

Viewpoints

Endoparasitic plants and fungi show evolutionary convergence across phylogenetic divisions

Summary

Endoparasitic plants are the most reduced flowering plants, spending most of their lives as a network of filaments within the tissues of their hosts. Despite their extraordinary life form, we know little about their biology. Research into a few species has revealed unexpected insights, such as the total loss of plastome, the reduction of the vegetative phase to a proembryonic stage, and elevated information exchange between host and parasite. To consolidate our understanding, we review life history, anatomy, and molecular genetics across the four independent lineages of endoparasitic plants. We highlight convergence across these clades and a striking trans-kingdom convergence in life history among endoparasitic plants and disparate lineages of fungi at the molecular and physiological levels. We hypothesize that parasitism of woody plants preselected for the endoparasitic life history, providing parasites a stable host environment and the necessary hydraulics to enable floral gigantism and/or high reproductive output. Finally, we propose a broader view of endoparasitic plants that connects research across disciplines, for example, pollen–pistil and graft incompatibility interactions and plant associations with various fungi. We shine a light on endoparasitic plants and their hosts as under-explored ecological microcosms ripe for identifying unexpected biological processes, interactions and evolutionary convergence.

Introduction

Parasitic plants have evolved at least 12 times from their free-living ancestors, constituting *c.* 1.2% of flowering plant species (Nickrent, 2020). They include the world's largest flowers in the genus *Rafflesia*, acclaimed 'the greatest prodigy of the vegetable world' (Nikolov & Davis, 2017), economically important pests, and ecosystem engineers (Press & Phoenix, 2005). These plants are united by possessing an organ called the haustorium, which attaches the plant to a host, penetrates its tissues, and establishes a direct connection to its vascular system, forming a living physiological bridge between the two plants (Teixeira-Costa, 2021). Whilst some haustorial parasitic plants are photosynthetic (hemiparasites), others lack chlorophyll completely and have become dependent

entirely on their host plants for carbon (holoparasites). The most extreme holoparasites are the endoparasites – those that spend their entire life cycles within the tissues of other plants, except when briefly emerging to flower and set seed. These extraordinary plants are united by the production of an endophyte that composes undifferentiated cell filaments or cell masses embedded within the host, and a common developmental trajectory in which cell division leads to the formation of extensive parasitic tissue masses within their hosts' cortex and bark tissues (Teixeira-Costa *et al.*, 2021). The endophyte can grow extensively throughout the host, even upwards to 13 m (Barkman *et al.*, 2017). Its cell filaments often anastomose to form extensive pockets of parasitic tissue in the host cortex and bark from which 'sinkers' then penetrate the host's vascular tissue (Watanabe, 1933; Teixeira-Costa *et al.*, 2021). These sinkers have been inferred to be analogous to roots, as they carry out a similar function of absorbing water and mineral nutrients (Amaral & Ceccantini, 2011). This complex process, characterized by haustorial penetration and endophytic growth within host tissues, shows a striking pattern of cross-kingdom convergence with diverse groups of fungi that live within the tissues of plants either mutualistically or as parasites.

Here we unite the various isolated lines of research into endoparasitic plants to identify gaps in our understanding and avenues for future work. In particular, we place focus on convergence among independently evolved clades of endoparasitic plants; furthermore, we highlight striking patterns of convergence among endoparasitic plants and various groups of fungi, hitherto underestimated. Our synthesis suggests that endoparasitic plants and their hosts are under-explored as living laboratories for identifying new and unexpected processes and interactions, and convergent evolution.

The evolution of endoparasitism in plants

Of the 4750 parasitic plant species described (Nickrent, 2020), the four convergent endoparasitic lineages include over 80 recognized species, belonging to eight genera (Fig. 1). These convergent lineages parasitize numerous woody host genera from disparate families but each within a defined host range (Table 1). They have a developmental process distinct from other parasitic plants (see the Patterns of convergence in life history among endoparasitic plants section) including the endophytic mistletoes (Teixeira-Costa *et al.*, 2021) which are not covered in our article. The number of species of endoparasitic plant recognized is growing steadily each year as new species are discovered. This has been especially so in the genus *Rafflesia* (Pelser *et al.*, 2019), probably due to interest in the genus because of its enormous flowers. Species diversity in each of the other, neglected, genera may be underestimated.

Phylogenetic analyses brought the obscure evolutionary origins of the 80 known endoparasitic plants into focus at the start of the

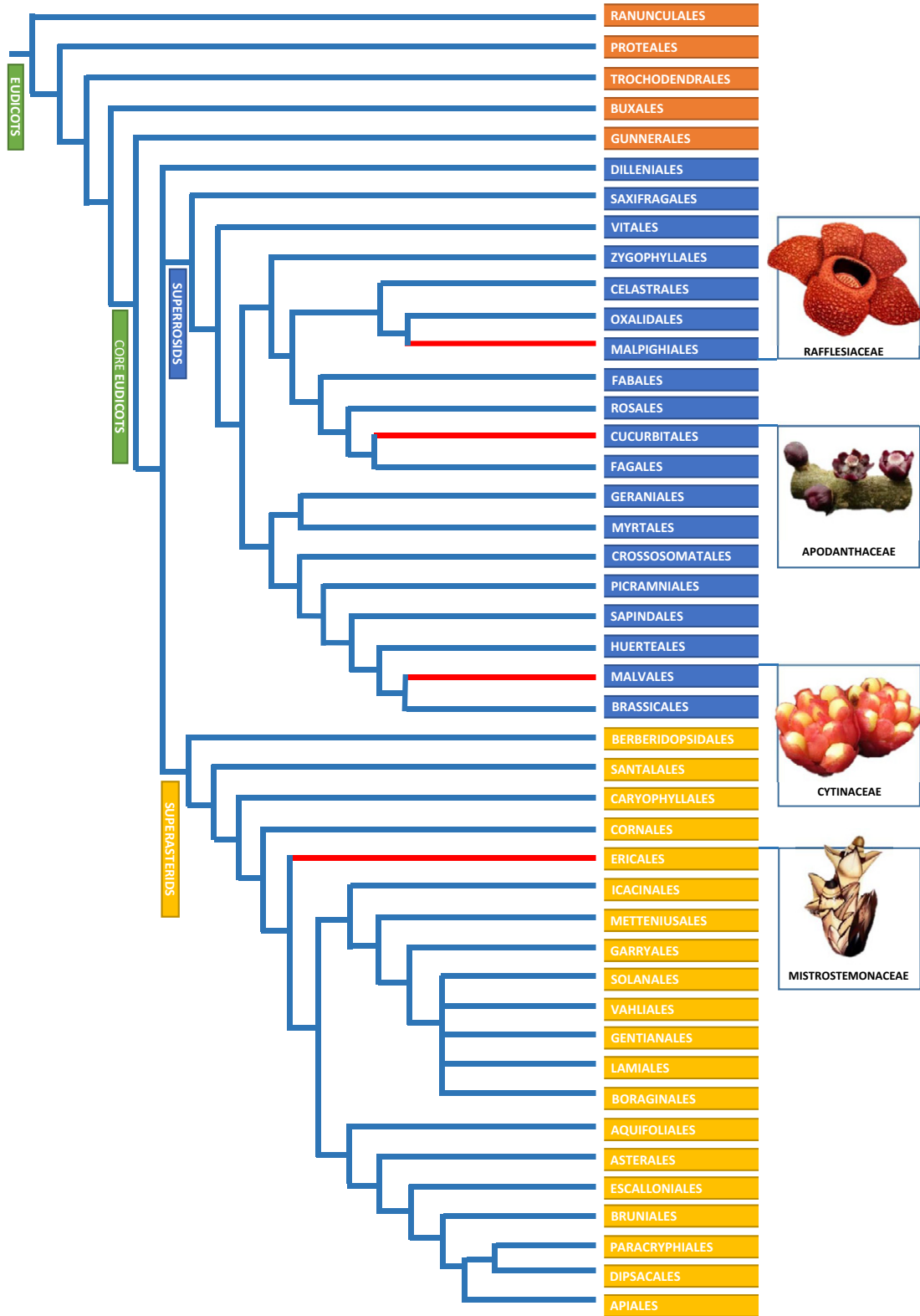


Fig. 1 The four independent evolutionary origins of clades exclusively comprising endoparasitic plants that spend their entire life cycles within their host tissues except when flowering. Lineages in which endoparasitism has evolved (indicated in red) are distributed across the Core Eudicots in both the Superrosids (indicated in blue: Malpighiales, Cucurbitales and Malvales) and the Superasterids (indicated in yellow: Ericales). Basal Eudicots are indicated in dark orange.

Table 1 An estimation of the diversity of endoparasitic plants.

Family	Genus	Number of species recognized	Principle host families
Apodanthaceae	<i>Apodanthes</i>	1	Salicaceae
	<i>Pilostyles</i>	10	Fabaceae
Cytinaceae	<i>Bdallophytum</i> (inc. <i>Sanguisuga</i>)	4	Burseraceae
	<i>Cytinus</i>	8	Cistaceae (Mediterranean) Asteraceae and others (South Africa and Madagascar)
Mitrastemonaceae	<i>Mitrastemon</i>	2	Fagaceae
Rafflesiaceae	<i>Rafflesia</i>	50 ^a	Vitaceae
	<i>Rhizanthus</i>	4	Vitaceae
	<i>Sapria</i>	4	Vitaceae

^aNumbers of accepted species were estimated by Plants of the World Online (POWO) (2019). Estimates are conservative, for example authorities on the genus *Rafflesia* recognize 30+ species (Pelser *et al.*, 2019) and the true number is likely to exceed 50.

21st century. Lacking nearly all recognizable plant structural features (Nikolov & Davis, 2017) and long-thought to be monophyletic, molecular phylogenetic analyses finally revealed their four independent origins (Nickrent *et al.*, 2004). Then astonishingly, the closest photosynthetic relatives of the enigmatic genus *Rafflesia* were revealed to reside in the Euphorbiaceae – a family of typically minute-flowered plants (Davis *et al.*, 2007). Similarly unexpected was the placement of Cytinaceae, the only clade of endoparasitic plants to form inflorescences rather than single flowers, as sister to Muntingiaceae – a family composed of trees with solitary flowers (Nickrent, 2007). Since the elucidation of the systematics of these cryptic plants, interest has returned to their unique and peculiar anatomy and development.

Convergent evolution in endoparasitic plants and biotrophic fungi

Parallels may be drawn between the life cycles of endoparasitic plants and various endophytic fungi (those that live in plant tissues) and biotrophic fungi (pathogens that derive nutrients from living tissues) in particular, at the physiological and molecular level (summarized in Fig. 2). Both endoparasitic plants and biotrophic fungi produce organs for host plant penetration and nutrient abstraction, and show filamentous growth within host tissues. Analogous structures have also evolved independently across groups of biotrophic fungi, including rusts (Basidiomycota), powdery mildews (Ascomycota), as well as symbiotic arbuscular mycorrhizas (Glomeromycota), and in downy mildews (Oomycota) – a different kingdom altogether. The architecture for attaching to and penetrating host plant tissues is clearly a widespread adaptation for host infection (Garnica *et al.*, 2014). The intimate association between plant and fungal pathogens has led to a high level of communication, for example, the exchange of small RNAs (Weiberg *et al.*, 2013; Wang & Dean, 2020) just as it

has in endoparasitic plants (summarized in the Host–parasite communication section). Another shared feature at the molecular level is the loss of genes associated with redundant pathways, for example, genes encoding enzymes for nitrate and sulphate assimilation in wheat biotrophic fungi (Tang *et al.*, 2018), broadly analogous to the loss of genes involved in photosynthesis in endoparasitic plants. Indeed, although gene losses or contractions are lineage specific, they occur across clades of fungal pathogens. For example, the genome of powdery mildew only has about half the total number of genes as the genomes of closely related ascomycete fungi (Wu *et al.*, 2018); meanwhile in the oomycetes, grapevine-downy mildew (*Plasmopara viticola*) has lost genes involved in nitrogen metabolism and γ -aminobutyric acid catabolism (Brilli *et al.*, 2018). Furthermore, as a common phenotypic solution to the challenge of infesting new hosts that may be rare or specific, biotrophic fungi and endoparasitic plants produce large numbers of tiny spores and seeds, respectively. Like parasitic plant seeds, the spores of biotrophic fungi germinate and penetrate host tissues in response to specific cues and host cell topology (Solanki *et al.*, 2019).

Patterns of convergence in life history among endoparasitic plants

In the past two decades, refreshed assessments of endoparasitic plant anatomy have progressed our understanding of the parasite–host interface (de Vega *et al.*, 2007; Amaral & Ceccantini, 2011; Nikolov *et al.*, 2014a; Nikolov & Davis, 2017). However, a comparative analysis of the anatomy and development of the four independent endoparasitic plant lineages was achieved only recently (Teixeira-Costa *et al.*, 2021). This work demonstrates that species in the four exclusively endoparasitic families, the Apodanthaceae, Cytinaceae, Mitrastemonaceae, and Rafflesiaceae, show a remarkably similar developmental trajectory. Unlike other plants with a reduced vegetative plan, the ontogenesis of these plants is characterized by a pattern of late cell differentiation which culminates in direct connections to host vessels, and profound changes to host xylem and phloem development. Furthermore, alterations to host vasculature and host–parasite xylem connectivity have important implications for the physiology of parasite–host relations; understanding their physiology (summarized in Fig. 3 and developed within the host) can, in turn, shed light on the evolution of these extraordinary plants. Here, we summarize key findings in endoparasitic plant life history and development to date, and highlight areas of convergence.

Germination and establishment

Seeds of holoparasitic (nonphotosynthetic) plants that have been researched to date (predominantly in the family Orobanchaceae), require exposure to host signal molecules to germinate. Data on germination for the majority of endoparasites are scant (Nikolov & Davis, 2017) but it appears that host-derived stimulants may also be required (Wicaksono *et al.*, 2020). Whilst *Rafflesia* and its closest relatives are among the most researched endoparasitic plants, details of how their seeds germinate and infect the host vine remain a mystery (Nikolov *et al.*, 2014a). Furthermore, most attempts to

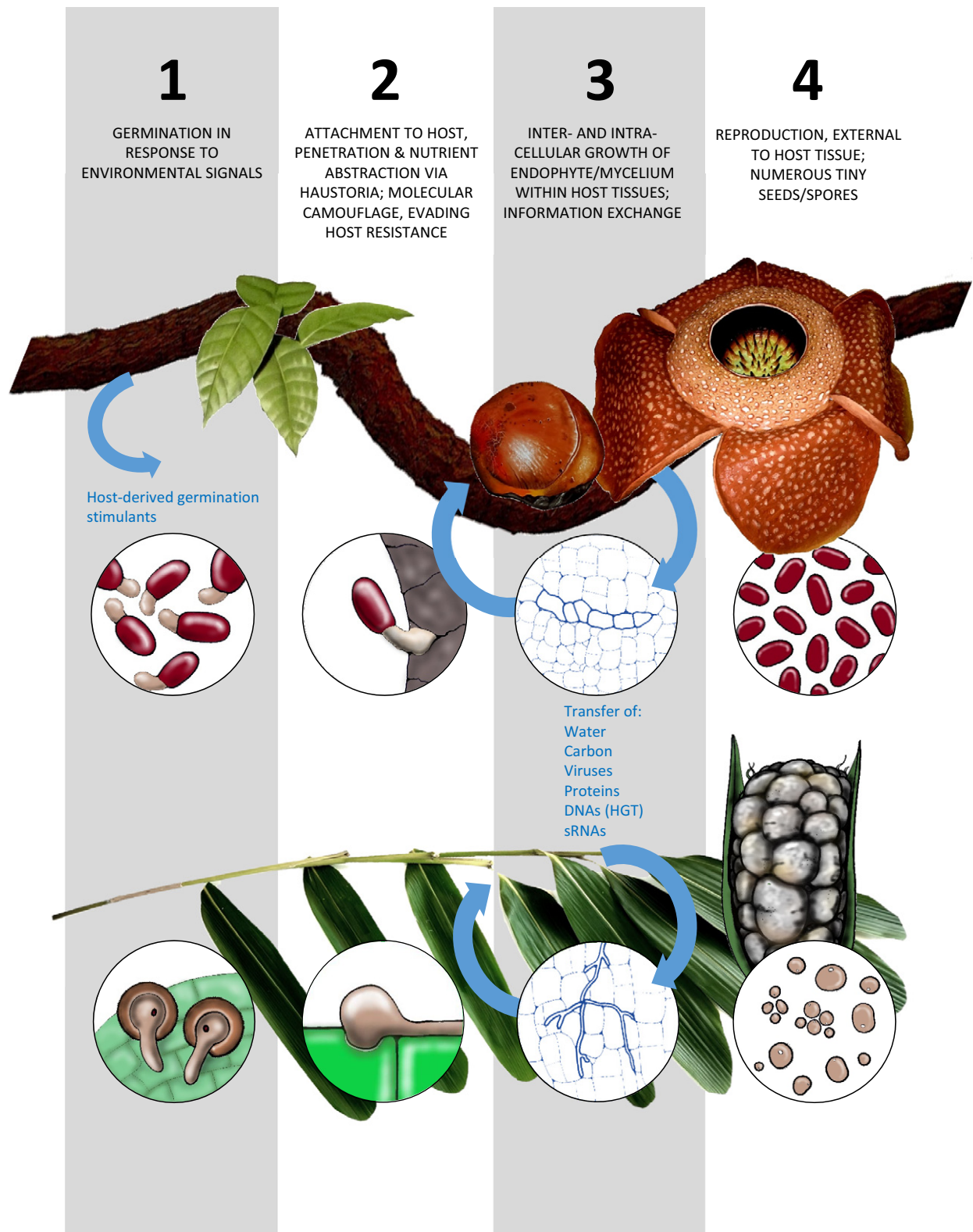


Fig. 2 The simplified life cycle of an endoparasitic plant (above; *Rafflesia*), and a biotrophic fungus (below: *Ustilago maydis*) in which the key phases are highlighted and patterns of convergence summarized. An important distinction between endoparasitic plants and biotrophic fungi can be seen in stage 3: the endophyte of endoparasitic plants shows intercellular growth, most likely leading to the formation of secondary plasmodesmata; by contrast the endophyte of the fungus invades the host cells, that is, it shows intracellular growth. Note that the diagrammatic representation in Fig. 2 is illustrative and not to scale.

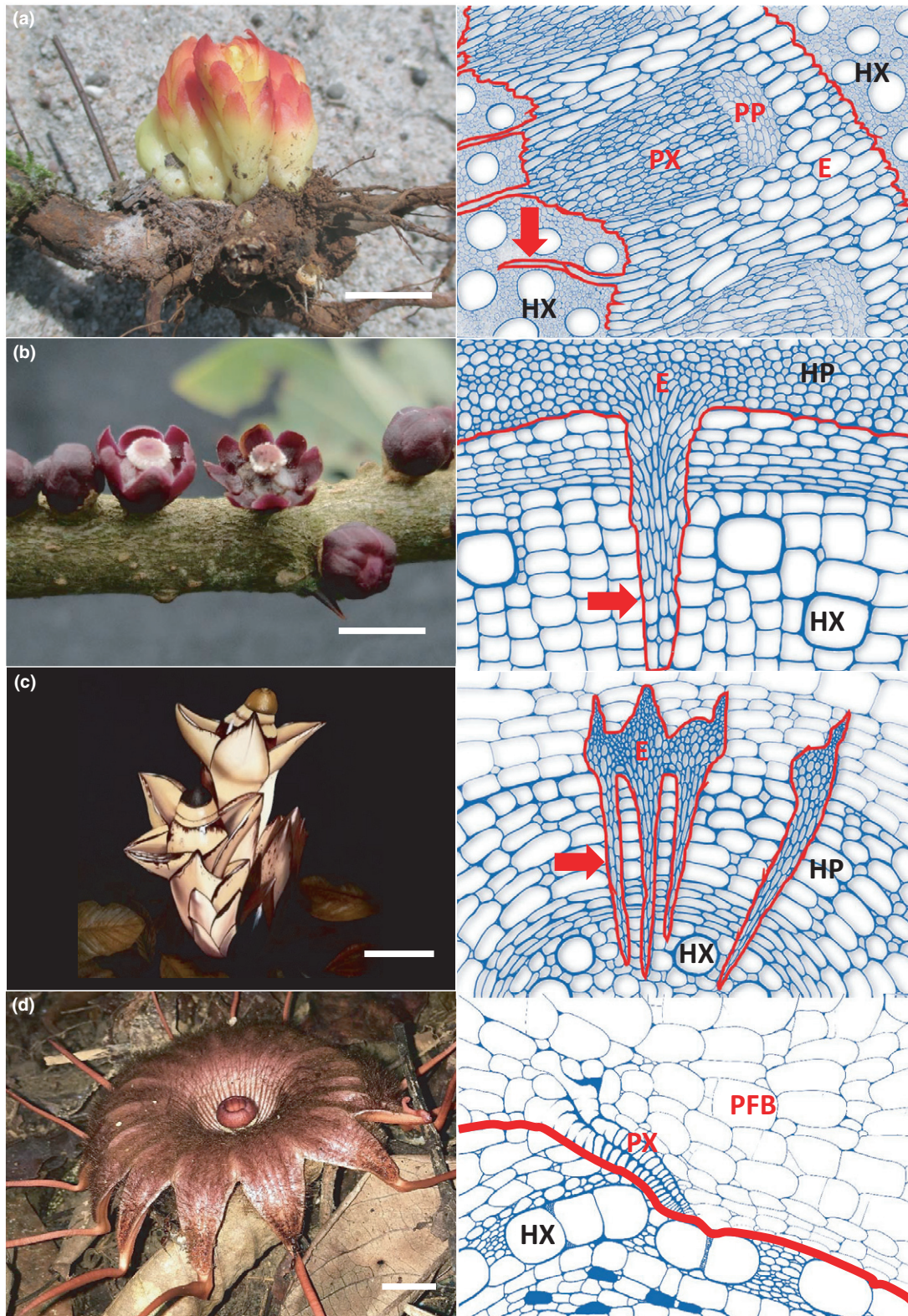


Fig. 3 The gross morphology (left) and endophyte anatomy (right) of the four independent lineages of endoparasite. Endophytes are demarcated in red: (a) Cytinaceae (*Cytinus hypocistis*); bar, 2 cm; note the presence of host vascular tissue. Cytology informed by de Vega *et al.* (2007). (b) Apodanthaceae (*Pilosyles*, photograph by Danilo Soares Gissi); bar, 1 cm; note the presence of the sinker traversing host phloem and xylem tissues. Cytology informed by Amaral & Ceccantini (2011). (c) Mitrostemonaceae (*Mitrastemon matudae*); bar, 1.5 cm. Cytology informed by Watanabe (1933). (d) Rafflesiaceae (*Rhizanthus lowii*, photograph by Syarifah Haniera); bar, 4 cm. Cytology informed by Nikolov *et al.* (2014a). Scale bars are not included for endophyte anatomy as these are representations rather than cytology slides. Red arrows, sinkers; E, endophyte; HP, host phloem; HX, host xylem; PP, parasite phloem; PX, parasite xylem; PFB, parasite flower bud.

germinate the seeds of endoparasitic plants artificially using approaches successful in other parasitic plants have failed, for example, the use of plant growth regulators (Molina *et al.*, 2017). The scant available reports of artificial germination of Rafflesiaceae seed have been summarized by Wicaksono *et al.* (2020), highlighting that successful attempts involve spreading and/or rubbing parasite seeds upon the stems of suitable hosts. It is also possible that mycorrhizas are involved in germination and establishment; these have been observed to form a tripartite interaction in the endoparasite *Cytinus hypocistis* and its host (de Vega *et al.*, 2010). The evolution of the endoparasitic life history may be more complex than considered previously, and involve multi-species interactions, and multidirectional flows of influence, as suggested for the evolution of (mutualistic) endophytic fungi (Saikkonen *et al.*, 2004). It should be noted that attempts to germinate *Rafflesia* seed using peptone, known to yield 100% germination in other mycorrhiza-dependent plants, have all failed (Molina *et al.*, 2017). The processes of host-triggered germination and early establishment in most species of endoparasitic plants remain a mystery.

Following attachment to the host, anecdotal observations suggest that the epicotyl of the endoparasite seedling dies, after which the endophyte spreads invasively and cryptically through the host, eventually losing contact with the initial site of penetration (Wicaksono *et al.*, 2020). Again, this mode of infestation bears great resemblance to endophytic fungal penetration, in which endophytes also grow undetected in their host tissues and often exhibit episodic growth, for example, a prolonged, inconspicuous period in which growth may cease and then resume after a maturational change in the host (Stone *et al.*, 2004).

Endophyte development and vascular connections with the host (Fig. 3)

The developing endophyte is intertwined with the tissues of its host. During the early development, endoparasite growth cannot be discerned macroscopically (Nikolov *et al.*, 2014a) and in most species, remains unexamined. Astonishingly, in the Rafflesiaceae, the endophyte appears to develop directly from a proembryo, like within the Orchidaceae, rather than an embryo proper (Nikolov *et al.*, 2014a). In all four endoparasitic lineages, differentiation of vascular cells is only detected among endophyte cells once flower bud formation begins (Teixeira-Costa *et al.*, 2021). At this stage, substantial alterations to the host xylem micromorphology occur. In general, a proliferation of host vascular tissue made up of small vessels at high density occurs during endoparasite bud development (García-Franco *et al.*, 2007; Amaral & Ceccantini, 2011; Teixeira-Costa *et al.*, 2021). These observations suggest that while endoparasite infestation reduces host efficiency in sap-conductivity, it may also optimize xylem 'hydraulic safety' in the longer term. We hypothesize that this right shift in the efficiency-certainty trade-off could be a strategy for endoparasitic plants, as they tend to be long-lived and slow growing (Wicaksono *et al.*, 2020).

These parasite-induced alterations in wood micromorphology indicate that endoparasitic plants divert significant volumes of

water from their hosts. Apodanthaceae, Cytinaceae, and Mitrastemonaceae all typically develop many (often hundreds) of small flowers/inflorescences that form extensive clumps among their hosts' stems or roots. By contrast, Rafflesiaceae produce solitary, enormous flowers; all lineages are characterized by a potentially high reproductive output. Flowering and fruiting in each family can also last for many weeks or even months, potentially increasing the demand for host-derived water. Species in the Rafflesiaceae parasitize *Tetrastigma* (Vitaceae), which form long and woody vines with large vessels and anatomical characteristics that confer both stem strength and flexibility (Pace *et al.*, 2018). This combination of traits may enable the necessary water flow and mechanical support to sustain such gigantic and heavy flowers. Vessel-size also appears to be an important feature determining host susceptibility and infestation in some endophytic fungi (Pouzoulet *et al.*, 2014).

It is noteworthy that endoparasitic plants parasitize exclusively woody hosts. A woody, slow-growing host may be a prerequisite to the endoparasitic habit, perhaps offering the parasite a stable, sheltered environment within which to develop gradually and (at least initially), undetected.

Floral shoot development

The floral shoot is the most conspicuous part of all endoparasitic plants. In *Rafflesia*, it is connected to the host vasculature by a chimeric structure known as the cupule, produced in part by the host, as a wound response caused by the burst of the parasite buds, as well as by the parasite itself (Nikolov *et al.*, 2014a). Nikolov *et al.* (2014a) show that the incipient floral shoots of species of Rafflesiaceae, known as the protocorm, originate from endophyte strands located among the parenchymatous rays of the secondary xylem. A detailed account of floral development is given by Nikolov *et al.* (2013). *Cytinus* and *Pilostyles* also trigger massive distortions of their host tissues when the flowering shoot emerges from the continuous sheath of the endophyte (de Vega *et al.*, 2007; Amaral & Ceccantini, 2011). Indeed, distortion of host tissue is common across endoparasitic lineages, characterized by the differentiation of new vessel elements towards the parasite flower or inflorescence (Teixeira-Costa *et al.*, 2021). Given the convergent life history of plant and fungal endophytes, interesting parallels may also be drawn with the emergence of fungal fruiting bodies from their hosts. An unusual convergent feature observed across the four endoparasitic families is endogenous flower/inflorescence development, in which the reproductive organs differentiate from a secondary morphological surface (Nikolov *et al.*, 2014a). Unlike most angiosperms, in which the reproductive apex forms from the primary morphological surface, the reproductive apex of Rafflesiaceae forms secondarily via internal cell separation (schizogeny) along the distal boundary of the host–parasite interface (Nikolov *et al.*, 2014b). This process has also been observed in *Bdallophytum*, *Pilostyles*, and *Mitrastemon* (Teixeira-Costa *et al.*, 2021) as well as in *Rafflesia* and *Rhizanthus* (Nikolov *et al.*, 2014b); a similar ontogenetic process is known only from *Balanophora* – a root holoparasite (not an endoparasite); here this unusual development was suggested to have evolved as a means of protecting the emergent floral shoot (Shivamurthy *et al.*, 1981).

Host–parasite communication

Host–parasite systems are ideal for exploring information exchange among plants because of the intimacy of the interactions involved. The recent availability of genomic and transcriptomic data has accelerated the rate of discovery of the molecular mechanisms underpinning plant parasitism (Clarke *et al.*, 2019). Horizontal gene transfer (HGT) – the rare exchange of genetic materials between distantly related, nonmating organisms has been shown to be astonishingly rampant in parasitic plants, especially in the mitochondrial genome (Xi *et al.*, 2013). Interestingly Rafflesiaceae contain horizontally transferred genes from the Cucurbitaceae and Apiaceae, revealing historic parasitism of species from families beyond the Vitaceae (Xi *et al.*, 2013). HGTs also revealed extinct host associations in *Sapria* (Rafflesiaceae) recently (Cai *et al.*, 2021). The authors demonstrated that at least 1.2% of the *Sapria* genome, including both genic and intergenic content, is derived from host-to-parasite HGTs and even includes genes potentially adaptive for parasitism. In the absence of plant endoparasite–host associations in the fossil record, this work shows the value of HGTs as ‘DNA fossils’ for investigating extinct associations among plants.

Besides HGTs, haustoria in parasitic plants can also facilitate the bidirectional movement of viruses, proteins, and RNAs (Kim & Westwood, 2015), and this has been especially well documented in some weedy parasites. For example, recent work shows that the parasitic plant dodder (*Cuscuta campestris*) can even synchronize its flowering with that of its host. The FLOWERING LOCUS T (FT) protein which induces flowering in plants, is synthesized in the leaves; therefore it is highly unlikely that leafless dodder has fully functional FT genes. Rather, host-synthesized FT protein moves from host tissues into dodder stems, where it interacts with the dodder FD transcription factor, activating flowering in the parasite (Shen *et al.*, 2020). Indeed large-scale inter-plant protein translocation involving over 1500 proteins has been reported in *Cuscuta* and its host plants (Liu *et al.*, 2020). The extent to which molecular exchange across the haustorium may affect gene expression in nonweedy species is unknown. However, it is likely that the transfer of RNAs and other molecules are significant in endoparasitic plants, given the particularly intimate association with their hosts.

Advances in our understanding of small RNA (sRNA) transfer from fungi to plants may offer insights into the life history and infection process of endoparasitic plants. Some fungal pathogens deliver sRNAs into host cells to suppress host immunity. Indeed, fungal pathogens have evolved various strategies to successfully establish infection through RNA-based communication and molecular camouflage. For example, the aggressive fungal pathogen grey mould *Botrytis cinerea*, can secrete small RNAs to silence host *Arabidopsis* and tomato plant genes involved in immunity (Weiberg *et al.*, 2013). Recently the wheat fungal pathogen *Zymoseptoria tritici* was shown to secrete LysM effector proteins to disarm fungal chitin-triggered plant immunity (Tian *et al.*, 2021). Similar mechanisms by which fungi grow undetected in their hosts’ tissues may exist in other fungal lineages; however, just as most research into parasitic plants is focussed on weedy species, most research into fungi has been directed at pathogenic species.

Host resistance responses that have been well-documented in other host–parasitic plant systems are, again, unknown in endoparasitic plants. In other parasitic plants, incompatibility interactions ‘kick in’ at early and late developmental checkpoints. The penetration of root tissues by haustorial cells has been compared with pollen tube growth in the transmitting tissue of styles (Thorogood & Hiscock, 2010) in which self-incompatible pollen–pistil interactions are also categorized by early-acting and late-acting mechanisms of pollen tube rejection. Moreover, graft compatibility and incompatibility in fruit cultivars is defined as success or failure of a graft union between rootstock and scion, and underpinned by complex anatomical, physiological, and biochemical interactions, which could also provide insight into the biology of parasitic plants (Melnik, 2017).

Convergent aspects of molecular evolution

Holoparasites are of particular interest to evolutionary biologists because they often possess reconfigured plastid genomes (plastomes) attributable to the loss of photosynthesis and housekeeping genes, and a general trend towards larger genome size, as well as an increasing occurrence of HGT, together making them a unique system for examining genome evolution (Davis & Xi, 2015; Nikolov & Davis, 2017). Aspects of the underlying genetics and genomics in holoparasites remain elusive, as recent findings have indicated that the plastomes of these rootless, leafless plants are virtually absent in some cases (Bellot & Renner, 2015; Roquet *et al.*, 2017) and entirely absent in others (Molina *et al.*, 2014; Cai *et al.*, 2021), owing to their highly derived parasitic life history. Cai *et al.* (2021) advanced our understanding by revealing that 44% of conserved eukaryotic orthogroups are lost in *Sapria*, dwarfing levels of gene loss in vascular plants reported to date. Moreover, of the extensive (44%) loss of conserved genes in *Sapria*, 13% are common across independently evolved parasitic clades (including the Orobanchaceae, Convolvulaceae and Rafflesiaceae). But the degree of loss in *Sapria* is exceptional. Intriguingly, these convergently lost genes are involved in photosynthesis, defence, and stress response, and point to a common genetic response to shared physiological modifications of the parasitic life history (Cai *et al.*, 2021). The *Sapria* genome reflects its unique biology in terms of its gene content, intron size, and level of HGT, and challenges our understanding of plant genome architecture. This work shows just how drastically endoparasitic plants’ genomes can be altered in line with their extraordinary life history.

Endophytic fungi and mycoparasitic fungi (i.e. fungi that parasitize other fungi) may also be especially prone to HGT in a way similar to endoparasitic plants in their hosts. For example, phylogenomic analyses have revealed high levels of HGT between the mycoparasitic mould *Trichoderma* (Hypocreales, Ascomycota) and plant-associated filamentous fungi belonging to different classes of Ascomycota. *Trichoderma* shows unique versatility, forming biotrophic interactions with many other organisms and an ability to decompose plant biomass. This may, in part, be a consequence of HGTs involving genes encoding plant cell wall-degrading enzymes from its plant-associated hosts (Druzhinina *et al.*, 2018). This substrate versatility appears to be analogous to the host range of parasitic plants. The transfer of fungal nuclear genes

from an ancestral species of endophytic fungus to a grass host is a further example of an HGT; an extinct grass lineage acquired the gene from a fungal endophyte *c.* 9–13 million years ago (Shinozuka *et al.*, 2017). So, just as in endoparasitic plants (Cai *et al.*, 2021), HGTs are important DNA fossils for investigating extinct associations. Colonization of plants by fungal endophytes long predates endoparasitism in plants. Indeed evidence from the fossil plant *Nothia aphylla* in association with three endophytic fungi indicates that the mechanisms causing host responses in extant plants were in place 400 million years ago (Krings *et al.*, 2007). The underlying infection mechanisms used by fungi and well-studied examples of parasitic plants have been considered to be distinct from one another (Mayer, 2006). However, it is tantalizing to hypothesize that plants may have hijacked some of the host colonization mechanisms first established by fungi during the evolutionary shift to parasitism, given the conspicuous parallels in their life cycles emphasized earlier.

In summary, our understanding of both endoparasitic plants and various lineages of fungi points to a striking pattern of convergence: (1) a high level of HGT as a consequence of intimately associated life histories; (2) the potential for reconfigured genomes as a consequence; and (3) the importance of HGT in informing extinct associations.

Toward a more complete understanding of endoparasitic plants

Endoparasitic plants have astonished biologists repeatedly. Our observations point to an under-appreciated degree of convergence, spanning phylogenetic divisions across the tree of life. Patterns of convergence among endoparasitic plants across disparate angiosperm lineages are striking from the molecular and physiological levels, to their life histories and ecologies. Meanwhile, cross-kingdom, both endoparasitic plants and endophytic fungi both grow ‘undetected’ in their hosts’ tissues for much of their life cycles and penetrate host tissues using haustoria. Both systems also show a common pattern of molecular convergence characterized by massive HGT events, and this shows great promise for unravelling extinct associations across biological kingdoms. We propose that future research would profit from insights in other fields concerning intimate associations at the cellular level including: (1) pollen–pistil compatibility/incompatibility interactions in angiosperms; (2) graft compatibility/incompatibility interactions between rootstock and scions, as well as (3) plant–fungal associations across fungal groups including endophytes, endomycorrhizas, biotrophic fungi and mycoparasites; here we suggest a potential role of secondary plasmodesmata in endoparasitic plants in contrast to the intracellular growth shown by fungi that requires further investigation. But given the intractability of endoparasitic plants in cultivation we must also look to new approaches for testing hypotheses in these ‘living laboratories’ *in situ*. And finally, when scientists look beyond the better-studied examples of endoparasitic plants such as *Rafflesia* and *Sapria*, to those that have evaded research, we will move the needle on our understanding of their unique and fascinating biology once again. Further astonishing discoveries must surely lie ahead in the plant kingdom’s greatest evolutionary enigmas.






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Author contributions

CJT conceived the work. LT-C contributed significantly to the preparation of the manuscript throughout. CD, SJH and GC contributed to the development of the manuscript and helped write particular sections.

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