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Endosymbiosis: The feeling is not mutual

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ABSTRACT

Endosymbiosis is an idea that provided a remarkable amount of explanatory power about the origins of eukaryotic organelles. But it also promoted a number of assumptions that have also been influential, but are less well-examined. Here we look at two of these to see whether or not they fit current evidence. The assumption we first address is that endosymbiotic relationships such as nutritional symbioses and eukaryotic organelles are mutualisms. We argue instead that they are more one-sided associations that can be regarded as context-dependent power struggles like any other ecological interaction. The second assumption is that during endosymbiotic interactions (such as the origin of organelles), the host genomes will acquire a great many genes from endosymbionts that assume functions in host systems (as opposed to the well-documented genes whose products are simply targeted back to the endosymbiont or organelle). The idea that these genes exist in large numbers has been influential in a number of hypotheses about organelle evolution and distribution, but in the most carefully-examined systems no such mass migration of genes is evident. Overall, we argue that both the nature and impact of endosymbiosis need to be constantly re-evaluated to fully understand what roles it really plays in both cell biology and evolution.

1. Introduction: untangling what we know and what we assume about endosymbiosis

The idea is old and appealingly simple: dissimilar organisms live together and by doing so become more than they were as individuals: they become a symbiosis (De Bary, 1879). The depth to which this idea would come to impact our understanding of cell biology and evolution was not immediately clear, nor was it immediately embraced by all biologists, but the landmark synthesis by Margulis (as Sagan, 1967) began to change that. It is now widely accepted that symbioses have propelled associations of organisms into environments where the individuals alone could not survive, and by doing so have significantly affected the evolution of life (Archibald, 2014). But what is less clear is how entering into symbiosis affects the participating organisms. Symbioses are often described as mutualisms, or relationships where both partners benefit. (In fact, the word symbiosis itself is sometimes used interchangeably with mutualism.) But the benefits for both partners are sometimes hard to see. Indeed, mutualisms have been previously likened to symbioses that have simply found a way to manage the inherent conflicts of interests between organisms (Herre et al., 1999). This is especially true in endosymbioses, which can often look remarkably one-sided (Bennett and Moran, 2015; Garcia and Gerardo, 2014; Kiers and West, 2016).

In this paper we use symbiosis in its most general sense. That is, we define it simply as any sustained organismal interaction somewhere on the pathogenic-beneficial continuum (Lewis, 1985). We highlight several recent examples that expose how the evolutionary interests of endosymbionts and hosts can become misaligned, and how endosymbioses that seem extremely interdependent and stable (even "permanent") can break down under the right circumstances. In particular, we focus on two aspects of endosymbiosis that affect our thinking of evolution more broadly: the idea that endosymbiosis is often a mutualistic relationship, and the idea that endosymbiosis has had a deep and lasting impact on the genome evolution through endosymbiotic gene transfer.

2. Endosymbiosis as an antagonistic relationship: context matters

Putting aside the classic organelles, the mitochondria and plastids, the vast majority of endosymbioses are probably pathogenic. That is, the presence of a microbe inside a host cell imposes a cost from the host perspective. If this is true, it follows that most endosymbioses that are beneficial from the host perspective likely evolved from interactions that were originally pathogenic, or at

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least mildly so. This idea is supported by analyses of the origins of proteobacterial symbionts with various hosts, which shows that the vast majority of beneficial proteobacterial symbionts have evolved from pathogenic ancestors (Sachs et al., 2014). What is required for an endosymbiosis to shift from costly to beneficial from the host perspective? Quite simply, the ecological context must change so that the benefits of the interaction outweigh the costs. The ecological context shifts that seem most common in endosymbiosis are those involving hosts gaining access to previously inaccessible nutrition or energy, or those where hosts defend themselves in ways not possible without the presence of the symbiont.

In endosymbioses where the microorganism provides energy or nutrition for the host, such as the mitochondrion, plastid, and many nutritional symbionts in insects, the context shift is absolute and (nearly, seemingly) permanent: the host cannot survive without its symbiont. These sorts of massive ecological context shifts drive the most spectacular and long-term types of symbiosis, because the host must preserve the symbiont at all costs, or get a new endosymbiont (Toenshoff et al., 2012; Koga and Moran, 2014; Bennett and Moran, 2015; Husník and McCutcheon, 2016; Meseguer et al., 2017) acquire the function in some other way such as HGT (Husník et al., 2013; Sloan et al., 2014; Luan et al., 2015; Nowack et al., 2016) or move to a new, richer environment that makes the symbiont expendable (Bennett and Moran, 2015). The long-term and nearly obligate nature of these symbioses can make the context dependency hard to see, because loss of an endosymbiont without a change in host context results in extinction of the entire symbiosis. The evolution of the yeast petite phenotype, where selfish evolution at the level of the mitochondrion eliminates its respiratory function to the detriment of its host cell when the population genetic context is appropriate, is one such example (Taylor et al., 2002). However, the context dependency of symbiosis is often more clear in symbioses that are relatively recent associations, such as protists and their photosynthetic symbionts (Lowe et al., 2016) or amoebae and their bacterial symbionts (DiSalvo et al., 2015). But recent work provides more instances where long-term endosymbioses—the type perhaps more naturally thought of as mutualisms—seem to be in the process of breaking down or have actually proceeded to eliminate their endosymbiont.

We can gain some insight into the way we instinctively think about endosymbiosis by considering the case of an insect endosymbiont called *Hodgkinia cicadicola. Hodgkinia* is in many ways a typical insect nutritional endosymbiont. It provides cicadas with two of the ten amino acids that they cannot make on their own and that are not provided at high levels in the strict plant sap diet of the insect (the remaining eight essential amino acids are provided by another bacterial endosymbiont called *Sulcia*; (McCutcheon et al., 2009). In many cicada species, this clean narrative is preserved: *Hodgkinia* provides two essential amino acids, *Sulica* provides the other eight, and the host gives them a nice place to live. Everyone is happy, and from some perspectives it looks like a three-way mutualism.

But this tidy story starts to break down in other cicada species. In some cicadas, the single ancestral *Hodgkinia* lineage has fragmented into two new distinct cell types, each with a distinct genome that has lost genes so that *both* are required by the host to provide the nutrition required by the ancestral single lineage (Van Leuven et al., 2014). Put another way, a host that used to have to keep track of two bacterial symbionts (*Sulcia* and a single *Hodgkinia* lineage) now is required to keep track of three. Why does this happen? It isn't clear yet, but we suspect that it is related to the unusual, long, and variable life cycles of cicadas. Documented cicada life cycles are between 2 and 17 years, and we know that in a short-lived species there is one *Hodgkinia* lineage, and that in the longest-lived cicada species there are *several dozen Hodgkinia* lineages (Campbell et al., 2015). These long-lived cicadas must therefore cope with numerous *Hodgkinia* lineages, each one encoding just a few genes.

While vertical transmission normally promotes cooperation between host and symbiont (Bull et al., 1991), this cooperation seems to be breaking down in some cicada groups despite an unchanged vertical transmission route. What has changed? We suspect that the high mutation rate of *Hodgkinia* combined with the increased symbiont generations that become possible in long-lived cicadas allows less fit symbiont genotypes to rise to high frequency during long cicada generations and occasionally get fixed, in an event we see as a split lineage (Van Leuven et al., 2014). From the host perspective, this process is probably nonadaptive. It is not better for the host to transmit dozens of *Hodgkinia* lineages, each encoding just a subset of original gene complement of the single lineage, to each egg instead of one. But the host has little recourse (outside of symbiont replacement) because its ecological context requires the two amino acids that *Hodgkinia* still produces. The cicada is stuck in a symbiont rabbit hole of its own making (Bennett and Moran, 2015).

What makes *Hodgkinia* different from a classical intracellular parasite, for example the malaria parasite, *Plasmodium*? No one would claim that *Plasmodium* is a mutualist, but we argue the difference between it and *Hodgkinia* is primarily context—or, different only in the direction in which the hostility is aimed. For *Plasmodium*, the simple narrative is the infectious agent is forcing itself into a cell, disrupting its normal function and subverting it to its own purpose. Ultimately the infectious agent kills and discards its host to move on to take over the next hapless victim. In the case of *Hodgkinia*, the story initially seems more like a nurturing embrace of one organism by another, the bacterium enveloped by its host, allowing it to shed functions that are no longer needed because essential nutrients and energy are all readily donated by its new benefactor. But when the context changes—in this case, the host life cycle—the host's ability to keep its symbionts in check breaks down. The evolutionary autonomy of *Hodgkinia* was always there, it was just constrained by the host.

We suggest that endosymbiotic interactions are best thought of not as mutualistic "happily ever-after" stories, but instead as "use it up and cast it off" situations that are stable for variable lengths of time. Endosymbiosis nearly always produces dead ends for one of the two partners—in the case of *Plasmodium* and other traditional parasites, the host is the partner that is cast off in the short term, but in the case of *Hodgkinia* and other beneficial endosymbionts it is the symbiont that is cast off in the longer term. In one case the symbiont is exploiting the host, while in the other the host is exploiting the symbiont. But neither one is mutualistic: they are both power relationships that differ simply based on whether the internal or external partner is in control.

This logic suggests that endosymbiotic relationships will always be temporary and they will be lost or replaced, but don't we already know this is not true? The answer depends on the time scale one considers, and the amount of diversity one has studied. For example, taxonomically narrow views of the mealybug or dilated protist symbioses might lead one to conclude that these complex relationships evolved only once, but studies with wider taxonomic breadth and depth reveals frequent endosymbiont turnover (Thao et al., 2002; Kono et al., 2008; Husník and McCutcheon, 2016). A similar situation is now also seen in essential bacterial endosymbionts of dilated protists (Boscaro et al., 2017). But what about eukaryotic organelles? Are they not the classic case of endosymbiosis leading to "happily ever after"? It's clear that the host has control, so why has the organelle not burned itself out like *Hodgkinia* seems to be doing? Why have organelles not been replaced with fresh symbionts?

It could be that organelle degeneration has stabilized due to large amounts of gene transfer and protein-targeting. But it is

also clear that if the core function of the organelles is acquired independently or side-stepped somehow, that even this "permanent" relationship can be lost. Indeed, photosynthesis has been lost scores of times in plastids and oxidative phosphorylation and electron transport has been lost many times in mitochondria when the host's ecological context has changed such that these functions were no longer required (Burki, 2016; Müller et al., 2012; van der Giezen, 2009; Williams and Keeling, 2003). As highly valuable as these functions are, they are not core functions of these organelles. Instead, the core function of mitochondria, the last function to be retained in even the most reduced organelle, is iron-sulfur cluster assembly (Müller et al., 2012; van der Giezen, 2009; Williams and Keeling, 2003). In the case of plastids, the core function is likely different in different lineages, but fatty acid, amino acid, heme, and isoprenoid biosynthesis are all candidates in different groups which have lost photosynthesis (Foth and McFadden, 2003; Williams and Keeling 2003; Keeling, 2013; Janouškovec et al., 2015; Ralph et al., 2001). But recent studies that have considered eukaryotic diversity more broadly show that *even these* core functions have sometimes been lost, and when they are, the association breaks down and the organelle is eliminated entirely. For example, the parasitic dinoflagellate *Hematodinium* is known to descend from photosynthetic relatives, but it has now completely lost not only photosynthesis but also the actual plastid organelle, apparently because the other core functions are satisfied in other ways (Gornik et al., 2015). Similarly, the flagellate *Monocercomonoides* has been found to have lost not only oxidative phosphorylation (it is anaerobic), but also lost the entire mitochondrial organelle since this lineage has acquired an iron-sulfur cluster biosynthetic pathway by horizontal gene transfer from bacteria (Karnkowska et al., 2016) Moreover, in the dinoflagellates, where photosynthesis and even plastids are particularly prone to loss, we see occasional cases of plastid replacement: apparently when one plastid is discarded, another one acquired to replace it (Keeling, 2013; Archibald, 2015). These studies show that even organelles have not been frozen into permanence, it just seems that way because we have not looked broadly enough (or waited long enough). The degenerative ratchet of endosymbiosis is still slowly turning, even in organelles.

When we mentally distinguish mutualistic from pathogenic symbioses, it is a mistake to apply the kind of thinking that we intuitively glean from the relationship within macroorganism symbioses such as plants and pollinators or cleaner wrasses and fish to the rather more abstract relationship we observe between one cell living within another (although they may be more similar after all, since context-dependent breakdown of the wrasse-fish mutualism is observed: Gingins et al., 2013). Instead, we argue that endosymbioses are rarely, if ever, mutualistic. Endosymbioses are about subordination, where the vector of control points in different directions with different magnitudes depending on the context. One partner is always in control, or fighting to increase control. Coexistence can occur for long periods of time, but if conditions change the partnership can quickly tip towards extinction for either the subordinate member or the entire symbiosis. The interesting questions for long term endosymbiosis, like eukaryotic organelles and insect endosymbionts, therefore shift from why and how these partnerships form, to why and how these partnerships have so far avoided extinction.

3. Genomic impacts of endosymbiosis

The endosymbiotic origin of mitochondria and plastids primed us to accept similar explanations for other phenomena. At the cellular level, this initially led to a rush to explain other organelles in endosymbiotic terms, for example the flagella and cilia, peroxisomes, endoplasmic reticulum, and even the nucleus (Cavalier-Smith, 1987; Gupta, 1999; Lake and Rivera, 1994; Margulis, 1970; Sagan, 1967). Endosymbiotic explanations for these organelles has gone out of fashion due to an ongoing absence of evidence (Martin, 1999), but, as we will outline below, the fashion has made a comeback to explain genomic data. The acceptance that mitochondria and plastids were indeed derived from endosymbiotic bacteria came at an auspicious time in the early days of molecular biology and subsequently genomics. These technologies were revolutionary, broke down a lot of long held ideas, and lead to an intellectual vacuum to be filled with new explanations. At least some of this vacuum was naturally filled by explanations involving endosymbiosis (Keeling, 2014); some of these explanations have now formed the foundations for other assumptions, but have not been subjected to serious critical examination.

Most important of these is a prevalent idea that by looking at the evolutionary history of genes in a genome we can "see" an ancient endosymbiosis based on the presence of genes in the host that were acquired from that endosymbiont. This presupposes that an endosymbiont will donate genes to the nucleus of its host, an idea with a complex history. Almost simultaneously with Margulis' influential paper in *The Journal of Theoretical Biology* (Sagan, 1967), Goksøyr outlined a similar hypothesis in *Nature* (Goksøyr, 1967), and went further suggesting that the endosymbiont would have moved some of its genes to the host, and that their products would then be targeted back to the endosymbiont. Weeden (1981) developed this idea further, going as far as to say it was a necessary corollary to the endosymbiont hypothesis because organelle genomes were insufficient to encode the necessary genes to support organelle function. Although the exact origin of all nucleus-encoded organelle genes has been questioned (Larkum et al., 2007; Keeling, 2013), these two ideas have provided enormous explaining power when looking at organelle biology and evolution. However, beyond this well-tested core is a less-well-examined idea that is nevertheless influential. The thinking goes that if a large number of genes were transferred to the host for proteins now targeted back to the organelle, then probably a lot of *other* genes were transferred as well. Many of these proteins, if not most, are now *not* targeted back to the organelle but acquired functions in the host.

This seems reasonable enough—genes were flowing, and if they are potentially useful then it stands to reason the host should keep some to function in cytosolic pathways, and maybe even keep a lot. Early studies supported this conclusion based on genomic data from model systems (e.g., Martin et al., 1998). Naturally, the implications of this conclusion can be extrapolated to touch on other, more complex problems. Most importantly, if organelle endosymbionts donated a lot of genes for now-cytosolic proteins, then we should be able to "see" evidence for now-lost organelles in the nuclear genomes of their erstwhile hosts. This idea rests on the assumption that, because these genes have acquired a function independent of the organelle, they will be retained even when the organelle is lost or replaced.

If true, this would be a powerful tool in the reconstruction of evolutionary history, and has formed the logical basis for a number of claims for ancient endosymbiotic events and cryptic or now-lost organelles. For example, in work on plastid organelles, such studies have concluded that non-photosynthetic lineages like oomycetes or ciliates once had a red algal plastid (Reyes-Prieto et al., 2008; Tyler et al., 2006), or that red algal plastid-containing lineages once had green algal plastids (Moustafa et al., 2009; Woehle et al., 2011). These conclusions have been challenged on the basis of the veracity of the phylogenetic results (Burki et al., 2012; Deschamps and Moreira, 2012; Moreira and Deschamps, 2014), but the idea itself has not been challenged particularly, and has had a major impact on models for the evolution of organelles and on how we perceive the impact of endosymbiosis on the host genome and cellular function. Indeed, it emphasizes the importance of endosym-

biosis on both counts: it predicts more endosymbiotic organelles in evolution and ascribes more functional impact to them. However, these conclusions are dependent on an assumption (that organelle derived genes will be kept in large numbers when the organelle is lost) that is itself built on another assumption (that those genes were transferred and retained in the first place), and neither has been thoroughly tested. In the almost two decades since the original analyses supporting the presence of large-scale transfers of genes from the organelle endosymbiont for proteins that do not function in the organelle (e.g., Martin et al., 1998), there have been significant advances that would allow this important conclusion to be reexamined with more confidence. We are now awash with recent genomic data from a variety of eukaryotes, and phylogenetic methods and computational power both now allow for significantly better tests of a gene's origin. Some studies support an overall episodic influx of genes that is consistent with this idea (e.g., Ku et al., 2015), but other studies on relatively recent secondary and tertiary endosymbiotic events find the number of endosymbiont-derived genes in the host nucleus that are not functionally linked to the organelle to be few, or even potentially zero (Deschamps and Moreira, 2012; Burki et al., 2012; Curtis et al., 2012; Hehenberger et al., 2016; Moreira and Deschamps, 2014; Patron et al., 2006). Different endosymbiotic events may have had different impacts, but if the assumption is untrue, or even if significant variation is found in different organelle origins, then it will limit the extent that we can interpret the presence or absence of such genes from a nuclear genome. This in turn impacts how much weight can be given to endosymbiosis to explain eukaryotic diversity.

4. Concluding remarks

Endosymbiosis is a mechanism that is unique in biology in the strength of ideas, or perhaps feelings, that it elicits: our view of this as a positive interaction with profound consequences for the partners is powerful, but vague in some important details. Aspects of both its nature and its impact are in need of deeper, critical examination as the body of evidence from a variety of endosymbiotic systems grows, particularly genomic data. Here we focussed on two ideas that form the basis for how we interpret endosymbiotic systems more generally and, we hope, make a case that there are reasons to re-think both assumptions.

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