Rethinking convergence in plant parasitism integrating molecular and population genetic processes

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Running Heads
Origins of convergent evolution in parasitic plants

ABSTRACT

Photosynthesis has shaped the body plan, physiology, as well as gene repertoire of all plants. Shifts to a parasitic lifestyle evolved at least twelve times, leading to more than four thousand extant parasitic plant species. This transition has consistently left a major evolutionary footprint among these parasites. Otherwise rare features have evolved repetitively at the molecular level and beyond, including reduced vegetative bodies, carrion mimicking during reproduction, and incorporation of alien genetic materials. Here, I propose an integrated conceptual model, referred to as "the bottleneck model", to describe the general evolutionary trajectory of parasitic plants and provide a mechanistic explanation for their convergent evolution. This model connects our empirical understanding of the regulatory network in flowering plants with classical theories in molecular and population genetics. It emphasizes the cascading effects brought by the loss of photosynthesis to be a major force bottlenecking the physiological capacity of parasitic plants and shaping their genomic landscapes. Here, I review recent studies on the anatomy, physiology, and genetics of parasitic plants in support of this photosynthesis-centered bottleneck model. Focusing on non-photosynthetic holoparasites, I elucidate how they inevitably reach an evolutionary terminal status, and highlight the utility of a general, explicitly described, and falsifiable model for future studies of parasitic plants.

Key words

ABA; floral pigments; gene loss; holoparasites; horizontal gene transfer; macroevolution; mycoheterotrophy; photosynthesis; transposable elements

The ca. 4,750 haustorial parasitic plants live intimately with their host plants, stealing water, nutrients, metabolites, and often genetic material through a specialized organ, the haustorium (Nickrent, 2020). These plants display a variety of lifeforms from canopy-forming sandalwoods to thread-like dodder vines, and from stem-feeding mistletoes to root-dwelling *Rafflesia*. This morphological diversity also spans a wide spectrum of photosynthetic capacity and host dependence. Based on these two axes, parasitic plants can be classified as hemi- or holo-parasites (photosynthetic vs. non-photosynthetic), and as facultative or obligate parasites (complete lifecycle with vs. without host plants). The formal recognition of parasitic plants traces back to the 18th century. In particular, Micheli (1729) and others first realized that Cynomoriaceae, Balanophoraceae, and Hydnoraceae are root parasites instead of fungi (Thunberg, 1775; Blume, 1858). This interesting historical twist epitomizes the extensive morphological modification in parasitic plants, often manifested in a highly convergent manner. Famous examples of convergence include the resemblance between dodders (*Cuscuta*, Convolvulaceae) and woe vines (*Cassytha*, Lauraceae), which are both thread-like parasitic vines separated by more than 100 million years of evolution (Ramirez-Barahona et al., 2020). Early morphology-based classification similarly grouped Rafflesiaceae, Apodanthaceae, and Cytinaceae under the order Rafflesiales, which was later demonstrated to be a polyphyletic assemblage based on molecular phylogenetic studies (Barkman et al., 2007).

The advent of modern genetic and phylogenetic era has greatly advanced our understanding of the mechanism and evolution of plant parasitism. Unlocking genomic resources from multiple parasitic lineages in recent years has led to revolutionary discoveries in systematics (Nickrent, 2020), anatomy and development (Teixeira-Costa, 2021; Teixeira-Costa et al., 2021), host interactions (Mutuku et al., 2021; Nelson, 2021), and the genomes themselves (Lyko and Wicke, 2021; Yoshida and Kee, 2021). Across all disciplines, convergence is a repeated theme. This is manifested not only at the phenotypical level such as shared features of haustoria anatomy and embryological development, but also at the molecular level, including consistent patterns of gene loss and horizontal gene transfer (Yang et al., 2019; Cai et al., 2021; Teixeira-Costa et al., 2021). On the other hand, the evolution of plant parasitism is also envisioned to progress in an irreversible manner. The best example of this comes from Orobanchaceae, the only family that retains all transitional stages from free-living taxa to facultative hemiparasites and obligate holoparasites. In this family and all other parasitic lineages, the unidirectional evolution of parasitism is evidenced by decreasing photosynthetic capacity, increasing host reliance, as well as other hallmarks of regressive evolution such as low diversification rate.

These predictable evolutionary changes highlight one fundamental question in the study of parasitic plants: What constrains the evolutionary landscape and drives convergence? To address this question, a general framework describing the evolutionary trends in parasitic plants is needed. One such framework is the “Three Phase Hypothesis”, where parasitic plants are expected to go through innovation–relaxation–optimization as time passes (Searcy and MacInnis, 1970; dePamphilis, 1995). This model has been successfully combined with molecular data to explain the genome evolution of the hemiparasites *Striga* (Orobanchaceae; Yoshida et al., 2019). However, it has several limitations. First, the concept of “phase” in the original model is misleading because it suggests a sequential order of these three evolutionary processes, while they are demonstrated to simultaneously shape the genetic landscape of parasitic plants (Lyko and Wicke, 2021). Moreover, while the three-phase model provides important insights into the transition from hemi- to holoparasitism, it offers very limited explanatory and predicative power beyond this point. For example, it struggles to explain the convergence in highly modified endoparasites, which live almost entirely inside host tissue.

To improve on this model and provide a formal, falsifiable framework for the study of parasitic plant biology, I propose a new integrated conceptual model—"the bottleneck model”—to guide the evolutionary and genetic investigation of parasitic plants. This model bridges our empirical understanding of the genetic architecture and metabolic network in angiosperms with theories of molecular genetics and population genetics. It is suited to explain the irreversible nature of plant parasitism and highlights the dynamics of the evolutionary forces shaping parallelism at different stages of plant parasitism. I highlight novel and emerging trends of convergence predicted by this model and discuss critical evolutionary forces driving these changes.

The bottleneck model for the evolution of parasitic plants

Plant parasitism can arise through multiple independent trajectories that diverge in different lineages during the early phase of evolution. As they develop a higher level of reliance on host-derived resources, relaxed selection on the corresponding pathways will lead to the accumulation of deleterious mutations and eventually loss of genes and functions. A milestone in this gene loss process is the deprivation of photosynthesis in holoparasites. This is because photosynthesis is both supported by and a major manufacturer for numerous metabolic and developmental products. Its loss marks a profoundly shrunken gene repertoire and the initiation of a series of predictable cascading effects, leading to the widely recognized convergence among independently evolved lineages. Therefore, I propose that the evolution of parasitism arises in a funnel-like process, bottlenecked by the loss of photosynthesis and constrained by molecular and population genetic processes, leading to convergent outcomes (Fig. 1A).

This model focuses on photosynthetic capacity as the major axis along which various stages of parasitism can be mapped. The constraining molecular and population processes are crucial components of the model (Fig. 1B), the identification of which provides an explicit framework for a mechanistic understanding of the evolution of plant parasitism. I propose that the molecular origins of convergence consist of three aspects: (1) shared ancestry, including a ubiquitous metabolic and genetic roadmap in green plants, the center of which is photosynthesis; (2) shared selective pressure to optimize parasitism; and (3) unidirectionality of regressive evolution due to gene loss and relaxed selection. Here, a common genetic/metabolic roadmap determines the convergence in which functional modules co-evolve (e.g., through co-degeneration). Specifically, photosynthesis related functions are overrepresented in highly conserved pathways and are tightly linked with numerous metabolic modules such as circadian rhythm, pigment production, and lipid synthesis. Dysfunction of the photosynthesis module will therefore overspread into connected modules in a cascading manner. These clues lead to the predictions that functional modules directly associated with photosynthesis are more likely to be impacted in similar ways among independently evolved lineages of parasites.

Superimposed on this molecular genetic force are population processes, which may have a stronger influence during the later phases of parasitism (Fig. 1B). Molecular changes initiated in photosynthesis-related functions can indirectly impact reproductive traits and thus influence population processes. For example, the loss of photosynthesis pigments such as chlorophylls and carotenoids can cause shifts in flower color and pollination syndrome (see more discussion in the section “Bold reproductive strategies” below). The establishment of specialized pollination services may increase population vulnerabilities to environmental oscillations (Wilcock and Neiland, 2002), causing repeated local extinctions and founder effects. Such reduction in the effective population size (N_e) will then lead to increased drift, inefficient purifying selection, and decreased fixation of adaptive changes. These phenomena will greatly inflate the genetic load of the species, and in turn bring about more deleterious mutations and selfish elements in the genome. Collectively, the mutational burden and population size limitation suggest that the fitness landscape of any parasitic organisms deteriorates overtime.

Verification and refinement of this model requires a thorough understanding of the genomic architecture and functional regulatory networks across multiple parasitic plant lineages, which is being actively constructed (Kösters et al., 2021). Using available resources, I show that this model is not only compatible with all existing data, but also provides additional power to elucidate the molecular mechanisms of enigmatic convergent trends among parasites.

From photosynthesis to ABA metabolism

Under this bottleneck model, early phases of plant parasitism are largely driven by selection to optimize parasitism and to dispense with now-useless traits. At the morphological level, Kuijt (1969) pointed out the systematic reduction in leaf size, complexity, and stomata density in parasites. At the molecular level, the minuscule plastid genomes are one of the most well studied genetic features among parasitic plants, and continue to be a captivating area of research (e.g., Wolfe et al., 1992; Wicke et al., 2013). Recent genomic studies also enabled comprehensive surveys of the nuclear genomes in *Cuscuta*, Orobanchaceae, and Rafflesiaceae. They consistently reported significant gene losses in photosynthesis related functions, including plastid organization and pigment metabolism, which aligns well with the prediction of the bottleneck model (Vogel et al., 2018; Cai et al., 2021; Xu et al., 2022). Meanwhile, unexpected gene losses were found in the biosynthesis of abscisic acid (ABA) in *Sapria* (Rafflesiaceae; Cai et al., 2021). While the classic three-phase model struggles to explain the loss of this conserved plant hormone, the bottleneck model provides an explanation for this potentially widespread trend among holoparasites.

Biosynthesis of ABA is reliant on the precursor carotenoids, which also serve as photosynthetic pigments and photoprotector (Cazzonelli, 2011). In *Sapria,* genes involved in the biosynthesis of carotenoids are completely absent and so is the organellar factory for ABA synthesis—the plastids (Cai et al., 2021). Thus the molecular basis for the absence of ABA biosynthesis can be explained by the cascading effect after the loss of photosynthesis (Fig. 2). Meanwhile, the reduced need for drought response and the possible access to host-derived ABA provide a further selective drive for such loss in parasites. One important function of ABA in flowering plants is to combat water loss by triggering stomatal closure (Wasilewska et al., 2008). However, holoparasites acquire the bulk of their water from the host and have very few to absent stomata (e.g., Kuijt and Dong, 1990; Mursidawati et al., 2020). The need to regulate these abiotic stresses is thus diminished (Zagorchev et al. 2021). Moreover, parasitic plants are known to enrich a wide range of metabolites from their hosts (Guo et al., 2022), potentially including ABA or its precursor through direct phloem–xylem transfer. In *Sapria,* despite the loss of native ABA biosynthesis pathways, downstream ABA signaling genes such as AREB/ABF, CPK, and PP2C remain intact and expressed (Cai et al., 2021), which suggests the possible use of host-derived ABA. Finally, these molecular and selective constraints accommodating the loss of ABA are not restricted to Rafflesiaceae. In fact, insensitivity to ABA has also been reported in *Cuscuta australis* (Li et al., 2015). Future studies focusing on other holoparasitic lineages are likely to find similar patterns.

On the other hand, the apparent dilemma between the absence of stomata and the demand for efficient water transportation in parasitic plants opens a new avenue for hydraulic evolution research: how do holoparasites effectively stream fluid and nutrient iron from the host when transpiration is limited? This question is particularly relevant for Rafflesiaceae, which produces the largest flowers in the world, through thread-like vegetative tissue, without any stomata (Nikolov and Davis, 2017; Mursidawati et al., 2020). Along these lines, Heide-Jørgensen (2008) commented on the possibility of an energy-consuming water pumping system in *Lathraea* (Orobanchaceae) to explain its water excreting hydathode glands under the moist subterranean habitat. However, the presence and significance of these active water transporters remain to be characterized in Orobanchaceae and other stomata-lacking parasites.

Bold reproductive strategies

While extensive effort has been dedicated to the comparative studies of vegetative organs in parasites, the evolutionary trends in reproduction have not been well studied across parasitic plants. These plants often exhibit specialized strategies that facilitate rapid regional colonization but may limit their evolutionary opportunities in the long run (e.g., specialized pollination syndrome). As predicted by the bottleneck model, many of these convergent features arise from the cascading effect of gene loss, but are also retained for their adaptive value.

The first example comes from the synchronized phenology between the host and the parasites. This phenomenon has been reported in *Cuscuta,* Loranthaceae, and Orobanchaceae (Holdsworth and Nutman, 1947; Fratianne, 1965; Candia et al., 2014; Teixeira-Costa et al., 2017) and is hypothesized to be achieved by the translocation of bio-active molecules (Smith et al., 2013). In *Cuscuta*, such a molecule has been pinpointed to a flowering signaling protein FT (Shen et al., 2020). Here, hijacking host-derived signal is a necessity because the native FT pathway in *Cuscuta* has been disrupted due to the loss of key genes in its upstream circadian clock and vernalization pathways (e.g., *CO*, *FLC*, and *SVP*; Sun et al., 2018). These losses can be traced back to relaxed selection on light sensing and abiotic stress response when the species transitions to a holoparasitic lifestyle (Fig. 2). On the other hand, such synchronization may confer an increased reproductive success when hosts are short-lived, therefore be favored by selection. In addition to *Cuscuta*, a very similar pattern of flowering synchronization is also observed in the lesser broomrape *Orobanche minor* (Holdsworth and Nutman, 1947). As more genomic resources become available in Orobanchaceae (Xu et al., 2022), comparative genomic tools can be used to identify the pathways modulating life-cycle events in *Orobanche* and test whether the same underlying molecular mechanism applies.

Another more widespread, yet poorly characterized trend is the presence of the intense red colorants in holoparasites (Fig. 2), whose composition, biosynthesis, and eco-evolutionary function remain largely unknown. A reasonable hypothesis for such convergence is that holoparasites do not produce (or produce at very low level) photosynthetic pigments, including chlorophylls and carotenoids, leaving flavonoids, especially anthocyanin, as the sole option for pigmentation. Indeed, a number of ethnobotanical studies have reported the abundance of anthocyanins in *Sapria* (Iwashina et al., 2020)*, Cynomorium* (Cynomoriaceae; Harraz et al., 1996; Zheng et al., 2021), *Hydnora* (Hydnoriaceae; Wintola and Afolayan, 2015), and *Ombrophytum* (Balanophoraceae; Nina et al., 2020), which often demonstrate in vitro antioxidant and antimicrobial properties. Compared with mycoheterotrophic plants, which are also non-photosynthetic and ground dwelling but generally lighter in coloration (Leake, 1994), the pigments present in holoparasites must provide additional adaptative value specific to this lifestyle. But how? Reasonable hypotheses include the demonstrated antimicrobial and antioxidant properties, but may also come from their role in photoprotection, pollinator attraction, or as carbon storage sinks (Steyn et al., 2002; Hughes et al., 2005; Cisowska et al., 2011; Bustos et al., 2012; Narbona et al., 2021). Future studies focusing on this question will need to investigate systems exhibiting intra- or inter-species variation in pigment composition and then look for molecular and environmental factors that covary with this phenotype. Interesting outliers also exist in holoparasitic Orobanchaceae, whose floral color ranges from pale white, yellow, to purple and red. Such diverse array of floral color could be constrained by their bee pollination syndrome but may also originate from the presence of native carotenoids. This is because strigolactone, a derivative metabolite of the carotenoid pathway, is essential for host detection and post-germination development in the broomrapes (Matusova et al. 2005; Das et al., 2015).

Finally, parasites are inevitably faced with a series of reproductive challenges. In holoparasites, the altered floral coloration and structure, the ephemeral inflorescence, and the ground dwelling habit do not fit with the search image for a rewarding flower in the eye of a nectar-seeking pollinator. As a result, these plants come up with creative alternative solutions. These include the carrion mimicking in *Rafflesia, Cynomorium,* and possibly *Pilostyles* (Apodanthaceae), the catch-and-release of hide beetles in *Hydnora*, and the recruitment of ants and roaches in *Mitrastemon* (Mitrastemonaceae) and *Balanophora* (Balanophoraceae; Fig. 2) (Bolin et al., 2009a; Bellot and Renner, 2013; Sipes et al., 2014; Wee et al., 2018; Suetsugu, 2019; Suetsugu and Hisamatsu, 2020; Wang et al., 2021). In all systems, distinct scent profiles are likely catered to the taste of their pollinators. Among them, carrion scents including indole and dimethyl disulphide are frequently produced (Jürgens et al., 2013; de Vega et al., 2014; Wee et al., 2018; Wang et al., 2021). Whether their biosynthesis also follows convergent pathways remains to be explored.

Besides pollination, seed dispersal and germination are another major challenge. Many parasites have a strong bearing on signals from host exudates for germination. These species have become r-strategists that produce numerous tiny seeds to hedge their investments in each individual offspring. As a result, a sharp contrast in seed size and number exists between species with host-dependent or host-independent early life strategies (e.g., holoparasitic Hydnoraceae vs. hemiparasitic Olacaceae in Fig. 3; Kuijt, 1969). To take an example from Orobanchaceae (Fig. 3), the facultative hemiparasite *Melampyrum* can complete its life cycle without a host. Its single seeded capsules produce heavily invested large seeds with nutritious endosperms and even elaiosomes for ant-vectored seed dispersal (Chlumský et al., 2013). In contrast, the obligate hemiparasite *Striga* is reliant on the host root exudate strigolactone for germination (Toh et a., 2015), and produces hundreds of small seeds with minute embryos in one fruit. The holoparasite *Orobanche* packs thousands of even smaller dust-like seeds because their survival is conditioned on instant connection with host plants. While seed size generally exhibit higher evolutionary plasticity, the number of seed per fruit is more likely to be constrained by ancestry. For example, Balanophoraceae has one of the smallest dust seeds in the world, but one seed is contained in each tiny flower due to the ancestral drupe fruits in Santalales (Fig. 3). The most extreme cases of dust seeds though, come from *Rafflesia* and *Hydnora,* whose big fleshy fruits contain tens of thousands of minute seeds. Given the odds of successful reproduction in these genera—distantly located dioecious flowers, highly skewed sex ratio, animal vectored pollination and seed dispersal, and host stimulated germination—fecundity is a necessity (Bolin et al., 2009b; Pelser et al., 2013).

Comparison to mycoheterotrophic (MHT) plants

Besides parasitic plants, MHTs are another group of heterotrophic plants who rely partially or entirely on their fungal partners for carbon and nitrogen (Leake, 1994). Among them, non-photosynthetic full MHTs exhibit a number of characters similar to holoparasites (Table 2). Many of these shared phenotypic and genetic traits can be explained by the bottleneck model of parasitic plant evolution. These include the degeneration in vegetative organs and loss of organellar and nuclear genes in alignment with the loss of photosynthesis. Besides, insect-pollinated flowers and dust seeds are also common among full MHTs (Leake, 1994), which could be similarly driven by the "seed bet hedge" strategy to enhance the success of their fungal-dependent germination. At the genetic level, gene loss and lineage-specific elevation in substitution rates are also reported in MHTs (Lemaire et al., 2011; Schelkunov et al., 2018; Yuan et al., 2018; Li et al., 2022), but generally less extensive compared to holoparasitic plants (Bromham et al., 2013). For example, 20.4% highly-conserved BUSCOs (benchmarking universal single-copy orthologs) were missing in the full MHT orchid *Gastrodia elata,* whereas up to 55.4% of these genes are lost in the holoparasitic *Sapria* (Yuan et al., 2018; Cai et al., 2021). In addition, unlike the prevalent horizontal gene transfer characterized in parasitic plants, fungus-to-MHT gene transfers are never identified. I hypothesize that the reduced level of gene loss and the lack of gene transfer in MHTs can be attributed to their cross-kingdom symbiotic relationship—a limited assemblage of fugus-derived carbon and nitrogen are available to MHT plants, while in haustorial parasites a much wider range of plant-derived bioactive molecules including DNA/RNA and metabolites can be directly used. As a result, haustorial parasites experience more widespread relaxed selection and gene losses in these related pathways. Future comparative genomic studies involving both MHTs and parasitic plants should focus on differentiated gene loss patterns to identify molecular resources uniquely utilized by these two groups of plants.

Macroevolutionary implications

So, is parasitism a cursed strategy? From a macroevolutionary perspective, my answer is yes. A comprehensive evaluation of parasitic plant diversification across all clades, time, and space is currently lacking. But even without such rigorous evaluations, it is clear that parasitism is negatively associated with diversification rates—parasitic plant lineages are less speciose than their sister groups, and older lineages have been left with much fewer extant species compared to younger clades such as *Cuscuta* and Orobanchaceae (Table 1; Hardy and Cook, 2012; Naumann et al., 2013). This reverse time–diversity relationship suggests a high extinction rate especially in holoparasites, which could be formally tested using state dependent speciation and extinction models (e.g., Mortimer et al., 2022).

In hemiparasites, rates of diversification are likely comparable to free-living plants. Rapid radiation is well-documented in *Pedicularis* (Orobanchaceae) and Loranthaceae, primarily fueled by ecological speciation (Ree, 2005; Liu et al., 2018). However, hemiparasitism is evolutionarily unstable. This is evidenced by the lack of transition series in all parasitic lineages except Orobanchaceae and arguably Santalales. Here, the reliance on host-derived water and nutrient relaxes the selective pressure maintaining autotrophic functions. The accumulation of deleterious mutations will eventually shut down photosynthesis and reversion to full autotrophy has never been reported even in facultative hemiparasites (Watson et al., 2022). This transition takes around thirty million years in *Cuscuta* and Orobanchaceae, but may take longer in Krameriaceae, *Cassytha,* and Santalales (Table 1). However, both Krameriaceae and *Cassytha* have crown group age estimates around 12 Ma despite their old stem group divergences (Renner & Schaefer 2010; Ramirez-Barahona et al. 2020). Hemiparasitism may have evolved only recently in these clades.

The transition to holoparasitism is a hallmark of a steeper evolutionary landscape, leading to a series of extensive and widespread changes that reshape their morphology, physiology, and reproduction. At the molecular level, deleterious mutations such as gene loss, selfish element proliferation, and non-coding region expansion are constantly accumulated in the genome. At the population level, high levels of host specificity and specialized pollination and seed dispersal mechanisms may make them vulnerable to environment upheavals and local extinctions. Founder effect may therefore repetitively bottleneck the genetic diversity, which contributes to the accumulation of more deleterious mutations. In fact, the overrepresentation of functional dioecy in holoparasites (Bellot and Renner, 2013) could be driven by the need for outcrossing to maintain genetic diversity. Nonetheless, this vicious circle will eventually reach a terminal point of extinction where it is no longer sustainable.

Routes to genetic innovations

Despite overpowering gene losses and isolated populations, parasites like Rafflesiaceae are still able to persist over dozens of millions of years and invade novel habitats (Pelser et al., 2019). This raises the question of how genetic variation as the raw material for adaptation is generated to offset deleterious mutations and open new ecological opportunities.

In eukaryotes, horizontal gene transfer (HGT) and tandem/genome duplication are two common ways for genetic innovation. HGT is especially well known in parasitic plants where alien genetic materials from the host(s) are fixed in the genome of parasites (Davis and Xi, 2015). The introduction of HGT across species boundaries can bring game-changing innovations, such as antibiotic resistance in bacteria, detoxification ability in whiteflies, and phytophagous insect resistance in ferns (Groisman and Ochman, 1996; Li et al., 2018a; Xia et al., 2021). In parasitic plants, despite the ubiquitous presence of HGT, few studies have attempted to address their functionality beyond gene ontology (GO). The best evidence towards the adaptive role of HGT perhaps comes from the convergent HGTs in *Cuscuta* and Orobanchaceae, where the same genes are repetitively acquired from the host and are highly expressed in the haustoria (Yang et al., 2019). Such parallelism may be shaped by selection or genome architecture, which I term as the “functional necessity” vs. “mechanistic convenience” hypotheses, respectively (Fig. 4). Here, the functional necessity scenario posits selection as a mechanism for convergence—HGTs rescue the loss of housekeeping functions that are susceptible to disruption in all parasites. Therefore, convergent HGT is more common in essential pathways closely linked with photosynthesis or other autotrophy related pathways. On the other hand, the mechanistic convenience scenario proposes that some genes are more likely to be transferred because they are structurally more mobile (e.g., hitchhiked to transposable elements, TEs). Their degree of parallelism is thus determined by conservation in genome architecture across species.

In addition to HGT, gene duplications can bring novelty through sub- and neofunctionalization (Ohno, 1970). In parasitic plants, the most well-known example comes from the strigolactone receptor gene *KAI2d* in Orobanchaceae, which proliferated in the genome via tandem and genome duplication and contributed to host range expansion (Conn et al., 2015; Toh et al., 2015; Yoshida et al., 2019; de Saint Germain et al., 2021). A recent comprehensive survey across six Orobanchaceae genomes reported that 11% of the highly expressed genes in the haustoria originated from a shared genome duplication event in the common ancestor of Orobanchaceae and *Mimulus* (Xu et al., 2022). In *Sapria,* however, GO analysis on expanded gene families only identified broad categories including chromosome organization, DNA metabolism, and cell cycle (Cai et al., 2021), suggesting that the creation and retention of duplicated genes are likely to be a lineage-specific process. Meanwhile, the proliferation of TEs is a non-neglectable force shaping the anatomy and function of the parasitic plant genome. In *Cuscuta,* LTR and satellite DNA drove a 102-fold variation in genome sizes within 23 Myr (Neumann et al., 2021). In both *Sapria* and *Phelipanche* (Orobanchaceae), TEs account for ~90% of the total genome sizes (Cai et al., 2021; Neumann et al., 2021; Xu et al., 2022). As more genomic resources become available, the general correlation between genome-wide relaxed selection (e.g., increased d_N/d_S rate) and TE abundance can be tested. Besides being a constant threat to genome integrity, TEs also generate adaptive phenotypes in the face of stressful conditions (Capy et al., 2000; Kazazian Jr, 2004). No studies have attempted to comprehensively evaluate the adaptive value of TEs in parasites. As more comparative genomic datasets become available, it will be possible to look for signatures of adaptation such as selective sweep of TEs (Dazenière et al., 2022; Li et al., 2018b), flanking genes promoting phenotypical innovation (Studer et al., 2011), and signs of differential evolutionary rates and N_e in TE islands (Schrader et al., 2014).

Concluding remarks

The bottleneck model offers an explicit framework to describe and understand the evolutionary trajectory of parasitic plants. It underscores the enormous value of these plants as an emerging system to study the battle between chance and necessity in evolution. If we replay the tape of parasitic plant evolution, will it culminate at the same scene? Numerous questions in comparative genomics, population genetics, and macroevolution emerge from this general framework. These questions are well suited to be tested as more genetic resources become available. Here, the application of medium-coverage genome sequencing can quickly and economically survey the gene spaces of multiple parasitic lineages. This can serve as the proof-of-concept for the bottleneck model and guide future investigations. Chromosome level assemblies, on the other hand, have immense potential to address fine-scale genetic mechanisms of host specificity or ecological adaptation among recently diverged clades. Perhaps a more important value of the framework is to connect the community of parasitic plant biologists with various branches of ecology-evolutionary disciplines. The study of parasitic plants has been hindered by their scarce occurrence in the wild and the failure to cultivate them under laboratory conditions. Consequently, the bulk of our knowledge towards parasitic plants is limited to *Cuscuta* and Orobanchaceae, which are established systems for *in vitro* manipulation. An exciting future direction in these established systems involves the application of single cell and spatial expression techniques to provide a cell-level resolution for host–parasite interaction. How different parasite and host cell subpopulations respond to this nuanced interaction? How are these cell types arranged spatially and through developmental series? Single-cell technology is well suited to address these questions and it can also be used to characterize the extensive exchange of RNAs in the haustoria at fine-scale. In addition to model systems, it is also crucial to incorporate insights from families representing more “derived” stages of parasitism. Like the example from Rafflesiaceae, these species provide revolutionary insights on how biological rules can be bent. The application of techniques from population genetics, comparative methods, and herbarium-based sciences will be essential to understand the evolution of these most unusual branches on the Tree of Life.

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Author contributions

L.C. designed the study and wrote and edited all drafts.

Data Availability Statement

No new data have been generated.

Supporting Information

Appendix S1 Seed dimension database in parasitic plants. The length and width of each seed is measured in mm and is directly cited from two published databases. Please see the ‘Reference’ column for data source. The number of seeds per fruit is obtained from literature or by counting herbarium specimens in TEX-LL. These counts should be interpreted as estimations.

Literature Cited

Barkman, T. J., J. R. McNeal, S.-H. Lim, G. Coat, H. B. Croom, N. D. Young, and C. W. Depamphilis. 2007. Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evolutionary Biology* 7: 1-15.

Baskin, J. M., and C. C. Baskin. 2022. Germination and seed/embryo size in holoparasitic flowering plants with "dust seeds" and an undifferentiated embryo. *The Botanical Review* 88: 1-49.

Bellot, S., and S. S. Renner. 2013. Pollination and mating systems of Apodanthaceae and the distribution of reproductive traits in parasitic angiosperms. *American journal of botany* 100: 1083-1094.

Blume, C.-L. 1858. *Flora Javae, nec non insularum adjacentium. Sumptibus Librariæ* J. Frank.

Bolin, J. F., E. Maass, and L. J. Musselman. 2009a. Pollination biology of *Hydnora africana* Thunb.(Hydnoraceae) in Namibia: brood-site mimicry with insect imprisonment. *International Journal of Plant Sciences* 170: 157-163.

Bolin, J. F., E. Maass, K. U. Tennakoon, and L. J. Musselman. 2009b. Host-specific germination of the root holoparasite *Hydnora triceps* (Hydnoraceae). *Botany* 87: 1250-1254.

Bromham, L., P. F. Cowman, and R. Lanfear. 2013. Parasitic plants have increased rates of molecular evolution across all three genomes. *BMC Evolutionary Eiology* 13: 1-11.

Bustos, D. V., R. Riegel, and D. F. Calderini. 2012. Anthocyanin content of grains in purple wheat is affected by grain position, assimilate availability and agronomic management. *Journal of Cereal Science* 55: 257-264.

Cai, L., B. J. Arnold, Z. Xi, D. E. Khost, N. Patel, C. B. Hartmann, S. Manickam, et al. 2021. Deeply altered genome architecture in the endoparasitic flowering plant *Sapria himalayana* Griff.(Rafflesiaceae). *Current Biology* 31: 1002-1011. e1009.

Candia, A. B., R. Medel, and F. E. Fontúrbel. 2014. Indirect positive effects of a parasitic plant on host pollination and seed dispersal. *Oikos* 123: 1371-1376.

Capy, P., G. Gasperi, C. Biémont, and C. Bazin. 2000. Stress and transposable elements: co-evolution or useful parasites? *Heredity* 85: 101-106.

Cazzonelli, C. I. 2011. Carotenoids in nature: insights from plants and beyond. *Functional Plant Biology* 38: 833-847.

Chlumský, J., P. Koutecký, V. Jílková, and M. Štech. 2013. Roles of species-preferential seed dispersal by ants and endozoochory in *Melampyrum* (Orobanchaceae). *Journal of Plant Ecology* 6: 232-239.

Cisowska, A., D. Wojnicz, and A. B. Hendrich. 2011. Anthocyanins as antimicrobial agents of natural plant origin. *Natural Product Communications* 6: 1934578X1100600136.

Conn, C. E., R. Bythell-Douglas, D. Neumann, S. Yoshida, B. Whittington, J. H. Westwood, K. Shirasu, et al. 2015. Convergent evolution of strigolactone perception enabled host detection in parasitic plants. *Science* 349: 540-543.

Das, M., M. Fernández-Aparicio, Z. Yang, K. Huang, N. J. Wickett, S. R. Alford, E. K. Wafula, et al. 2015. Parasitic plants *Striga* and *Phelipanche* dependent upon exogenous strigolactones for germination have retained genes for strigolactone biosynthesis. *American Journal of Plant Sciences* 6: 1151-1166.

Davis, C. C., and Z. Xi. 2015. Horizontal gene transfer in parasitic plants. *Current Opinion in Plant Biology* 26: 14-19.

Dazenière, J., A. Bousios, and A. Eyre-Walker. 2022. Patterns of selection in the evolution of a transposable element. G3 12: jkac056.

dePamphilis, C. W. 1995. Genes and genomes. *Parasitic Plants*: 176–205.

de Saint Germain, A., A. Jacobs, G. Brun, J.-B. Pouvreau, L. Braem, D. Cornu, G. Clavé, et al. 2021. A Phelipanche ramosa KAI2 protein perceives strigolactones and isothiocyanates enzymatically. *Plant Communications* 2: 100166.

de Vega, C., C. M. Herrera, and S. Dötterl. 2014. Floral volatiles play a key role in specialized ant pollination. *Perspectives in Plant Ecology, Evolution and Systematics* 16: 32-42.

Harraz, F., A. Pedersen, and Q. Andercen. 1996. Anthocyanins from the parasitic medicinal plant *Cynomorium coccineum*. *Alexandria Journal of Pharmaceutical Sciences* 10: 158-160.

Fratianne, D. G. 1965. The interrelationship between the flowering of dodder and the flowering of some long and short day plants. *American Journal of Botany* 52: 556-562.

Ganhão, E., and L. S. Dias. 2019. Seed volume dataset—An ongoing inventory of seed size expressed by volume. *Data* 4: 61.

Graham, S. W., V. K. Lam, and V. S. Merckx. 2017. Plastomes on the edge: the evolutionary breakdown of mycoheterotroph plastid genomes. *New Phytologist* 214: 48-55.

Groisman, E. A., and H. Ochman. 1996. Pathogenicity islands: bacterial evolution in quantum leaps. *Cell* 87: 791-794.

Guo, C., L. Qin, Y. Ma, and J. Qin. 2022. Integrated metabolomic and transcriptomic analyses of the parasitic plant *Cuscuta japonica* Choisy on host and non-host plants. *BMC Plant Biology* 22: 1-16.

Hardy, N. B., and L. G. Cook. 2012. Testing for ecological limitation of diversification: a case study using parasitic plants. *The American Naturalist* 180: 438-449.

Heide-Jørgensen, H. 2008. *Parasitic Flowering Plants*. Brill.

Holdsworth, M., and P. Nutman. 1947. Flowering responses in a strain of *Orobanche minor*. *Nature* 160: 223-224.

Hughes, N., H. Neufeld, and K. Burkey. 2005. Functional role of anthocyanins in high-light winter leaves of the evergreen herb *Galax urceolata*. *New Phytologist* 168: 575-587.

Iwashina, T., N. Tanaka, M. M. Aung, H. P. Devkota, and T. Mizuno. 2020. Phenolic compounds from parasitic *Sapria himalayana f. albovinosa* and *Sapria myanmarensis* (Rafflesiaceae) in Myanmar. *Biochemical Systematics and Ecology* 93: 104179.

Jacquemyn, H., and V. S. Merckx. 2019. Mycorrhizal symbioses and the evolution of trophic modes in plants. *Journal of Ecology* 107: 1567-1581.

Jürgens, A., S. L. Wee, A. Shuttleworth, and S. D. Johnson. 2013. Chemical mimicry of insect oviposition sites: a global analysis of convergence in angiosperms. *Ecology Letters* 16: 1157-1167.

Kazazian Jr, H. H. 2004. Mobile elements: drivers of genome evolution. *Science* 303: 1626-1632.

Kösters, L. M., S. Wiechers, P. Lyko, K. F. Müller, and S. Wicke. 2021. WARPP—web application for the research of parasitic plants. *Plant Physiology* 185: 1374-1380.

Kuijt, J. 1969. *The Biology of Parasitic Flowering plants.* University of California Press, Berkeley.

Kuijt, J., and W.-X. Dong. 1990. Surface features of the leaves of Balanophoraceae—A family without stomata? *Plant Systematics and Evolution* 170: 29-35.

Leake, J. R. 1994. The biology of myco-heterotrophic ('saprophytic') plants. *New Phytologist* 127: 171-216.

Lemaire, B., S. Huysmans, E. Smets, and V. Merckx. 2011. Rate accelerations in nuclear 18S rDNA of mycoheterotrophic and parasitic angiosperms. *Journal of Plant Research 124*: 561-576.

Li, F.-W., P. Brouwer, L. Carretero-Paulet, S. Cheng, J. De Vries, P.-M. Delaux, A. Eily, et al. 2018a. Fern genomes elucidate land plant evolution and cyanobacterial symbioses. *Nature Plants* 4: 460-472.

Li, J., C. Hettenhausen, G. Sun, H. Zhuang, J.-H. Li, and J. Wu. 2015. The parasitic plant *Cuscuta australis* is highly insensitive to abscisic acid-induced suppression of hypocotyl elongation and seed germination. *PLOS One* 10: e0135197.

Li, Y.-Y., M. Boeraeve, Y.-H. Cho, H. Jacquemyn, and Y.-I. Lee. 2022. Mycorrhizal switching and the role of fungal abundance in seed germination in a fully mycoheterotrophic orchid, *Gastrodia confusoides*. *Frontiers in Plant Science* 12: 3286.

Li, Z.-W., X.-H. Hou, J.-F. Chen, Y.-C. Xu, Q. Wu, J. González, and Y.-L. Guo. 2018b. Transposable elements contribute to the adaptation of *Arabidopsis thaliana*. *Genome Biology and Evolution* 10: 2140-2150.

Liu, B., C. T. Le, R. L. Barrett, D. L. Nickrent, Z. Chen, L. Lu, and R. Vidal-Russell. 2018. Historical biogeography of Loranthaceae (Santalales): Diversification agrees with emergence of tropical forests and radiation of songbirds. *Molecular Phylogenetics and Evolution* 124: 199-212.

Lyko, P., and S. Wicke. 2021. Genomic reconfiguration in parasitic plants involves considerable gene losses alongside global genome size inflation and gene births. *Plant Physiology* 186: 1412-1423.

Matusova, R., K. Rani, F. W. Verstappen, M. C. Franssen, M. H. Beale, and H. J. Bouwmeester. 2005. The strigolactone germination stimulants of the plant-parasitic Striga and Orobanche spp. are derived from the carotenoid pathway. Plant physiology 139: 920-934.

Micheli, P. A. 1729. *Noua plantarum genera iuxta Tournefortii methodum disposita.*

Mortimer, S. M., J. Boyko, J. M. Beaulieu, and D. C. Tank. 2022. Synthesizing existing phylogenetic data to advance phylogenetic research in Orobanchaceae. *Systematic Botany* 47: 533-544.

Mursidawati, S., A. Wicaksono, and J. A. Teixeira da Silva. 2020. *Rafflesia patma* Blume flower organs: histology of the epidermis and vascular structures, and a search for stomata. *Planta* 251: 1-10.

Mutuku, J. M., S. Cui, S. Yoshida, and K. Shirasu. 2021. Orobanchaceae parasite–host interactions. *New Phytologist* 230: 46-59.

Narbona, E., J. C. d. Valle García, M. Arista Palmero, M. L. Buide del Real, and P. L. Ortiz Ballesteros. 2021. Major flower pigments originate different colour signals to pollinators. *Frontiers in Ecology and Evolution, 9, 743850.*

Naumann, J., K. Salomo, J. P. Der, E. K. Wafula, J. F. Bolin, E. Maass, L. Frenzke, et al. 2013. Single-copy nuclear genes place haustorial Hydnoraceae within Piperales and reveal a Cretaceous origin of multiple parasitic angiosperm lineages. *PLOS One* 8: e79204.

Nelson, D. C. 2021. The mechanism of host-induced germination in root parasitic plants. *Plant Physiology* 185: 1353-1373.

Neumann, P., L. Oliveira, J. Čížková, T. S. Jang, S. Klemme, P. Novák, K. Stelmach, et al. 2021. Impact of parasitic lifestyle and different types of centromere organization on chromosome and genome evolution in the plant genus Cuscuta. *New Phytologist* 229: 2365-2377.

Nickrent, D. L. 2020. Parasitic angiosperms: how often and how many? *Taxon* 69: 5-27.

Nikolov, L. A., and C. C. Davis. 2017. The big, the bad, and the beautiful: Biology of the world's largest flowers. *Journal of Systematics and Evolution* 55: 516-524.

Nina, N., C. Theoduloz, A. Giménez, and G. Schmeda-Hirschmann. 2020. Phenolics from the Bolivian highlands food plant Ombrophytum subterraneum (Aspl.) B. Hansen (Balanophoraceae): Antioxidant and α-glucosidase inhibitory activity. *Food Research International* 137: 109382.

Ohno, S. 1970. *Evolution by Gene Duplication*. Springer Science & Business Media.

Ogura-Tsujita, Y., T. Yukawa, and A. Kinoshita. 2021. Evolutionary histories and mycorrhizal associations of mycoheterotrophic plants dependent on saprotrophic fungi. *Journal of Plant Research* 134: 19-41.

Pelser, P. B., D. L. Nickrent, J. R. C. Callado, and J. F. Barcelona. 2013. Mt. Banahaw reveals: The resurrection and neotypification of the name *Rafflesia lagascae* (Rafflesiaceae) and clues to the dispersal of *Rafflesia* seeds. *Phytotaxa* 131: 35-40.

Pelser, P. B., D. L. Nickrent, B. W. van Ee, and J. F. Barcelona. 2019. A phylogenetic and biogeographic study of *Rafflesia* (Rafflesiaceae) in the Philippines: limited dispersal and high island endemism. *Molecular Phylogenetics and Evolution* 139: 106555.

Ramirez-Barahona, S., H. Sauquet, and S. Magallon. 2020. The delayed and geographically heterogeneous diversification of flowering plant families. *Nature Ecology & Evolution* 4: 1232-1238.

Ree, R. H. 2005. Phylogeny and the evolution of floral diversity in *Pedicularis* (Orobanchaceae). *International Journal of Plant Sciences* 166: 595-613.

Renner, S., and H. Schaefer. 2010. The evolution and loss of oil-offering flowers: new insights from dated phylogenies for angiosperms and bees. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365: 423-435.

Schelkunov, M. I., A. A. Penin, and M. D. Logacheva. 2018. RNA-seq highlights parallel and contrasting patterns in the evolution of the nuclear genome of fully mycoheterotrophic plants. *BMC Genomics* 19: 1-16.

Schrader, L., J. W. Kim, D. Ence, A. Zimin, A. Klein, K. Wyschetzki, T. Weichselgartner, et al. 2014. Transposable element islands facilitate adaptation to novel environments in an invasive species. *Nature Communications* 5: 1-10.

Searcy, D. G., and A. J. MacInnis. 1970. Measurements by DNA renaturation of the genetic basis of parasitic reduction. *Evolution*: 796-806.

Shen, G., N. Liu, J. Zhang, Y. Xu, I. T. Baldwin, and J. Wu. 2020. *Cuscuta australis* (dodder) parasite eavesdrops on the host plants' FT signals to flower. *Proceedings of the National Academy of Sciences* 117: 23125-23130.

Sipes, S. D., K. E. H. Hartz, H. Amin, and D. L. Nickrent. 2014. Floral scent and pollinators of the holoparasite *Pilostyles thurberi* (Apodanthaceae). *Journal of Pollination Ecology* 12: 31-39.

Smith, J. D., M. C. Mescher, and C. M. De Moraes. 2013. Implications of bioactive solute transfer from hosts to parasitic plants. *Current Opinion in Plant Biology* 16: 464-472.

Stevens, P. F. 2016. *Angiosperm Phylogeny Website.* Version 13. http://www.mobot.org/MOBOT/research/APweb/

Steyn, W. J., S. Wand, D. Holcroft, and G. Jacobs. 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *New Phytologist* 155: 349-361.

Studer, A., Q. Zhao, J. Ross-Ibarra, and J. Doebley. 2011. Identification of a functional transposon insertion in the maize domestication gene tb1. *Nature Genetics* 43: 1160-1163.

Suetsugu, K. 2019. Social wasps, crickets and cockroaches contribute to pollination of the holoparasitic plant Mitrastemon yamamotoi (Mitrastemonaceae) in southern Japan. *Plant Biology* 21: 176-182.

Suetsugu, K., and S. Hisamatsu. 2020. Potential brood-site pollination mutualism between *Balanophora tobiracola* Makino (Santalales: Balanophoraceae) and the sap beetle *Epuraea ocularis* Fairmaire, 1849 (Coleoptera: Nitidulidae). *The Coleopterists Bulletin* 74: 652-655.

Sun, G., Y. Xu, H. Liu, T. Sun, J. Zhang, C. Hettenhausen, G. Shen, et al. 2018. Large-scale gene losses underlie the genome evolution of parasitic plant *Cuscuta australis*. *Nature Communications* 9: 1-8.

Teixeira-Costa, L. 2021. A living bridge between two enemies: haustorium structure and evolution across parasitic flowering plants. *Brazilian Journal of Botany* 44: 165-178.

Teixeira-Costa, L., F. M. Coelho, and G. C. T. Ceccantini. 2017. Comparative phenology of mistletoes shows effect of different host species and temporal niche partitioning. *Botany* 95: 271-282.

Teixeira-Costa, L., C. C. Davis, and G. Ceccantini. 2021. Striking developmental convergence in angiosperm endoparasites. *American Journal of Botany* 108: 756-768.

Timilsena, P. R., C. F. Barrett, A. P. Nelson, E. K. Wafula, S. Ayyampalayam, J. R. McNeal, T. Yukawa, et al. 2022. Phylotranscriptomic analyses of mycoheterotrophic monocots show a continuum of convergent evolutionary changes in expressed nuclear genes from three independent nonphotosynthetic lineages. *Genome Biology and Evolution* 15: evac183.

Thunberg, C. 1775. Beskrifning paa en ganska besynnerlig och obekant svamp, *Hydnora africana*. *Kongalia Vetenskaps Akademiens Handlingar* 36: 69-75.

Toh, S., D. Holbrook-Smith, P. J. Stogios, O. Onopriyenko, S. Lumba, Y. Tsuchiya, A. Savchenko, and P. McCourt. 2015. Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga*. *Science* 350: 203-207.

Vogel, A., R. Schwacke, A. K. Denton, B. Usadel, J. Hollmann, K. Fischer, A. Bolger, et al. 2018. Footprints of parasitism in the genome of the parasitic flowering plant *Cuscuta campestris*. *Nature Communications* 9: 1-11.

Wang, D., H. Yu, and G. Chen. 2021. Scent chemistry and pollinators in the holoparasitic plant *Cynomorium songaricum* (Cynomoriaceae). *Plant Biology* 23: 111-120.

Wasilewska, A., F. Vlad, C. Sirichandra, Y. Redko, F. Jammes, C. Valon, N. F. dit Frey, and J. Leung. 2008. An update on abscisic acid signaling in plants and more. *Molecular Plant* 1: 198-217.

Watson, D. M., R. C. McLellan, and F. E. Fontúrbel. 2022. Functional roles of parasitic plants in a warming world. Annual Review of Ecology, Evolution, and Systematics 53: 25-45.

Wee, S. L., S. B. Tan, and A. Jürgens. 2018. Pollinator specialization in the enigmatic *Rafflesia cantleyi*: a true carrion flower with species-specific and sex-biased blow fly pollinators. *Phytochemistry* 153: 120-128.

Wicke, S., K. F. Müller, C. W. de Pamphilis, D. Quandt, N. J. Wickett, Y. Zhang, S. S. Renner, and G. M. Schneeweiss. 2013. Mechanisms of functional and physical genome reduction in photosynthetic and nonphotosynthetic parasitic plants of the broomrape family. *The Plant Cell* 25: 3711-3725.

Wilcock, C., and R. Neiland. 2002. Pollination failure in plants: why it happens and when it matters. Trends in Plant Science 7: 270-277.

Wintola, O. A., and A. J. Afolayan. 2015. The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of *Hydnora africana* Thunb. used as antidysenteric in Eastern Cape Province, South Africa. *BMC Complementary and Alternative Medicine* 15: 1-12.

Wolfe, K. H., C. W. Morden, and J. D. Palmer. 1992. Function and evolution of a minimal plastid genome from a nonphotosynthetic parasitic plant. *Proceedings of the National Academy of Sciences* 89: 10648-10652.

Xia, J., Z. Guo, Z. Yang, H. Han, S. Wang, H. Xu, X. Yang, et al. 2021. Whitefly hijacks a plant detoxification gene that neutralizes plant toxins. *Cell* 184: 1693-1705. e1617.

Xu, Y., J. Zhang, C. Ma, Y. Lei, G. Shen, J.-J. Jin, D. A. Eaton, and J. Wu. 2022. Comparative genomics in Orobanchaceae provides insight into the origin and evolution of plant parasitism. *Molecular Plant* 8:1384-1399.

Yang, Z., E. K. Wafula, G. Kim, S. Shahid, J. R. McNeal, P. E. Ralph, P. R. Timilsena, et al. 2019. Convergent horizontal gene transfer and cross-talk of mobile nucleic acids in parasitic plants. *Nature Plants* 5: 991-1001.

Yoshida, S., and Y. J. Kee. 2021. Large-scale sequencing paves the way for genomic and genetic analyses in parasitic plants. *Current Opinion in Biotechnology* 70: 248-254.

Yoshida, S., S. Kim, E. K. Wafula, J. Tanskanen, Y.-M. Kim, L. Honaas, Z. Yang, et al. 2019. Genome sequence of Striga asiatica provides insight into the evolution of plant parasitism. *Current Biology* 29: 3041-3052. e3044.

Yuan, Y., X. Jin, J. Liu, X. Zhao, J. Zhou, X. Wang, D. Wang, et al. 2018. The *Gastrodia elata* genome provides insights into plant adaptation to heterotrophy. *Nature Communications* 9: 1615.

Zagorchev, L., W. Stöggl, D. Teofanova, J. Li, and I. Kranner. 2021. Plant parasites under pressure: Effects of abiotic stress on the interactions between parasitic plants and their hosts. *International Journal of Molecular Sciences* 22: 7418.

Zheng, Y., X. Sun, Y. Miao, S. Qin, Y. Jiang, X. Zhang, and L. Huang. 2021. A systematic study on the chemical diversity and efficacy of the inflorescence and succulent stem of *Cynomorium songaricum*. *Food & Function* 12: 7501-7513.

Figure Captions

Figure 1 The bottleneck model of parasitic plant evolution. (A) The overall architecture of the bottleneck model. The onset of parasitism can be induced by multiple independent pathways, as hinted by the diverse life history strategies in *Viscum* (stem parasites), *Cuscuta* (stem and leaf parasites), and *Castilleja* (root parasites). At this stage, weak evolutionary constraints largely come from the selective pressure to optimize parasitism, leading to moderate levels of gene loss in photosynthesis, stress response, and nutrient metabolism. Genetic innovations are also accumulated to promote adaptation. However, the loss of photosynthesis in holoparasites significantly increases the evolutionary constraints by triggering widespread gene loss. The smaller and more isolated populations typical of holoparasites also leads to the accumulation of deleterious mutations and prevents the fixation of adaptive mutations. (B) Dynamics of molecular and population constraints governing the bottleneck model. Compared to hemiparasites, more intense interactions between mutation, drift, and selection are present in holoparasitic plants, reducing their overall fitness. These negative impacts may necessitate the introduction of horizontal gene transfer (HGT) to rescue interrupted functions or the evolution of outcrossing to enrich the effective population size (N_e). Illustrations of plants are obtained from the Biodiversity Heritage Library (https://www.biodiversitylibrary.org/).

Figure 2 Phenotypical convergence in parasitic plants originating from concerted degradation of functionally linked modules. The loss of photosynthesis (PS) in holoparasitic plants triggers the disfunction of PS pigment biosynthesis and circadian rhythm, causing the parallel evolution of otherwise rare traits. In particular, traits affecting reproductive strategies, such as the convergent recruitment of anthocyanins for floral pigmentation, can further impact population and macroevolutionary processes. Photo panels highlight the red colorants commonly found in holoparasites. Their corresponding pollinators is shown on the top or bottom of each photo. Species from top left to bottom right: *Hydnora africana* (Hydnoroideae, Aristolochiaceae), *Mitrastemon kawasasakii* (Mitrastemonaceae), *Bdallophytum americanum* (Cytinaceae), *Pilostyles thurberi* (Apodanthaceae), *Balanophora laxiflora* (Balanophoraceae), *Cynomorium coccineum* (Cynomoriaceae), *Cytinus ruber* (Cytinaceae), and *Rafflesia kerrii* (Rafflesiaceae). Images are obtained under the Creative Commons license from iNatualist and Flickr. Please see Acknowledgement for specific contributors.

Figure 3 Evolutionary trend of seed size and number in parasitic plants. Holoparasites (orange) produce smaller seeds that are greater in number compared to hemiparasites (blue). This trend is consistent within Orobanchaceae (solid circles). Representative species with extreme seed size and number are highlighted. The box plot on the left shows the seed size from holoparasites (orange) and hemiparasites (blue). This dataset contains 113 representative species from 17 parasitic plant families, summarized primarily from two seed morphology databases (Ganhão and Dias, 2019; Baskin and Baskin, 2022). The relative seed dimension is calculated by a log-transformed area: Relative dimension = log(π x length x width). The x-axis (number of seeds per flower) should be interpreted as categorical rather than precise values, especially for species with more than 100 seeds per fruit. Please refer to Appendix S1 for data and reference (see Supplemental Data with this article).

Figure 4 The 'functional necessity' and 'mechanistic convenience' hypotheses for convergent horizontal gene transfer (HGT) in parasitic plants. (A) Under the functional necessity scenario, HGT can rescue the loss of essential functions that are susceptible to disruption in parasites. For example, HGTs are recruited to compensate the convergent loss of an essential gene triggered by the loss of photosynthesis in multiple species. (B) Under the mechanistic convenience scenario, convergent HGT is endowed by consistent structural mobility. For example, genes hitchhiked to transposable elements (TEs) can form long genetic blocks that translocate as HGT. When this genetic block nests within a conserved syntenic region across multiple species, convergent HGT can take place.

Table

Table 1 Age and diversity of twelve independently evolved parasitic lineages. The stem group age is cited from Nickrent (2020) and the species number is cited from the Angiosperm Phylogeny Website (Stevens, 2016).

Clade	Classification	Stem group age (Ma)	Species number
Cassytha (Lauraceae)	Hemiparasite	77	19
Hydnoroideae (Aristolochiaceae)	Holoparasite	133	15
Santalales	Hemi + Holoparasite	108-110	2428
Cynomoriaceae	Holoparasite	100	2
Krameriaceae	Hemiparasite	60.9	18
Rafflesiaceae	Holoparasite	101	26
Cytinaceae	Holoparasite	72	11
Apodanthaceae	Holoparasite	75	10
Mitrastemonaceae	Holoparasite	78.3	2
Lennooideae (Boraginaceae)	Holoparasite	88	4
Cuscuta (Convolvulaceae)	Hemi + Holoparasite	34.6	195
Orobanchaceae	Hemi + Holoparasite	35.68	2025

Table 2 Comparison of diversity, morphology, and genetic traits between holoparasitic and fully mycoheterotrophic (MHT) plants. Data on MHT is summarized from Leake, 1994; Graham et al., 2017; Schelkunov et al., 2018; Yuan et al., 2018; Jacquemyn and Merckx, 2019; Ogura-Tsujita et al., 2021; Li et al., 2022; and Timilsena et al., 2022 in addition to the literature on parasitic plants referenced in the main text.

	Trait	Similarities	Differences	
			Holoparasitic plants	Fully mycoheterotrophic (MHT) plants
Life history	Host	–	Seed plants	Arbuscular mycorrhizal, ectomycorrhizal, or saprotrophic fungi
	Host-derived resources	Water, carbohydrates, nitrogen	DNA/RNA, protein, signaling chemicals, and metabolites	Limited reports beyond basic nutrients
Diversity pattern	Diversity	Rare in general	Evolved at least 13 times in ca. 500 flowering plants	Evolved at least 50 times in ca. 800 land plants
	Distribution and habitat	Host dependent	Both tropical and temperate zones; from moist forest understories to arid deserts	Primarily tropics except Ericaceae and Orchidaceae; prefer dense overstory with deep shades
	Growth habit	Ephemeral	Ground dwelling or aerial parasite; subterranean or endoparasitic until reproductively mature	Never aerial, subterranean for most of their lives
Vegetative trait	Root	Reduced	Mostly rootless; haustorium connects the host	Rarely rootless; thickened root cortex used for carbon storage
	Stem		Highly truncated, only to support inflorescence axis	Aerial stem slender; underground stem often modified for storage
	Leaf		Floral bracts or scales on the stem	Veins reduced to a single trace or absent
	Stomata		Absent in Rafflesiaceae, Balanophoraceae, and Hydnoroideae	Absent in most MHT
Reproduction	Flower	Modified from ancestral design	Heavily pigmented in various shades of red	Lightly pigmented in epidermal cells; color ranges from pale white to yellow, crimson, and purple
	Seed	Dust seeds produced in large numbers	Germination depends upon host plant exudates	Germination depends upon infection by a symbiotic fungus
Genetics	Plastid genome	Reduced and restructured	Low GC content; complete loss of plastid genome in Rafflesiaceae	Less extensive gene losses
	Nuclear genome	Widespread gene loss in photosynthesis related pathways	Additional loss in development and metabolic pathways	Limited loss in pathogen resistance, antioxidant regulation, and other cellular processes
	Horizontal gene transfer	–	Widespread in motochondrial and nuclear genomes	None
	Substitution rate	Overall increased rates	Increased substitution rates in obligate parasitic plants	Lineage specific—genome-wide increasing in rates observed in Orchidaceae but not Ericaceae