extensive that all traces of ancient bifurcations in the cell tree as it developed have been irretrievably erased (see also Box 2)? With the development of many powerful genomics techniques (both with regard to massive generation of new sequences and their interpretation), this question can be answered. Many new key findings indicate that such a reconstruction is still feasible, with important strides being made in doing so.[46] Let us consider the facts of LGT: it has been thoroughly catalogued, and found to occur abundantly between different prokaryotes and, to a lesser extent, between eukaryotes.[47] On top of that, prokaryote to eukaryote transfer can occur.[48] Even cases involving combinations of eukaryotic donors and prokaryotic acceptors have been documented.[39] According to some publications, only 30% of prokaryotic genes seem to be linearly inherited,[49] with closely related species sometimes showing large genomic differences. But how are these organisms then identified as "closely related"? This is of course (partly) done by making use of the fact that many crucial genomic components (e.g., the genes for ribosomal RNAs) are almost never replaced by LGT. Such genes form a subset of the so-called core-genomes, present in all species and having essential functions. They differ from the extensive set of "accessory/dispensable genes," which, for example, play important roles in niche-specific adaptations and are exchanged by intense LGT. Of note, apart from the ones mentioned above, core genes *can* be replaced by homologous recombination (HR), and this might even occur more frequently in their case, when compared to accessory genes. Interestingly, this leads to lower mutational divergence for core genes, which suggests that HR might contribute to efficient maintenance of some conserved genome components.[50]

So far, increasing rates of prokaryotic genomic analysis have allowed the reconstruction of the archaeal and bacterial branches of the TOL; see amongst others.[33,51–53] This can indeed be depicted as a sequence of ever-growing branch diversification: for example, the last bacterial common ancestor seems to precede several splits, giving rise to broad groupings such as the so-called Gracilicutes and early branching Terrabacteria; for further details, see.[33] Interestingly, looking at gene transmission rates in this last analysis, a two-thirds vertical, and only one-third horizontal distribution was found.[31] Analyses of the most profound branches of the two prokaryotic domains are technically challenging and, especially in the case of the archaea, insights are still somewhat in flux.[33,54] The correct selection of a proper set of ancient vertically evolving genes is crucial,[33] as are the exact methods chosen for phylogenetic tree reconstruction starting with sequence alignments of this specific gene set. As a first criterium, such a proper set of ancient vertically evolving genes should give a tree coinciding with the tree derived using homologs of the 16S rRNA gene, which has proven quite robust against LGT-derived complications. From these examples we can conclude that also in the prokaryotic domains, with their quite extensive LGT, tree building has been successfully going forward, despite the theoretical criticisms described above. An organismal (cell) tree, influenced by, but independent of the individual gene trees, is taking shape.

COMPLICATIONS FOR ACCURATE TOL DESCRIPTIONS IN THE EUKARYOTIC DOMAIN

So far, we talked about the TOL as a "cell tree." Now that we have thoroughly distinguished between the concepts of a cell tree and a gene tree, we must face further complications associated with this organism (cell) tree. While LGT complicates TOL reconstruction chiefly in the primary, prokaryotic domains, these complications arose later with the advent of the eukaryotic domain. They stem from meiosis,[20–22] multicellularity and endosymbiosis.[23] Multicellularity can be dealt with by only taking germ-line cells into account, but meiotic sex itself has led to more confusion. Upon discussing (gamete) cell fusion in eukaryotes, Mallet and colleagues harshly conclude that based on this phenomenon: "A "tree of cells" justification for the *eukaryote* TOL is no longer possible." (our italics).[22] This is a grave exaggeration, as the pattern in the eukaryotic branch of the TOL overall also resembles a duplication-alteration pattern (as reflected in the branching diagrams Darwin started out with, see Figure 1), and not a "river-delta" pattern of splitting-combining. Splitting is the rule, merging is the exception. With the well-understood proviso of sexual cell fusion on board the TOL as a cell tree remains robust. However, is this the only instance of combination/fusion we have to account for while reconstructing the eukaryotic part of the TOL? No, because the evolution of the eukaryotes is deeply connected with (rare) instances of the most extreme form of fusion imaginable: those between completely different (i.e., taxonomically highly diverged) organisms. We will describe the most important one, the merger of two cell types at the basis of all extant eukaryotes (although this is disputed in ref. [55]), which probably enabled all later uptakes of other cells (bacterial as well as eukaryotic),[23,56] next.

THE CHALLENGE OF EUKARYOGENESIS AND LATER INSTANCES OF ENDOSYMBIOSIS

The second critical consideration we discussed above stems from a momentous occurrence in the evolution of life: the birth of the eukaryotes. The "Eukarya" form a quite exceptional domain,[12] as they came about due to a *merger* of an archaeon and a bacterium. Thus, when we reconstruct the TOL, concentrating on the origin of the eukaryotic branch, we are confronted with a unique situation: Parts of the two entirely separate, most basic, taxonomic branches (archaea and bacteria) combining to allow the emergence of a fundamentally new branch of organisms, characterized by a unique set of old (both archaeal and bacterial) and completely new attributes. These "new" attributes came about because of symbiogenic mechanisms, in which the new characteristics can be explained in the light of mutual adaptations of the two founding members[57–62] involved in the merger.[13,63–66] These considerations show that it would be highly erroneous to envision this singular development as extensive LGT of the proteobacterium to an (Asgard) archaeal "host" only. The mutual adaptation and progressive integration of two living organisms is much more challenging than integrating single genes and their products within the metabolism of an existing host cell. A lot of examples illustrate how eukaryotes combine biochemical and cellular features from archaea and bacteria.[63,67,68] Along came large-scale innovations in these attributes, for example, regarding ribosome and genome composition, organelle structures, accuracy of DNA replication and transcriptional control, lipid biochemistry, tRNA anticodon modifications, cytoskeletal proteins, histones and the organelle electron transfer complexes.[56,65,69–74] This portentous merger of an archaeal and bacterial prokaryote, giving rise to the singular hybrid and innovative cellular character of the eukaryotes, is stressed in Figure 1F. One such eukaryotic innovation is the capability of phagocytosis (though mechanistically unrelated forms are also found in prokaryotes[75]), which lay at the basis of the many instances of later organelle acquisitions. Do all these examples of uptakes resulting in organelles (none so momentous as the symbiogenic one[56]), fundamentally invalidate our TOL image? We recently argued that the merger of two lineages at the basis of the eukaryotes represents an unassailable problem for a strictly cladistic taxonomic approach, while still defending the "three domains system."[13] In the same fashion, a TOL concept not allowing examples of (overall quite rare) cell fusions/uptakes, seems to be counterproductive at best. It seems common-sense to just incorporate these few well-known instances in a more nuanced but basically correct TOL description. We conclude that endosymbiosis is both highly impactful and exceedingly rare (especially in the case of primary endosymbiosis). The event can be understood as a merging of two TOL branches.[76] Thus, the TOL concept is *not invalidated* by instances of endosymbiosis but *required* to properly understand its biological and evolutionary consequences.[56,77,78] In the same vein we can accommodate the species concept-related criticisms: we know about the nature and instances of problematic extrapolations of this concept, and thus allow for a certain fuzziness, because the fundamental nature of the historical development of life naturally fits with sequences of bifurcations. We will try to strengthen this common-sense approach by some "extrabiological" examples, next.

BROADER APPLICABILITY OF THE CONCEPT OF TREES GROWING BY DESCENT WITH MODIFICATION: COMPARING CELL/GENE TREES WITH LANGUAGE/WORD TREES

The Darwinian pattern of trees observed over the history of life, connecting every contemporary cell with the first cell (if it indeed existed, see the legend to Figure 1), every contemporary gene with a nucleic acid sequence existing very long ago, as well as every cell in multicellular organisms with its single-cell progenitor, is also seen in other systems which exhibit Darwinian behaviour. When studying gene trees (e.g., of protein-coding genes), we encounter the well-known general pattern of duplication and subsequent mutation/modification. A duplicated gene allows the two versions to slowly drift apart, giving material for the interplay of genetic drift and selection to generate novel protein functions (genes).[79,80] One of the basic series of events which often seems at play: an ancestral multifunctional protein becomes a set of more specialized proteins after gene duplication(s) allowed this to happen.[81] The mechanism involved was dubbed "subfunctionalization" and described in ref. [82] Importantly, as most proteins (especially in eukaryotes) seem to work by binding other proteins, changes in protein complex composition and subunit sequence also provide a lot of potentially new functions for certain gene products. Interestingly, in this case occasional gene loss might open up space for a duplicated gene to step in.[83] Duplications also allow more optimal "gene sharing," for example, letting a metabolic enzyme become an eye lens crystallin.[84] Reconstructed gene trees might thus come up with ancient ancestral forms of diverged proteins, helping us understand how certain structures and functions evolved, even in the case of proteins now having completely different functions.[85] In exciting new applications, gene phylogenies have even been used to try to reconstruct ancient gene consortia, to determine in what kind of environment the last universal common ancestor (LUCA) arose[35]; see also Box 2. Here we just want to stress that all this research is based on the tree pattern of recurring duplication and subsequent diversification.

One can think of many pedigrees showing Darwinian behaviour, but the tree we selected to discuss here concerns the pedigree of languages, because a comparison of cell/gene trees with language/word trees is enlightening.[86,87] We will see that, also in the case of many cultural phenomena, "trees" (as in "bifurcating graphs") are the best way to comprehend their history and evolution. An extensive body of linguistic research has resulted in trees proposed to describe how the wide spectrum of current languages came into being. Such phylogenetic trees of language evolution also have wide implications for understanding cultural evolution (e.g., the evolution of musical instruments or religious beliefs) as a whole.

The depiction of the Indo-european or Austronesian language trees is strikingly similar to a phylogenetic tree.[88,89] This is because the authors analyse linguistic data with new computational algorithms derived from evolutionary biology. But why were these specific algorithms used? It is here that the parallels between language trees and biological trees are particularly enlightening. Just as in evolutionary phylogenomics, earlier approaches were plagued by differences in the speed with which languages/lineages evolved,[90] and substantial borrowing of words between languages (compare LGT) complicates tree reconstruction and relative timing. To detect loanwords (such as "jaguar" and "piranha" from Tupi-Guarani or "boss" and "mannequin" from Dutch) and compensate for word transfer, algorithms are also being developed in linguistics.[91] Many other similarities, apart from loan words and transferred genes, exist: species varieties and dialects, highly reduced organisms and pidgin languages, cell fusions and "mixed languages," as well as the phenomenon of more rapid diversification upon prolonged isolation. There are important differences, but both language and cell evolution can best be depicted by trees that grow by descent with modification, in which some modifications involve LGT (in the broad sense of the meaning described here). Genetic and linguistic trees can then be checked against other data (e.g., of an ecological/geological or cultural/historical nature).

THE CONTOURS OF EVOLUTION: CONCLUSIONS

Here, we wish to cite a text fragment from our recent article in Biological Reviews regarding the necessary upgrade of Linnaean taxonomy[76]: "Finally, a fundamental aspect to be considered is that, as far as we know, ever since the emergence of the first cells on planet Earth, all subsequent cellular evolution proceeded by alternating cell divisions and mutations (with the highly notable exception of eukaryogenesis, where two cells lines merged): *omnis cellula e cellula*.[92] The present challenge is to map this (several billion year-long) uninterrupted chain of divisions and diversifications in a taxonomic system." (Reference number updated). We implicitly associated this taxonomic system with the TOL. In the current manuscript, we defend the TOL against alternatives such as pattern pluralism. In our introduction above we quoted from[4]: "Pattern pluralism (the recognition that different evolutionary models and representations of relationships will be appropriate, and true, for different taxa or at different scales or for different purposes) is an attractive alternative to the *quixotic* pursuit of a single true TOL." (our italics). The arguments that Doolittle and Bapteste present boil down to the assertions that, (i) the TOL cannot be represented by a unique hierarchical pattern. We contend that, upon interpreting hierarchy in the light of a relative timing sequence, it can. That (ii), the resulting historical picture cannot accurately be represented using a branching pattern. The arguments described above, a strict discrimination between cell and gene trees, and recent phylogenetic advances shows this to be in error. We are not insensitive to the valid points researchers such as Doolittle raise, for example, regarding the tremendous number of genes that have been exchanged back and forth between all prokaryotes, and later on with eukaryotes as well, which seem to make talk about "independent" lineages and prioritizing transcriptional and translational machinery over other cellular functions, somewhat arbitrary. However, in the process of reconstructing the cell tree we actually find that it is nature "prioritizing" the transcriptional and translational machineries or the entry of the (pre)mitochondrion....

We want to conclude by again stressing three points:

- (i) The main reason for the "it's not a tree but a web" position can be found in mixing the concepts of the cell tree and gene trees. Correctly integrating the Cell Tree (the TOL) and gene trees in the light of LGT is essential for a productive view of evolutionary biology.
- (ii) The concepts of endosymbiosis, meiosis, reticulate species, and species hybridization do not invalidate the tree concept. In the extensive, "deep time" view, the contours of evolution become clear: it is a tree.
- (iii) The continuing practice of phylogenetic research demonstrates that this is implicitly recognized by the large majority of professionals working in the field (see Box 2).

Following the general pattern, each individual human being (as well as each individual nonhuman multicellular animal or each plant) can be seen as a tree of cells developed from the fertilized egg or a plant zygote. All these individual trees could even be envisaged as twigs on the enormous four-billion-year-old cell tree. Trees everywhere: in the case of genes, of individuals, of species, of languages, of words. Here we analysed and described the general contours of (biological) phenomena evolving over time. Our verdict: *These contours show themselves to be trees, not webs, nets or bushes.*

AUTHOR CONTRIBUTIONS

All three authors conceived and wrote the article.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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