

## Primer

# The real 'kingdoms' of eukaryotes

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The diversity of life has generally been divided into a few — four to six — fundamental 'kingdoms'. The most influential system, the 'Whittaker' five kingdom structure, recognises Monera (prokaryotes) and four eukaryotic kingdoms: Animalia (Metazoa), Plantae, Fungi and Protista. Whittaker's system, somewhat modified, was presented as more realistic than the traditional division of life into animals and plants. Fungi, as well as prokaryotes, were separated from the plants, to which they are not related, while various protists were also released from artificial 'plant-like' or 'animal-like' designations.

Yet, the improvement is limited. In five kingdom systems, Protista is a grab-bag for all eukaryotes that are not animals, plants or fungi. This grab-bag is sometimes considered as an interim convenience or, very dubiously, to recognise a distinct 'grade' of organisation. In any case, most systematists and biology textbooks now hold that only monophyletic groups — an ancestor and all of its descendants — should be formally classified as higher taxa, overriding notions of convenience or grades. Nevertheless, the five kingdom system is still presented in some form in most biology textbooks.

The long popularity of the five kingdom system may be due more to the pedagogical and rhetorical utility of simply dividing life into a few groups than any biological realism. Summarising all organisms in five points on a slide or five chapters in a textbook is of great appeal in traditional, eukaryote-centric, biology (remember, four of these kingdoms are eukaryotic). With our historically poor knowledge of the deep-level relationships amongst eukaryotes,

biologically realistic alternatives would have involved literally dozens of eukaryotic 'kingdoms' — unpalatable for many communication purposes.

The last few years have seen dramatic improvements in our understanding of deep-level evolutionary relationships, thanks to advances in molecular phylogenetics, particularly in analytical methods, and in the diversity of organisms for which data are available, as well as a maturing of knowledge about some key features of eukaryotic cells. Eukaryotes at least can now be divided into just a few major groups that are probably all monophyletic. For the first time systems that reflect the actual relationships amongst eukaryotes can also be useful for communication. The following sections introduce each of these major groups.

### Opisthokonta

Opisthokonta contains animals and true fungi, as well as several unicellular groups, including the free-living choanoflagellates, a diverse range of parasitic forms called Ichthyosporea or Mesomycetozoa, and a group of free-living amoebae called the nucleariids. Two significant groups of spore-forming parasites, myxozoa and microsporidia, often considered as protists, turn out to be animals and fungi, respectively.

Some 19th and 20th century morphologists had suspected that choanoflagellates were involved in the evolution of animals, given their similarity to the choanocytes of sponges (both trap food particles using a microvillar collar surrounding a single flagellum). Choanoflagellates, most animal sperm and the zoospores of chytrids (the only fungi with flagella) all swim with their single flagellum emerging from their posterior end; surprisingly, this arrangement is nearly unique, and appears to have been inherited from the common ancestor of opisthokonts. In the early 1990s, several molecular phylogenies demonstrated that animals, choanoflagellates and fungi are specifically related. The other groups have been added

incrementally since then, largely through analyses of ribosomal (r)RNA gene sequences. Recent molecular studies indicate that choanoflagellates and Ichthyosporea are more closely related to animals than to fungi, but the precise highest-level relationships within Opisthokonta are still under investigation.

Animals are essentially the only multicellular predators on Earth, and although small forms overlap ecologically with some larger unicellular eukaryotes, they monopolise large heterotroph niches in all environments. Fungi are dominant osmotrophs, especially in terrestrial systems, playing crucial roles as decomposers and as symbionts or parasites of plants. Microsporidia infect a wide range of animals, including insects, and are probably amongst the most biodiverse of parasite groups.

### Amoebozoa

Most of the cells that move and feed using broad or finger-like pseudopodia are grouped together as the Amoebozoa. This group includes classical amoebae with broad pseudopodia, such as *Amoeba* itself, as well as the major groups of slime moulds and some mitochondrion-lacking organisms — pelobionts and entamoebae. Most Amoebozoa are free-living heterotrophs that engulf other cells using their pseudopodia (some large amoebae eat small animals!). The role of such amoebae in benthic ecosystems is probably very significant, but is still poorly understood. There are several facultative or obligate parasites, for example the amoebic dysentery agent *Entamoeba histolytica*, responsible for 40,000–100,000 deaths per year worldwide.

The slime moulds are amoebae that periodically form a stalked spore-producing phase called a fruiting body. For this reason alone slime moulds used to be treated as fungi, when other Amoebozoa were considered to be animals. In cellular slime moulds, such as *Dictyostelium*, many individuals aggregate to form a super-organism, in which some cells are sacrificed to create the stalk of the fruiting body — a form of

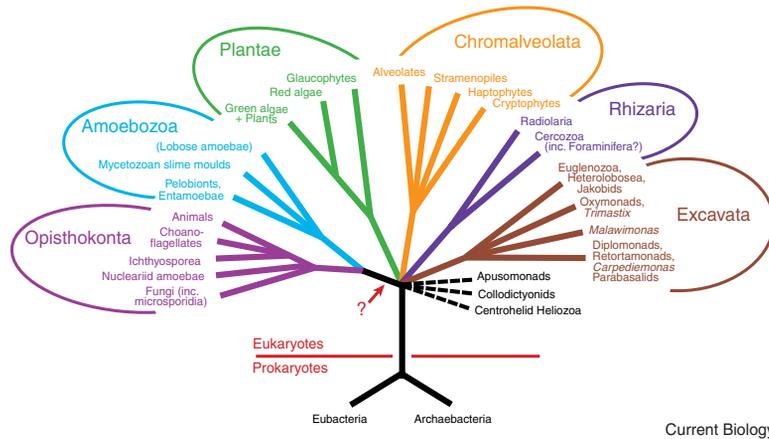


Figure 1. A diagrammatic tree depicting the organisation of most eukaryotes into six major groups. The relationships amongst most of the major groups and the position of the 'root' of the tree are shown as unresolved (note however, the grouping of Opisthokonta and Amoebozoa). The arrow shows a possible precise placement of the root, based on gene fusion data (see text).

multicellularity and differentiation, derived independently of animals, plants and fungi. Myxogastrid slime moulds, such as *Physarum*, form giant multinucleate super-cells (plasmodia) which are commonly found in terrestrial ecosystems.

Evidence that Amoebozoa is a monophyletic group has emerged only recently. Traditional classifications grouped diverse amoeboid organisms together; this united most or all Amoebozoa, but grouped them also with unrelated forms such as Radiolaria, Foraminifera and 'heliozoa' (see below). By contrast, early phylogenetic studies of rRNA sequences suggested the various Amoebozoa are independent groups. This now appears to have been an artefact of the simplistic analytical methods available at the time — improved analyses and sampling of more amoebae tend to show that these organisms are related, as do analyses of individual protein sequences (especially actin) and sophisticated studies of multiple proteins.

### Plantae

'Primary endosymbiosis' describes the origin of a eukaryotic organelle by the engulfment, enslavement and genomic reduction of a prokaryotic cell. Three photosynthetic groups have plastids (chloroplasts) that originated by primary endosymbiosis: land plants

(embryophytes) and green algae such as *Chlamydomonas*; red algae (rhodophytes); and an obscure group called the glaucophytes. Phylogenies of several plastid genes and the organisation of plastid genomes suggest that the plastids of these groups form a single lineage specifically related to cyanobacteria. So primary plastid endosymbiosis seems to have happened just once in eukaryotic evolution, with the host being a common ancestor of these three groups. Phylogenetic analyses, particularly some centred around the gene for elongation factor 2 indicate that 'reds' and 'greens' are closely related, with glaucophytes perhaps being their sister group. At present we follow many in the field, and refer to this whole group as 'Plantae', but we caution that most botanists use 'Plantae' for subsets of this group, such as green algae plus land plants.

The incorporation of the primary plastid had a huge effect on the genetic potential and basic biology of the host organisms. Almost all Plantae are specialist phototrophs; a few are non-photosynthetic parasites, but even these organisms retain plastids in a reduced form. Plantae are the only one of the major groups that may lack entirely the ability to engulf particulate food.

Multicellularity has evolved on several occasions within Plantae: probably once in red algae, but

multiple times within green algae. One particular multicellular assemblage, the 'charophytes', actually gave rise to the embryophytes that dominate land habitats, but are of very minor importance in the ocean (where, in fact, extremely small unicellular green algae are significant).

### Chromalveolata

In 'secondary endosymbiosis' a eukaryote already containing a primary plastid is engulfed by another host eukaryote, and over time is reduced to an organelle. The new plastid-containing host is termed a 'secondary alga'. Secondary endosymbiosis has happened more than once in eukaryotes, but mounting evidence from plastid gene trees and a distinctive gene replacement event suggests that most groups of secondary algae descend from one particular endosymbiosis involving a red algal symbiont. These organisms, plus their many non-photosynthetic relatives, comprise the group Chromalveolata.

The chromalveolates unite four major groups of eukaryotic algae: dinoflagellates, cryptophytes, haptophytes and stramenopiles (~heterokonts), and many non-photosynthetic forms (see below). The first three groups are unicellular, with a few colonial forms. Stramenopiles, however, range from tiny unicells, through to elaborate unicells and colonies, for example diatoms, and truly multicellular and massive life forms, such as kelps. Dinoflagellates and diatoms are the dominant 'large' phytoplankton in the ocean.

Dinoflagellates and stramenopiles also include a wide diversity of heterotrophic forms (and mixotrophs, organisms that subsist by both photosynthesis and heterotrophy). Heterotrophic stramenopiles are very important consumers of bacteria in aquatic environments, but also include some animal parasites/commensals, and a diversity of fungal-like forms. For example, the Irish potato famine pathogen, *Phytophthora infestans*, is an oomycete stramenopile. Heterotrophic and mixotrophic dinoflagellates are important

micro-predators in the plankton, and there are numerous parasitic forms.

Despite its apparent algal origins, Chromalveolata includes two of the best known groups of non-photosynthetic microbial eukaryotes. Ciliates are dominant micro-predators in many habitats, and include several important laboratory models, such as *Paramecium* and *Tetrahymena*. Apicomplexa are arguably the most successful group of specialist parasites on earth, and include the agents of toxoplasmosis, cryptosporidiosis, coccidiosis and, of course, malaria (*Plasmodium*). Although Apicomplexa live inside their hosts, usually in total darkness, most have retained a non-photosynthetic plastid, betraying their algal ancestry and providing a tempting target for novel drug therapies.

#### Rhizaria

Over the last decade, this novel major grouping of eukaryotes has been delimited mainly thanks to improvements in the sampling of rRNA genes. Rhizaria unites a wide diversity of free-living unicellular organisms, many of which feed using fine 'filose' pseudopodia, together with some fungi-like plant parasites, such as plasmodiophorids, and some animal parasites, for example Haplosporidia. The best-known free-living Rhizaria are Foraminifera and Radiolaria. The exact relationships amongst all of these organisms is still uncertain — most likely Radiolaria are the sister group to all the others, most or all of which form a large subgroup called Cercozoa.

Foraminifera are planktonic or benthic marine amoebae, many of which are large and construct external mineralized shells that fossilize well and are important geological indicators. Radiolaria are large planktonic marine cells, generally with beautiful radially symmetrical spiked internal skeletons. Most other free-living Rhizaria are heterotrophic flagellates or amoebae that consume other microbes associated with surfaces, and are probably extremely important in understudied benthic and soil

habitats. The chlorarachniophytes, however, are mixotrophic secondary algae that contain a plastid of green algal origin, stemming from a completely separate secondary endosymbiosis from that of chromalveolates. Many Foraminifera and Radiolaria contain algal symbionts.

#### Excavata

Excavata are unicellular eukaryotes, most of which are heterotrophic flagellates. They include several groups that cause significant disease, such as trypanosomatids, diplomonads and parabasalids, which include the agents that cause sleeping sickness, giardiasis and trichomoniasis, respectively. But each parasitic group has free-living relatives that consume other microbes, often capturing them out of suspension using a distinctive feeding groove. Many excavates have greatly modified mitochondria that are not used for oxidative phosphorylation, and these cells are common in low-oxygen habitats, including animal guts. Members of one group, Heterolobosea, have evolved as broad-pseudopod-forming amoebae independently of Amoebozoa, and even include their own group of slime moulds, the acrasids. Another group, the euglenids, includes another independent lineage of secondary algae; the laboratory standard *Euglena* is an example.

Excavata is the most contentious of the major groups of eukaryotes. Early molecular phylogenetic analyses had suggested that many groups of excavates are separate early branches in the eukaryotic tree; however, more sophisticated analyses have deflated this model. The most recent phylogenetic studies, some using multiple proteins, divide excavates into about four strongly supported subgroups, but are equivocal as to whether those subgroups are specifically related. However, contemporary electron microscopy studies indicate that members of each strong subgroup do indeed share a common ancestor with a distinctive morphology.

#### Other groups

The six major groups include all eukaryotes that can be considered 'well-known'. It is possible, however, that a few poorly known microbial forms might represent one or more additional distinct groups. There are several small groups of free-living heterotrophs — centrohelid heliozoa, apusomonads and collictyonids, for example — for which both morphological and molecular studies have failed, so far, to reveal close evolutionary affinities. There is a longer list of even more obscure 'mystery taxa' that have not been examined using molecular techniques, and must remain candidate representatives of novel major groups.

Recently, several research teams have examined eukaryote biodiversity by obtaining rRNA gene sequences directly from the environment, rather than from cultures. Some studies report several uncultured 'kingdom-level' groupings, suggesting that the 'familiar' eukaryotes might represent only a fraction of the high-level diversity in nature. But careful re-analyses incorporating data from more cultured organisms indicate that most genuine environmental sequences are actually related to known groups; some of the most distinct types turn out to be undetected artificial fusions of two unrelated sequences! Increasingly, it appears that the 'six major groups' will encompass the bulk of extant eukaryotic biodiversity, however it is measured.

#### Relationships amongst the major groups

Identifying six natural groups of eukaryotes raises the question: what are the relationships amongst them? Molecular phylogenetics could provide the answer in principle, but there are tremendous practical difficulties. As we look further back in time, most historical signal is lost from present day molecular sequences, so that non-historical (artefactual) signals in the same data can easily obscure the true relationships.

There are two main solutions being explored. The first is to analyse many — potentially

hundreds — of genes together, aiming to combine the numerous weak historical signals into a strong signal. Misleading signals could then be over-ridden, or 'weeded out' from the larger data sets. The second approach is searching for discrete 'evolutionarily unlikely' characters that are shared by major groups, such as large insertions or deletions in coding sequences, or fusions between genes. Of course, individual characters of this sort might themselves mislead, because of convergence, differential loss or even lateral gene transfer between distantly related eukaryotes, so multiple lines of evidence will still be required.

In this vein, both protein phylogenies and a complex fusion of pyrimidine synthesis genes suggest that Opisthokonta is most closely related to Amoebozoa. This grouping has been called 'unikonts'. Interestingly, a different fusion character, involving the genes for dihydrofolate reductase and thymidylate synthase, suggests that all other major groups of eukaryotes might be specifically related to each other. Pending confirmation from other evidence (see the caveat in the previous paragraph), these data imply that the root of eukaryotes falls between unikonts and everything else, along the branch indicated by an arrow in Figure 1.

#### When did eukaryotes diversify?

While there is increasing agreement on the deep-level structure of the tree of eukaryotes, placing this diversification in time remains contentious. The earliest fossils very widely accepted as assignable to a living eukaryote group are 1.2 billion year old red algae (Plantae). From our current understanding of eukaryote phylogeny, this implies that much of the diversification into the six major groups happened before 1.2 billion years ago, perhaps before 1.7 billion years ago if more uncertain fossils and chemical biomarkers are believed. Yet even the red algal fossils predate by ~400 million years the next material that is clearly assignable to a living eukaryote group. This either

reflects extreme patchiness of the fossil record, or, according to some, indicates that the 1.2 billion year old fossils are actually not red algae, and that eukaryotes diversified within the last ~800 million years, perhaps co-incident with the 'Snowball Earth' glaciations. There is also debate as to whether the living eukaryotes radiated over a very short time, in a rapid 'big bang' precipitated by some key evolutionary innovation, or whether the six major groups diverged over many hundreds of millions of years.

Recently multi-gene 'molecular clock' analyses have been used to estimate the timing of eukaryote diversification. Studies with different gene sets, phylogenetic trees and analytical methods give estimates ranging from ~1 billion years to some 2.8 billion years. This huge disparity is perhaps not surprising given that such analyses are extrapolations based on much younger fossil dates and assume that no genome-wide and sudden 'clock' speed-changes occurred in ancestral lineages. These and other methodological problems still seriously compromise deep molecular clock dating. Without new fossil (or ancient biomarker) finds, the precise age of eukaryotes and the tempo of their divergence are unlikely to be resolved in the near future.

#### Ancestral eukaryotic cells

Eukaryotic cells are drastically different from their presumably prokaryotic ancestors. With the limited fossil record, researchers have tried to understand the evolution of the eukaryotic cell by identifying living eukaryotes that are 'primitive' in some aspects. The primitive status of a group is untenable, however, if phylogenetic studies indicate that it is closely related to 'complete' eukaryotic cells. In fact, all the groups of eukaryotes seriously suggested to be primitive eukaryotes now seem to be related to 'complete' forms (most fall within Excavata). The last common ancestor of living eukaryotes now appears to have been a 'complete' eukaryotic cell. It had a nucleus, endoplasmic reticulum and Golgi apparatus, and underwent mitosis

and meiosis. It had mitochondria capable of oxidative phosphorylation, amongst other functions, and was presumably aerotolerant. It had a complex eukaryotic cytoskeleton including eukaryotic flagella (most likely a pair of them), and was heterotrophic, consuming food particles by phagocytosis. The only major eukaryotic features that seem to be of later origin are plastids. We are now left with an intriguing and difficult question: did living eukaryotes diverge shortly after the rapid and drastic evolution of the eukaryotic cell, or was this cell assembled gradually, but with modern eukaryotes then replacing all intermediate forms?

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