

The evolution of parasitism in plants

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The multiple independent origins of plant parasitism suggest that numerous ancestral plant lineages possessed the developmental flexibility to meet the requirements of a parasitic life style, including such adaptations as the ability to recognize host plants, form an invasive haustorium, and regulate the transfer of nutrients and other molecules between two different plants. In this review, we focus on the Orobanchaceae, which are unique among the parasitic plants in that extant member species include the full range of host dependence from facultative to obligate parasites. The recent emergence of genomic resources for these plants should provide new insights into parasitic plant evolution and enable the development of novel genetic strategies for controlling parasitic weeds.

Parasites provide a framework for understanding specialization

Parasitism is a highly successful life strategy and a theme that bridges all kingdoms of life [1]. The high selection pressure associated with host co-evolution can drive a parasite's morphology and physiology in new directions, making parasites valuable subjects for studying diversification and the evolution of phenotypic changes associated with heterotrophy [2]. Parasitic plants are characterized by the ability to feed directly on other plants, invading either the roots or shoots of their hosts through parasitic structures called haustoria [3]. Haustoria function in host attachment, invasion and in the physiological redirection of host resources into the parasite.

In angiosperms, parasitism has independently evolved at least 12 or 13 times, and by recent estimates approximately 1% of angiosperm species are parasitic (Figure 1). The degree of host dependence varies among parasitic genera. Facultative parasites can live autotrophically and reproduce without host contact, but will opportunistically parasitize neighboring plants when available. In contrast, obligate parasites must parasitize a host in order to complete their life cycles. Seeds of some obligate parasites require exposure to host signal molecules in order to germinate. Parasitic plants can also be classified as to their photosynthesis status: hemiparasites are photosynthetically competent, though the efficiency of photosynthesis varies considerably between different species, while holoparasites lack photosynthetic activity and obtain all their reduced carbon through haustorial connections with a host [4]. Among the recognized major lineages of parasitic plants, three contain only hemiparasitic species and eight

are entirely holoparasitic. Only the Orobanchaceae contain all classification stages (Figure 1). This provides a unique system for using extant species to investigate the evolutionary origins and consequences of plants evolving heterotrophic capacity and for this reason is the subject of this review.

Three genera of Orobanchaceae are illustrative of the range of host dependence in this family (Figure 2). *Triphysaria* is a facultative hemiparasite that is identified as a common springtime wildflower throughout the Pacific Coast of the United States. *Striga* is a hemiparasite that requires host factors from seed germination through maturity and *Orobanche*, an obligate non-photosynthetic holoparasite (including members of the synonymous *Phelipanche*). These genera have been characterized anatomically, physiologically and ecologically [5], and are currently the subject of a massive transcriptome sequencing and gene discovery project (<http://ppgp.huck.psu.edu/>). Together they provide a comparative framework for exploring the underlying mechanisms – and consequences – of parasitism in plants.

This review summarizes our current understanding of parasitism in the Orobanchaceae and discusses current models for parasite evolution. We will focus on the contributions of host chemistry and metabolism to parasite development and consider how recent insights into parasite biology can lead to new approaches for controlling weedy parasitic plants.

Economic impact of parasitic Orobanchaceae

Any study of plant parasitism naturally begins with a review of the economic impact of the parasites. Members of Orobanchaceae, in particular *Striga* (witchweeds) and *Orobanche* (broomrapes), are among the most agronomically destructive parasitic plants globally. *Striga* species are found mainly in Africa, India and Southeast Asia where they are a significant constraint to crop productivity. At present, over 50 million hectares of the arable farmland under cultivation with cereals and legumes in sub-Saharan Africa are infested with one or more *Striga* species, resulting in annual losses of yield estimated to be in excess of US\$10 billion [6,7]. *Striga* species fall into two groups based on host preference: most *Striga* parasitize grasses (Poaceae) including several species (e.g. *Striga hermonthica*, *Striga aspera* and *Striga asiatica*) that specialize on important food and forage grains such as maize (*Zea mays*), sorghum (*Sorghum bicolor*), rice (*Oryza sativa*) and millet. Only *Striga gesnerioides*, the most morphologically diverse and widely distributed witchweed, preferentially attacks dicot plants, including cultivated and wild legumes [8].

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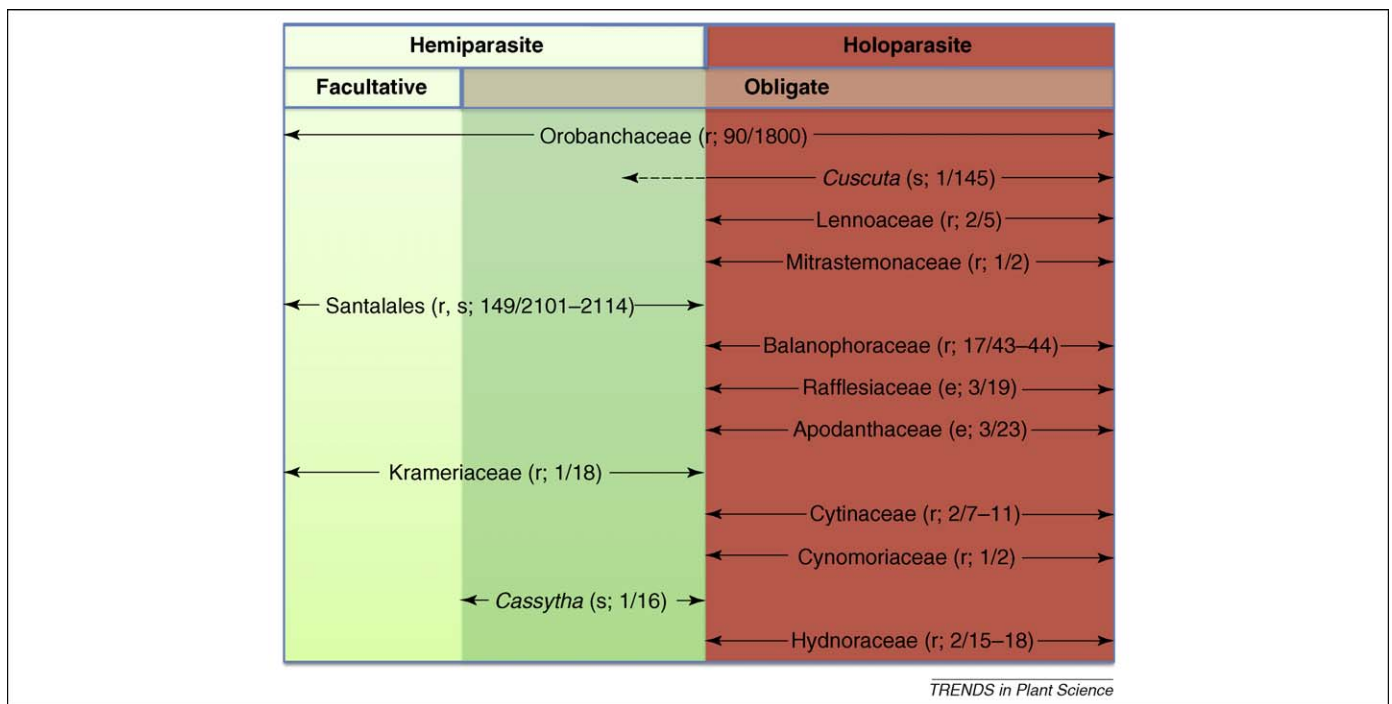


Figure 1. The diversity of parasitism in flowering plants, reflecting a likely 12 or 13 surviving origins of parasitism in angiosperm evolutionary history; using data from Ref. [4], the Angiosperm Phylogeny Website (<http://www.mobot.org/MOBOT/research/APweb>) and the Parasitic Plant Connection (<http://www.parasiticplants.siu.edu/>). Several studies suggest that Balanophoraceae, a family of holoparasitic root parasites, could be closely related to Santalales [59,78]; however, it is not yet clear if Balanophoraceae represents a derived holoparasitic lineage within the parasitic Santalales (consistent with 12 parasitic origins), or a basal lineage that has independently evolved parasitism (13 parasitic origins). Thirteen lineages are indicated, with mode of feeding (r, root; s, stem; e, plant is principally an internal endophyte), whether individual species are facultative (optional) or obligate parasites, if species are hemiparasitic or holoparasitic, and the estimated number of genera and species (Parasitic Plant Connection, <http://www.parasiticplants.siu.edu/>). Only parasites that directly invade the tissue of a photosynthetic host plant via a haustorium are included; parasitism of mycorrhizal interactions has also evolved on numerous occasions [79]. Most of the lineages are fully holoparasitic (8 of 13), although three contain only facultative and/or obligate hemiparasites. Orobanchaceae has the full trophic range of parasitic plants from facultative hemiparasites through obligate hemiparasites and completely heterotrophic holoparasites. Within Orobanchaceae, hemiparasitism has progressed to holoparasitism on at least five [80] and possibly more occasions [81,82]. Most *Cuscuta* (dodder) species produce chlorophyll and perform photosynthesis, but photosynthesis in at least some *Cuscuta* species serves to recycle host-derived carbon rather than fix carbon from the atmosphere (see discussion in Ref. [83]) suggesting that these *Cuscuta* species could be considered 'photosynthetic holoparasites'. Members of traditional Santalales (mistletoe) families range from free-living non-parasitic trees to species that maintain only minimal photosynthesis at narrow points in the life cycle [84].

Orobanche spp. are similarly destructive, estimated to threaten 16 million hectares in the Mediterranean and West Asia regions in 1991 [9]. Seven *Orobanche* species are problem weeds in Europe, and crops impacted include important food legumes, such as faba bean (*Vicia faba*), chickpea (*Cicer arietinum*) and pea (*Pisum sativum*), as well as other important vegetable [tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*) and carrot (*Daucus carota*)] and oilseed [sunflower (*Helianthus annuus*)] and *Brassica* crops. The impact of *Orobanche* parasitism on yields typically ranges from 20 to 100% crop loss, depending on the infestation [10]. Actual losses are difficult to estimate because a common response by farmers to heavily infested fields is to abandon the culture of affected crops, thus eliminating crop damage at the expense of area planted. For example, Egypt is now importing faba bean because *Orobanche crenata* in the upper Nile region has contributed to farmers abandoning this staple crop [11].

Control of *Striga* and *Orobanche* is challenging, partly as a result of the highly coordinated life-cycle of the parasites with their host species, but also because of the economic limitations that exist in the developing world where these parasites are most devastating. Over the past several decades extensive research has been aimed at practical, low-cost methods for limiting *Striga* and *Orobanche* infestation and parasitism [12,13]. Although some

favorable progress has been achieved, no approach has been entirely successful. Understanding how parasitic plants function is an important step toward generating new resistant varieties.

The first step toward parasitism: evolution of a haustorium

Lateral and terminal haustoria

Parasitism originated in non-parasitic plants and the key evolutionary event in this transition was the origin of invasive haustoria. Haustoria made by the earliest parasitic Orobanchaceae were probably similar to those of hemiparasitic, facultative species. These species develop lateral haustoria on the sides of their roots (Figure 3). In *Triphysaria* the genetic pathway responsible for lateral haustorium development is active for about 12 hours, after which the parasite root resumes normal growth, resulting in a lateral haustorium positioned proximal to the tip that looks like a hairy bump (Figure 3). Development of lateral haustoria does not permanently alter root meristem development and a single parasite root can have multiple lateral haustoria. In obligate parasites, including *Striga* and *Orobanche*, evolutionary events subsequent to lateral haustorium development resulted in the evolution of terminal (or primary) haustoria [14]. Terminal haustoria develop at the tip of the seedling radicle and there is no

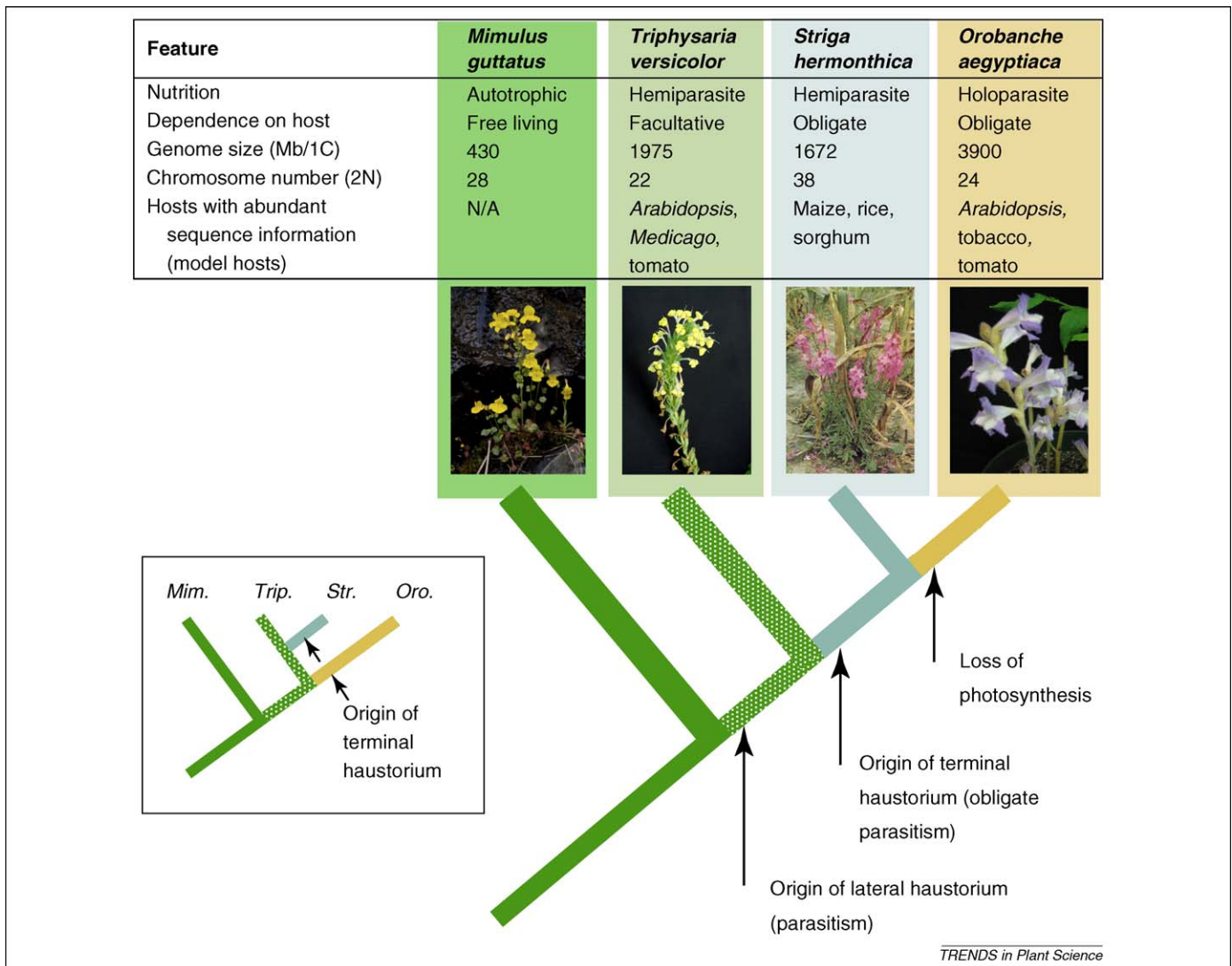


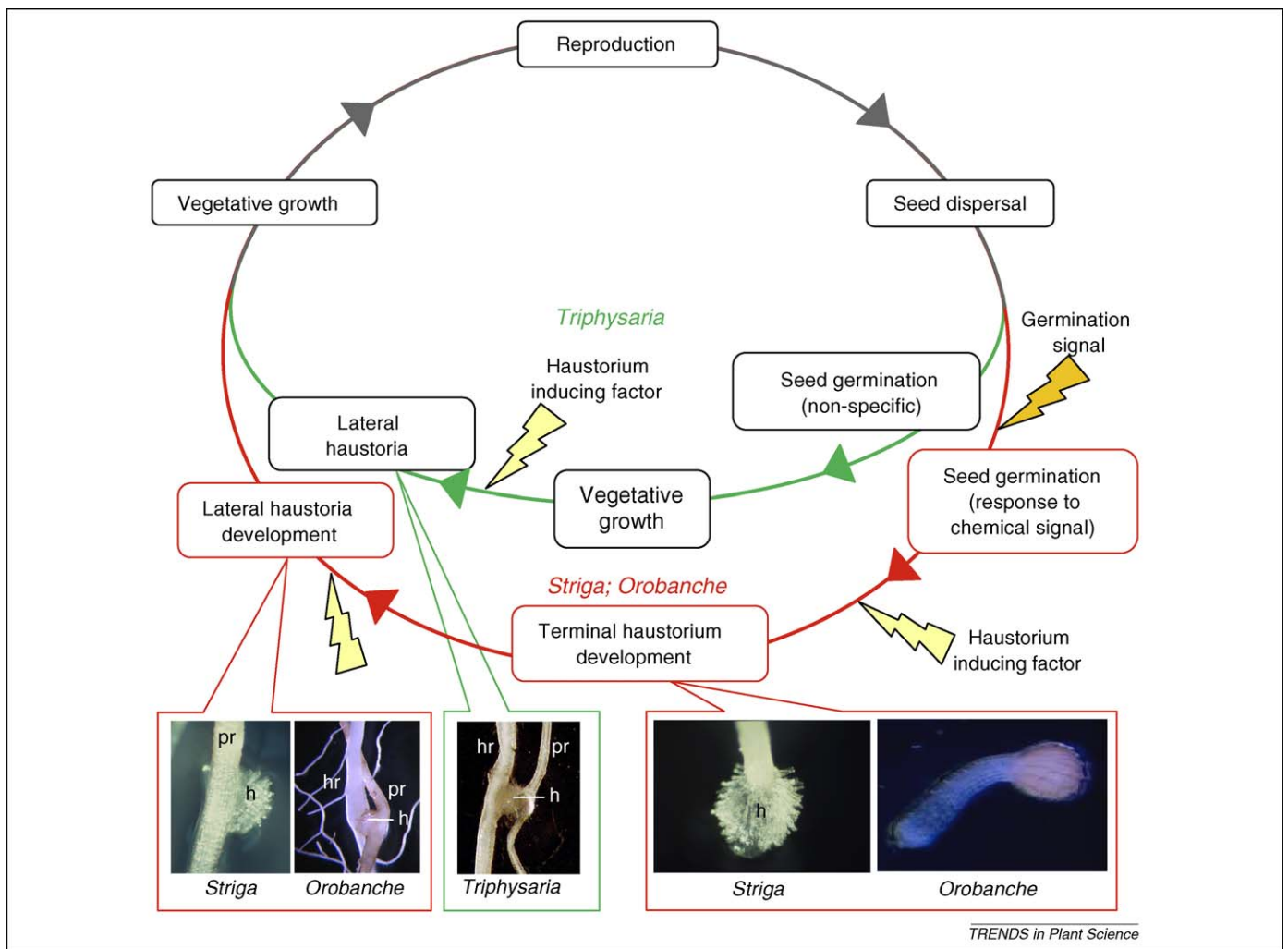
Figure 2. Comparison of features and evolutionary relationship among Orobanchaceae species discussed in this review. The three focal species represent the full range of parasitic ability from facultative parasites that can live and grow without a host (*Triphysaria*) to obligate hemiparasites (*Striga*) to obligate holoparasites (*Orobanche*). Along with the non-parasitic outgroup *Mimulus*, key evolutionary events can be captured through comparative analyses of these species, including the origin of lateral and terminal haustoria, and genomic changes associated with the evolution of obligate parasitism and the loss of photosynthesis. Inset: alternative evolutionary topology (consistent with Bennett and Mathews [82]) in which the terminal haustorium is suggested to have evolved independently in *Striga* (*Str.*) and *Orobanche* (*Oro.*). Genome size and chromosome numbers for *Mimulus* are from MimulusEvolution (<http://www.mimulusevolution.org/>). Parasite species genome sizes are from C.W. dePamphilis, K. Arumuganathan, J.H. Westwood, M.P. Timko, and J.I. Yoder (unpublished), and chromosome numbers are from Refs. [85–88]. *Mimulus* photograph courtesy of Jennifer L. Modliszewski, Duke University.

further root development until the terminal haustorium has successfully invaded and established itself in the host [15,16] (Figure 3). Once vascular connections are established the parasite commences development of a shoot (*Striga*) or a tubercle (*Orobanche*), which lead to above-ground structures. Both genera may produce additional roots that are capable of forming lateral haustoria (Figure 3).

Cell structural changes, leading to early events in haustorium development, use existing plant mechanisms for altering cell shape. Expansin genes are transcriptionally regulated during this period as are several other cell wall modifying enzymes [17,18]. In *Triphysaria*, the cell elongation and expansion characteristic of early haustorium development is associated with a localized accumulation of auxin [19]. These studies show that genetic pathways associated with autotrophic plant development have been recruited for parasite-specific functions.

Induction of early haustorium development

It has been known for many years that Orobanchaceae generally only develop haustoria when grown in the presence of another plant. It is now known that Orobanchaceae develop haustoria in response to chemical and tactile stimuli provided by a host root. Biochemical fractionation of plant materials identified two flavonoids, xenognosin A and B, and the quinone 2,6 dimethoxy-1,4-benzoquinone (DMBQ) [20]. This work motivated the analysis of various purified chemicals for their ability to induce haustoria and several haustoria inducing phenolic acids, quinones and flavonoids were identified, many of which have been identified in plant root exudates [21]. The same set of phytochemicals induced both lateral and terminal haustoria *in vitro* consistent with their having common origins [14,22]. DMBQ is a widespread plant secondary metabolite that is generated during the biosynthesis and degradation of lignin. DMBQ is often used in experimental studies



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Figure 3. Life cycles and haustorium morphologies of parasitic Orobanchaceae. Above ground, the facultative parasite *Triphysaria* looks like a typical herbaceous annual wildflower. However, when its roots are examined, there are clear lateral haustorial connections between parasite *Triphysaria* and neighboring roots. The obligate parasites *Striga* and *Orobanche* require a host-derived germination signal and produce a terminal haustorium that must connect to the host vascular tissue before further plant development can proceed. *Striga* and *Orobanche* have limited root systems, but the roots are capable of forming lateral haustoria upon encountering a host root or a haustorium-inducing factor. *Triphysaria* and *Striga* both have hairs associated with terminal and lateral haustoria, whereas *Orobanche* never produces haustorial hairs. Abbreviations: h, haustorium; hr, host root; pr, parasite root.

because it is a very active haustoria inducer and is commercially available. However, DMBQ was not originally identified in sorghum root exudates but was only identified after physical abrasion of the roots. This led the authors to propose a host honing mechanism in which *Striga* causes the release of DMBQ from the host cells by activating host peroxidases by H_2O_2 produced at the tip of the *Striga* radicle [23]. In this way, the parasite detects the HIF only upon close contact with the host root. However, this feature may be specific for terminal haustoria because lateral haustoria typically develop in response to exudates taken from undisturbed host exudates. *Triphysaria* populations selected for their inability to develop haustoria in response to *Arabidopsis thaliana* root exudates, suggesting that DMBQ is not the only active molecule in *Arabidopsis* exudates [24]. Given the number of phytochemicals that are potentially active haustorial inducers in root exudates, it is likely that parasites have evolved to respond to multiple signals from the host roots.

One model to account for the haustorium-inducing activity of different phenols proposes that the developmen-

tal signal initiating haustorium development is a reactive semiquinone intermediate formed during redox cycling of the HIF between quinone and hydroquinone states [25]. This model is consistent with similarities in redox potentials between different active quinones as well as the inhibition of haustorium development by chemical spin trap inhibitors [26]. Two distinct quinone oxidoreductase cDNAs, TvQR1 and TvQR2, have been isolated from *Triphysaria* root tips on the basis of their rapid transcriptional activation in response to exposure to HIFs [27]. Both TvQR1 and TvQR2 catalyze the NADPH-dependent reduction of plant quinones, including DMBQ, to the hydroquinone form [28] (T.A. Fillapova and J.I. Yoder, unpublished). However, their reduction mechanisms are different. TvQR2 encodes a highly conserved protein that catalyzes two electron reductions without generating a toxic radical intermediate and is hence considered a detoxification protein. By contrast, TvQR1 is likely to catalyze a univalent quinone reduction that is predicted to generate the unstable semiquinone hypothesized by Keyes and colleagues [25] as the haustorium signal. Homologs of TVQR1 are present in *Striga* and *Orobanche* but their

roles in terminal haustorium development have not yet been examined.

Host specificity

Self and non-self recognition

The competence to develop invasive haustoria in the earliest parasitic plants must have been soon followed by their ability to distinguish their own roots from those of a potential host. The ability to distinguish self from non-self is the first move towards developing host specificity. There are reports of self parasitism and the ability to auto parasitize varies between different parasitic species. In *Triphysaria*, this self-recognition system extends to the species level because haustorium initiation occurs much less frequently between conspecific *Triphysaria* than between different genera of *Triphysaria* or between *Triphysaria* and *Arabidopsis* [29]. The evolutionary rationale for a vegetative self-recognition system in root parasites seems obvious – a plant would receive few nutritional benefits by parasitizing its own roots or those of a closely related sibling. However, the molecular mechanisms acting on the systems are obscure. A generalist parasite such as *Triphysaria* gains benefits by recognizing a wide range of host plants from maize to *Arabidopsis*. Therefore, the host recognition signal(s) need to be conserved among different species, presumably because of positive selection for the molecules. However, these same conserved molecules must be distinguished, ignored, not produced, or not activated in the roots of the parasite. The mechanisms by which parasitic plants do not parasitize their own roots are not known.

Haustorial compatibility with host roots

Another layer of parasite–host interaction occurs between cells of the parasite haustoria and the host root. The ability of the parasite to form and maintain a union with the host ultimately determines its success, and host- and non-host-resistance mechanisms shed light on this balance. Plant species not parasitized by *S. hermonthica* under natural conditions are considered non-hosts and include *Arabidopsis*, cowpea (*Vigna unguiculata*), *Lotus japonicus*, and *Phtheirospermum japonicum* [30,31]. Penetration and xylem development occur on *Arabidopsis* and cowpea but full development of the parasite is not achieved. *Striga* penetration is aborted in the cortex of *L. japonicus*. *Striga* cannot penetrate *P. japonicum* due to an apparent inability to produce haustoria [30].

Plants capable of supporting parasite growth to maturity are considered hosts, and within host species, levels of resistance to parasitism can vary. One of the clearest examples of variation in host resistance responses is the interaction of *S. gesnerioides* with cowpea. In this case, resistant cowpea genotypes exhibit two different response mechanisms to *Striga* attack: a hypersensitive response in which necrosis occurs at the site of parasite attachment with subsequent rapid death of the *Striga* parasite within 3–4 days, and tubercle arrest, which is not as rapid and dramatic, but still results in failure of the parasite to grow and in most cases complete failure to expand their cotyledons. Tubercle arrest is also the most common response when *S. gesnerioides* isolates adapted to one species

attempt to parasitize a species outside their host range. Several cowpea genotypes have been identified that show race-specific resistance to *S. gesnerioides*. In all cases, resistance to the various races is inherited in a monogenic fashion. Furthermore, recent studies characterizing a cowpea gene encoding a CC-NBS-LRR resistance protein confirmed that resistance to *S. gesnerioides* in cowpea operates in a gene-for-gene manner similar to other host–pathogen associations [32].

Complete resistance to *Striga* infection has not been identified in the germplasm of most agronomically important grass hosts (maize, sorghum, rice and millet). In sorghum, highly tolerant varieties that differ in their sensitivity to infection have been developed and used with great success over the past two decades, and two sorghum cultivars and a wild sorghum accession have been shown to exhibit a hypersensitive-like necrosis at the site of *Striga* attachment [33]. The resistance response appears to be controlled by several quantitative trait loci (QTLs) that account for a significant portion of the resistance phenotype. In rice, seven major QTLs that account for 31% of the phenotypic variation in host resistance have been identified. *Striga* radicles penetrate the cortex of the resistant rice strain Nipponbare, but fail to breach the endodermis and do not penetrate the host vascular system [34,35]. Failure of the parasite to thrive after attachment to an incompatible host or non-host could be the result of metabolic uncoupling [36]. That is, parasite development could be blocked by the failure of the proper signals to be transferred from host to parasite, or the failure of the host to provide adequate nutrition.

One of the open questions in parasitic plant biology is what drives host selection and specialization by the parasite. Both intercrop specialization (i.e. strain specificity to a crop species) and intracrop specialization (strain specialization to a specific cultivar of a species) of *Striga* have been described, although the relationship between genetic variability of the parasite and its host range and virulence is not known at this time. Discrete races or pathotypes have been described for *Striga* and *Orobanchae* species. For example, at least seven distinct races of *S. gesnerioides* parasitic on cowpea are known [37], and six races of *Orobanchae* exist on sunflower [38]. The existence of host-specific races suggests that parasites have evolved sophisticated mechanisms to overcome potential host-plant resistance. The observation of R protein-mediated resistance to parasitic plant attack raises the question of what constitutes avirulence or virulence factors in these pathogens [32]. In other host–pathogen interactions, pathogens have evolved specific ‘effectors’ (e.g. virulence factors, phytochemicals, extracellular polysaccharides and proteins) to inhibit host recognition and activation of host defense responses, as part of effector-triggered immunity [39]. It remains to be determined if parasitic plants harbor such effectors and use these factors to overcome their hosts, but the potential for chemical interchange does exist. The exchange of biochemical information between host and parasite (in the form of metabolites – small molecules, sugars, sugar alcohols and amino acids) and perhaps even exchange of nucleic acids in the form of mRNA and microRNA (see below) are intriguing possibilities.

Refining parasitism: germination signals

Another evolutionary step toward obligate parasitism is an increased sophistication in mechanisms for locating a host. The facultative parasite *Triphysaria* will germinate without biological stimuli and hosts are detected by the roots as described in the discussion of haustorial initiation above. In *Striga* and *Orobanche*, where germination without a nearby host root would be risky, seed germination occurs in response to minute quantities of chemicals exuded by host roots. Many compounds have been demonstrated to act as germination triggers, but most research revolves around the strigolactones [40,41].

For years researchers have wondered why so many host plants would reveal their location to parasites by the exudation of germination stimulants, but this mystery has recently been solved, first by the finding that the germination stimulants are a signal to facilitate colonization by arbuscular mycorrhizal (AM) fungi [42], and then by the discovery that these signals are closely related to a newly discovered strigolactone-related plant hormone that contributes to regulation of branching in plants [43,44]. Thus, it now appears that strigolactones are important mediators of the plant's response to the environment, with levels being increased under phosphate starvation conditions and thus encouraging greater AM fungi colonization [41,45,46]. At the same time these compounds serve as mobile signals within the plant to regulate shoot architecture, which can also serve the plant by limiting branching under phosphate-limiting conditions [47,48].

Parasite use of the strigolactone system provides an elegant example of how signals can be modified to serve an alternative function. Because strigolactone-mediated regulation of branching is an ancient mechanism in angiosperms, parasitic plants are likely to have shared this signaling pathway ancestrally with non-parasitic relatives. The Orobanchaceae parasitic lineage has adapted this pathway for host detection by somehow modifying strigolactone signaling during the crucial seed germination stage. Means to this end could have arisen through parasitic species stopping their strigolactone biosynthesis either permanently through evolutionary loss of function or temporarily by downregulation during developmental steps leading to germination readiness. Parasite seeds could then detect strigolactones exuded from host roots to trigger germination, yet later in development the parasite could obtain strigolactones from the host through direct xylem connections or by activating its own biosynthetic pathway. Alternatively, parasitic species could have normal strigolactone biosynthesis, yet altered response to strigolactones might regulate germination. It is noteworthy that plants produce multiple variants of strigolactone, and parasitic species show remarkable levels of specificity to germination stimulants [49]. Thus, it is possible that the parasites can distinguish endogenous from exogenous strigolactones and respond accordingly. The mechanism by which strigolactone perception is transmitted into a germination cue is not understood, but likely candidates for receptors include an F-box protein [43,44] and a protein member of the α/β -hydrolase superfamily [50], both of which are related to proteins implicated in hormone signaling.

The ability of parasitic plants to adapt the function of strigolactones from branching factors to germination-stimulants might not be such a large jump. Strigolactones have been reported to break seed dormancy of non-parasites such as lettuce (*Lactuca sativa*) and wild oats (*Avena fatua*) [51,52]. Butenolide, a smoke-derived compound with structural similarities to strigolactones, promotes seed germination in a wide variety of plant species [53,54] including *Orobanche* and *Striga* [55]. Although a widespread role for strigolactones in regulation of dormancy and germination in plants has yet to be demonstrated [56], these observations suggest that the strigolactone-mediated regulator of germination in parasitic plants derives from a more common mechanism for control of plant development.

Consequences of parasite evolution: managing the exchange of information

A consequence of the transition to heterotrophy is the relaxation of evolutionary pressure to conserve photosynthetic processes, and the resulting loss of plastid genes associated with light harvesting in holoparasitic species [57,58]. Although this reduction has been well documented, a more intriguing side-effect of plant-plant connections is the transfer of nucleic acids that could have important effects on parasite evolution.

Horizontal gene transfer (HGT) involving parasitic plants appears to have occurred in many parasitic lineages, including Rafflesiaceae [59–61], Orobanchaceae [62], *Cuscuta* [63], Mitrastemonaceae [59,61], Santalales [64], and *Pilostyles* (Apodanthaceae) [59–61]. Such transfers have been detected during phylogenetic studies in which the gene from either the parasite (or host lineage) unexpectedly appears on a gene tree in a lineage where a host (or parasite) would be expected. Strong evidence ideally requires careful experimental controls from parasite, host, and non-host lineages, replicated sampling and detailed sequence analysis to assess the potential for phylogenetic artifacts such as long branch attraction or intra-genomic transfer [65], and evidence that other genes from the same organisms are generally not misplaced in phylogenetic analysis [59,66]. HGT appears to take place on an evolutionary time scale, and it is not known how frequently genes pass between host and parasite, or whether there is a selective advantage to this gene acquisition. However, multiple apparent horizontal acquisitions of the mitochondrial *atp1* gene in holoparasites and an association between holoparasitism and the occurrence of the invasive *cox1* intron [59,67] suggest that mitochondrial HGT events could be surprisingly frequent. Because the putatively xenologous *atp1* in *Rafflesia* is transcribed and subject to RNA editing, this also suggests that the sequence does encode a potentially functional copy of the gene [59].

Host RNA molecules can be detected in some parasites after functional host invasion [68–70]. Because only a subset of host mRNAs are present in the parasite, there is likely to be some selectivity in the molecules that are translocated [71]. Whether host encoded mRNAs function in the parasite is not known, but there is evidence that silencing RNA molecules that are transcribed in the host can affect gene expression in the parasite. For example, in

Box 1. Pressing research questions in parasitic plant research

- How have gene structures or functions been modified during the evolution of parasitism?
- What new regulatory patterns have been imposed on existing genes to accommodate new structures or developmental activities in parasitic plants?
- Do lateral and terminal haustoria differ in their regulation?
- How has strigolactone signaling been modified in parasitic plants to enable host-specific germination?
- How has host specificity evolved in parasites?
- What factors define self-recognition by parasites?
- Do specific effectors (e.g. avirulence genes) exist to overcome host defenses?
- How much gene transfer occurs between hosts and parasites? Does it have an impact on parasite genome evolution and host specificity?
- To what extent do RNAs move between host and parasite and are these exchanges essential to parasite growth and differentiation?
- Can host plants be engineered to shut down parasite processes through gene silencing?

one case, the GUS reporter gene, which had been transformed into *Triphysaria* roots, showed visual evidence of silencing upon parasitizing a host lettuce plant bearing a hairpin GUS construction [69]. In a second case, expression of the mannose 6-phosphate reductase gene was reduced in the parasitic weed *Orobanche aegyptiaca* following parasitism of transgenic tomato bearing a hairpin construction [70]. Inactivating vital parasite genes *via* cross-family gene silencing could be an effective approach for engineering resistance against parasitic weeds. Gene silencing RNA-based strategies have been developed for viruses [72], bacteria [73], nematodes [74] and insects [75], and could be equally effective as a resistance mechanism in agronomically important susceptible host plant species.

The future of parasitic plant research

As more comparative transcriptomic information becomes available from parasitic plants, it should become possible to identify genes whose expression is limited to specific stages of parasite development and whose products are unique to parasitic angiosperms (e.g. genes involved in haustoria formation, and functioning of transfusion cells). Identifying the function of these genes will require parasite transformation systems, such as those that have been developed for *Triphysaria* [76].

The coevolution of parasitic plants with their hosts has enforced selective pressures resulting in great diversity and specialization among the parasites. From plants that lack roots, stems, and leaves, and yet produce the world's largest flowers (*Rafflesia*) [77], to minute seedlings with exquisite gene-for-gene interactions with host plants (*Striga* and *Orobanche*), parasitic plants exhibit extremes of what a plant genome is capable of doing. The mechanisms of host- and self-perception, host integration and nucleic acid transfer described above are further examples, but each of these derives from modifications of ancestral processes in non-parasitic plants. It will be interesting to reconsider autotrophic plants in light of our understanding of parasites and ask whether similar mechanisms (e.g. recognition of neighbor roots in the rhizosphere) exist in

more subtle forms in all plants. But ultimately, as plant science endeavors to discover and understand functions for all plant genes, few species will provide as fertile ground for new insights as parasitic plants (Box 1).

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