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The mother of all parasites

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Evaluation of: Moore RB, Obornik M, Janouškovec J *et al.*: A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* 451(7181), 959–963 (2008).
Malaria and related apicomplexan parasites contain a relict plastid (apicoplast) that is a promising drug target. The apicoplast has been argued to derive from either an engulfed red or green alga. The discovery of the first photosynthetic apicomplexan, dubbed *Chromera velia*, with a fully functional plastid resolves the debate, clearly showing that the relict plastid is derived from a modified red alga. Intriguingly, *C. velia* is a coral symbiont and thus reminiscent of the closely related dinoflagellate symbionts (zooxanthellae) vital to corals and many other invertebrates. Symbiosis and parasitism are thus wide-spread in both the dinoflagellates and apicomplexans, suggesting that modern parasites like *Plasmodium* spp. and *Toxoplasma* likely started out as mutualistic symbionts that initially nourished their animal hosts before turning to parasitism. These symbiotic/parasitic relationships thus extend back in evolutionary time to the earliest origins of the animals, which means that either as parasites or symbionts, these protists have been interacting with the animal immune system since its inception. As a consequence of this protracted dance, malaria parasites are exquisitely well-equipped to evade our immune system: a sobering harbinger for malaria vaccine prospects.

A new phylum of algae discovered in Sydney harbor appears to be the ancestor of one of humanity's worst scourges [1]. Malaria has won and lost wars, contributed to the fall of the Roman Empire and remains a key obstacle to development and eventual prosperity in Africa [2]. The malaria parasite infects 500 million people annually and inflicts a tragic toll, particularly in sub-Saharan Africa where one child dies every 30 seconds from infection with mosquito-borne *Plasmodium* [2]. The identification of relict plastids (with the same evolutionary ancestry as the chloroplasts of plants and algae) in malaria and thousands of related parasites like *Toxoplasma* revolutionized our understanding of this parasite group and offered a novel line of attack, using herbicides to kill the parasites [3–5]. Although no longer photosynthetic, the plastids (known as apicoplasts, for apicomplexan plastids) are essential to parasites [6–8]. However, the origin of the parasite plastid has long been unresolved [9–13]. In a recent issue of *Nature*, Moore *et al.* describe a new photosynthetic organism, *Chromera velia*, that effectively ends a decade-long argument about how parasites came to be in possession of a plastid at all [1]. Moreover, the new alga provides a very revealing glimpse into what the ancestors of malaria parasites were like several hundred million years ago. The discovery of *Chromera* also reminds us that the current battle between our immune system and the malaria parasite goes a very, very long way back, perhaps explaining why the parasites are so effective at evading immune attack.

Since its discovery about 10 years ago the relict plastid of malaria parasites has been the subject of much research focused on exploiting the organelle as a drug target to fight disease [8,14]. The malaria parasite plastid is nonphotosynthetic but is clearly homologous to the light-catching plastids of plants and algae [3]. Our best guess has been that the ancestors of the malaria parasite, and indeed the entire phylum Apicomplexa to which malaria and some 5000 other parasites belong, were originally photosynthetic but converted to parasitism and abandoned the ability to synthesize food from light and CO_2. Why the parasites retained the plastid after having jettisoned photosynthesis is still not entirely clear. Like plant and algal plastids, the apicoplast harbors a small circular genome, a set of gene expression (housekeeping) machinery [15] and a collection of metabolic synthesis pathways [6]. It is assumed that these anabolic pathways make components on which the parasite is dependent, as the apicoplast is essential for parasite survival: genetic or pharmacological perturbation leads to parasite death [6]. Therefore the apicoplast, whose metabolism is more similar to plants than human hosts, is considered to be a promising drug target.

Keywords: apicoplast, *Chromera velia*, endosymbiosis, malaria, *Plasmodium*, plastid evolution, *Toxoplasma*, vaccine

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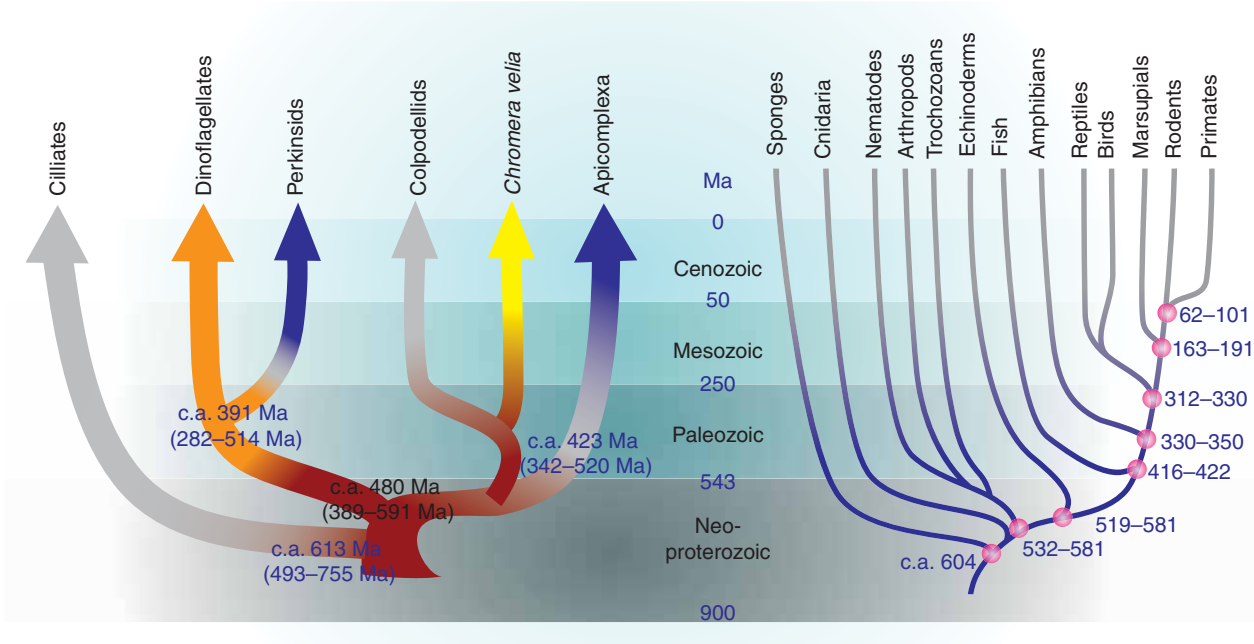
Importance as a potential drug target notwithstanding, the evolutionary origin of the apicoplast has remained elusive. Everyone agrees that the apicoplast is derived by secondary endosymbiosis of a eukaryotic alga to create a cell-within-a-cell, but there has been a protracted debate as to whether the engulfed cell was a red or a green alga [9–13]. Because apicoplasts are vestigial and lack pigments and genes for photosynthesis, it has been difficult to compare them with green and red algae using traditional biochemical and molecular approaches. To compound the problem, it seems that those genes still retained in the apicoplasts have undergone a tremendous burst of evolution, masking their origins in evolutionary reconstructions.

Even the plastids in organisms such as dinoflagellates, which are closely related to apicomplexan parasites (Figure 1), have been difficult to compare. Apicomplexans, along with dinoflagellates and ciliates, belong to the supergroup Alveolata (Figure 1). Ciliates apparently lack a plastid, but dinoflagellates are photosynthetic and contain a secondary endosymbiotic plastid of red algal origin. Even so, comparison between the apicoplast and the dinoflagellate plastid shows two markedly different plastids (Table 1). Apicoplasts are bound by four membranes and have little, if any, substructure. Conversely, the typical dinoflagellate plastid has three membranes and triple-stacked thylakoids (membranes carrying the photosynthetic machinery) (Table 1). Comparing plastid genes is also fraught with difficulties. The apicoplast has a fairly standard, although highly reduced, circular genome [15], but the plastid genome of dinoflagellates underwent significant fragmentation, keeping each photosynthesis gene on a small separate mini-circle [16], which prevents the usual genome architecture comparison. To make matters worse, these single gene mini-circle genes in dinoflagellate plastids have diverged even more rapidly than apicoplast genes, rendering the inference of their relationships virtually impossible with existing phylogenetic reconstruction tools [16].

Results from the paper: new alga demonstrates a common origin for apicoplasts & dinoflagellate plastids

The discovery of a novel photosynthetic alga, *C. velia*, by Moore *et al.* has provided the exact intermediate or transition form that allows us to bridge the difference between parasite apicoplasts and dinoflagellate plastids [1]. *C. velia* is

Figure 1. Evolutionary trees of the alveolate protists and the animals with comparable timelines.



The new alga, *Chromera velia*, described by Moore *et al.* is a photosynthetic symbiont of corals and sister to colpodellids, a group of predatory apicomplexans [1]. *Chromera* is thus a relative of malaria and *Toxoplasma* parasites. Apicomplexa and dinoflagellates, also symbionts of corals and invertebrates, diverged some 400–600 million years ago, suggesting their ancestors were likely symbionts/parasites of the earliest animals at the point when the immune system evolved.

Table 1. Features of the plastids of dinoflagellates, apicomplexans and *Chromera velia*.

	Dinoflagellates	*Perkinsus*	Apicomplexans	*Chromera velia*
Plastid membranes	3	4	4	4
Major pigments	Chl. *a*/*c*, peridinin	Nil	Nil	Chl. *a*, Vaucheriaxanthin, novel carotenoids
Plastid genome	Numerous mini-circles	?	Single, circular 35-kb genome	?
UGA codon	Stop	?	Tryptophan	Tryptophan

Chl. a: Chlorophyll a; Chl. a/c: Chlorophyll a/c.

superficially very similar to some symbiotic dinoflagellates of corals. However, molecular phylogeny of nuclear genes shows that *C. velia* is more closely related to the apicomplexans than the dinoflagellates [1]. Moreover, the plastid of *C. velia*, although photosynthetic, is distinct from that of dinoflagellates in that it lacks two of the major pigments characteristic of dinoflagellates (chlorophyll *c* and peridinin), and it is bound by four membranes (Table 1). Another feature of the *C. velia* plastid shared with parasite apicoplasts is an alternate genetic code; the *psbA* gene of *C. velia* utilizes UGA codons (normally a stop codon) for tryptophan [1]. Apicoplasts also use this noncanonical code (some mitochondria also evolved this code independently). Thus, the numbers of plastid membranes, and the unique codon usage, make us confident that the plastid of *C. velia* is a photosynthetic representative of the apicoplasts of apicomplexan parasites. What can we infer from its genes?

The *C. velia* plastid rRNA gene confirms its apicomplexan affinity and, significantly, the photosystem protein (PsbA) of *C. velia* is closely related to that of the dinoflagellates [1]. *Chromera* thus corroborates the hypothesis that dinoflagellate plastids and apicoplasts share a common origin from an engulfed red alga [17].

Significance of the results: ancient origin of symbiosis & parasitism?

We previously suggested that apicomplexan plastids and dinoflagellates were ancestrally photosynthetic and that their common ancestor likely formed symbiotic associations with animals, probably similar to the symbiotic relationships between modern invertebrates and zooxanthellae (dinoflagellates including the genus *Symbiodinium*) [18]. *C. velia* is thus an example of a classic transition form supporting this hypothesis as it is photosynthetic, a symbiont of animals and an apicomplexan [1].

The existence of *C. velia* allows us to speculate on the ancient origin of symbiosis and parasitism. Dinoflagellate symbionts inhabit corals, foraminifera, radiolarians, flatworms, anemones, jellyfish and bivalve mollusks [19]. Are there yet-to-be-discovered *C. velia*-like symbionts in other invertebrates? Just as parasitic apicomplexa infect the majority of invertebrates [20], photosynthetic symbiotic ones may also be widespread. Molecular prospecting in invertebrates for *Chromera*-like sequences is probably worthwhile.

In a broad sense, symbiosis and parasitism are similar phenomena. Both require three key underpinning biological processes: host/symbiont recognition, a mechanism for invasion/uptake and transport of metabolites and small molecules between partners. If we look at the distribution of these two interactions among extant alveolates, we see symbiontism and parasitism throughout the members, which suggests that these lifestyles are an ancestral trait and that the prevalence of parasitism in apicomplexa is a relatively recent trend (in evolutionary terms). As *C. velia* is a coral symbiont [1], it is tempting to speculate that the common ancestor of *C. velia* and dinoflagellates was also a symbiont in corals (and probably other invertebrates). Latter emerging lineages of apicomplexans could then have converted to parasitism, abandoning photosynthesis but retaining the plastid (apicoplast). How does this hypothesis fit with evolutionary timescales?

A recent molecular clock study estimates the divergence of the common ancestor of apicomplexans and dinoflagellates from ciliates to have occurred approximately 613 million years ago [21]; contemporaneous to the establishment of cnidarians some 604 million years ago (Figure 1) [22]. Thus, we can hypothesize that the invertebrate fauna of the Precambrian ocean hosted symbionts/parasites ancestral to modern alveolates. Indeed, it would be naive to think that these animals were parasite/symbiont-free, and the alveolates were present at the time. If this scenario is true, apicomplexan parasites have infected animals for hundreds of millions of years; their ubiquity among animal phyla is consistent with this. Indeed, one scenario suggests

that apicomplexan parasites evolved in parallel with animal evolution [20], and this means they have probably been interacting with the animal immune system since its inception [23–25]. Theories on the origin of the immune system in metazoa describe its development from simple systems occurring in sponges and other early animals [24]. The importance of these systems in managing interactions with symbionts, as well as invaders, has been stressed [23]. The presence of symbionts/parasites ancestral to modern alveolates likely had a profound influence on the origins of innate immunity. Looking at this from a different perspective, we may have to face the fact that pathogens like malaria have been evading the best efforts of the animal immune system for hundreds of millions of years and have seen every innovation and new method of attack and avoided them all. Such an arms race puts the challenge of developing vaccines against malaria and other apicomplexan parasites into perspective.

Conclusion & future perspective

The discovery of *C. velia* all but finishes the long argument about the origin of the apicoplast, providing firm evidence for a common origin with dinoflagellate plastids from a single red algal endosymbiont. It will be interesting now to determine how widespread *C. velia* is as an endosymbiont, to investigate its plastid genome, and look at its apical complex. The little alga from the bottom of Sydney harbor may eventually be enlisted in the fight against malaria.

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Executive summary

Methods

- Microbes were extracted from corals and cultured in a marine algal culture medium under lights.
- Gene phylogenies were used to identify a photosynthetic apicomplexan.
- Electron microscopy confirmed the presence of alveoli (sub-plasmalemmal membrane sacs diagnostic of apicomplexa and related protists).

Insights

- *Chromera velia* is a symbiont of coral closely related to apicomplexans.
- *C. velia* confirms a single origin of plastids of apicomplexans parasites and dinoflagellates (photosynthetic algae).
- The apicoplast derives from an endosymbiotic red alga.
- The plastid of *C. velia* and apicomplexan plastid share a four-membrane-bound plastid and noncanonical UGA codon for tryptophan.

Conclusion

- Photosynthetic apicomplexa with fully functional plastids (apicoplasts) exist. Symbiosis and parasitism are widespread in Alveolata, suggesting an ancient origin.

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