

# How the endosymbiont got its cell

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*Despite fundamental advances in cellular, molecular and genome biology, there is still surprisingly little consensus concerning the evolutionary origins of the eukaryote cell. While it is clear that the mitochondrion (responsible for generating much of the energy requirements of the eukaryote cell) has evolved from an endosymbiont cell of bacterial origin, the recent literature has borne witness to a tidal wave of speculative theories regarding the nature of the cell in which this bacterium took up residence. David Penny and I recently argued that much of this confusion can be avoided if models are grounded in known biological processes, and if speculation is tempered by formulating testable hypotheses. The most fanciful hypotheses are an inevitable casualty of a pragmatic approach, but what remains is a productive framework wherein biologically plausible alternatives can be evaluated without the need to invoke ad hoc events or processes, such as biological 'big bangs' or hitherto unobserved cell biological phenomena.*

## Introduction

The eukaryote cell is traditionally distinguished from its simpler archaeal and bacterial relatives by a list of 'haves': eukaryotes have a cell nucleus, introns (intervening sequences that must be removed from mRNA during gene expression) and numerous organelles (subcellular structures visible under the microscope) (Sapp 2005). That eukaryotes possess so many features that appear absent from bacteria and archaea begs the question: how did these features evolve? In the case of one organelle — the mitochondrion (responsible for respiration and energy production) — we now have a clear picture of its origin and evolution; it is beyond doubt that mitochondria have evolved from once free-living bacteria (Embley & Martin 2006; van der Giezen & Tovar 2005).

However, many unicellular eukaryotes apparently lack mitochondria. As these species were scattered around the base of the eukaryote tree, but did not form a single group, the conclusion was that only some eukaryotes (e.g. plants, animals, fungi) evolved from an ancestor that picked up a bacterial symbiont; the other 'earlier-diverging' eukaryotes were given the name 'archezoa' (Cavalier-Smith 1983; Keeling 1998).

Advances made in recent years have turned the field of eukaryote evolution on its head. Significantly, the archezoan hypothesis is dead — far from never having had mitochondria, the mitochondria of archezoan eukaryotes have either been reduced to vestigial forms, such as hydrogenosomes (that generate energy via fermentation and evolve hydrogen as by-product) and mitosomes (van der Giezen & Tovar 2005), or they have been lost secondarily.

The first cracks in the received view came from evolutionary trees. One group of deep-branching archezoa — the microsporidia — in fact turned out to be related to fungi (Germot *et al.* 1997; Hirt *et al.* 1997, 1999). This derived position in the tree meant that microsporidia probably once possessed mitochondria but had lost them during their evolutionary history. Moreover, archezoa such as *Trichomonas vaginalis* and *Giardia lamblia*, appeared to contain genes of mitochondrial origin, despite not apparently possessing mitochondria (Hashimoto *et al.* 1998; Roger *et al.* 1998). Closer inspection of the cellular ultrastructure of these species and microsporidia yielded a surprise: all these lineages either carry hydrogenosomes or mitosomes, and these organelles all share a common ancestry (Embley *et al.* 2003; Tovar *et al.* 2003; van der Giezen & Tovar 2005; Williams *et al.* 2002).

Since all eukaryotes that have been examined in sufficient detail carry one of these organelles, archezoa are not 'living fossils' that pre-date mitochondria. Rather, the inescapable conclusion is that the common ancestor of all eukaryotes possessed a mitochondrion (or some variant thereof) — no such missing links exist.

These new insights have created a problem in the minds of many biologists: if the mitochondrion is a universal feature of the eukaryote cell and the missing-link status of the archezoa is incorrect, fully evolved eukaryotes cannot have existed to engulf the bacterial endosymbiont. The 'death' of the archezoa left in its wake a vacuum of uncertainty regarding the nature of the cell in which the mitochondrial ancestor took up residence. This vacuum was rapidly filled with a diverse array of theories,



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including the return of several previously disfavoured ones (Martin *et al.* 2001; Poole & Penny 2007b). The most frequent (but by no means the only) answer to this was that the host must have been a prokaryote cell, specifically an archaeon.

There are numerous reasons to suspect a close affinity between archaea and eukaryotes. They seem to share key features of their fundamental molecular machinery, which sets them apart from bacteria. In particular, archaeal and eukaryotic DNA replication and transcription machineries share greater similarity to one another than either does to bacteria (Edgell & Doolittle 1997; Forterre *et al.* 2004; Leipe *et al.* 1999; Cramer 2002). And in fact, genome wide comparisons suggest that for these and other ‘informational’ processes, archaea and eukaryotes share strong similarities, whereas for ‘operational’ processes (e.g. energy metabolism) there is greater similarity between eukaryotes and bacteria (Penny & Poole 1999; Rivera *et al.* 1998). The latter makes intuitive sense given that ATP generation via the citric acid cycle is performed in the mitochondrion, not the cytosol. Genomically then, eukaryotes appear to be part bacterium, part archaeon (Rivera & Lake 2004; Horiike *et al.* 2001).

### Habits of indefinite argumentation

In the post-archezoan world, so many hypotheses for the origin of the eukaryote cell have been tabled that substantial reviews are required simply to cover them (Martin *et al.* 2001), and hypotheses can even be classified into general categories (Poole & Penny 2007b)! The numerous recipes for making a eukaryote cell range from low-calorie (archaeon + bacterium) to three-course (archaeon + bacterium + bacterium), from contaminated (archaeon + viral infection + bacterium) to imaginary (archaeon + bacterium + hypothetical RNA cell). It is hardly surprising that parallels have been drawn to the parable of blind men describing an elephant (Minkel 2001). What is significant is that all new theories avoid archezoa (I will return to this point), and most are in fact at odds with the view that modern cellular life evolved into three distinct domains (archaea, bacteria, and eukaryotes) by descent with modification – the reason for this is that, in most accounts, the origin of eukaryotes can be traced back to a symbiosis and subsequent genetic integration between archaea and bacteria.

Evolutionary biologists have been known to cast aspersions on one another by labelling each other’s theories on the origin of various features ‘just-so’ stories – after Kipling’s comical explanations of the origins of animal traits. In biology, key advances have had speculative origins – a good example is the endosymbiotic theory, where early speculation can be traced back to the early 1880s (Martin *et al.* 2001), and yet definitive tests of this hypothesis were not published until the 1980s (Douglas & Turner 1991; Yang *et al.* 1985). However, that does not mean speculation should be without bounds; no compelling observations support the emergence of modern eukaryotes by the new routes being suggested. Bacteria are not known to reside within archaea, and there are no documented mechanisms by which bacteria can enter archaeal cells; this is likewise the case for archaeal entry into bacterial cells. Similarities between viruses and the nucleus are preposterously few, and there are no RNA cells. Yet these scenarios are postulated without embarrassment, and there is no shortage of skill among those who defend these theories; the just-so epithet seems inescapable.

When discussing the state of the field with David, he rather characteristically dug out a quote from Charles Lyell’s *Principles of Geology* (Lyell 1830–1833), which beautifully illustrates how speculation can all too easily devolve into polemics:

*The system of scholastic disputations encouraged in the Universities of the middle ages had unfortunately trained men to habits of indefinite argumentation, and they often preferred absurd and extravagant positions, because greater skill was required to maintain them... No theory could be too far-fetched or fantastical not to attract some followers... [who] were not at all restricted in building their systems, to the agents of known causes,*

To beat a path through the jungle of speculative ideas on eukaryote origins, two things were needed. One was to remind practitioners of the merits actualism, which is to favour the interpretation of past events by reference to mechanisms in action in the present. The other was to encourage the inclusion of testable predictions. In other words, while speculation can be an important facet of the scientific process, theories need to be grounded in fact, and bolstered through the formulation of testable predictions (Poole & Penny 2007a).

### Stems, crowns, tests & teapots

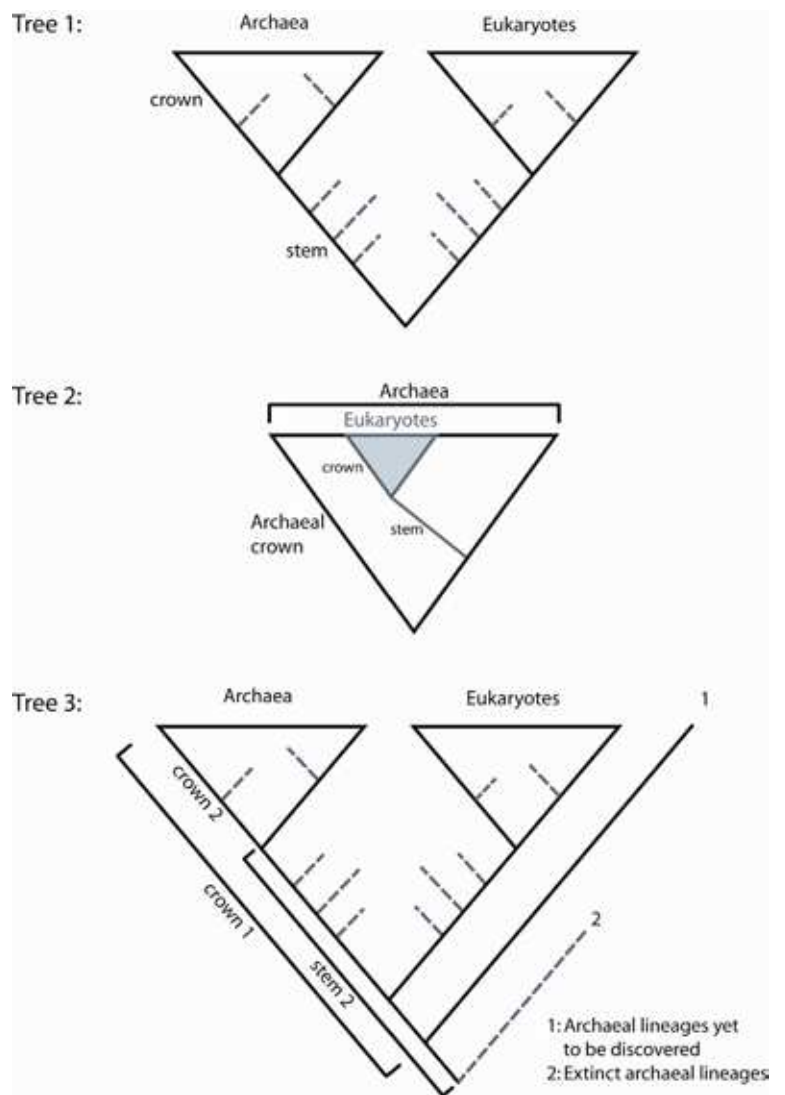
The killer observation that put the endosymbiotic origin of mitochondria and chloroplasts beyond doubt came from evolutionary trees. Both these organelles contain DNA distinct from the DNA housed in the nucleus, and it was reasoned that if chloroplasts and mitochondria are bacterial in origin, their evolutionary history could be traced using phylogenetic methods. Indeed, genes from both compartments have been shown to belie a bacterial origin in that evolutionary trees demonstrate that they are more closely related to genes in bacteria than they are to genes in the eukaryote nucleus; taxonomically, mitochondria and chloroplasts group within the wider diversity of bacteria (Douglas & Turner 1991; Yang *et al.* 1985). For eukaryotes to be considered part archaeon (in addition to a bacterial contribution) the same should apply; nuclear genes should likewise group within the diversity of modern archaea (Cavalier-Smith 2002; Poole & Penny 2007b). This amounts to an important phylogenetic test: either archaea and eukaryotes are related by descent from a common ancestor, or else eukaryotes evolved directly from archaea (Figure 1). The second issue is how these two possibilities impact on the various hypotheses.

That similarity measures have in the past been used instead of phylogenetic analyses has contributed to ambiguity; if one asks whether a gene found in eukaryotes is more similar to genes in bacteria or archaea, there are only three possible results:

1. The gene is most similar to genes in bacteria
2. The gene is most similar to genes in archaea
3. The gene is found in neither archaea nor bacteria (i.e. it is eukaryote-specific)

However, there are numerous ways in which a eukaryote gene with similarity to, say, an archaeal gene, might be related to that archaeal gene. Phylogenetic analyses can supply the additional information that permits different models to be distinguished. Of equal concern, similarity is known not to always equate with phylogenetic relationships (Koski & Golding 2001).

**Figure 1. Evolutionary trees depicting different possible relationships between Eukaryotes and Archaea. Tree 1 depicts Archaea and Eukaryotes as distinct monophyletic domains with a common ancestry. For both groups, the crown (triangle) represents the known modern diversity of the group. The stem illustrates that some evolutionary change has taken place since the two groups diverged. Early diversification cannot be observed along the stem as these groups have gone extinct (dashed lines), and no fossils exist. Note that extinction is likewise a feature of the crown. Tree 2 depicts Eukaryotes as a group within the diversity of modern crown group Archaea. Note that the existence of a eukaryotic stem is necessary since there are no extant intermediates ('living fossils') that can account for the large differences between modern archaeal and eukaryotic cell and genomic architecture. Tree 3 shows two alternative explanations that have been invoked to account for the topology of Tree 1 under models where Eukaryotes are proposed to have evolved directly from Archaea. In scenario 1, by invoking the existence of deep-branching archaeal lineages (which as yet have not been discovered), Tree 1 is converted into Tree 2. Scenario 2 seeks the same outcome as scenario 1, the difference being that in this case the deep-branching archaeal lineages are extinct (and hence unidentifiable).**



Perhaps the major problem that deep phylogeny suffers from is whether the results of a given analysis are correct. Indeed, Trees 1 & 2 in Figure 1 have both been reported, and it is as yet unclear which topology is correct (Daubin *et al.* 2002; Pisani *et al.* 2007; Rivera & Lake 1992; Tourasse & Gouy 1999; Woese & Fox 1977; Yutin *et al.* 2008). While this phylogenetic impasse is problematic, it is equally important to consider which hypotheses the alternative trees in Figure 1 could be claimed to support.

The literature is sloppy when it comes to interpretation of results, and several issues have become entangled. Perhaps the biggest difficulty is that there are considerable cell structural differences between archaea and eukaryotes, but no fossils or 'missing links' to indicate how these two different cell types might have diverged. As phylogenetic trees only include extant lineages, the picture is at best a partial one.

A clear example of how fossils can inform phylogeny is through the application of stem and crown groups. For instance, there are no extant intermediate forms between jawless and jawed vertebrates, making it difficult to establish evolutionary tempo and mode (e.g. Did jaws arise abruptly? Was there something special about this change?) from trees alone. Fortunately, informative fossils can be placed in the stem, illustrating that special explanations (e.g. genome duplication followed by a rapid burst of morphological evolution) are not necessary to

explain the stepwise evolution of jawed vertebrates (Donoghue & Purnell 2005).

This example illustrates the need to consider trees in terms of stem and crown groups. A crown group represents a monophyletic group of extant (and extinct) lineages, whereas the stem is by definition extinct: molecular phylogenies only cover the extant part of the crown. It is helpful to examine alternative phylogenetic relationships between archaea and eukaryotes within this framework because it clears up some of the misconceptions regarding eukaryote origins. Consider these different takes on eukaryote origins (Figure 1, Trees 1-3):

1. Eukaryotes and archaea are sister groups (Woese *et al.* 1990)
2. Eukaryotes group within the extant diversity of archaea (Pisani *et al.* 2007)
3. Eukaryotes appear as sister group to archaea because the deepest branching archaea have gone extinct; eukaryotes therefore have an archaeal origin (Yutin *et al.* 2008)

Trees 1 & 2 represent different phylogenetic results; whereas Tree 3 is an *explanation* of the result obtained for Tree 1 (i.e. extinction events preclude a complete picture from being realised). While the key point behind the application of stem and crown is that it enables the inclusion of fossil data, since no fossil data are available for the origin of eukaryotes and

archaea, how should a contention such as that illustrated in Tree 3 be dealt with?

Before addressing these three trees and their implications, a slight digression is required. As described above, the prevailing view on eukaryote origins is that eukaryotes evolved via some sort of union between, at minimum, an archaeon and a bacterium. Ignoring those models which invoke more than two partners (there is no good evidence for a three-way endosymbiotic origin for the eukaryote cell (Martin 1999; Poole & Penny 2007b)), we can focus on the most reasonable alternative to the archezoan hypothesis: namely, that the bacterial ancestor of mitochondria took up residence in an archaeon.

Returning to Figure 1, Trees 2 & 3 suggest on phylogeny that the ancestry of eukaryotes is archaeal; results of the form depicted in Tree 2 are taken as support of this because eukaryotes group within the diversity of modern archaea. In the case of Tree 3, the *phylogeny* (which is built from sequences from extant species) is the same as Tree 1, but unobservable extinction events are invoked that steer the interpretation towards that for Tree 2 (Yutin *et al.* 2008). This amounts to populating the eukaryote-archaeal stem with extinct and unobservable archaea. Under this model eukaryotes are phylogenetically archaeal, it's just that the archaeal lineages that would allow us to definitively conclude this have gone extinct. Tree 3 is thus an appeal to an archaeal origin, on the basis of apparently missing data. This type of argument has in fact appeared in two guises in the literature. The other is that, rather than early-diverging archaea being extinct, the full diversity of archaea is not as yet appreciated, and, with further exploration of microbial environments, deep-branching archaea will be identified that demonstrate an archaeal origin for eukaryotes (Davidov & Jurkevitch 2007). Both of these suggestions could of course be true, but the problem is that, without data, no case can be made. Curiously, however, the notion that eukaryotes evolved directly from archaea seems so deeply engrained, that many seem content to argue that we *ought* to accept this as true.

David and I disagreed with this on two levels. The first is a philosophical one, and involves teapots. The second relates to Lyell's lament (see above). We dubbed the form of explanation shown in Tree 3, the celestial teapot of phylogenetics (Poole & Penny 2007c), due to the clear parallel between the lack of evidence invoked in arguing archaeal origins for eukaryotes and Bertrand Russell's celestial teapot (which in its original form was an attack on religious belief (Russell 1952)). Said Russell,

*Many orthodox people speak as though it were the business of sceptics to disprove received dogmas rather than of dogmatists to prove them. This is, of course, a mistake. If I were to suggest that between the Earth and Mars there is a china teapot revolving about the sun in an elliptical orbit, nobody would be able to disprove my assertion provided I were careful to add that the teapot is too small to be revealed even by our most powerful telescopes. But if I were to go on to say that, since my assertion cannot be disproved, it is intolerable presumption on the part of human reason to doubt it, I should rightly be thought to be talking nonsense.*

The lesson here is that one can assert that deep-branching archaea exist (but have yet to be found), but a theory that requires this should not be preferred to theories which do not. Worse

is to propose that, rather than a sampling problem (for which proponents can take action by tirelessly searching for evidence), what we are faced with is an extinction problem. Aside from the minor issue that we cannot identify archaea from fossils, there would be no way of establishing whether they belonged to stem or crown.

Tree 1 does not suggest a direct archaeal origin for eukaryotes, so there is no requirement that archaea evolved into eukaryotes. However, were Tree 2 the correct tree topology – and this is our second point of disagreement – should such a result (i.e. an archaeal origin for eukaryotes) be taken to support unspecified mechanisms by which a bacterial cell gained entry into an archaeal cell (or by which an archaeal cell engulfed a bacterium)? Let's just separate these two points. The first concerns the tree topology. Tree 2 tells us that, phylogenetically, eukaryotes are a group within the wider diversity of archaea. If this result holds, there would be no denying an archaeal origin for eukaryotes. There is no reason *a priori* why this could not be the correct phylogenetic relationship. The second point concerns the *biological interpretation* of that tree topology, and this is where Lyell comes in. No archaea are known to carry bacterial endosymbionts, no archaea have been demonstrated to engulf bacteria, and no bacteria have been shown to be capable of invading archaeal cells. So while it would be phylogenetically correct to say that eukaryotes are a branch of archaea that picked up an endosymbiont, it is still necessary to explain how this could have occurred mechanistically.

Suggesting that the endosymbiont must have got in *somehow* is not sufficient; if we are to confine speculation to known causes, the key point we need to acknowledge is that the presence of endosymbiont-derived organelles is but one feature that can be placed in the common ancestor of eukaryotes. Other key features include the nucleus and endomembrane system, linear chromosomes, introns and a spliceosome. Without stem group fossils to separate the evolution of the eukaryote cell into a series of tidy steps, it is tricky to establish the order of emergence of these features. What we can do, however, is evaluate alternatives with respect to mechanisms in operation in the present. This still leaves us with a pile of unsolved mysteries, but the most important one – how the endosymbiont got its cell – can be resolved very easily.

## **Endosymbiosis by phagocytosis or by mechanisms unspecified?**

The propensity for one cell to engulf another is a trait specific to eukaryotes. Phagocytosis is observed in multicellular organisms (macrophages in the mammalian immune system eliminate pathogenic bacteria via this route), it is common amongst the algae and amoebae, and, importantly, every single organelle that has evolved from a free-living cell has been engulfed via this mechanism.

As outlined above, it is reasonable to expect that, regardless of the ultimate origin of the eukaryote cell (Figure 1, Trees 1 & 2), a mechanism of engulfment evolved before the engulfment of the mitochondrial ancestor. There is every reason to expect that that mechanism was phagocytosis (which evolved in some early eukaryote stem ancestor) (Poole & Penny 2007b), and not some special 'one-off' mechanism, which is no longer observable (Martin & Koonin 2006).

This is not an attempt to reinvigorate the archezoan hypothesis, which is clearly incorrect. However, this does not mean a protoeukaryote host (PEH) could not have engulfed the mitochondrial ancestor. These are two separate hypotheses and we need to be careful not to reject the PEH hypothesis simply because the archezoan hypothesis has been rejected. Although there are no extant PEH cells around (these would be archezoa), invoking the existence of extinct PEH cells is not the same as invoking an archaeal host for the mitochondrion. The reasoning is as follows. A PEH provides a mechanism for engulfment (phagocytosis) and numerous examples exist that illustrate how this process can lead to the formation of organelles from endosymbionts. With an archaeal host, no such mechanism or examples can be furnished. Even if eukaryotes evolved from archaea (e.g. Figure 1, Tree 2), there is still a requirement for a stem leading to modern eukaryotes, and on current knowledge it would be reasonable to expect that phagocytosis evolved in the eukaryote stem. The alternative, that it was a general feature of archaea, requires it has since been lost from all archaea, because no examples of archaea bearing bacterial (or other) endosymbionts are known, nor are there indications that archaea can engulf cells. Thus, an archaeal host cannot be mechanistically equated with phagocytosis.

In contrast, the varied forms of endosymbioses among extant eukaryotes suggest a series of steps by which a free-living cell can become a eukaryotic organelle (Poole & Penny 2007b):

**Step 1 — Predatory cells engulf bacterial prey via phagocytosis:** e.g. many amoebae are 'phagotrophic' (i.e. they engulf and digest other cells) (Greub & Raoult 2004).

**Step 2 — Prey cells evolve resistance to digestion:** this is well known among bacteria preyed upon by amoebae (Greub & Raoult 2004). Resistance is also known from human pathogenic bacteria, which can evade digestion by macrophages (Rosenberger & Finlay 2003).

**Step 3 — Emergence of a facultative symbiotic relationship between predator and prey:** a fascinating example is *Candidatus Odysella thessalonicensis*, an endosymbiont of *Acanthamoeba polyphaga*. At 22°C, this bacterium is a stable intracellular occupant, but will escape its amoebal host (through lysis) if the temperature is raised to 30–37°C (Birtles *et al.* 2000). Interestingly, there is evidence of cospeciation of bacterial endosymbionts and their amoebal hosts (their evolutionary histories mirror one another, as evidenced by congruent phylogenies (Beier *et al.* 2002)).

**Step 4 — The association becomes obligate:** the best studied example is *Buchnera*, a bacterial endosymbiont of aphids; both endosymbiont and host are obligately interdependent, and their evolutionary histories show clear evidence of cospeciation (Douglas & Raven 2003; Munson *et al.* 1991).

**Step 5 — The endosymbiont evolves into an organelle:** this has occurred on numerous occasions. The spread of phototrophy via phagotrophy has led to primary (i.e. bacterial ancestor of chloroplasts engulfed by a non-photosynthetic eukaryote), secondary (photosynthetic eukaryote engulfed by non-photosynthetic eukaryote) and tertiary endosymbioses (Archibald 2005; Raven 1997).

Clearly, none of these examples are directly linked to the establishment of the mitochondrion. However, they illustrate

that, by mechanisms in action in the present, past events can be explained without the need to invoke unknown processes (e.g. archaeal engulfment or bacterial invasion of archaeal cells). Given the ancestor of crown group eukaryotes possessed the capacity for phagocytosis, it is far more reasonable to argue that this feature evolved in the eukaryote stem, than that it evolved after the mitochondrion, which leaves us without a plausible mechanism for entry.

## Concluding remarks

The knee-jerk reaction to the rejection of the archezoan hypothesis has been to seek out an archaeal host for the mitochondrion. However, this is unnecessary, provided one understands how to read evolutionary trees. The PEH theory puts the biology back into the origin of eukaryotes by establishing a plausible mechanism by which an endosymbiont could have entered the intracellular milieu of an early eukaryote. There is no conflict between this and rejecting the archezoa hypothesis, and making this distinction is preferable to invoking *ad hoc* events or processes, which, in some formulations, are completely untestable. Clearly, the ideas of Darwin and Lyell are as relevant today as they were in the 19<sup>th</sup> century.

## Acknowledgements

Much of the material presented at this Symposium draws on papers I have published jointly with David Penny. David is a continuing source of inspiration and insight, and an exceptional collaborator. I gratefully acknowledge support from the Knut and Alice Wallenberg Foundation under the auspices of the Research Fellow programme of the Royal Swedish Academy of Sciences. Financial support from the Swedish Research Council is likewise gratefully acknowledged. Sincere thanks are also due to the School of Biological Sciences at the University of Canterbury for providing a stimulating intellectual environment during my sabbatical.

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