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Evolution: Untangling the mix of plastid endosymbiosis events

Patrick J. Keeling[1] and Fabien Burki[2]
[1]Botany Department, University of British Columbia, Vancouver, BC, Canada
[2]Department of Organismal Biology, Program in Systematic Biology, Uppsala University, Uppsala, Sweden
Correspondence: pkeeling@mail.ubc.ca (P.J.K.), fabien.burki@ebc.uu.se (F.B.)
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The demonstration that a plastid protein targeting system remained unchanged following the endosymbiotic transfer to a new host calls into question whether we can distinguish between different models commonly used to explain the distribution and origin of eukaryotic organelles.

It is generally agreed that plastids and mitochondria originated by endosymbiosis, but using the word 'endosymbiosis' may give a false impression of a specific mechanism, when it is really just shorthand for a number of complex processes that we are only beginning to understand. Endosymbiosis is 'what' happened, but to know 'how' it happened, one must look deeper into the mechanisms that underpin it. New insights published in a recent issue of *Current Biology* from Lewis *et al.*[1] do just that, describing the key mechanism of protein import in a particularly complex organelle, with implications that ripple through fundamental theories and models for endosymbiosis and the evolution of organelles.

Of all the outstanding mysteries surrounding endosymbiotic organelle origins, probably the most vexing is that we do not know how many times it took place. While mitochondria are in all eukaryotic lineages and arose once, plastids are scattered across the tree of eukaryotes higgledy-piggledy. This is because after plastids first originated through endosymbiosis with a cyanobacterium, they spread from one eukaryote to another through additional endosymbioses[2–4]. This process can have several layers: the original uptake of a cyanobacterium is called 'primary endosymbiosis', and when one of the resulting primary plastids is transferred and integrated into a new host, it is called 'secondary endosymbiosis'. This process has gone at least one step further, as secondary plastids have been taken up in 'tertiary endosymbiosis' events, but there has been increasing disagreement on whether a given plastid may be the result of secondary, tertiary, or even higher levels of endosymbiosis.

We do not wish to debate the merits of these arguments, so for clarity here we will refer to haptophyte plastids as secondary, and Kareniaceae plastids as tertiary, recognizing that some authors would count them differently[5–7]. Indeed, this uncertainty goes to illustrate how even fundamental facts about the number and nature of endosymbioses remain matters of debate, a debate which has coalesced around two main models that differ in the relative importance they assign to plastid gain *versus* loss[4]. These opposing forces can both explain the same distribution, for example in the hypothetical case in Figure 1. An ancient endosymbiosis followed by numerous plastid losses (left-hand case) can lead to the same patchy distribution as a series of more recent, recurring endosymbioses moving the plastid between lineages (right-hand case). On the face of it, these explanations are so fundamentally different that they should be simple enough to distinguish, but the reality is more difficult because we know so little about the two mechanisms that are core to both models: loss and recurring gain.

Lewis *et al.*[1] tackle one of the key questions in recurring endosymbiosis — how the host targets proteins to the organelle. This is fundamental to understanding how the organelle evolved because protein targeting is not only central to controlling the organelle function, but also one of the only novel systems that had to evolve to enable the integration. Using transcriptome sequencing and cryoelectron tomography, they show that the tertiary haptophyte-derived plastids in Kareniaceae dinoflagellates have plastid-targeting machinery and a plastid envelope that are virtually the same as they were in the original haptophyte host. This is more surprising than it may sound at first, because successive rounds of endosymbiosis are known in other cases to add membranes to the plastid envelope, and each new membrane represents another hurdle that targeted proteins must clear in order to make it into the plastid[8]. Primary plastids have two membranes and a protein-targeting complex in each, whereas secondary plastids have three or four membranes and additional targeting steps. The number of membranes surrounding the tertiary Kareniaceae plastid has never been clear, but there were thought to be more than the four membranes found in haptophyte plastids. Lewis *et al.* now show this is not the case — the Kareniaceae they examined have either four or three membranes, and they also possess the same set of protein targeting complexes found in haptophytes.

How these haptophyte plastids were so seamlessly 'plugged into' new hosts is a fascinating feat of cell and evolutionary biology, but it also impacts the way we think about the overall distribution of plastids in other important ways. If the protein targeting system and membrane arrangement of a tertiary plastid can be virtually identical to those of a secondary plastid, it makes it easier to explain recurring rounds of endosymbiosis — as this paper nicely argues — but it also means that secondary and tertiary plastids cannot reliably be distinguished by their import machinery. It is possible

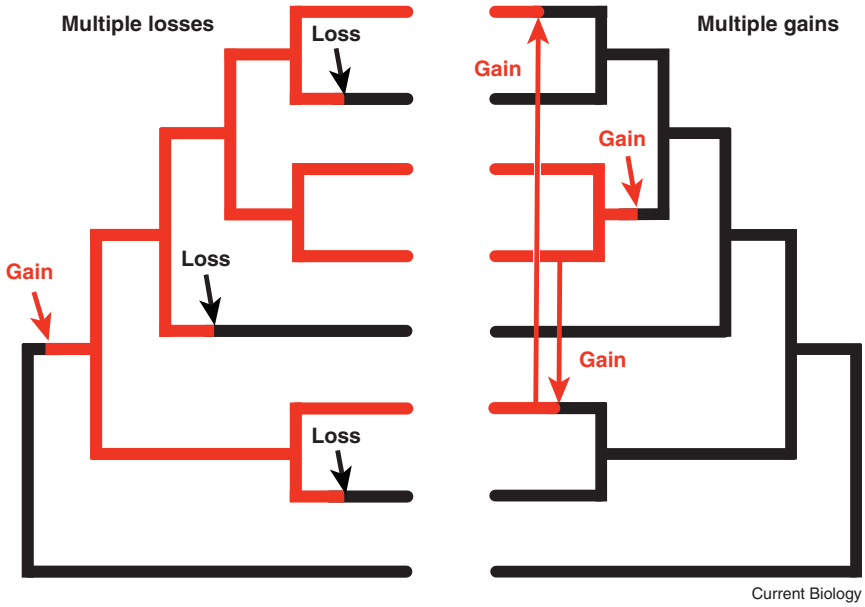

Figure 1. A hypothetical scheme to show how two different evolutionary processes can lead to the same pattern of plastid distribution.
On the left, a single ancient gain of a plastid is followed by multiple parallel plastid losses, leading to a patchy distribution. On the right, a more recent origin is followed by multiple secondary and tertiary endosymbiotic transfers of that plastid to different lineages, leading to the same patchy distribution. Though very different, these two processes are difficult to distinguish.

that they could be different, but this case shows we cannot assume they must be different. In the case at hand, we can still be confident that kareniaceans have tertiary plastids because of their evolutionary context: the host cells fall deep within dinoflagellates and yet they clearly have a haptophyte plastid. But this is only clear because the endosymbiosis took place rather recently — what if the endosymbiosis was much more ancient and the phylogenetic context much less clear? The current data suggest we can never really be certain whether an ancient plastid arose from secondary or tertiary endosymbiosis.

At the same time, it has also become increasingly apparent that we cannot distinguish a cell that lost a plastid from one that never had one[9]. It was long assumed that when a cell acquired a new plastid through endosymbiosis, it would undoubtably acquire many new genes from the endosymbiont that do not necessarily function in the organelle[10]. If the organelle was later lost, it was commonly assumed that these genes would remain and be detectable, serving as a molecular 'footprint' of past endosymbiosis[11]. However, genomic data do not always bear this out: there is little evidence from algal genomics of such a molecular footprint, and in the few clear cases of outright organelle loss, there is no evidence of the host retaining genes detectably derived from the organelle[9].

As a result, we find ourselves in a philosophical quandary, where two competing stories to account for the distribution of plastids in eukaryotes are mechanistically and evolutionarily distinct (Figure 1), but potentially indistinguishable. Phylogenetic context can make it clear when plastid loss or complex muti-layered endosymbioses have taken place, but without such context, in ancient lineages for example, it is possible that any data that existed could only be used to verify either hypothesis, and no data would ever be able to refute it. For example, tertiary plastids could conceivably evolve targeting systems that are distinguishable from secondary plastids (e.g., having extra membranes and targeting complexes), and identifying such differences could verify the hypothesis that the plastid arose by tertiary endosymbiosis. But if it is possible for them to share the same targeting system, as the current analysis indicates[1], then you can never refute the hypothesis that any apparently secondary plastid is actually tertiary. Similarly, an ancient lineage that had completely lost an ancestral plastid could conceivably retain genes to verify the hypothesis that it once was there, but if it is possible that such a cell retains no trace of the former organelle, then you can never refute the hypothesis that one was once there.

To make matters even more complex, we must also concede that we cannot be certain that either of these competing models explains the entire distribution of plastids. Biology and the evolutionary history that led to its diversity are complicated, chaotic, and messy. It is easy to believe that there should be laws that govern biological processes at levels where stochasticity and contingency play too large a role for this to be the case. There is often no reason why the events underlaying the distribution of characters like plastids in eukaryotes might not be a combination of several processes. In this instance, the distribution of secondary red plastids in some lineages might be due to multiple parallel losses of ancestral plastids, while in others they might have arisen by a series of recurrent endosymbioses. We need not hold up the two most extreme cases and argue about which is correct, because the actual answer may be that both explain different aspects of diversity.

Recognizing the quandary we are in is important, but because most of the vast diversity of protists has yet to be explored, there is still reason for optimism that a 'smoking gun' to unambiguously distinguish plastid types or plastid loss will emerge from further exploration. But in the meantime, we need to be keenly aware of the limitations of our evolutionary modelling.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Insect chemosensation: A grapefruit squeeze on mosquito-borne disease

Joshua U. Abel-Nwachukwu and Benjamin J. Matthews*
Department of Zoology, The University of British Columbia, Vancouver, BC, Canada
*Correspondence: ben.matthews@ubc.ca
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Widespread insecticide resistance has sparked the search for new strategies to combat vector-borne disease. Recent work uncovers the mechanism behind nootkatone, a grapefruit-derived compound, which works as a dual-action repellent and insecticide, highlighting its potential as a powerful tool for mosquito control efforts.

For centuries, humans have waged a relentless battle against mosquitoes — small yet formidable adversaries that have altered the course of history through the diseases they transmit[1]. Our chemical combat strategy typically takes two forms: repellents, which keep mosquitoes from biting, and insecticides, which eliminate them outright. Chemical insecticides have played a key role in mosquito control efforts for many years, but their continued use has produced a major unintended consequence — insecticide resistance[2]. In response, researchers have scurried to find alternative options, now including nootkatone, a fresh and herbal-smelling compound naturally occurring in grapefruits and cedar trees and which shows potential as both a repellent and insecticide in a variety of medically important arthropods[3]. But what exactly is the secret behind nootkatone's potency? New research by Fernandez Triana, Andreazza *et al.*[4] in a recent issue of *Current Biology* reveals a dual mechanism of action: nootkatone acts as both a spatial and contact repellent by targeting two key chemosensory pathways, the Orco-dependent olfactory receptors (ORs) and the ionotropic receptors (IRs). Additionally, nootkatone functions as an insecticide by enhancing GABA signaling, specifically through the broadly expressed GABA-gated chloride channel known as Resistant to dieldrin (Rdl), ultimately causing paralysis and mortality[4].

Nootkatone has been previously proposed as a rival to today's mosquito control heavyweights such as DEET and Picaridin[3,5,6], but its molecular targets and its efficacy in head-to-head comparisons against the current state of the art repellents remained unclear. Fernandez Triana, Andreazza *et al.*[4] launched their own investigation into nootkatone's multiple modes of action: spatial repellency, contact repellency, and insecticidal activity. The authors first placed mosquitoes in a dual-chamber setup devoid of host cues where one side was coated with nootkatone and the other left untreated. Remarkably, three species of mosquito — the yellow fever mosquito *Aedes aegypti*, the southern house mosquito *Culex quinquefasciatus*, and the African malaria mosquito *Anopheles gambiae* — were completely repelled by nootkatone, matching the performance of DEET, the current gold standard in repellents. However, the authors found that volatile nootkatone had no impact on mosquito flight trajectories towards a human hand, in contrast to DEET, which

