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Update

Genomic reconfiguration in parasitic plants involves considerable gene losses alongside global genome size inflation and gene births

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Introduction

Specialist plantsfeeding on other plants (haustorial parasites) result from the fierce competition for resources in the botanical world. A parasitic lifestyle in plants always coincides with a heterotrophic nutrient acquisition, which brings about a plethora of morphological, metabolic, and genetic changes. Most parasitic plants retain the ability to carry out photosynthesis (Nickrent, 2020). Some of these may even fulfill their lifecycle as free-living, autotrophic plants (facultative parasites). Others require a host plant during at least certain developmental stages (obligate parasites). Losing photosynthesis and relying entirely on heterotrophic nutrient supply from another plant is a derived stage of an obligate parasitic lifestyle. More lineages (orders) of parasitic angiosperms contain only holoparasites (Nickrent, 2020). We could view this as circumstantial evidence that the specialization on a (holo)parasitic lifestyle occurs rapidly by providing an, at least temporary, eco-evolutionary advantage.

Parasitic plants never become a dominating species in undisturbed environments, where they even profoundly benefit their habitat by keeping plant growth in check and maintaining species diversity (Press and Phoenix, 2005). However, a few parasitic plant species like witchweeds (*Striga* spp.), dodders (*Cuscuta* spp.), and broomrape

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- Parasitism in plants coincides with novel genes and newly rewired genetic pathways, whereby evolutionary novelty derives from changes in the parasitic plants' nuclear genomes.
- Genome evolution in parasites is an interplay of functional gain and plant tissue- or function-specific gene losses as parasitic specialization unfolds.
- Horizontal gene transfers are more frequent in obligate parasitic plants than in facultative parasites, potentially bringing about functional advantages.
- Besides polyploidy, the proliferation of transposable elements is a common mechanism of genome size increase in parasites, possibly providing a cradle for genetic novelty.
- Plastid genome evolution of nonphotosynthetic plants represents the most striking example of genomic convergence, signifying genetic streamlining under extreme selective regimes, while mitogenome evolution of parasites remains elusive

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(*Orobanche* spp. and *Phelipanche* spp.) have become dominant weeds in managed, agricultural ecosystems. These weedy parasites diminish the production of many cereal, oilseed, forage, and vegetable crops, inflicting annual crop losses worth billions of Euros worldwide (Parker, 2013).

Innovations in *omics*-technologies and high-throughput data analysis have accelerated research into parasitic plants' genetics. The Broomrape family (Orobanchaceae) and *Cuscuta* have become primary models for understanding parasitism in plants. Concerted efforts have established resources for in vitro experiments and genetic manipulations of model parasites (Tomilov et al., 2007, 2008; Fernandez-Aparicio et al., 2011; Ishida et al., 2011; Westwood et al., 2012; Pouvreau et al., 2013; Aly et al., 2014). Large-scale genomic, transcriptomic, and fine-grained molecular-genetic analyses have identified core gene sets for germination and parasitic attack (Wickett et al., 2011; Honaas et al., 2013; Conn et al., 2015; Ichihashi et al., 2015; Yang et al., 2015; Fürst et al., 2016; Bao et al., 2017; Ishida et al., 2017; Wakatake et al., 2018; Brun et al., 2019; Hegenauer et al., 2020; de Saint Germain et al., 2020; Su et al., 2020). Various studies have uncovered the extent of transfer of informational molecules like viruses, proteins, and nucleic acids between parasites and their hosts (Mower et al., 2004; Yoshida et al., 2010b; LeBlanc et al., 2012; Zhang et al., 2013; Kim et al., 2014; Yang et al., 2016, 2019; Hettenhausen et al., 2017; Shahid et al., 2018; Liu et al., 2020a). Recently, also a parasitic plant-specific web application was launched to facilitate data sharing (Kösters et al., 2021). Comparative surveys pioneered the unlocking of genomic causes and consequences of parasitism. Among others, they showed that reductive genome evolution in parasites is an interplay between the degree of parasitic specialization, micro- and macrostructural genomic modifications, and the molecular processes underlying molecular evolutionary rate shifts (Wicke et al., 2013, 2016; Barrett et al., 2014, 2018; Cusimano and Wicke, 2016; Feng et al., 2016; ; Chen et al., 2020b). Likely because many obligate parasites have highly complex genomes (Wicke, 2013), parasitic plants were the last major group of specialists for which complete genome resources became available. We are now beginning to gain the first detailed insights into parasitic plant genome landscapes, which offer exciting new research perspectives.

Here, the most recent advances in parasitic plant genomics are analyzed in order to disentangle the differences between nuclear, mitochondrial, and plastid genome evolution. This Update highlights the innovations and potentials of genome analyses of parasitic plants by integrating general and lineage-specific molecular-evolutionary trajectories.

Phases of genome evolution in parasites

The first models describing the trajectory of genomic evolution in parasitic plants were founded on organelle data and described up to five degradative phases (Barrett and Davis, 2012; Naumann et al., 2016; Wicke et al., 2016). Concepts describing the overall genomic evolution of parasites require the inclusion of adaptive characters. Therefore, we may consider the overall genomic evolution in parasitic plants to progress in three major phases (Searcy and MacInnis, 1970; dePamphilis, 1995; Yoshida et al., 2019):

Phase I, during which functional innovation confers the ability to exploit another plant.
Phase II, where parasitic specialization relaxes molecular-evolutionary constraints on dispensable, host-complemented molecular processes.
Phase III, during which parasitic plant–host interactions are tightened and optimized.

We must understand these phases as co- and interdependent rather than differentiated stages. Once functional novelty enables a heterotrophic lifestyle, the new parasite gains an ecological-evolutionary advantage in the competition for nutrients. As a result, increased fitness leads to a rapid specialization in heterotrophic nutrient acquisition. We assume that genes and proteins essential for parasitic lifestyles will experience selective sweeps on the molecular level, possibly showing coevolutionary effects on directly interacting elements, which act in the same molecular or metabolic pathways.

Other cellular processes will lose relevance alongside the optimization of parasite function and intensification of parasite–host interactions. Elements of phase II are detectable by their distinct (episodic) relaxations of purifying selection. The degradation of plastid genomes and losing many nuclear-encoded photosynthesis genes are phase II symptoms. We may expect that especially autotrophic energy gain processes are among the first to suffer notably from a relaxation of selective constraints. Relaxed purifying selection in autotrophic processes, irrespective of genomic loci of the genes involved, could reduce photosynthetic efficiency, further driving metabolic optimization towards heterotrophy (Wicke et al., 2016; Wicke and Naumann, 2018). As photosynthetic energy gain drops, the parasitic nutrient acquisition will gain relevance for the plant. Therefore, phase III genes are likely to show positive selection, and gene families containing genes that undergo optimization are probably expanded. To ensure that positive selection is not mistaken for signs of selection relaxation, large-scale scans for selective footprints require advanced molecular-evolutionary methods and a robust phylogenomic framework.

We should use caution to interpret genomic features and generalize results from parasite genomes as long as the taxonomic representation of genera and species with whole-genome data is still limited. Available genome sequences are all highly unusual in that they are all small-genome plants (see below). Thus, they might display considerably distinct patterns of genomic reconfiguration than the "average" parasitic plant—be it regarding genic, nongenic, repetitive, transposable, or foreign genetic material. Some genomic or functional effects might not directly relate to establishing a parasitic lifestyle as the expected loss of photosynthesis. Indirect changes in genomic compartments that contain

dispensable genes or those in proximity to those might benefit (or suffer) from location effects. Location (position) effects can be protective or destabilizing, in that they influence the rate of molecular evolution, gene expression, and gene clustering (Zhang et al., 2002; Yang and Gaut, 2011; Wicke et al., 2013, 2014; Chen and Zhang, 2016; Kustatscher et al., 2017; Xu et al., 2019). Should those effects occur as a (late) consequence of an overall system reconfiguration, we may assume them to follow other rearrangements and be relatively weak. In contrast, dispensable genes associated with essential elements might survive longer than their function would let us think. However, larger clusters of co-localized nonessential genes might be lost earlier during genomic reconfigurations. Several earlier studies support this genome evolutionary scenario for parasitic and nonparasitic angiosperms (Yang and Gaut, 2011; Wicke et al., 2013, 2014).

As a plant gains the ability to parasitize another, environmental constraints might drive a rapid eco-evolutionary specialization. This process might set off a feedback loop between trophic specialization, interdependent functional gains, losses, and large-scale genomic and metabolic reconfigurations. With all its genomic compartments, the entire parasite system would show footprints of a gradual functional reorganization and optimization. Comparative genomic approaches analyzing transition forms alongside target species like weedy taxa are therefore essential to understand the various links of lifestyle preferences and genomic features and infer the relative timing of genomic novelties.

Genomic reduction in organelles versus expansion in the nucleus

Parasitic plant genomes and transcriptomes show that all three phases coexist in the three cellular compartments at a lineage-specific magnitude. The complexity and overall sizes of parasitic plant genomes are puzzling, especially as it partly contradicts the widely assumed (genomic) reduction syndrome of many parasitic organisms. Physical and functional reductions are well studied in the parasites' plastid genomes (reviewed in Wicke and Naumann, 2018). They might even extend to mitochondrial genomes, as seen in mistletoes belonging to the Viscaceae (Petersen et al., 2020). However, nuclear genomes of parasitic plants seem to evolve on a genomically nonreductive route.

Genome size data are available for 174 representatives (254 accessions) of Apodanthaceae, *Cuscuta* (Convolvulaceae), Krameriaceae, Loranthaceae, Olacaceae, Orobanchaceae, Santalaceae, and Viscaceae (Supplemental Data Set S1), spanning five origins of parasitism in flowering plants and all parasitic specializations. These data reveal that the overall range of mean parasite genome sizes is higher than in nonparasitic flowering plants (mean genome size in angiosperms: 1C = 5.7 Gb Dodsworth et al., 2015; mean genome size of parasitic angiosperms: 1C = 9.3 Gb, $n = 254$ accessions from 174 species; Supplemental Data Set S1). Considering modes gives 1C = 0.6 Gb for genome sizes of nonparasitic angiosperms (Dodsworth et al., 2015) compared with 1 C = 9.89 Gb for parasitic species (Supplemental Data Set S1). Together, these data suggest that parasite families have larger genomes than nonparasites. This trend shows even more clearly in comparing parasites with their phylogenetically closest nonparasitic relatives (Figure 1). Besides *Krameria*, a lineage of photosynthetic parasites, with genomes seven-fold larger than in the closely related Zygophyllaceae (Veselý et al., 2013), the tiny endoparasitic Apodanthaceae, the stem-twiners of *Cuscuta*, and the diverse root-parasitic Orobanchaceae all exceed the average genome sizes of their most closely related autotrophs (Supplemental Data Set S1). There is also considerable evidence for dynamic genome-evolutionary histories, including whole or partial genome duplications (Schneeweiss and Weiss, 2003; Schneeweiss et al., 2004; Weiss-Schneeweiss et al., 2006; McNeal et al., 2007; Wickett et al., 2011; Piednoël et al., 2012; Veselý et al., 2013; Wicke, 2013; Ataei, 2017; Sun et al., 2018; Vogel et al., 2018). These findings raise the urgent questions of why parasites have larger genomes and if genome size inflation in parasites possibly relates to advanced functions or sophisticated adaptation to the parasitic lifestyle.

There may be a trend towards larger genomes within Orobanchaceae as the degree of heterotrophy increases (Supplemental Data Set S1; a statistical test with adequate corrections for phylogenetic nonindependence remains to be done). In Santalales, larger genomes characterize parasites of a mistletoe habit, which is the derived feeding mode, corresponding with parasitic specialization (Vidal-Russell and Nickrent, 2008; Su et al., 2015; Chen et al., 2020b). *Cuscuta* shows differences in plastid genome complexity and photosynthesis gene retention (e.g. Revill et al., 2005; Braukmann et al., 2013; Banerjee and Stefanović, 2019). It would be interesting to test whether these differences relate to a metabolic progression series towards holoparasitism. The lack of transition series in all other parasitic plant lineages allows only limited direct comparisons. The observation of convergent increases along trophic specialization invites us to wonder whether genome size increase has adaptative potential towards (holo)parasitism.

We would expect many gene losses in nonphotosynthetic plants. Many gene families in parasitic plants have experienced reductions in their diversity, whereas others show expansions. Gene predictions can vary considerably from species to species, with the model plant Arabidopsis (*Arabidopsis thaliana*) containing approximately 27,000 protein-coding genes (Sterck et al., 2007). The obligate hemiparasite *Striga asiatica* contains 34,577 predicted gene loci (Yoshida et al., 2019), and the facultative parasite *Phtheirospermum japonicum* encodes 30,337 protein-coding genes (Cui et al., 2020). These genes numbers are similar to the number of genes in the closely related nonparasitic *Mimulus*. Under phase II, gene classes involved with environmental stimulus–response, plant hormones, water relations, and some photosynthesis pathways, among others, show

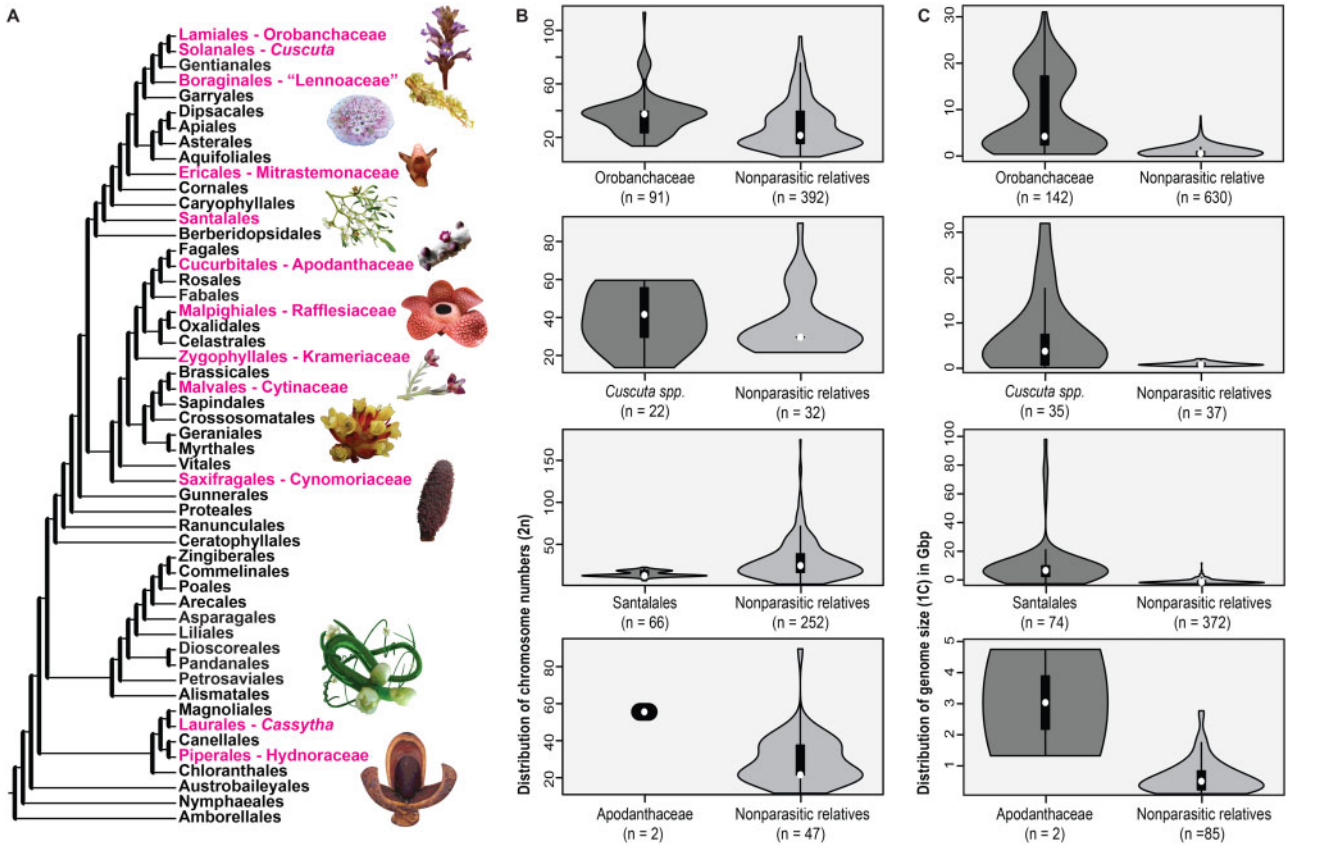

Figure 1 Trends of genomic evolution in parasitic angiosperms. A, Phylogeny (cladogram) of flowering plants, highlighting the 12 independent origins of haustorial parasitism (pink); branch lengths are meaningless. Illustrations to the right of the tree tip labels show a characteristic representative for each parasite lineage. Graphical summaries of B, chromosome count data and C, genome size information for parasitic plants (dark gray) and their closest nonparasitic relatives (light gray) highlight distinct patterns of karyological variation and a trend towards larger genomes in parasitic plants. A white dot per data group indicates the median, and a black bar shows the interquartile range. The 95% confidence intervals are indicated by slim lines. The curvature of each plot expresses the density and distribution of the individual data points, with the number of considered accessions given below group names. Original data underlying this graphical summary are presented in Supplemental Data Set S1.

notable contractions in *Striga*. However, gene families associated with germination stimulant detection or cellular transport are notably expanded (Yoshida et al., 2019).

Recent work reported extreme differences in gene content for *Cuscuta australis* and *Cuscuta campestris*, both (physiological) holoparasites. Traditionally, the absence of chlorophyll defined holoparasites. Today, we know that holoparasites may contain chlorophyll but cannot photosynthesize. While *C. australis* has only 19,671 predicted protein-coding genes (Sun et al., 2018), *C. campestris* contains 44,303 genes in its first annotation (Vogel et al., 2018). Different gene model prediction procedures may explain the observed discrepant coding capacity of the two closely related dodder species. Therefore, an independent comparative genomic analysis of both species is desirable. The possibility of recent ploidization events should be tested carefully as well. Both *C. australis* and *C. campestris* appear to contain $2n = 56$ chromosomes (Goldblatt and Johnson, 1979 and reference therein; Pazy and Plitmann, 1995; García and Castroviejo, 2003). However, *Cuscuta* spp. show extensive genome size variation, with frequently occurring ploidy changes (Neumann et al., 2020). Yet, the number of studied individuals per species and the taxa's geographic representation is limited.

The two dodder species with complete genome have lost several plastid- and nuclear-encoded photosynthesis-associated genes. Several more gene families showed extensive contractions, including gene families implicated in (root) developmental processes, reflecting *Cuscuta*'s unusual body plan. Similarly, nutrient transporters and resistance genes were reduced (Sun et al., 2018; Vogel et al., 2018). The parasite may compensate for such losses by rampant incorporation and expression of genetic material, and the uptake of macromolecules from its host (Kim et al., 2014; Zhang et al., 2014; Westwood and Kim, 2017; Shahid et al., 2018; Vogel et al., 2018; Yang et al., 2019; Liu et al., 2020a).

Endoparasitic Rafflesiaceae might contain the most extreme genomic reconfigurations (Cai et al., 2021), reflecting theses plants' aberrant morphology, physiology, and lifestyle. Although ~60% of the estimated total genome size remain unassembled for *Sapria*, 42,512 transcribed genes were identified and sorted into 56% of the universally conserved orthogroups of eudicots. Gene losses are enriched in gene families associated with photosynthesis, plastid biology,

defense, and stress response genes (as described in the sequenced Orobanchaceae species and *Cuscuta* spp.). However, *Sapria* also exhibits gene losses in abscisic acid biosynthesis, protein degradation, and purine metabolism, not seen (to such extent) in other parasites (Sun et al., 2018; Vogel et al., 2018; Yoshida et al., 2019; Cui et al., 2020; Cai et al., 2021). Genomic structure is also extremely aberrant, in that the genome of *Sapria* encodes proteins shorter than those of close relatives and genic regions contain fewer introns than other eudicots (Cai et al., 2021). Intron sizes in nonhousekeeping genes are largely inflated due to an influx of transposable elements (Cai et al., 2021). To understand better the significance of the observed gene losses and genome restructuring, it will be important to analyze the remaining, currently unassembled fraction of the *Sapria* genome and compare it with other species of Rafflesiaceae.

Expanding the genetic repertoire with host-derived information and selectively using it to its advantage fits phases II and III of parasite genome evolution. The optimization and specialization of the host–parasite interaction coincide with the relaxation or intensification of selection pressures. Several lines of evidence suggest that horizontal gene transfer (HGT) itself increases as heterotrophic specialization unfolds (Yang et al., 2016). Two *Cuscuta* studies independently concluded that HGT is exceptionally diverse in *C. campestris*. However, the nature of genes identified to derive from other plants differs (Vogel et al., 2018; Yang et al., 2019), perhaps due to differences in taxon sampling and the employed methods' sensitivity. Despite the observed discrepancies, both Orobanchaceae and *Cuscuta* may have benefitted from functional HGT in nuclear genomes and might have adopted foreign gene fragments to their advantage (e.g. Zhang et al., 2013, 2014; Shahid et al., 2018; Yang et al., 2019). However, functional evidence regarding the relevance of host-derived genes is still missing.

Parasites are genomic innovators that partly rewire the genetic repertoire known from ordinary plants. For example, parasitic Orobanchaceae recruit genes otherwise expressed in floral tissue during the stage of haustorium formation (Yang et al., 2015). Parasites may have also "learned" to re-use genetic material from their hosts, including miRNA that apparently target host defenses (reviewed in Hudzik et al., 2020). Plus, many taxonomically restricted, parasite-specific orthogroups imply genic novelty, potential "parasitism genes" (Yoshida et al., 2010a, 2010b, 2019; Wickett et al., 2011; LeBlanc et al., 2012; Zhang et al., 2013, 2014; Yang et al., 2016, 2019; Su et al., 2020; Cai et al., 2021). Even though non-native or lineage-specific genes are expressed in parasites, functional evidence substantiating their relevance is missing. Those new genes of parasitic plants appear to have arisen from the genetic repertoire shared with nonparasitic angiosperms. Especially gene duplication seems to have created many new genes with novel domain combinations (e.g. Yang et al., 2015), whereas HGT has brought in new genes to differing scales. More fine-scaled transcriptome and genome sequencing should determine how many of the new parasite-specific elements are of a genomic de novo origin, i.e. arisen from random, nongenic DNA sequences (Carvunis et al., 2012; Bornberg-Bauer et al., 2015; Neme et al., 2017).

The C-value paradox, which describes the enigma that genome size is uncorrelated with organismal complexity and the number of genes, holds up in parasitic plants. Different causes, mechanisms, and selectional pressures drive genome size evolution. Polyploidy is frequent in plants, including in parasites, as an analysis of chromosome evolution in parasite lineages compared with close relatives exemplifies (Figure 1). In Orobanchaceae, we must assume at least three ancient whole-genome duplication events. However, the only thorough examination of whole-genome duplications based not only on the distribution of synonymous substitutions in paralogous genes (K_S plots) in Orobanchaceae is limited to *S. asiatica* (Yoshida et al., 2019). *Striga* experienced one unique ploidization event, one shared with *Mimulus* (and most likely other Orobanchaceae), and shows footprints of the more ancient gamma (eudicot), epsilon (angiosperm), and zeta (seed plant) duplications. Differing K_S values, phylogenetic relationships, and karyological analysis suggest that the *Striga* duplication is independent of whole-genome duplication in *Phelipanche aegyptiaca* (Schneeweiss et al., 2004; Wicke, 2013; McNeal et al., 2013; Li et al., 2019; Yu et al., 2018). The facultative parasite *Triphysaria* seems to share neither of these duplications (Wickett et al., 2011). Therefore, (micro)synteny data from whole-genome sequences of many more Orobanchaceae should bring clarity. Chromosome data from the C-value Database (Pellicer and Leitch, 2020; last accessed in May 2020) merged with recently published work (Supplemental Data Set S1) strongly suggest that many more polyploidization events occurred during the Broomrape family's diversification. Palaeopolyploidy and recent ploidization events may also be essential drivers of species divergence in the *Orobancheae* clade alone (Schneeweiss et al., 2004; Ataei, 2017).

The functional and ecological implications of those whole-genome duplications and whether they contribute an explanation for parasitic Orobanchaceae's evolutionary success remains elusive. With the few whole-genome and some more comprehensive transcriptomes at hand, we may assume that functional diversification in the aftermath of a genome duplication aided the occurrence of a parasitic lifestyle (see above). Specifically, polyploidy could provide a cradle for gene duplication for sub- and/or neofunctionalization and the repurposing of genes for the development of new plant structures (e.g. Yang et al. 2015). Both *Cuscuta* genomes exhibit footprints of past genome duplications (Sun et al., 2018; Vogel et al., 2018), and many "parasite genes" in Orobanchaceae originate from the *Striga–Mimulus* duplication (Yang et al., 2015; Yoshida et al., 2019). Together, these data fit the radiation time-lag model (Schranz et al., 2012), where paleopolyploidy advances the evolution of new

genes and novel phenotypes. However, the radiation of the lineage would occur later.

Mechanisms and selection pressures for genome size inflation

Recent genome duplications are not uncommon in both hemiparasites and holoparasites. Orobanchaceae but also *Cuscuta* spp. exhibit extreme ploidy levels. In *Cuscuta*, there is ample evidence for multiple rounds of poly- and disploidization based on chromosome counts (García and Castroviejo, 2003; McNeal et al., 2007; Neumann et al., 2020). Genome synteny analysis of *C. australis* revealed an ancient whole-genome triplication shared with its nonparasitic relative *Ipomoea* (Sun et al., 2018). A similar analysis of the *C. campestris* genome showed a duplication after the split of the parasite lineage from the autotroph (Vogel et al., 2018). These results hint at differences in the ploidization history of *C. australis* and *C. campestris*, which is unsupported by currently available chromosome count data for the two species (see above). Therefore, further karyological studies for each of the sequenced individuals of *C. australis and C. campestris*, or their progeny, are desirable to clarify the issue.

Polyploid parasite genomes experienced a subsequent downsizing (e.g. in several species of *Orobanche*; Weiss-Schneeweiss et al., 2006), meaning that the monoploid genome size (1 Cx value) can be lower than in nonpolyploid relatives (Leitch and Bennett, 2004). Whatever the biological significance and evolutionary advantage of this eventual loss of genetic information following polyploidization events is (e.g. transposable element elimination, heterochromatin reduction), it appears to act in parasites in the same way as it selects for smaller base genomes in ordinary plants (Supplemental Data Set S1). Parasites with giant genomes might differ in their mechanisms of genome downsizing. Other nutrient stoichiometry-based mechanisms known to control genomic obesity might play a role as well. In nonparasitic plants, limitation of certain nutrients results in smaller genomes because it biases the composition of N-/P-rich to N-/P-poor nucleotides and/or amino acids (Acquisti et al., 2009; Guignard et al., 2016; Bales and Hersch-Green, 2019). In parasites, nutrient-limiting effects could play a less critical role because they withdraw the nutrients they need to survive from their host. The pressure to delete temporarily unused parts of the genome might be reduced in the absence of a need for nutrient stoichiometry-dependent genome downsizing. Therefore, micro-evolutionarily only sporadically used genetic information might survive longer than usual in parasites. Perhaps, this genetic information becomes even useful to overcome ecological bottleneck in that it provides an occasional eco-evolutionary advantage in acclimation and adaptation.

Polyploidy alone does not explain the extensive genomic variation between parasitic species, which exhibit a remarkable intrageneric variation of genome sizes. Extensive genome size variation by several gigabases in the absence of chromosome number alterations is well known in (parasitic) plants. For instance, holoparasitic broomrapes of the genus *Phelipanche* range in haploid genome size between 3.4 Gb (*P. nana*) and 5.5 Gb (*P. iberica*), yet, all taxa possess a stable chromosome number of $2n = 24$. Those species of *Orobanche* with a chromosome number of $2n = 38$ vary in haploid genome size from 1.4 Gb (*O. cernua* var. *cumana*) to 4.6 Gb (*O. anatolica*; Supplemental Data Set S1; Weiss-Schneeweiss et al. 2006). Species of *Cistanche*, which are the record holders of Orobanchaceae concerning haploid genome size, range between 8.4 Gb (*C. ambigua*) and 31.2 Gb in a specimen of *C. tubulosa* (Supplemental Data Set S1; Ataei, 2017). Outsides Orobanchaceae, *Cuscuta* shows extensive genome size variation within even single species (McNeal et al., 2007; Neumann et al., 2020). Proliferation (or loss) of transposable elements seems like the most probable mechanism underlying such genome size variation.

Keeping mobile elements in check by effectively silencing them might be challenging for parasite cells when they experience physiological or environmental stress (e.g. upon host switches or (a)biotic disturbances). Novel transposons entering the genome via HGT may expand wildly until the genome can control them. The proliferation of noncoding DNA and mobile elements affects many plant species under stress (reviewed in Hou et al., 2019). A side effect of the far-reaching genomic shock around the massive genetic, functional, and metabolic reconfigurations alongside lifestyle changes represents another potential hypothesis for the repeat proliferation-dependent genome size inflation in parasites. On the other hand, parasites conquering new niches, be it geographic or biotic (host) niches, often result in small (founder) populations, in which genetic drift can outweigh that of selection. Thus, progenitors might inherit temporary repeat proliferation with no adaptive benefits, as long as it provides no eco-evolutionary disadvantage.

An adaptive value of genome size inflation, be it from polyploidy or the proliferation of transposable elements, might also lie in their power to restructure genomes (Morgante et al., 2005; Dodsworth et al., 2015; Galindo-González et al., 2017; Sahebi et al., 2018). Transposon-mediated exon shuffling can form new genes by joining exons from different genes or modifying genic regions by altering intron–exon structures. Long interspersed nuclear elements, helitron transposons, miniature inverted-repeat transposable elements, and long-terminal repeat retrotransposons commonly associate with genic transductions and exon shuffling, resulting in a continuous change of coding and noncoding regions. Insertions of transposable elements near or in genes raise the potential for alternative splicing, epigenetic control, duplication, and recombination. The insertion of transposable elements into promoter regions could alter gene (co)expression networks, thereby leading to developmental modifications.

Chromosomal and transposable element landscapes are tightly connected and turn over rapidly in plants. There is no evidence that parasitic plants are an exception. Compared with the nonparasitic *Lindenbergia* and the

hemiparasite *Schwalbea*, nonphotosynthetic *Orobanche* and *Phelipanche* species contain higher proportions of repetitive DNA (Piednoël et al., 2012), in line with larger genomes in the holoparasites. Among broomrapes, transposable elements in *Orobanche* species diversify extensively and more so than in *Phelipanche* (Piednoël et al., 2012, 2013, 2015), which is also less dynamic concerning ploidy variation (Schneeweiss et al., 2004). In sum, the combined evidence lets us assume that a mechanism of genomic and functional innovations along lifestyle transitions in parasitic plants involves changes of ploidy and transposable element diversity.

Genomic reconfiguration in organelles of parasitic plants

Plastomes of nonphotosynthetic plants represent *the* most striking example of convergence in genome evolution yet described. A recent synthesis of conceptual models integrates plastid genome data from nearly all nonphotosynthetic plant lineages. It describes reductive genome evolution in parasites as lifestyle-associated reconfigurations that proceed with interdependent changes in plastome structure, function, and rates of molecular evolution (Naumann et al., 2016; Wicke et al., 2016; Wicke and Naumann, 2018). Lineage-specific patterns of gene losses are not uncommon, reflecting the independent transitions into heterotrophy. The only heterotrophic gymnosperm *Parasitaxus* as a potential chimera of a mycoheterotroph and haustorial parasite is unique in its parasitic mode (Feild and Brodribb, 2005). It also represents the only case in which plastid genome reduction occurs with no parallel loss of plastid housekeeping genes and involves the re-gain of a new pair of inverted repeats (Lam, 2016; Qu et al., 2019). In contrast, many angiosperm parasites and mycoheterophs lose or reduce their inverted repeat regions alongside plastome reduction (Wicke et al., 2013, 2016; Bellot and Renner, 2015; Schelkunov et al., 2015, 2019; Feng et al., 2016; Logacheva et al., 2016; Naumann et al., 2016; Braukmann et al., 2017; Petersen et al., 2019; Jost et al., 2020).

The resemblance of patterns regarding gene losses and other structural modifications to the plastid chromosome is remarkable between haustorial parasites and mycoheterophs. Comparative studies based on dense intrageneric taxon representations show that both functional and physical reductions are evolutionarily ongoing processes that proceed at lineage-specific rates (Petersen et al., 2015b; Cusimano and Wicke, 2016; Feng et al., 2016; Barrett et al., 2018; Banerjee and Stefanović, 2019; Chen et al., 2020b). However, our understanding of microevolutionary (i.e. intraspecific) dynamics of plastome degradation is limited. Similarly, functional validations of plastid gene or pseudogene models of parasitic plant lineage are still widely lacking, opening interesting routes for future research of plastid genomics in heterotrophs.

Reductive plastome evolution proceeds with a gradually increasing nucleotide compositional bias, favoring A/T over G/C bases (Wicke and Naumann, 2018) and might include the complete loss of a plastome (postulated by Molina et al., 2014 and Cai et al., 2021). The most extreme case of AT-richness pertains to the plastomes of some Balanophoraceae, where less than ∼15% of nucleotides are guanines or cytosines (Nickrent et al., 1997; Schelkunov et al., 2019; Su et al., 2019; Chen et al., 2020b). Despite the low complexity in DNA composition, the plastome of *Balanophora* species encodes genes that are actively transcribed and evolving under purifying selection, suggesting that these genes are functionally relevant (Su et al., 2019). At least some species in Balanophoraceae have even evolved a novel genetic code to maintain plastid gene function, allowing them to deal with the extreme nucleotide compositional bias. Among others, holoparasitic Balanophoraceae use alternative start codons and read over one of the three stop codons, which newly serves as the codon for tryptophan (Su et al., 2019). The extreme modification in a holoparasitic *Balanophora* species emphasizes the remarkable plasticity of the plastid genetic system. Importantly, it opens questions as to whether DNA-based evidence like missing start or stop codons or extreme sequence drift suffices to classify genes as pseudogenes. It also undermines the need for considerably more plastid gene expression data from parasitic plants (Wicke and Naumann, 2018).

Plant cells and tissues differ in the proportion of plastid DNA (Golczyk et al., 2014; Kumar et al., 2014; Oldenburg et al., 2014; Ma and Li, 2015; Morley and Nielsen, 2016). Several studies have reported lower proportions of plastid DNA in holoparasites than in their photosynthetic relatives (Wicke et al., 2013, 2016; Feng et al., 2016; Braukmann et al., 2017). The reasons underlying an underrepresentation of plastome DNA in genome sequencing data of holoparasites remain unknown. On the one hand, underrepresentation of plastid DNA in holoparasites could found on a reduced need for high plastome copy numbers due to a reduced or no more turnover of subunits for the photosynthetic machinery. On the other hand, technical artifacts arising from compositional biases could cause or further reduce a (biologically) low genomic representation of plastid DNA. Extreme compositional biases significantly hamper genome sequencing at various technical procedures and during bioinformatic data analysis (Chevet et al., 1995; Oyola et al., 2012; van Dijk et al., 2014). Already specific DNA extraction and purification methods eliminate AT- or GC-rich fractions of a genome, and sequencing technologies that require a PCR-based amplification might further increase the bias (Kozarewa et al., 2009). Attempting to retrieve an extremely reduced plastome in bulk data resembles the search for the needle in a haystack, especially if a plastome would be present at low copy numbers and genome assemblies remain incomplete. Our new knowledge of the extremes of genetic reconfigurations of parasitic plant genomes justifies and requires additional research into plants' nuclear and organellar genomic evolution at micro- and macroevolutionary scales.

Plant mitochondrial genomes' intractable nature explains why our understanding of mitogenomic evolution in

parasitic plants lags behind plastid genomics. The mitogenomes in the *Viscum-Phoradendron* lineage of the sandalwood order (Santalales) have experienced unprecedented (functional) gene losses, including all genes for complex I (*nad* genes), maturase R (*matR*), the cytochrome c-type biogenesis protein (*ccmB*), and various ribosomal protein genes (*rpl* and *rps* genes). These losses coincide with a general elevation of substitution rates in the rest of the mitogenome (Petersen et al., 2015a, 2020; Skippington et al., 2015, 2017; Zervas et al., 2019). Other heterotrophic plants show no apparent loss of core mitochondrial genes and little to no divergence in coding sequences (Molina et al., 2014; Bellot et al., 2016; Schneider et al., 2018; Yuan et al., 2018; Sanchez-Puerta et al., 2019; Zervas et al., 2019; Shtratnikova et al., 2020).

The most striking feature of parasitic plant mitogenomes is their frequent incorporation of foreign DNA. These newly gained fragments might originate from both other cellular genome compartments (Cusimano and Wicke, 2016; Chen et al., 2020a; Liu et al., 2020b) and/or host plants (Nickrent et al., 2004; Barkman et al., 2007; Mower et al., 2010; Fan et al., 2016; Sanchez-Puerta et al., 2017, 2019; Cusimano and Renner, 2019; Garcia et al., 2020; Roulet et al., 2020). To which extent those non-native DNAs influence the processes in parasite mitochondria is unknown. Similarly, the trajectories of mitogenome evolution along the transition to parasitism remain to be clarified.

Concluding remarks and perspectives

The past three decades of parasitic plant genome research have shaped our understanding of how parasitism and the loss of photosynthesis affect (plastid) gene loss. Less is known about the nuclear genes involved in or impacted by the transition to a parasitic lifestyle, which plays a role in the functional adaptation to this extraordinary way of life in plants. Genome-evolutionary novelty pertains to the parasitic plants' nuclear-genomic-coding capacity, whereas organellar genomes exhibit various stages of physical and functional reduction. Genes required for host–plant penetration and efficient nutrient uptake are likely to have experienced selectional sweeps along the way to rewiring existing developmental pathways by recruiting newly evolved gene functions. So far, the most apparent evidence of the adaptive evolution of parasite genes derives from the patterns of molecular evolutionary rate variation in protein-coding regions and gene expression shifts (Yang et al., 2015; Sun et al., 2018; Vogel et al., 2018; Yoshida et al., 2019), the latter of which implying that promoter evolution plays an as yet unexplored role. While single gene duplication is considered a prerequisite for the birth of novel genes via neo- and sub-functionalization, the duplication of the whole genome creates the potential to spur massive reorganizations, shifts in life history traits, and ecological tolerance (Levin, 1983; Otto, 2007). Associated with these are a number of puzzles for future research.

What are the biological significance of larger genomes in parasitic plants and the dominant mechanisms that shape the architecture of these genomes? It remains to be tested whether genome size increase reflects relaxed selection on genome size in parasitic organisms, if it has adaptive potential, or if it is a result of (invading host) transposons and their uncontrolled proliferation. Besides polyploidy, the expansion of the transposable element landscape represents a common mechanism of genome size increase. In parasites, it may even be tolerated evolutionarily as a genomic cradle for functional and structural novelty.

Another conundrum around the complex adaptation of parasites is whether and how genetic networks differ in parasites and how much and which steps it takes to rewire if it is not single genes or proteins that underlie the switch to parasitism? Moreover, how different are the genetic networks are among the various lineages of parasitic plants, in general, is not presently known. Which are the sets of genes and molecular processes involved in adaptation to the parasitic lifestyle in general and for enhancing the fitness of parasites under changes of their biotic and abiotic environment? Furthermore, as some genomic compartments shrink and might even be lost entirely en route to holoparasitism, how is organelle–nucleus communication secured and the control of organelle-based molecular processes? It will also be essential to clarify the role of HGT in the origin and early evolution of parasites. Compared with nonparasites, parasitic plant lineages show enrichment of ancestral and functional HGT, without which the parasite ancestors might not have survived. Addressing and answering many of these open questions requires more genome sequences covering various taxonomic levels. As we cannot go back to the earliest parasites, this might require sophisticated combinations of micro- and macroevolutionary approaches in lineages with abundant and closely related parasites and nonparasites, which are genomically tractable. Especially, chromosome-scale assemblies are needed in the near future to address the hypotheses regarding parasitic plant genomes.

OUTSTANDING QUESTIONS

- What is the biological and evolutionary significance of larger genomes in parasitic plants?
- How different are genetic networks between parasites and ordinary plants?
- Which molecular processes and mechanisms drive environmental adaptation in parasites?
- What are the biological consequences of the loss of a genomic compartment like the plastome?
- How has HGT contributed to parasitic plant genome evolution and adaptation?

Supplemental data

The following materials are available in the online version of this article.

Supplemental Data Set S1. Chromosome and genome size data for parasitic plants and nonparasitic relatives.

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