

Review

The practice of classification and the theory of evolution, and what the demise of Charles Darwin's tree of life hypothesis means for both of them

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Debates over the status of the tree of life (TOL) often proceed without agreement as to what it is supposed to be: a hierarchical classification scheme, a tracing of genomic and organismal history or a hypothesis about evolutionary processes and the patterns they can generate. I will argue that for Darwin it was a hypothesis, which lateral gene transfer in prokaryotes now shows to be false. I will propose a more general and relaxed evolutionary theory and point out why anti-evolutionists should take no comfort from disproof of the TOL hypothesis.

Keywords: tree of life; lateral gene transfer; horizontal gene transfer; prokaryote genome evolution; phylogenetics

If the tree of life (TOL) was the thesis and the web of life (WOL) its antithesis, then we are now in the period of disciplinary synthesis. A more realistic (less idealistic and dogmatic) microbial systematics and evolutionary theory will inevitably emerge. WOL advocates (WOLers) will be unable to claim total victory, however, and TOLers will be tempted to redefine what it was they were defending in order to avoid the appearance of defeat (e.g. Galtier & Daubin 2008). Preferable to this, epistemologically and ontologically, would be the adoption of a pluralist perspective, from which this controversy can be seen as but a stage in the development of a more powerful and *general* reading of Darwin's theory.

1. WHAT IS AT STAKE HERE

The papers in this special edition of *Transactions B* attest to the traction that the idea that lateral (or horizontal) gene transfer (LGT or HGT) might invalidate the TOL has, particularly in microbial phylogenetics. Moreover, this debate is no longer confined to academia, and much is at stake socio-politically. Anti-evolutionists, whose influence on the public misunderstanding of science is increasing worldwide and is astonishingly broad and deep in the USA, think they have found another nail for a coffin in which to bury the theory of evolution.

For instance, in his recent book *The Politically Incorrect Guide to Darwinism and Intelligent Design*, Jonathon Wells (2006), an 'intelligent design' advocate, asserts

that 'molecular phylogeny has failed, utterly and completely, to establish that universal common ancestry is true' (p. 46). He justifies this by quoting several evolutionists with contributions in this issue of *Transactions B*, for instance me (Doolittle 2005, p. 131).

We have, for several decades, thought that our job was to uncover the structure of a Tree of Life, whose reality we need not question. But really, what we have been doing is testing Darwin's hypothesis that a tree is the appropriate representation of Life's history, back to the beginning. Like any hypothesis, it could be false.

Indeed, I wrote that and will say pretty much the same here, and the other authors are also correctly quoted by Wells, as far as I know. Still, Wells' assertion is a willfully (or at best ignorantly) misleading summation of what we actually mean. We (some of us) do doubt that there ever was a single universal common ancestor (a last universal common ancestor or LUCA), if by that is meant a single cell whose genome harboured predecessors of all the genes to be found in all the genomes of all cells alive today. But this does not mean that life lacks 'universal common ancestry'—no more than the fact that mitochondrial DNA and Y-chromosome phylogenies do not trace back to a single conjugal couple named Eve and Adam whose loins bore all the genes we humans share today means that members of *Homo sapiens* lack common ancestry (Hein 2004).

That 'common ancestry' does not entail a 'common ancestor' is perhaps a subtle point—one that Woese and co-authors recently explicated very nicely in the case of the genetic code by the way (Vestigian *et al.* 2006). Wells might be forgiven for missing it, except that one suspects that he does so on purpose. But another recent book from what should be our side of the culture wars, Mark Isaak's *The Counter-Creationism*

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One contribution of 11 to a Theme Issue 'The network of life: genome beginnings and evolution'.

Handbook also misses the point, in asserting that ‘Horizontal gene transfer does not invalidate phylogenetics. Horizontal gene transfer is not a major force affecting modern life, including all macroscopic life’ (Isaak 2005, p. 87). No one writing in this issue doubts that horizontal (lateral) gene transfer is a major force (especially for microscopic life), and all would probably agree that whether or not LGT ‘invalidates phylogenetics’ depends on what we think is the purpose of that practice.

There is a natural impulse for evolutionists to circle their wagons in making common cause against the creationists. I think we should resist it. Evolutionary theory itself evolves, but creationists attack it as a dogma of a revealed religion of which Darwin was the prophet, celebrating debate within the field as evidence for doctrinal weakness. In fact, it is evidence for disciplinary strength. At the end of this essay I will argue that many of us have been defending a too-narrow form of neo-Darwinism, and that accepting a more pluralistic view of evolutionary pattern as well as evolutionary process will put us in a better position to defend ourselves against the forces of unreason, as well as advancing our science.

2. CLASSIFICATION, ARTIFICIAL AND NATURAL

There is a rich and dense literature in the philosophy of classification and the meaning of biological categories, in particular, whether they are artificial or natural, cutting Nature at joints that exist independently of human thought. I am inclined to accept Ian Hacking’s position, which is that ‘Some classifications are more natural than others, but there is no such thing as a natural kind’ (Hacking 2007, p. 203). That natural-kind thinking continues to inform systematics, perhaps especially microbial systematics, I illustrate by two examples.

The first is the comment of a referee concerning a submitted manuscript in which Eric Bapteste and I had asserted that ‘our job as systematists is not to discover what an organism truly is (in some essentialist sense) but rather to decide what we should call it’. To this the referee objected that ‘most systematists would be in complete disagreement with this idea. This is an absolute negation of the essence of a natural classification system and a very depreciative view of the systematist’s work. Our work is *to try to know what organisms are*, not to put them into a catalogue with gratuitous names’. I am not sure how many systematists would phrase it quite so baldly, but certainly most publications in phylogenetics are written as if the phylogenetic position of a species is something to be *discovered*—a proveable fact of Nature—and not something to be *negotiated* after consideration of the relative merits in consequential terms of placement here versus there in some larger hierarchical scheme.

My second example of natural-kind thinking is the position taken by Norm Pace in his continued efforts to eradicate the word ‘prokaryote’ from the language of biology. He argued recently (Pace 2006) that ‘The lessons of the three-domain tree are profound. Instead of two kinds of organism, prokaryotes and eukaryotes, there are three: bacteria, eukarya (eukaryotes) and

archaea’. The second ‘are’ here is strongly ontological: for Pace there seems no doubt that the three domains are real in the same sense that, say, a particular species or even an individual cell might be real.

A comparison that illustrates through contrast the special naturalness we attribute to biological classifications can be made with the Dewey Decimal system. This wondrous scheme divides all knowledge into 10 main classes (the first number), 100 divisions (the second number) and 1000 sections (the third). Dogs and cats, for instance, are subsumed in the hierarchy...

- (i) 600 Technology
- (ii) 630 Agriculture and related technologies
- (iii) 636 Animal husbandry
- (iv) 636.7 Dogs
- (v) 636.8 Cats

A revisionist librarian might argue for moving the cat and dog books to the shelves where we could also find books on recreation, or even ‘activity therapy’ (therapeutic uses of animals is 615.81.58) but no matter where they wind up, he or she would admit that the final decision has an arbitrary character—it had to be negotiated. Indeed, the principles under which negotiation takes place are negotiable social constructs as well.

Not so, we think, if we are dealing with the actual cats and dogs themselves, in the context of the global biological classification that is underwritten by the still-dominant Linnaean system. Even the supposedly revolutionary Phylocode (which admits to arbitrariness of higher taxonomic ranking) grants each organism (or each ‘species’) but one true position in the hierarchy of life, discoverable through phylogenetics (Donoghue & Gauthier 2004).

So I think there are three beliefs, variously strongly held, about biological classification that inform the two examples of natural-kind thinking I gave earlier, and microbial systematic practices generally. First, species, though hard to define operationally, are real entities in Nature: we discover them. Second, some higher taxonomic categories (bacterial divisions and Pace’s domains) are also real: there can be final, evidence-based answers to questions about them. Third, each species has a unique phylogenetically defineable relationship to all others: we might quibble about the ontological status of higher taxa, but there is only one tree and once we know what it is, we will know exactly where every species belongs on it.

3. DARWIN AND HIS TREE OF LIFE HYPOTHESIS

Why do we think this way? It would be silly to deny that large tracts of observable life are indeed *naturally* clustered, independently of our desire to impose order on our experience. Animal species, in particular, seem like natural kinds, and there is cross-cultural support for this. From a study of the Ketnegban people of Indonesian New Guinea, Diamond and Bishop conclude, for instance, that it is ‘striking that the units into which the Ketengbans and western scientists classify birds correspond for the most part

on a one-to-one basis. This can only mean that their and our shared classification describes a mutually perceived reality in the biological world' (Diamond & Bishop 1999, p. 41).

Why we believe in supra-specific hierarchies may be less obvious. Folk taxonomies are seldom extensively elaborated at multiple higher ranks (and of course never address microbes). The practice of constructing multi-rank inclusive hierarchies (each group a member of one and only one grouping of the next higher rank, the whole representable as a tree) has an honoured place in Western natural history and philosophy, however. According to Panchen (1992), there are deep and complex historical connections between neo-Platonist practices of logical division (often illustrated by the Tree of Porphyry, whose successive bifurcations take us from Substance down to Socrates) and systematists' beliefs about the ontological status of supra-specific taxa. These varied: even Linnaeus (1707–1778) had doubts about the naturalness of taxa higher than genera. But for the devout Christian systematist, the diversity, plentitude and orderliness of Nature, as much as the watch-like perfection of her individual creatures, were evidence of some divine plan, however obscure. John Ray (1627–1705), whose botanical systematics prefigured Linnaeus', accordingly titled his major work, embracing inanimate as well as animate Nature, *The Wisdom of God Manifested in the Works of the Creation*.

Darwin replaced such beliefs with his two-part theory, which offered: (i) natural selection as a credible and testable hypothesis for the phenomena of biological adaptation and (ii) descent with modification and a tree-like pattern of successive speciations as an explanation for biological diversity and a reason for existing hierarchical classification schemes being (in principle) 'natural'. Evolutionary theory (and current intelligent design creationist attacks) has focused much more on the former than the latter aspects of Darwin's contribution, but both were essential elements in the 'one long argument' that was *The Origin*. After a disquisition in chapter 13 of its first edition on the existing practices and problems of systematics, Darwin (1859, p. 420) wraps up ...

All the foregoing rules and aids and difficulties in classification are explained, if I do not greatly deceive myself, on the view that the natural system is founded on descent with modification; that the characters which naturalists consider as showing true affinity between any two or more species, are those which have been inherited from a common parent, and, in so far, all true classification is genealogical; that community of descent is the hidden bond which naturalists have been unconsciously seeking, and not some unknown plan of creation, or the enunciation of general propositions, and the mere putting together and separating objects more or less alike.

And elsewhere (Darwin 1859, p. 129) he articulates his view that there is a universal genealogy, possibly knowable, that relates all life in a single hierarchy, which he was happy to call a tree ...

The affinities of all the beings of the same class have sometimes been represented by a great tree. I believe

this simile largely speaks the truth. The green and budding twigs may represent existing species; and those produced during each former year may represent the long succession of extinct species.... The limbs divided into great branches, and these into lesser and lesser branches, were themselves once, when the tree was small, budding twigs; and this connexion of the former and present buds by ramifying branches may well represent the classification of all extinct and living species in groups subordinate to groups.

As Eric Baptiste and I have argued elsewhere (Doolittle & Baptiste 2007), very much following Panchen (1992), what Darwin presents in *The Origin* is a hypothesis, not an observation or claimed-to-be true fact. The observation or fact to be explained (the *explanandum*) is hierarchical or tree-like classifications, their apparent robustness and their potential naturalness. The cause of or explanation for this observation (the *explanans*) is the fundamentally tree-like (genealogical) character of an underlying (causal) process of successive speciations that comprises Darwin's model for 'descent with modification'. In other words, patterns of classification are tree-like because the process that produces the traits on which they are based, evolution, is tree-like. Simply enlarging the scope of the observations it was meant to account for (growing the *explanans* by adding more 'groups subordinate to groups') cannot prove this hypothesis to be true. We need an independent test. After all, evolution itself could have been (as far as data available to Darwin could show) web-like, or comb-like, with 'the characters which naturalists consider as showing true affinity between any two or more species' owing their dispositions among living things to interspecies transfer or parallel and convergent evolution. These too could be considered 'descent with modification', but not Darwin's version of it. And, since trees will inevitably emerge from the data when we order them with tree-building algorithms, we might be just fooling ourselves about such order being naturally embedded in the data.

4. A POTTED HISTORY OF PHYLOGENETICS

For almost 150 years, most biologists have ignored the possibility that they are fooling themselves. We have taken the TOL as an established fact in need only of elaboration, that is the branch-by-branch reconstruction of a unique true universal tree. Each new branch seemed to us to lend more credibility to the construct, and yet really has only served to expand the dataset for which the Tree was proposed as an explanation.

And for the first 100 of those years, the data with which we sought to recreate the Tree and the methods we used to do it were not radically different from those employed by preDarwinian systematists. As De Queiroz (1988, p. 238) notes 'Taxonomies of living things and the methods used to produce them changed little with the institutionalization of evolutionary thinking in biology. Instead, the relationships expressed in existing taxonomies were merely reinterpreted as the result of evolution, and evolutionary concepts were developed to justify existing methods'. Although disputes between pheneticists and cladists,

and both with evolutionary taxonomists, raged in mid-last-century (Mayr 1981) patterns of relationships, whether they were taken as genealogical or not, had to be derived from similarities and differences between the phenotypes of the organisms being classified. Panchen argues that the fossil record and biogeographic distributions offered some independent confirmation of descent with modification, but neither can address the whole of the Tree (or microbes, for that matter).

Molecular phylogenetics, invented in the early 1960s by Emile Zuckerkandl, Linus Pauling, Walter Fitch and Emmanuel Margoliash (and others), offered a way out of such circular pseudo-confirmation of the TOL hypothesis. Zuckerkandl & Pauling (1965, p. 102) argued that...

...the topology of branching of molecular phylogenetic trees should in principle be definable in terms of molecular information alone. It will be determined to what extent the phylogenetic tree, as derived from molecular data in complete independence from the results of organismal biology, coincides with the phylogenetic tree constructed on the basis of organismal biology. If the two phylogenetic trees are mostly in agreement with respect to the topology of branching, the best available single proof of the reality of macroevolution would be furnished.

For much of the living world, especially the macroscopic animals and plants with which Darwin was (and most anti-evolutionists still are) most concerned, the molecular approach has lived up to this promise (Cracraft & Donoghue 2004). Incongruencies between molecular phylogenies and evolutionary reconstructions based on 'organismal biology' do remain, but a pattern of ongoing resolution suggests that these result from inadequate sequence or taxon sampling or wrong models of sequence evolution, not some fundamental deviation from the successive branching speciation process represented by the 'simile' of a 'great tree'.

But the molecular approach also offered the possibility of extending phylogenetics downwards, into the prokaryotes, an effort first pursued with a variety of protein sequences (cytochromes especially) but soon superseded by Carl Woese's heroic effort to unite all of life in a single universal tree using a single 'universal molecular chronometer', small subunit ribosomal RNA (SSU rRNA) and the genes encoding it. There was no prior basic agreement on even the rudiments of an 'organismal tree' (a tree of species based on organismal traits) with which the molecular tree could be compared (Woese 2005), however. So the faith that prokaryotic evolution could also be properly represented as a tree came largely, I contend, from a misplaced notion that the universal TOL was already an established fact (and perhaps a logically essential element of any theory of evolution), with only its topology being in doubt. Woese (1987) himself acknowledged the lack of an accepted organismal phylogeny for prokaryotes, and suggested that with them we might look to the congruence between trees made with different genes for support for the TOL hypothesis.

5. DOES LGT INVALIDATE PHYLOGENETICS?

It is now a tedious truism to say that there is much more *incongruence* between prokaryotic gene trees than we would have imagined there could be two decades ago. And more of this incongruence is due to LGT than we would have guessed then—although the potential for that process to render evolution non-tree-like was already known (in the guise of plasmid-borne antibiotic resistance among human pathogens) at the time Zuckerkandl and Pauling wrote, 20 years earlier still. It is also not surprising that there remain vigorous and possibly unresolvable disagreements between TOLers and those WOLers favoring a web-like representation, about just how much LGT there has been, and whether or not its extent 'invalidates phylogenetics'.

Before attempting to defuse this question (about the invalidation of phylogenetics) and sideline the debate by endorsing pluralism, I will point out some of the several ways in which the very idea of measuring the extent of LGT in realistic models (those which recognize that this process is frequent) remains problematic and vexing.

1. *We must go all the way back to LUCA if we want to know how many genes a genome has acquired by LGT.* It is customary in publications first describing a newly completed genome sequence (for instance Nelson *et al.* 1999) to estimate the fraction of its genes that are likely lateral transfers, using one of three methods—gene compositional heterogeneity (GC content, codon usage and so forth), phylogenetic incongruence or gene content comparisons with closely related taxa, for instance using BLAST (Doolittle *et al.* 2007). The first method has, because of 'amelioration', a limited look-back time, the second suffers from weak phylogenetic signal at depth, the third can mistake differential loss for LGT, and all three beg the question—*lateral transfers since when?* It is reasonable to suppose that many transfers are recent and ephemeral (Marri *et al.* 2007) but even stable genes will have been subject to orthologous replacement, so the further back in the history of a lineage we probe, the more of its current crop of genes should reveal themselves to have been acquired by LGT, not vertical descent (VD). The sum total requires that we go all the way back to LUCA. But this is impossible in practical terms because of inadequate signal, and in theoretical terms, because LUCA is a profoundly problematic entity.

2. *In fact, LUCA is illusory.* If there is as much LGT as we WOLers think there is, the proper way to model prokaryotic evolution over 4 Gyr is as a single, albeit highly structured, recombining population, not an asexual clade. To accept this is to abandon the concept of a single tree with a single root (or at least a single rooted tree of genomes) because it means that different gene families trace to gene-family-specific ancestors that existed in different genomes at different times in the past. There will have been no single common ancestral *cell* whose genome harboured a direct ancestor (either the last common ancestor or their lineal predecessors) of all the genes present in all genomes today. Indeed such an ancestral cell need not have contained lineal ancestors of any of the genes around today, and is thus basically unknowable. The many attempts one still sees to reconstruct its genome are

groundless, and to say the LUCA was a population is meaningless. Of course populations such as ‘all prokaryotes today’ have populations (all prokaryotes Y years ago) as their ancestors in this loose sense, but there is no principled way to designate the population at some particular value of Y as ‘LUCA’.

3. *Many studies use a false null.* Attempts to measure LGT as incongruence among gene family datasets across the prokaryotes often founder on the patchy distributions and weak phylogenetic signals that characterize most of them. It is thus common to look among such datasets for strongly supported incongruence with respect to reference trees (scored as LGT), while assuming that genes that statistically ‘do not reject’ the reference topology exhibit VD. It would be reasonable to do this if LGT were a dubious process whose very occurrence needed to be proven, but not if we know it to be common and wish to measure its extent compared with VD: then the same standards of detection and proof should be applied to both. Lerat *et al.* (2003) failed in this regard in 2003 (see Baptiste *et al.* 2004) and Daubin (one of the authors of Lerat *et al.* 2003, writing now with Galtier (Galtier & Daubin 2008)), persists in such thinking. He writes that ‘even in the most highly self-conflicting data set, more than 75 per cent of the genes do not significantly reject the consensus tree’, as if this somehow proved the unimportance of LGT. There will be many other trees that these genes do not reject, as well (Baptiste *et al.* 2004; Susko *et al.* 2006), and no way to choose between these trees—other than to *assume* a single topology. Absence of evidence for LGT must not be taken as evidence of absence of LGT, as it still too often is. ‘Fails to reject’ does not mean ‘supports’.

4. *Measuring the frequency of LGT does not measure its importance.* When, in such studies, equal standards are applied to both support and reject a reference tree, nodes at which support can be inferred predominate in number (as in Beiko *et al.* 2005), and this might be taken as evidence for the unimportance of LGT. However, deep transfers will result in the phylogenetic ‘misplacement’ of all descendant genes, and the genomic databases (in which most sampled taxa are relatively closely related to—and increasingly conspecific with—each other) systematically undersample deep nodes. The relevant question about any genome in this context, I think, is how many of its genes have an unbroken history of events of VD, back to whatever early date we chose (let us say 3.5 Gyr ago, if that is when we think Archaea and Bacteria began to diverge). This number probably represents less than 10 per cent of its genes (for reasons I discuss next) and may be zero. The average frequency of LGT could be vanishingly small, of the order of one in every trillion generations (a billion years, with an 8 h generation time) and still all truly deep phylogenetic signals would be erased.

5. *The core of potentially untransferred genes is tiny, over all evolution.* Few gene families (fewer than 50; Charlebois & Doolittle 2004) are shared by all genomes, and these are characteristically genes of the translational system, which is thought for other reasons (the ‘complexity hypothesis’; Jain *et al.* 1999) to be recalcitrant to LGT. Even when the requirement for

ubiquity is relaxed to exclude hyper-reduced and poorly annotated genomes (requiring presence in only 80% of the genomes in any phylum, for instance), this number will not likely rise much above 100. It is clear why: prokaryotes have many different ways of making energy, interacting with their environments and structuring their cells—although they have only one way to make proteins, hence the shared translational ‘core’. The same energy, interaction and structuring components are often found widely and patchily distributed across the Bacteria and Archaea, in a way that makes sense only if loss and gain (through LGT) of the genes encoding them were frequent over evolutionary time.

6. *The core is not large even within species.* That the core of genes shared by all of a set of related genomes is surprisingly small is true at all levels of taxonomic inclusiveness, although the number does go up the more phylogenetically restricted the set. Among 13 gamma-proteobacterial genomes, there are approximately 200 shared genes, for instance (Lerat *et al.* 2003), while a recent comparison of 17 *Escherichia coli* strains finds approximately 2200 shared genes (about half the number of genes in the average *E. coli* genome and one-sixth of the 13 000 genes found in at least one of them (the *E. coli* ‘pangenome’; Rasko *et al.* 2008)). The non-universal genes in any such dataset comprise: (i) genes invented *de novo* in one of the lineages, (ii) genes present in the common ancestor but lost in one or more lineages, and (iii) genes acquired by LGT in some of the lineages. If genome sizes are to remain on average roughly constant over long evolutionary times (which seems a necessary assumption) then (i) and (iii) must cancel out (ii), and (iii) can be distinguished from (i) by BLASTING genes outside the taxon (accepting some false negatives). By almost anyone’s analysis of almost any such dataset, (iii) is a substantial number. However, such variably present genes are generally excluded from estimates of the extent of phylogenetic incongruence between gene families.

7. *Core genes need not have the same phylogeny.* It is a temptation, not often enough resisted, to use the shared genes in residual genomic ‘cores’ to make trees, with the assumption that they have the same phylogeny, and concatenating them to compensate for their individually weak phylogenetic signals. Two problems with this are: (i) that core genes often do not have the same signal, or cannot safely be concluded to do so (although they may not ‘reject’ the VD hypothesis) and (ii) concatenation gives increased bootstrap values and thus false confidence even when the concatenated genes provide no additional (or even conflicting) signal (Jeffroy *et al.* 2006). Thus, the 31-gene universal tree from Bork’s group (Ciccarelli *et al.* 2006; derided by Dagan and Martin (2006) as ‘The Tree of One Percent’) clearly comprises mixed signals, even after all the obvious LGTs were algorithmically eliminated. Why, when we know that so many genes have different histories should we place special importance on so few, simply because we *think* they have the same history?

8. *The tree of cells (TOC) is not Darwin’s tree of life.* The TOC seems to be the construct for which trees based on such core (and especially translational) genes strive and on which TOLers now pin their

hopes (Lerat *et al.* 2003; Ciccarelli *et al.* 2006; Soria-Carrasco & Castresana 2008). The idea here is that a residue of never-transferred genes tracks the history of each successive speciation (and for preceding asexual lineages, each successive cell division), back to LUCA. This history represents a tree of organismal identity for those with essentialist leanings or at least a framework against and with which LGT events can be assessed and evolutionary hypotheses can be formulated, for the more practical-minded. Enthusiastic TOLers see this TOC, no matter how little of the actual phenotype-determining information or history the organisms they wish to classify it encompasses, or how extreme the algorithm used to derive it, as a triumph of the Darwinian method and a vindication of their belief in the TOL. It is, in their view, the genealogy upon which Darwin thought classification could safely, and ultimately must, rest. This is I think a misreading of history and a non-trivial re-formulation of the goals of phylogenetic practice. And it is unnecessary in the pluralistic perspective that comes from an understanding of evolution grounded in process rather than pattern.

6. PHENETICS, CLADISTICS AND THE REFUTATION OF THE TOL HYPOTHESIS

In his 1981 Science essay *Biological classification: toward a synthesis of opposing methodologies*, Ernst Mayr (1981) defines the two pure ideologies of systematics, phenetics and cladistics, with admirable clarity. Of the former, he writes . . .

From the earliest preliterary days, organisms were grouped into classes by their outward appearance, into grasses, birds, butterflies, snails, and others. Such grouping 'by inspection' is the expressly stated or unspoken starting point of virtually all systems of classification. Any classification incorporating the method of grouping taxa by similarity is, to that extent, phenetic.

while of the latter he notes . . .

This method of classification, the first statement of which was published in 1950 by Hennig, bases classifications exclusively on genealogy, that is on the branching pattern of phylogeny. For the cladist, phylogeny consists of a sequence of dichotomies, each representing the splitting of a parental species into two daughter species . . .

The enduring beauty of Darwin's TOL hypothesis is that it promises to align the outcome of these two classificatory practices, at least much of the time. Genealogy should always take precedence in a Darwinian classification (all true classification is genealogical), but most often there will not be a conflict with phenetics, basically because 'like begets like'. Thus, as Padian (1999) notes . . .

For Darwin, the only real difference between these traditional [preDarwinian] systems and what he regarded as the proper basis of classification was that descent with modification replaced divine plan or other philosophical considerations. But he still needed a practical way to discover genealogical relationships. He recognized that many of the traditional criteria, such as

degree of similarity, embryological resemblances, and so on, reflected genealogical proximity. In these respects, he appreciated that traditional taxonomy had probably been replicating more or less faithfully the pathways of diverging parentage.

The destructive power of LGT lies in its ability to disrupt this workable alignment of phenetics and cladistic practices, this more or less faithful replication of the 'pathways of diverging parentage'—at least as far as prokaryotes are concerned. Evolution of prokaryotic genomes is not tree-like and there is no guarantee that a classification based on similarities and differences in phenotype determined by the genes in those genomes must reflect whatever tree-like behaviour some minor fraction of the genetic repertoire might or might not have. Traits most relevant to a practical and flexible phenetic or ecological classification have often been acquired by LGT, not through VD. This is obviously so for many pathogenic bacteria (Deurenberg *et al.* 2007; Vilas-Boas *et al.* 2007), but is also true of such seemingly ancient and stable groups as the cyanobacteria (Coleman *et al.* 2006) or haloarchaea (Walsh *et al.* 2006). Phenetic classifications will of course remain tree-like if we persist in using methods that produce trees, but an underlying tree-like evolutionary process is not needed to explain them. Nothing in Nature requires that we make of these complex evolutionary relationships a single tree.

This does not mean, of course, that trees constructed from that minor fraction of genes found in many genomes, and possibly refractory to LGT, are devoid of interest in themselves, or that they *might* not trace cellular (if not genomic) history. We are never likely to know that, because it seems impossible to imagine an independent test. But the TOC cannot fulfil the explanatory role that Darwin assigned to the TOL: it is not the *explanans* for an *explanandum* that is hierarchical classification. To equate TOL with TOC is to accept as Darwinian a seriously watered-down version of his hypothesis.

Whether or not LGT also 'invalidates phylogenetics' depends on what we think phylogenetics is for and is. If its goal is to elucidate the origin of phyla (phylogenesis) and if phylogenetics is taken—as it now invariably is—to be the construction of bifurcating trees, then this goal is not achievable. Phylogenesis is a much more complex, reticulating, process. If the goal of *molecular* phylogenetics in particular was to provide, through its agreement with traditional classification, what Zuckerkandl and Pauling hoped would be 'the best available single proof of the reality of macroevolution', then that goal too cannot be achieved. Indeed, for prokaryotes, molecular data have falsified the TOL hypothesis. If the goal of phylogenetics was the re-creation of the true TOC, then the verdict is not yet in, and may never be.

7. PLURALISM, A GENERAL POST-NEO-DARWINIAN EVOLUTIONARY THEORY, AND COLD COMFORT FOR CREATIONISTS

Steven Jay Gould, making his usual case for an expanded view of the forces driving evolution with his usual eloquence, wrote . . .

We live in a world of enormous complexity in organic design and diversity—a world where some features of organisms evolved by an algorithmic form of natural selection, some by an equally algorithmic theory of unselected neutrality, some by the vagaries of history's contingency, and some as byproducts of other processes. Why should such a complex and various world yield to one narrowly construed cause?

This attitude, by which Gould meant to dethrone natural selection as the perceived near-exclusive cause of evolution, we call 'process pluralism'. A parallel effort, aimed at relieving the TOL of its responsibility as the sole proper representation of the history of living things (and of the interspecies relationships that it is meant to depict), we call 'pattern pluralism'. One sees a gradual move in this direction in several articles in this issue. In a pluralistic perspective, it may be admitted that even a 'Tree of One Percent' tells us something about the history of cells, without having to accept it as the TOL. One can agree that LGT will likely not interfere with a robust phylogeny for primates that indeed recreates ancient splittings of their populations, without buying in to the notion that the supposed sisterhood of Thermotogales and Aquificales revealed by a small subset of their shared ribosomal protein genes reflects an even remotely similar evolutionary process. We know the mechanisms by which prokaryotes evolve, and like the driving forces listed by Gould, they are various in their frequency, intensity and consequences for the evolution of genomes. Why should we expect them together to produce a single pattern, a fractal one at that, good over all time and for organisms of all types?

Such relaxed views of process and pattern require a relaxation of what I believe to be the version of evolutionary theory that we are defending from antiscientific attack (although it is admittedly not easy to say what that is). A general formulation might look like this: *genetic mechanisms (broadly construed) and population and ecological process (broadly construed) that we already for the most part understand, operating over enormous time, are responsible for the diversity of life we see around us, and for the adaptedness of living things.* Such a broadly uniformitarian formulation leaves no gaps for miracles, and pays adequate homage to the quite versatile and powerful mechanistic toolkit we now have on hand to explain the history of life, with independent corroboratory evidence in some cases and plausibility in all. Acknowledging a prominent role for LGT more specifically eases the evolution of complex adaptations, by making it unnecessary for each unlikely step to occur in sequence in a single genomic lineage, seen by some anti-evolutionists as a stumbling block for our theory.

For us TOLers and WOLers, endorsing pluralism would let us get back to doing science at the only level at which it can actually be done, applying our explanatory toolkits to individual problems and refraining from wrangling about reified concepts like species, domains (or whatever the highest taxa might be called) and the TOL, which I suspect Darwin himself might now eschew, and that should never have been imported without due consideration of its

relevance into microbiology. I take to heart these words of Maynard Smith (1982, p. 5), written a quarter of a century ago but even more relevant in this post-genomic and post-phylogenetic era . . .

The modern synthesis of the 1940s was concerned with eukaryotes . . . Its essential achievement was to bring together two previously separate disciplines – the chromosome theory of heredity and the study of natural populations. The same synthesis is now required for the prokaryotes. There is an abundant knowledge of their genetics, but as yet no adequate synthesis of that knowledge with a study of the natural history of bacteria. For example, we have little idea of the significance of conjugation for bacterial populations; it is as if we had no idea of the significance of sexual reproduction for populations of birds and insects. Population thinking has been well developed for fully half a century, but has yet to be adopted by microbiology.

We would have to give something up, of course, and that is the notion that there is a uniquely natural hierarchical classification scheme for prokaryotes. And if placement in such a uniquely natural hierarchy is what our disapproving referee meant by knowing what 'organisms are', we must abandon hope of such unambiguous knowledge. We might entertain different sorts of hierarchical, multidimensionally clustered or reticulated classifications for different sorts of purposes (Ereshefsky 1992; Baptiste & Boucher 2008). Alternatively, we could stick with current hierarchical rRNA-based or total-proteome classifications, with the full admission that at the very best they are only just 'more natural' than other systems, in that more (or more 'fundamental') data support them. But as with the placement of books on library shelves, there is in principle, no final truth of such matters.

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