Horizontal gene transfer (HGT) between species has been a major focus of plant evolutionary research during the past decade. Parasitic plants, which establish a direct connection with their hosts, have provided excellent examples of how these transfers are facilitated via the intimacy of this symbiosis. In particular, phylogenetic studies from diverse clades indicate that parasitic plants represent a rich system for studying this phenomenon. Here, HGT has been shown to be astonishingly high in the mitochondrial genome, and appreciable in the nuclear genome. Although explicit tests remain to be performed, some transgenes have been hypothesized to be functional in their recipient species, thus providing a new perspective on the evolution of novelty in parasitic plants.

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Introduction
Parasitism has evolved multiple times across the tree of life. Parasitic plants obtain some or all of their water and nutrients, including carbohydrates and minerals, through a specialized feeding apparatus called the haustorium, which attaches to roots or shoots of their hosts (Figure 1). Parasitic plants exhibit a range of diversity, including species with the ability to photosynthesize (hemiparasites) and those that cannot (holoparasites). Despite the direct haustorial connection parasitic plants establish with their hosts, most grow predominantly exterior to their hosts. The exceptions include a small group of endophytic holoparasites, which emerge only during sexual reproduction (Figure 1). These species otherwise grow embedded in their hosts and have no discernable roots, shoots, or leaves, persisting largely as a mycelium-like body consisting of a greatly reduced strand of cells [1].

Owing to their extreme vegetative reduction and modified reproductive morphology, the phylogenetic placement of parasitic plants among their free-living relatives was long a mystery. This is especially true for the endophytic holoparasites, most of which have been historically grouped together to include species variously placed today in Apodantheaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae. Recent phylogenetic investigations, however, have greatly challenged this traditional view of classification by demonstrating that these families are not closely related. Instead, the most comprehensive analyses indicate that parasitism in angiosperms has evolved at least 11 times from free-living ancestors [2].

These insights have greatly stimulated research relating to genome evolution in parasitic plants [3,4], and the investigation of the adaptations that have enabled the origin of parasitism [5,6,7]. One of the most exciting discoveries to emerge from this body of research is the finding that parasitic plants and their hosts undergo horizontal gene transfer (HGT)—the exchange of genetic materials between distantly related, non-mating organisms. More generally, the hypothesis of HGT in autotrophic plants was invoked in two landmark studies [8,9], in which gene phylogenies were identified as strongly incongruent with well-established species relationships based on various lines of molecular and morphological evidence. In this regard, phylogenies that are incongruent with accepted species relationships have been deemed the ‘gold standard’ for deducing HGT [10] (Figure 2). Since that time, HGT has been identified in a variety of autotrophic plant clades [10,11,12], most notably in bryophytes [13], ferns [14,15], basal angiosperms [16,17], and grasses [18].

Parasitic plants are exemplary systems for studying HGT
Following the two initial discoveries of HGT in autotrophic plants, investigations in parasitic plants have provided important insights into HGT [14,19–21]. Several studies ranging from single to hundreds of genes demonstrate that the parasitic mode of life may enable HGT in plants, which is thought to be facilitated by the intimate physical association between the parasite and its host [19]. This intimacy makes parasitic plants a potential model system for HGT related research. Here, the donor and recipient lineages are often well characterized, making it much easier to discern HGT. By contrast, explanations of HGT in autotrophic plants tend to be less clear from a mechanistic perspective. Up until now, both host-to-parasite [e.g., 19] and parasite-to-host gene transfers [e.g., 14,20] have been identified in parasitic plants (Table 1). In addition, the possibility that parasitic plants act as bridges for gene flow between diverse autotrophic
Parasitic plant diversity. (1) Holoparasitic Cuscuta europaea (Convulvulaceae); (2) Cuscuta campestris (Convulvulaceae) penetrating host tissue via a haustorium (highlighted with dotted yellow lines); (3) Holoparasitic Rafflesia arnoldii (Rafflesiaceae), which produces the world’s largest flowers; (4) Holoparasitic Rhizanthes lowii (Rafflesiaceae) showing its very reduced endophyte (marked with dotted yellow lines) in the phloem of the host — the only remnants of a vegetative body; (5) Hemiparasitic Taxillus delavayi (Loranthaceae, Santalales); (6) Hemiparasitic Krameria argentea (Krameriaaceae); (7) Holoparasitic Pilostyles hamiltonii (Apodanthaceae); (8) Holoparasitic Mitrastema yamamotoi (Mitrastemonaceae); (9) Holoparasitic Pholisma sonorae (Lennoaceae); and (10) Holoparasitic Cistanche phelyphaea (Orobanchaceae). Images copyright Dave, M. Costea, J. Holden, L. Nikolov, J. Lundberg, J. Medeiros, K. Thiele, C. Tada, J. Bartel, and P. Precey, respectively.

Figure 2

Using phylogeny to infer horizontal gene transfer (HGT). (a) Inferred gene tree on the left depicts transgene in parasite (placed with host clade), coexisting with its vertically inherited homolog (i.e., duplicative HGT). Outer species tree (in grey) on the right with inner gene tree demonstrating host-to-parasite HGT. (b) Inferred gene tree on the left depicts transgene in parasite (placed with host clade). Outer species tree on the right with inner gene tree demonstrating host-to-parasite HGT, followed by loss of vertically inherited homolog (red ‘x’; i.e., replacement HGT).
results from intergenic transfer, first involving plastid-to-mitochondrion transfer within the donor lineage, followed by mitochondrion-to-mitochondrion host-to-parasite transfer. With regard to the second possibility, a clearer example was demonstrated in Rafflesiaceae, where Sapria himalayana [3**] and Rafflesia lagascae [26] acquired an astonishing 14 and 15 plastid genes from their grapevine hosts (Tetrastigma, Vitaceae), respectively. Based on the availability of a previously assembled mitochondrial genome of the host lineage [28], it was confidently determined that these transgenes were likely resident in the host’s mitochondrial genome at the time of transfer. These results collectively establish that most transgenes to date have been acquired from the mitochondrial genome rather than the plastid genome [11**].

Although a larger picture of rampant HGT involving mitochondrial DNA in parasitic plants is emerging, our knowledge of HGT involving nuclear DNA is still in its infancy. This is partly due to the paucity of deeply sequenced genomes available to address this topic in a broad, comparative framework. Yoshida et al. [29] used genome scanning approaches to demonstrate that the gene ShContig9483 in the purple witchweed, Striga hermonthica (Orobanchaceae), was acquired from a member of the grass family (Poaceae). Similar host-to-parasite transfers have subsequently been identified in Cuscuta australis and Orobanche aegyptiaca (Convulvulaceae and Orobanchaceae, respectively; involving the strictosidine synthase-like gene) [30] and Phelipanche aegyptiaca (involving the allelin 1 gene) [31**]. More focused efforts investigating hundreds of genes indicate that host-to-parasite transfer involving nuclear genes may be prevalent. For example, several dozen genes were identified via phylotranscriptomics as likely candidates for nucleus-to-nucleus transfers between Rafflesiaceae and their hosts [4]. These authors additionally sought to establish background rates of non-HGT causes of phylogenetic incongruence (e.g., gene duplication, gene loss, and incomplete lineage sorting). This is likely to become especially relevant as more genomic data become available. Promising new analytical approaches have been developed to identify HGT in the face of these confounding effects using parsimony [32,33] or Bayesian methods [34,35*].

In summary, these results suggest that while the relative magnitude of HGT involving the nuclear genome in parasitic plants is appreciable, transfers involving the mitochondrial genome are likely to be potentially much more frequent.

### Mechanisms and functionality of transgenes in parasitic plants

One outstanding question involves the uptake mechanisms of foreign DNA. Although vectors remain unclear, fungi, bacteria, and viruses have been invoked [9,10]. More recently, transposable elements have also been
implicated as a vector for HGT in autotrophic plants [36]. Although many nutrients and macromolecules, including mRNAs, are trafficked between host and parasite [5**,37,38], the evidence points primarily to direct uptake of DNA, rather than mRNA. This is demonstrated in various studies of parasitic plants [3**,4,22,30,31**], as well as in autotrophic ones. In Rafflesiaeaceae, evidence for direct DNA uptake is supported by the presence of introns in transgenes and the inclusion of cytosine-to-uracil RNA editing sites in mitochondrial transgenes. One exception is in Striga where the putative transgene mentioned above possesses no intron and has consecutive adenine nucleotides at the 3’ end, suggesting that this transgene was RNA-mediated and then incorporated into the genome via reverse transcription [29]. Given the unique properties of the mitochondrial genome and recent observation of exchange of whole plastid genomes in plant grafting experiments [39,40], it appears that the intimate association between parasites and their hosts greatly facilitates HGT via the direct incorporation of large fragments of DNA, rather than shorter mRNAs. Finally, as broader comparative data becomes available on the sizes of plasmodesmatal connections between hosts and parasites, we may gain insight into which symbioses are likely to confer more or less HGT.

A second outstanding question relates to the functionality of transgenes in parasitic plants. Although the magnitude of HGT involving nuclear genes is lower, on average, than that of the mitochondrial genome, nuclear transgenes may nevertheless confer key evolutionary advantages in their recipient lineages. Most of the acquired DNAs identified in mitochondrial genomes are introns or pseudogenes. Whether these pseudogenes are inherited as such at the time of transfer, or whether gene death takes place subsequent to their transfer via deterioration of the foreign DNA remains unclear [12]. However, several recently identified nuclear transgenes maintain reading frame, and in some cases have been shown to be actively transcribed. The Rafflesia cantleyi transcriptome, for example, exhibits dozens of expressed transgenes that represent a wide range of cellular functions as determined by gene annotation data, including roles related to respiration, metabolism, mitochondrial translation, and protein turnover [4]. Although the extent to which these transgenes are functional awaits further exploration, these results raise the possibility that transcripts acquired via HGT may express host-like genes to better manipulate their host. Similarly, a transgene closely related to the albumin 1 gene was discovered in Phelipanche aegyptiaca [51**]. Albumin 1 genes encode seed storage proteins and insect toxins, and appear to have been acquired via HGT in the ancestor of Phelipanche from one of its legume hosts. In this case, the transfer appears to have given rise to a family of duplicated, differentially expressed genes in their recipient lineage. This suggests the intriguing possibility that differential function of these copies may have arisen in the parasites subsequent to their transfer. In these cases, the functional relevance of transgenes remains unclear, but parasitic plants need to avoid host defenses as well as resist herbivores, bacteria, fungi, and abiotic environmental stresses. Follow-up studies may reveal that these transgenes provide an evolutionary advantage in this regard.

Finally, an additional discovery to emerge is that codon usage patterns of native genes in some parasites demonstrate a very strong genome wide bias towards coding properties more like those of their hosts rather than their closest relatives [4]. This apparent form of molecular convergence may help to explain why HGT is so high in some parasitic lineages: the similarity in their genetic machinery relative to their hosts may greatly facilitate gene uptake. Why this convergence exists remains an open topic for investigation, and should be investigated in other lineages.

Detecting former host associations with HGT

Perhaps one of the most exciting possibilities offered by HGT in parasitic systems is their ability to provide insights into current and past host associations. In a recent broad survey of Rafflesiaeaceae mitochondrial genomes [3**], gene transfers fell within two distinct categories—transfers that were ancient, perhaps dating back to the late Cretaceous, and those that were more recent. For the ancient transfers, transgenes were broadly shared and maintained synteny across related parasitic plants. This combination of factors suggests that they were likely the result of a single, ancient host-to-parasite transfer event. In other cases, however, transgenes from these parasitic plants were broadly dispersed among more recently derived host lineages, suggesting recent transfers. Interestingly, in the case of the ancient transfers, the transgenes did not group near to their current host lineages, suggesting that HGT involved former host associations, subsequently followed by a host shift. This is further supported by molecular divergence estimates, which demonstrate that the origin of the parasitic plants greatly predates that of their extant hosts [3**]. By contrast, the more recent transfers tended to group with their current hosts. In these regards, HGT can serve as the ‘ghost of transfers past’, and will aid us in identifying former host associations. As more and better comparative genome data become available we will be able to time these HGT events with greater precision to better identify former host associations. In addition, by identifying these former associations we can begin to clarify fundamental host requirements of these parasitic plants.

Conclusions and future directions

Studies to date indicate that parasitic plants represent an active area of HGT. These symbioses are ideal for studying this phenomenon owing to the intimacy of their symbiotic interactions, and our ability to more confidently
invoke HGT when phylogenies are incongruent with accepted species relationships. In general, HGT in parasitic plants is reflective of what we see in autotrophic plants [10,11]**; gene transfers involving mitochondrial genomes are high, and appreciable in nuclear genomes. The functionality of these transgenes is unknown, but seems likely in at least some instances. Along these lines, establishing functionality of transgenes in their recipient genomes will be a key goal going forward. Despite lower overall rates of HGT in the nuclear genome, these events could have a major influence on the evolution of novelty in their recipient lineages. The difficulty in elucidating function is not trivial, however, owing to their two-parted symbiosis and the challenges of growing these plants in controlled environments. In this regard, some taxa, such as *Cuscuta*, are likely to be more suitable for this purpose [41]. These species possess a simple anatomical structure, seed germination requiring only water, and a wide range of host tolerances. In addition to these more phylogenetically focused experiments, HGT remains largely unexplored for numerous parasitic clades at the genomic level. Future explorations should generate genomic data for these neglected clades to further discern the magnitude of HGT, especially by sequencing regions outside of transgenes to identify putative vectors from genomic signatures. These exercises in genomic surveillance should prove useful for understanding differential rates of HGT among genomes, common patterns of genes that are transferred (and lost in recipient genomes following transfer), and the prevalence of HGT among different modes of parasitism.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest

** of outstanding interest

1. Nikolov LA, Tomlinson PB, Manickam S, Endress PK, Kramer EM, •<br> Davis CC: Holoparasitic Rafflesiaceae possess the most reduced endophytes and yet give rise to the world’s largest flowers. *Ann Bot* 2014, 114:233-242.<br> This study characterizes the anatomy and morphology of the greatly reduced endophyte of the parasitic plant clade, Rafflesiaceae, which also produce the world’s largest flowers. It provides a good introduction to holoparasites more generally.


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17. Rice DW, Alverson AJ, Richardson AO, Young GJ, Sanchez-• Puerta MV, Munzinger J, Barry K, Boone JL, Zhang Y, dePamphilis CW et al.: Horizontal transfer of entire genomes via mitochondrial fusion in the angiosperm *Amborella*. *Science* 2013, 342:1468-1473.<br> This paper reports the nearly 4-megabase mitochondrial genome of Amborella, which acquired six genome equivalents of foreign DNA via HGT from donors as diverse as algae and mosses. How this occurred is unclear but may have been facilitated by the close interaction between Amborella and its diverse leaf epiphyte community.


This study characterizes the likely loss of the plastid genome in the holoparasitic plant clade, Rafflesiacae.


This study traces a nuclear transgene from the parasitic plant Phelipanche to their distant legume donor. The authors identify the evolutionary fate of this transgene in its recipient lineage, which goes on to evolve and possibly specialize to achieve new function within the parasite.


This is a new Bayesian method for assessing gene duplication and gene loss versus horizontal gene transfer within a phylogenomic data set.


This study uses analyses of whole genome sequences to establish that HGT involving transposable elements may be common in plants.


