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On the evolutionary significance of horizontal gene transfers in plants

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Summary

Horizontal gene transfer (HGT) has long been seen as a crucial process in the evolution of prokaryotic species, but until recently it was thought to have little, if any, effect on the evolution of eukaryotic life forms. Detecting and describing HGT events in eukaryotes is difficult, making this phenomenon at times controversial. However, modern advances in genomics and bioinformatics have radically altered our view of HGT in eukaryotes, especially in plants. It now appears that HGT to and from plant lineages is more common than previously suspected. Importantly, the transfer of functional nuclear genes with adaptive significance has been reported in numerous taxa. Here we review several recent studies that have found evidence of the horizontal transfer of nuclear genes, and argue that HGT has undoubtedly had profound impacts on plant evolution as a whole.

I. Background

Horizontal gene transfer can be defined as the transmission of genetic material between evolutionary lineages by some means other than fertilization. Evidence of horizontal gene transfer (HGT) was initially observed as early as 1928 (Griffith, 1928) when virulence was shown to be transferred between pneumococcal strains in mice. Tatum & Lederberg (1947) later provided incontrovertible proof of bacterial transformation by characterizing the offspring of mixed colonies of *Escherichia coli*, in which subsequent generations expressed a combination of traits from each of the parent. While HGT rapidly became central to any study regarding evolution in prokaryotes, it was not known to occur in eukaryotes until decades later.

Research into HGT has expanded rapidly in the 21st century. As next-generation sequencing and published genomes have become more accessible, large-scale analyses of genomic data have resulted in the discoveries of numerous horizontally acquired genes in a broad range of eukaryotic taxa (Soucy *et al.*, 2015). However, HGT in eukaryotes has not been uncontroversial. One of the primary concerns is sample contamination in genomic studies, particularly in cases involving putative transfer between eukaryotes and associated microbes. This issue is exacerbated by the short reads produced by many modern sequencing methods which can result in fragmented assemblies, making it difficult to distinguish true nuclear genomes from bacterial contaminants (e.g. Richards & Monier, 2016). In addition, rapid gene family turnover (via duplications and losses) can create gene tree–species tree

discordance that might be mistaken as evidence of HGT (Ku & Martin, 2016).

Nevertheless, despite the difficulties in accurate detection, HGT could, in theory, have a disproportionately large effect on species' evolutionary trajectory. Unlike the gradual process by which newly duplicated genes diverge, horizontally acquired genes can have immediate and significant consequences for host fitness. Indeed, multiple HGT genes have been identified that appear to confer a significant selective advantage in diverse taxonomic groups (e.g. Li *et al.*, 2014; Dunning *et al.*, 2019; Yang *et al.*, 2019).

The goal of this perspective article is to review recent discoveries on HGT in plants, and argue that HGT does have the potential to have a drastic impact on plant adaptation. We will not discuss transfers involving organelles (e.g. Taylor *et al.*, 2015) or genetic materials of unclear evolutionary significance (e.g. transposable elements; Roulin *et al.*, 2008). Instead, we will focus specifically on cases where the transfer of nuclear genes is likely to have imparted a selective advantage.

II. The role of HGT in the evolution of parasitic plants

Much of the modern research regarding HGT in plants has focused on several lineages of parasitic plants, including *Cuscuta* (Vogel *et al.*, 2018), Orobanchaceae (Yang *et al.*, 2016; Kado & Innan, 2018), *Striga* (Yoshida *et al.*, 2010), *Rafflesia* (Xi *et al.*, 2012) and *Santalales* (Davis *et al.*, 2005). The prevalence of HGT in heterotrophic plants is perhaps unsurprising given their close physical association with their hosts. Many parasitic plants form intimate intercellular attachments with their hosts via haustoria. It is through these haustoria that they acquire nutrients from the host and possibly hosts' genetic material (Fig. 1a). Indeed, the transfer of entire nuclear genomes (Fuentes *et al.*, 2014) has been observed across graft junctions in unrelated taxa. Therefore, it stands to reason that heterotrophic plants could have a higher than usual HGT incidence as compared with autotrophic plants. Consistent with this, the number of horizontally acquired genes in a given species appears to be positively correlated with the degree of

heterotrophy (Yang *et al.*, 2016); in other words, obligate parasites are more likely to possess horizontally acquired genes from their host than closely related facultative species.

Several studies have found evidence of the movement of mRNA transcripts from host plants in Orobanchaceae (David-Schwartz *et al.*, 2008), implying that such mobile mRNA could act as an intermediate for the horizontal transfer of genes (Yoshida *et al.*, 2010). However, subsequent research in Orobanchaceae and *Cuscuta*, in particular, has shown that the vast majority of transfers seem to be derived from genomic fragments of DNA rather than an mRNA intermediate (Yang *et al.*, 2016; Kado & Innan, 2018).

There are several lines of evidence suggesting that the parasites may have repurposed the host-derived genes for a heterotrophic lifestyle. Studies in Orobanchaceae, *Cuscuta* and *Rafflesia* have found that many horizontally transferred genes are being expressed (Xi *et al.*, 2012; Yang *et al.*, 2016; Vogel *et al.*, 2018; Yang *et al.*, 2019), and in many cases preferentially in haustorial tissues. Furthermore, of the identified HGT cases in *Cuscuta* and Orobanchaceae, 18 of them came from the same gene families (Yang *et al.*, 2019). This convergence suggests that the retention of horizontally transferred genes may be nonrandom and adaptive.

III. The role of HGT in the evolution of ferns

Ferns were one of the early groups to provide evidence of plant–plant HGT involving the transfer of a mitochondrial gene from a parasitic angiosperm in Santalales to the partially myco-heterotrophic fern *Botrypus virginianum* (Davis *et al.*, 2005). This finding supports the correlation of HGT with intimate physical contact and heterotrophic lifestyle. The 'weak link' model (Huang, 2013) proposes that a foreign gene would be more likely to be transmitted to future generations if organisms' germline cells are more exposed to the environment (Fig. 1b). A prime example can be found in the gametophytes of seed-free plants where eggs and sperm are weakly protected from the external environment, possibly making them more prone to HGT. Indeed, more recent

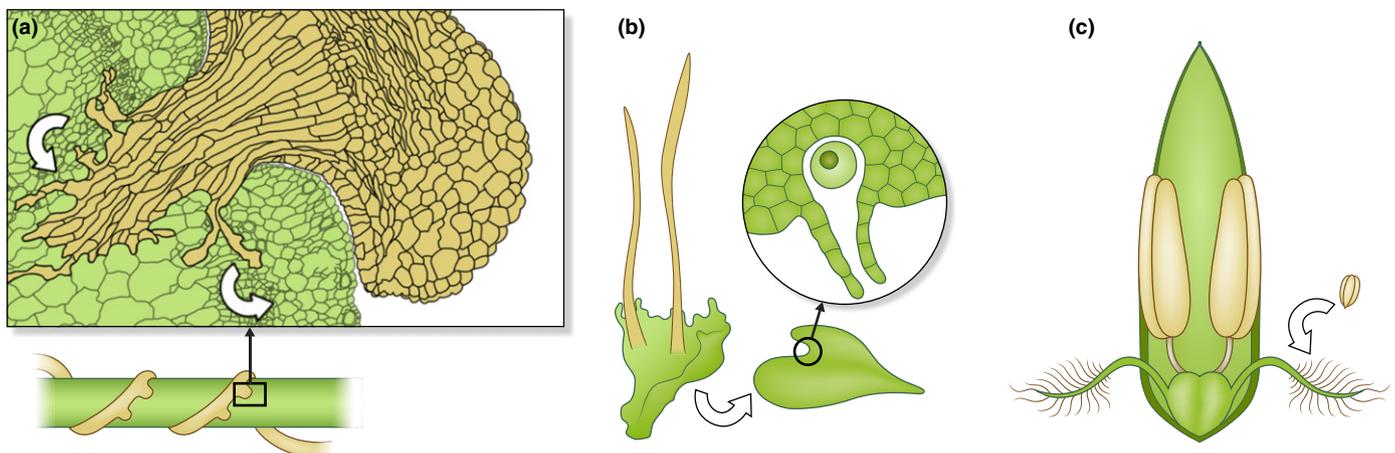


Fig. 1 Putative mechanisms for horizontal gene transfer (HGT) between: (a) *Cuscuta* and its host; (b) a hornwort and fern gametophyte; and (c) C_4 grasses. White arrows indicate the direction of transfer.

examples of HGT in ferns are not necessarily associated with heterotrophy.

The presence of the photoreceptor neochrome in ferns was something of a mystery when it was initially discovered in *Adiantum capillus-veneris* (Nozue *et al.*, 1998). Neochrome is a chimeric protein, having the N-terminus derived from the red light-sensing phytochrome and the C-terminus from the blue-sensing phototropin. Neochrome was found to significantly enhance the phototropic response in *Adiantum* (Kawai *et al.*, 2003; Kanegae *et al.*, 2006). As such, it presumably had a role in ferns' adaptation to low light conditions, and represents a key innovation leading to their modern diversification under the Cretaceous/Tertiary angiosperm canopy (Schneider *et al.*, 2004; Schuettpelz & Pryer, 2009). While neochrome had been thought to be unique to ferns, upon inspecting multiple hornwort transcriptomes, a copy of neochrome was discovered that strongly resembled that found in ferns (Li *et al.*, 2014). Further phylogenetic analyses showed that fern neochromes are nested within the hornwort clade, a pattern indicative of HGT. The hornwort-to-fern HGT event was dated *c.* 178 million yr ago (Ma), roughly coinciding with the radiation of ferns under the canopy of modern angiosperms.

Further evidence highlighting HGT's role in the evolution of ferns came during the course of assembling the first fern genomes (Li *et al.*, 2018). The fern genus *Azolla* has an obligate symbiotic relationship with the nitrogen-fixing bacteria *Nostoc azollae*. Because of the species' intimate association, they were identified as probable candidates for HGT. Surprisingly, no gene was found to be horizontally transferred between *Azolla* and the cyanobiont (Li *et al.*, 2018). Nevertheless, Li *et al.* (2018) did find an HGT gene derived from cyanobacteria, but one that is shared with many other fern and lycophyte species (i.e. a more ancient HGT). This gene encodes squalene-hopene cyclase (SHC) and is exclusively found in seed-free plants. Interestingly, the plant SHC genes are not monophyletic. Instead, there are three clades – corresponding to mosses, liverworts and lycophytes/ferns – interspersed among bacterial SHCs. This phylogenetic relationship indicates three independent HGT events, each with a different bacterial donor and a different plant recipient. In bacteria, the products of SHC – hopanoids – are important plasma membrane constituents and required for various types of stress tolerance (Siedenburg & Jendrossek, 2011; Ricci *et al.*, 2017). It is possible that plant hopanoids have similar functions, and that repeated acquisition of bacterial SHC was instrumental for the early plant evolution.

Investigations into the fern genomes also revealed a possible link between HGT and ferns' well-known resistance to insect herbivory. Ferns' underutilization as a food source by phytophagous insects as compared with angiosperms has been thoroughly documented (Cooper-Driver, 1978). A chitin-binding protein, Tma12, was recently isolated which may have contributed to such reduced herbivory (Shukla *et al.*, 2016). Interestingly, Tma12 has no close homolog in any other land plants, and all the fern sequences are nested within bacterial chitin-binding proteins. This points to yet another case of putative HGT that may have provided particular fern lineages with an evolutionary advantage.

IV. The role of HGT in the evolution of C₄ grasses

C₄ photosynthesis is a complex process by which certain angiosperm lineages concentrate and fix carbon by way of a four-carbon intermediate. By increasing primary productivity in warm, arid climates, it has aided in the expansion and diversification of a wide range of taxa across grasslands and deserts (Sage, 2004). It appears to have arisen at least 45 times in over 7500 species (Sage, 2004). As such, the evolutionary history of C₄ plants is a convoluted one involving phenotypic convergence on a massive scale.

In the course of disentangling this complicated history in the grass genus *Alloteropsis*, Christin *et al.* (2012) found evidence that multiple genes encoding key enzymes in the C₄ cycle (PEPC and PCK) had been inherited via HGT. Importantly, these horizontally acquired paralogs have functionally significant amino acid residues that are characteristic of C₄ plants, whereas the native, vertically transmitted ones do not (Christin *et al.*, 2012). In other words, HGT probably jumpstarted the capacity of C₄ photosynthesis in certain *Alloteropsis* species.

These C₄ genes do not appear to come from associated heterotrophic or symbiotic taxa. Instead, they were derived from multiple other grass lineages, possibly via illegitimate pollination (Christin *et al.*, 2012). Illegitimate pollination or partial hybridization is a process that has been observed in multiple taxa, including oats, maize and sunflowers, whereby single chromosomes can be transferred between different species (Riera-Lizarazu *et al.*, 1996; Faure *et al.*, 2002). It is possible that illegitimate pollination (Fig. 1c) may represent an evolutionarily significant source of novel genes in certain lineages. Indeed, further studies have identified additional HGT events in *Alloteropsis* beyond those involved in C₄ photosynthesis. A chromosomal-level assembly of the *Alloteropsis semialata* genome uncovered 26 genomic fragments containing 59 genes that were transferred from nine distinct clades of grasses (Dunning *et al.*, 2019). Interestingly, 12 of the HGT genes have a higher expression level than the native homolog, and in one case the native copy became pseudogenized and replaced by the foreign one.

V. The future of HGT research in plants

The research discussed here demonstrates that HGT could have been a significant force driving evolution and adaptation in several groups throughout the plant kingdom, and was probably instrumental in the adaptation to a parasitic lifestyle, light-sensing and insect resistance in ferns, as well as C₄ photosynthesis in grasses. However, despite the recent expansion of HGT research, there is still considerable room for improvement. Specifically, there are two areas upon which the advancement of HGT research relies: methods for identifying HGT and the quality of genomic data to which those methods are applied.

All modern studies of eukaryotic HGT employ a combination of BLAST searches and gene tree–species tree reconciliation. Although this approach has been highly effective in identifying HGT between distantly related species (e.g. ferns and hornworts), its use at shallow phylogenetic scales is problematic, owing to the complexity introduced by recent introgression, gene duplication/loss (GDL), and incomplete lineage sorting (ILS) (Fig. 2). Models have been

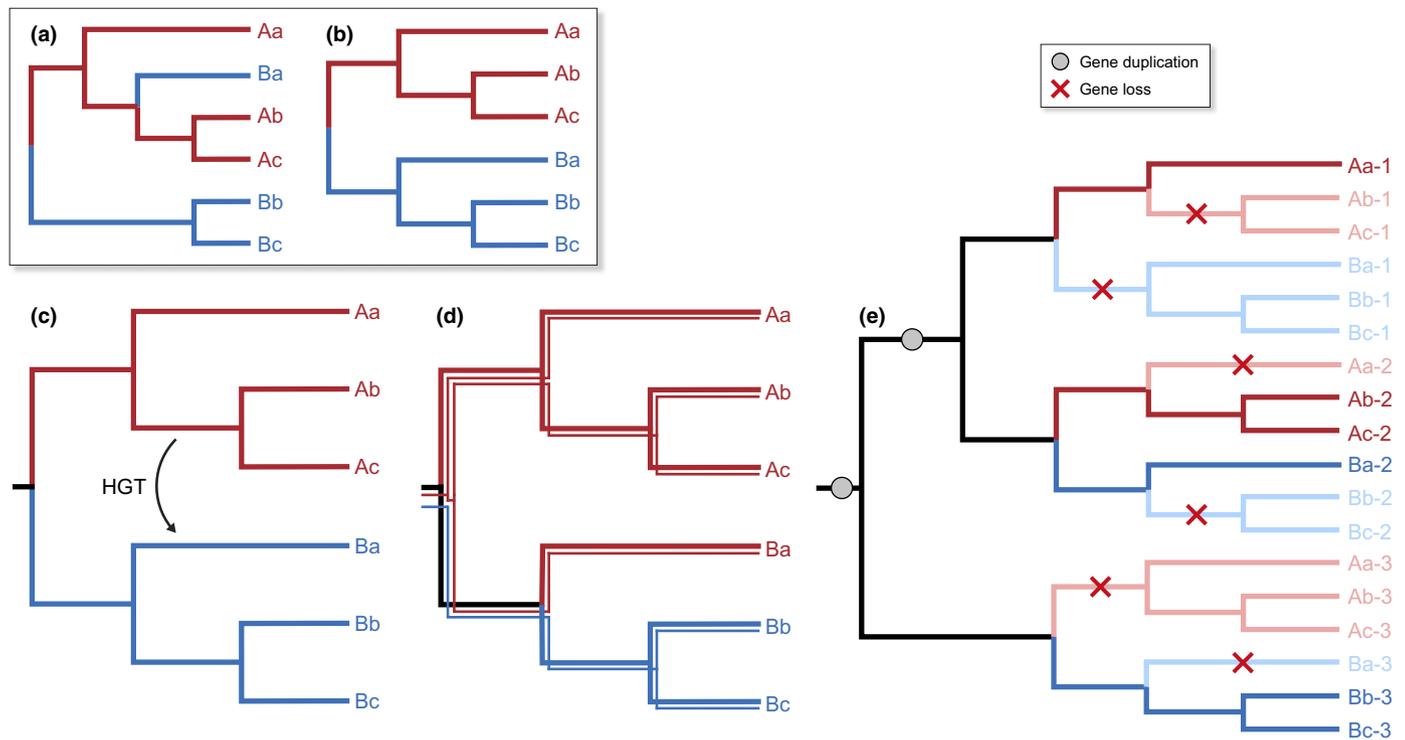


Fig. 2 Three separate evolutionary mechanisms that could yield an identical gene tree topology shown in (a). (b) A hypothetical species tree with two genera, 'A' in red and 'B' in blue, each having three species. (c) Horizontal gene transfer (HGT) from Ab–Ac lineage to Ba, followed by a loss of the original copy in Ba. (d) Incomplete lineage sorting with separate alleles represented by thin red and blue lines. (e) Gene duplications followed by recurrent losses shown by circles and crosses, respectively.

devised for prokaryotic systems that specifically consider GDL and HGT, but these methods still fail to consider ILS (Dupont & Cox, 2017). In addition, as a result of the relative rarity of HGT in eukaryotes, few, if any, studies have evaluated the efficacy of these models outside of bacterial lineages. To fill this gap, researchers have begun using coalescent statistics to model reticulate evolution, including HGT in the context of both GDL and ILS in eukaryotic taxa (Du *et al.*, 2019). Nevertheless, there is still considerable work to be done in developing and fine-tuning phylogenetic reconciliation methods.

Some of the most controversial claims of HGT have involved the transfer of genetic material from prokaryotic to eukaryotic lineages. A recent case in point involved a purported large-scale HGT of functional genes in tardigrades (Boothby *et al.*, 2015). In this instance, HGT inference was based on a fragmented genome assembly, and a more complete assembly established that most, if not all, of the previously identified HGT events were a result of bacterial contamination (Koutsovoulos *et al.*, 2016). In fact, a broader analysis of 55 eukaryotic genomes found little to no evidence of recent HGTs between prokaryotes and eukaryotes, leading researchers to propose that eukaryotic genes with >70% sequence identity to prokaryotic lineages almost always result from endosymbiotic origin or contamination, suggesting a natural barrier to prokaryote–eukaryote HGT (Ku & Martin, 2016). This raises two points that are critical to the ongoing study of HGT. First, that great care must be taken during DNA extraction, assembly and annotation of genomes to rule out sources of contamination. Second, high-quality genome assemblies can

enable comparisons of flanking genes and synteny, thereby providing additional lines of evidence for HGT. The presence of transferred genes alongside native ones in contiguous scaffolds is useful in ruling out contamination, while detailed syntenic analyses can aid in sorting out paralogy, particularly in closely related species where the arrangement of genes is likely to be conserved (e.g. Dunning *et al.*, 2019).

In summary, we believe that the plant HGT cases reported to date represent only the tip of the evolutionary iceberg. As more high-quality genomic data become available from all branches in the plant tree of life, we anticipate that future research will provide us with a better picture of both the extent and the evolutionary significance of HGT in plants.

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Author contributions

DAW and F-WL reviewed the literature and wrote the manuscript.

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