Current Biology 21, 2098–2104, December 20, 2011 ©2011 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2011.11.011

Report

Transcriptomes of the Parasitic Plant Family Orobanchaceae Reveal Surprising Conservation of Chlorophyll Synthesis

Norman J. Wickett,[1,5,*] Loren A. Honaas,[1] Eric K. Wafula,[1] Malay Das,[2,6] Kan Huang,[3] Biao Wu,[4] Lena Landherr,[1] Michael P. Timko,[3] John Yoder,[4] James H. Westwood,[2] and Claude W. dePamphilis[1,*]

[1]Department of Biology, Institute of Molecular Evolutionary Genetics, and Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA 16802, USA

[2]Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

[3]Department of Biology, University of Virginia, Charlottesville, VA 22904, USA

[4]Department of Plant Sciences, University of California, Davis, Davis, CA 95616, USA

Summary

Parasitism in flowering plants has evolved at least 11 times [1]. Only one family, Orobanchaceae, comprises all major nutritional types of parasites: facultative, hemiparasitic (partially photosynthetic), and holoparasitic (nonphotosynthetic) [2]. Additionally, the family includes *Lindenbergia*, a nonparasitic genus sister to all parasitic Orobanchaceae [3–6]. Parasitic Orobanchaceae include species with severe economic impacts: *Striga* (witchweed), for example, affects over 50 million hectares of crops in sub-Saharan Africa, causing more than $3 billion in damage annually [7]. Although gene losses and increased substitution rates have been characterized for parasitic plant plastid genomes [5, 8–11], the nuclear genome and transcriptome remain largely unexplored. The Parasitic Plant Genome Project (PPGP; http://ppgp.huck.psu.edu/) [2] is leveraging the natural variation in Orobanchaceae to explore the evolution and genomic consequences of parasitism in plants through a massive transcriptome and gene discovery project involving *Triphysaria versicolor* (facultative hemiparasite), *Striga hermonthica* (obligate hemiparasite), and *Phelipanche aegyptiaca* (*Orobanche* [12]; holoparasite). Here we present the first set of large-scale genomic resources for parasitic plant comparative biology. Transcriptomes of above-ground tissues reveal that, in addition to the predictable loss of photosynthesis-related gene expression in *P. aegyptiaca*, the nonphotosynthetic parasite retains an intact, expressed, and selectively constrained chlorophyll synthesis pathway.

Results and Discussion

The transcriptomes of three parasitic plants representing three nutritional strategies in Orobanchaceae were sequenced: *Triphysaria versicolor*, a facultative hemiparasite (here grown autotrophically); *Striga hermonthica*, an obligate hemiparasite; and *Phelipanche aegyptiaca*, a nonphotosynthetic holoparasite. Although previous studies have reported cDNA sequences and expressed sequence tag (EST) analyses of single parasitic plant species [13, 14], the results here represent the first comparative transcriptome analysis of parasitic species that differ in their photosynthetic capacity and significantly advance the genomic resources available to study parasitic plant biology.

EST Assembly and Annotation

In total, 1,278,054 454 FLX reads and 102,521,594 Illumina GAIIx paired reads were generated from above-ground, reproductive, and vegetative shoots of *T. versicolor*, *S. hermonthica*, and *P. aegyptiaca* (see Table S1 available online). Combined assemblies for each species resulted in 51,479 to 67,794 unigenes (>200 bp) with N_{50} values ranging from 663 to 952 (Table S2) and are available from the Parasitic Plant Genome Project (PPGP) database (http://ppgp.huck.psu.edu). Unigenes were sorted into an objective classification scheme (http://fgp.bio.psu.edu/tribedb/10_genomes) [15] that assigns each unigene to a SuperTribe, Tribe, and Orthogroup along with associated annotations. Membership in Tribes (global approximations of gene families) and Orthogroups (potentially narrower lineages stemming from a single ancestral gene in the reference species set) were used to investigate the overall classification of the transcriptomes.

The functional classification of genes in these three parasites revealed two primary patterns: first, a general, global similarity of functional categories across all three species (Figure 1; Figure S1), and second, numerous fine-scale differences among them (Figure 1; Figure 2). A large degree of similarity is expected in the transcriptomes of different plant cells regardless of function or photosynthetic ability; whether the large number of Tribes and Orthogroups shared between the two hemiparasites reflects their nutritional strategies requires a more careful dissection, as we have done here, of the functional annotations assigned to overlapping sets of unigenes. The complete summary of annotated unigenes with GO terms is provided in Table S3A, Table S3B, and Table S3C.

Read Mapping, Evolutionary Constraints, and Chlorophyll Synthesis

For each set of overlapping and uniquely expressed Orthogroups (Figure 1A), the proportion of the total number of Orthogroups assigned to each term for each Gene Ontology (GO) was plotted in order to scan for GO terms that are over- or underrepresented in each overlapping set (Figures 1B–1D). If the set of Orthogroups expressed in all species (sector A in Figure 1A) is taken as the "background" distribution of Orthogroups across the GO Slim terms, then extreme deviations from this can be easily visualized. The most obvious of these is the Chloroplast term of the Cellular Component ontology, for which the Orthogroups expressed in both the autotrophically grown hemiparasite and the obligate hemiparasite (*S. hermonthica* and *T. versicolor*; sector D in Figure 1A)

[5]Present address: Chicago Botanic Garden, Glencoe, IL 60022, USA

[6]Present address: Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, 85764 Neuherberg, Germany

*Correspondence: nwickett@chicagobotanic.org (N.J.W.), cwd3@psu.edu (C.W.d.)

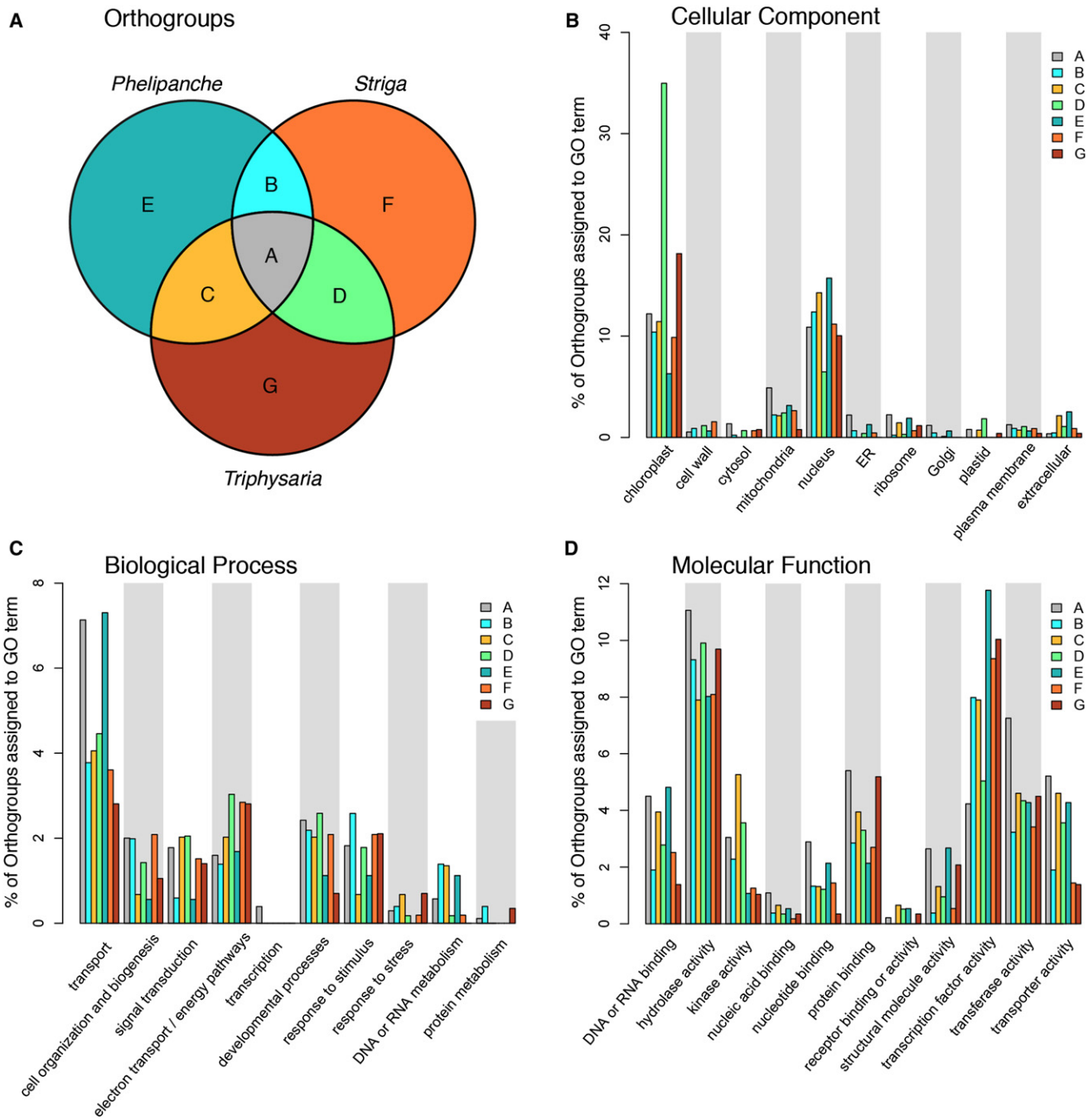

Figure 1. Proportion of Orthogroups Assigned to Gene Ontology Terms for Unigenes Expressed in One, Two, or Three of the Parasitic Plants *Phelipanche aegyptiaca*, *Striga hermonthica*, and *Triphysaria versicolor*

(A) Proportion of PlantTribe's Orthogroups assigned to GO Slim terms for assembled cDNA sequences (unigenes) expressed either uniquely in one of three parasitic plants (sectors E, F, and G; *Phelipanche aegyptiaca*, *Striga hermonthica*, and *Triphysaria versicolor*), expressed in two of three plants (sectors B, C, and D), or expressed in all three plants (sector A).

(B–D) For each section of the Venn diagram in (A), the proportion of Orthogroups assigned to a specific GO Slim term (per total number of Orthogroups in that section) was plotted for the Cellular Component (B), Biological Process (C), and Molecular Function (D) ontologies. See also Table S3A, Table S3B, and Table S3C.

account for three times the proportion of total unigenes as in the background.

For each species, Illumina reads were mapped to the combined assembly in order to approximate expression levels. Using the mapping information for each unigene, a normalized number of reads (counts per million total mapped, cpm) per Orthogroup was calculated (Table S4). Because the three plants sequenced represent three points along a photosynthetic spectrum, Orthogroups corresponding to structural components of the photosynthetic apparatus were extracted from the read mapping results. Of the Photosystem I and II reaction center proteins, structural

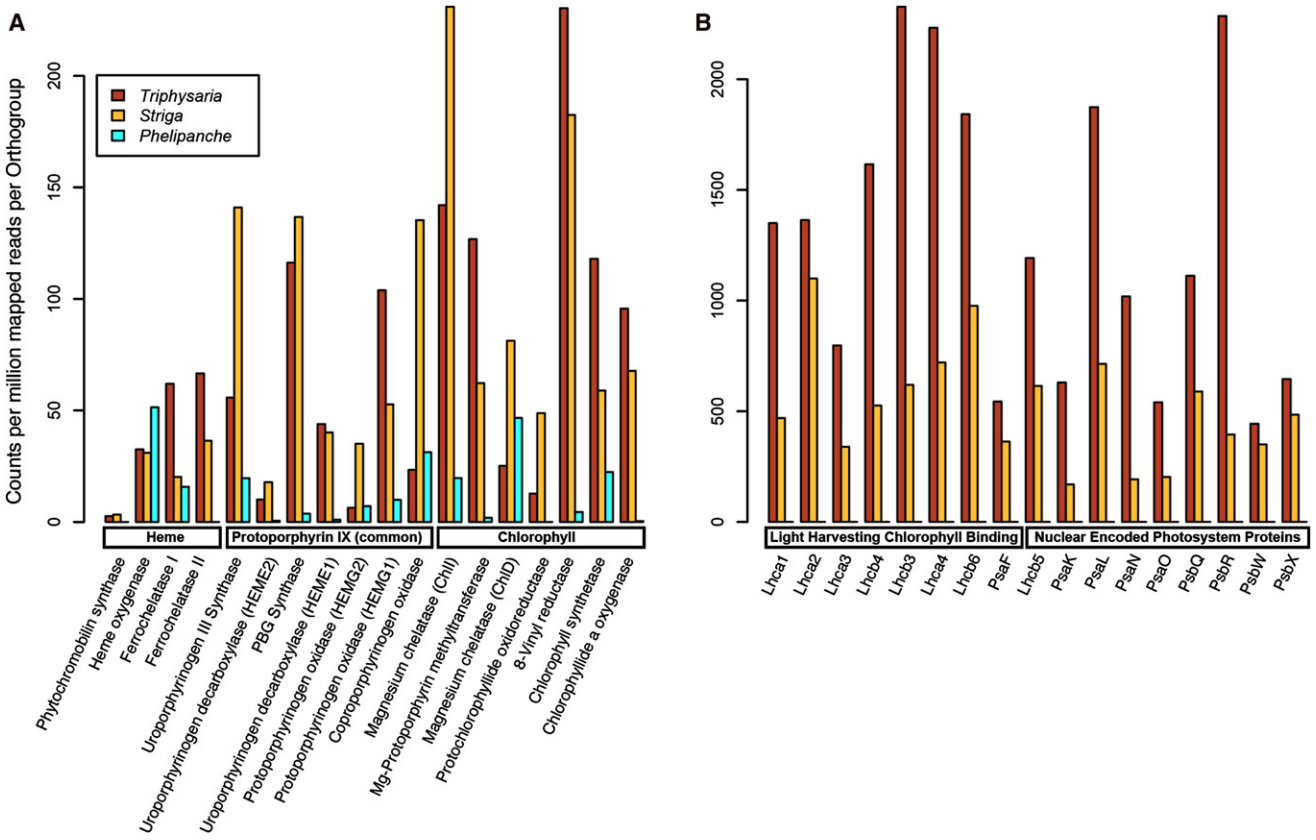

Figure 2. Normalized Number of Illumina Reads Mapped per Orthogroup for the Chorophyll Synthesis and Photosynthetic Pathways

Normalized number of reads mapped (number of reads per million total mapped reads) to PlantTribes Orthogroups representing genes of the chlorophyll synthesis (A) and photosynthesis (B) pathways for three parasitic plants: *Triphysaria versicolor*, *Striga hermonthica*, and *Phelipanche aegyptiaca*. Chlorophyll is synthesized from a precursor (protoporphyrin IX) that is also an intermediate in heme biosynthesis. Illumina GAIIx sequence reads were mapped to the unigenes from the combined (454 + Illumina; vegetative + reproductive) assemblies. The numbers of reads mapped were normalized for the number of total mapped reads and then summed for each Orthogroup (narrow lineage of genes potentially descending from a single ancestral gene in the reference genome set). See also Table S4.

components of the cytochrome b6/f complex and photosynthetic electron transport, and F-type ATPases identified from the *Arabidopsis* reference pathway (http://www.genome.jp/kegg/), all Orthogroups showed reduced representation in *S. hermonthica* compared to *T. versicolor*, with no expression detected in *P. aegyptiaca* (Figure 2B). The absence of detectable transcripts in *Phelipanche* may be due either to the absence of expression or to large-scale gene losses and pseudogenization as has been observed in parasitic plant plastid genomes [8–10, 16, 17].

In contrast to what was observed for structural components of the photosynthetic reaction centers and electron transport chain, expression of the chlorophyll biosynthesis pathway was detected in all three species. The number of mapped reads is reduced for many genes in the nonphotosynthetic *P. aegyptiaca* (Figure 2A) but is still represented by measurable read depths of between 5 and 615 cpm. Furthermore, reconstructed transcripts of chlorophyll biosynthesis genes include a full-length reading frame that can be aligned to their closest homologs in the hemiparasites. In order to look for evidence of evolutionary constraint in these protein-coding sequences in each species, the value of dN/dS (ω) was calculated for ten genes encoding enzymes in either the shared heme/chlorophyll synthesis pathway or the branch of this pathway dedicated to chlorophyll synthesis (Figure 2A; Table 1). Using a likelihood ratio test to determine whether the likelihood of the data is significantly better when ω is free to vary relative to nonholoparasites, we see that only coproporphyrinogen oxidase is characterized by a (weakly) significant relaxation of purifying constraint (increase in ω).

Our data demonstrate that the major chlorophyll binding proteins of the photosynthetic apparatus (photosystem reaction centers, light-harvesting antenna complexes; Figure 2B) are likely not expressed in the above-ground tissues of *P. aegyptiaca*. If chlorophyll synthesis is occurring, the lack of photosynthetic components necessary to stabilize it could be the basis for its exceptionally low abundance. Thus, our findings are consistent with previous studies reporting that some nonphotosynthetic Orobanchaceae produce an extremely low but detectable amount of chlorophyll a [18]. Additionally, we show that the enzyme responsible for chlorophyll b synthesis, chlorophyllide a oxygenase [19], is expressed in all three parasites, although at a low level in *P. aegyptiaca*. Detection of chlorophyll b has not been demonstrated by high-performance liquid chromatography in Orobanchaceae [18]. In the absence of producing structural components for light harvesting (e.g., reaction center light-harvesting complex [LHC] proteins), selective pressure may be imposed against expressing the chlorophyll biosynthesis pathway enzymes and expending energy to make chlorophylls

Table 1. Ratio of Nonsynonymous to Synonymous Substitutions Calculated Both Globally and Independently for Nonphotosynthetic and Photosynthetic Plants

Gene Description	PlantTribes Orthogroup	Background ω	*Phelipanche* ω	p Value	Significance
PBG synthase	4439	0.0548029	0.100076	0.11631	NS
Uroporphyrinogen III synthase	8895	0.189247	0.283278	0.340545	NS
Uroporphyrinogen decarboxylase II	9434	0.0367646	0.0817034	0.101072	NS
Protoporphyrinogen oxidase	7621	0.184048	0.248049	0.42842	NS
Coproporphyrinogen oxidase	6991	0.0898638	0.220987	0.0117435	**
Magnesium chelatase I	4619	0.0442333	0.0905171	0.0777454	NS
Magnesium chelatase II	7885	0.0964938	0.162689	0.0750581	NS
Protoporphyrin methyltransferase	6868	0.0388797	0.108856	0.0516701	NS
Protochlorophyllide a 8-vinyl reductase	7184	0.0840586	0.127192	0.153202	NS
Chlorophyll synthetase	8582	0.0486038	0.0938493	0.148466	NS

The ratio of nonsynonymous substitutions to synonymous substitutions per nonsynonymous and synonymous site (ω or omega) calculated independently for *Phelipanche aegyptiaca* and related photosynthetic taxa (*Triphysaria versicolor* [facultative parasite, grown autotrophically], *Striga hermonthica* [obligate parasite], *Mimulus guttatus*, and *Lindenbergia philippensis*). For each gene, the assembled cDNA sequences (or genomic protein coding sequences for *M. guttatus*) were translated and aligned. The likelihood of the aligned data, given a hypothesis of relationships (trichotomy for the three parasites, sister to *L. philippensis*, together sister to *M. guttatus*), was calculated under a global model (ω is constant for all taxa and internal branches) and for a partitioned model (ω allowed to vary in *P. aegyptiaca* only). Subsequently, a likelihood ratio test was used to determine whether one model better described the data. ** indicates $0.001 < p < 0.01$; NS indicates not significant.

that cannot be utilized. If chlorophylls a and b are produced in *P. aegyptiaca* and are not participating in photosynthesis, one might wonder whether chlorophylls are serving an alternative role in the plant.

In addition to giving rise to chlorophylls, the porphyrin biosynthetic pathway produces hemes and their derivatives, which function in photosynthesis and as cofactors in enzymatic reactions and components in nonphotosynthetic electron transport. The biosynthetic steps from aminolevulinic acid (ALA) formation through protoporphyrin IX (ProtoIX) are in common for both heme and chlorophyll biosynthesis, with the Fe and Mg chelation steps marking the branchpoint between the two [20]. Six enzymatic reactions are required to convert ProtoIX, the last common intermediate of heme and chlorophyll formation, into chlorophyll a (Chla). Transcripts encoding all six of these enzymes are expressed in all three parasites. Although this is not surprising in *Striga* and *Tryphysaria*, given that both are photosynthetic upon emergence, the detection of transcripts encoding all of the enzymes involved in chlorophyll formation in the holoparasite *P. aegyptiaca*, generally considered nonphotosynthetic, is unexpected. Moreover, our analysis of transcript assemblies is consistent with purifying selection, strongly suggesting that the *P. aegyptiaca* transcripts could be translated into functional enzymes for chlorophyll synthesis, raising several intriguing questions.

It is well documented that the buildup of porphyrins and chlorophyll intermediates, such as protochlorophyllide (Pchlide), can be deleterious as a result of their strong photooxidizing properties [21]. In dark-grown plants, the ratio of bound Pchlide (photoconvertible pigment-protein complexes formed with the protochlorophyllide oxidoreductase [POR] isoforms) to free Pchlide (F631) is important because the bound form is photoprotective and suppresses photooxidative damage. If *P. aegyptiaca* accumulated even small amounts of Pchlide during its growth in the dark, when exposed to light on emergence this would be deleterious to growth. The capacity to avoid energy transfer from Pchlide to triplet oxygen is through the transformation of Pchlide into Chlide as part of Chlide-POR-NADPH ternary complexes, which are strong energy quenchers, leading to avoidance of reactive oxygen species (ROS) formation. Thus, having expression of all steps up to POR formation and photoconversion would be important.

The tetrapyrrole pathway is regulated by metabolic intermediates at the transcriptional and posttranslational levels [22], and tetrapyrrole synthesis must be regulated to prevent damage to the photosynthetic machinery. When photooxidative stress accelerates the degradation of pigment-protein complexes, the synthesis of chlorophyll must slow down to compensate. It may be that because all of the steps are present, but the end-product chlorophyll is not stabilized, the pathway runs at the bare minimum. But why maintain it at all and risk photooxidative damage if it is not being used for photosynthetic activity? One possibility is that expression of the pathway is essential for functions other than photosynthesis. Experimental evidence has accumulated for the specific involvement of plastid-derived signals including tetrapyrroles, redox status, and ROS controlling nuclear transcriptional activities (retrograde signaling) for plastid development as well as antiretrograde responses, where the nucleus is controlling plastid genes [23]. If porphyrin intermediates were important for signaling, then the plant would have to deal with the presence of these molecules in the absence of their use in photosynthesis.

Although the detection of an expressed chlorophyll synthesis pathway is surprising, it is notable that some (not all) holoparasitic Orobanchaceae have also been shown to retain and express conserved plastid *rbc*L genes [24–26]. Although functions for *rbc*L that are alternative to its primary role of CO_2 fixation have yet to be uncovered, the pattern of evolutionary conservation [24, 25] and evolutionary constraint following the loss of photosynthesis [27] presents a compelling case that some genes in holoparasites appear to have unexpected or additional functions beyond those normally associated with their primary role in photosynthetic plants. In this case, we do not detect any expression of *rbc*S in *P. aegyptiaca*, whereas tens of thousands of *rbc*S reads are detected in *T. versicolor* and *S. hermonthica*. Additionally, transcripts of *rbc*L are found in our data from both *T. versicolor* and *S. hermonthica*, but not from *P. aegyptiaca*.

Evolution of Parasitism

The species in our study represent three distinct points along a range of parasitic ability, from facultative (*Triphysaria*) to obligate hemiparasite (*Striga*) to holoparasite (*Phelipanche*). However, the phylogenetic relationships between these

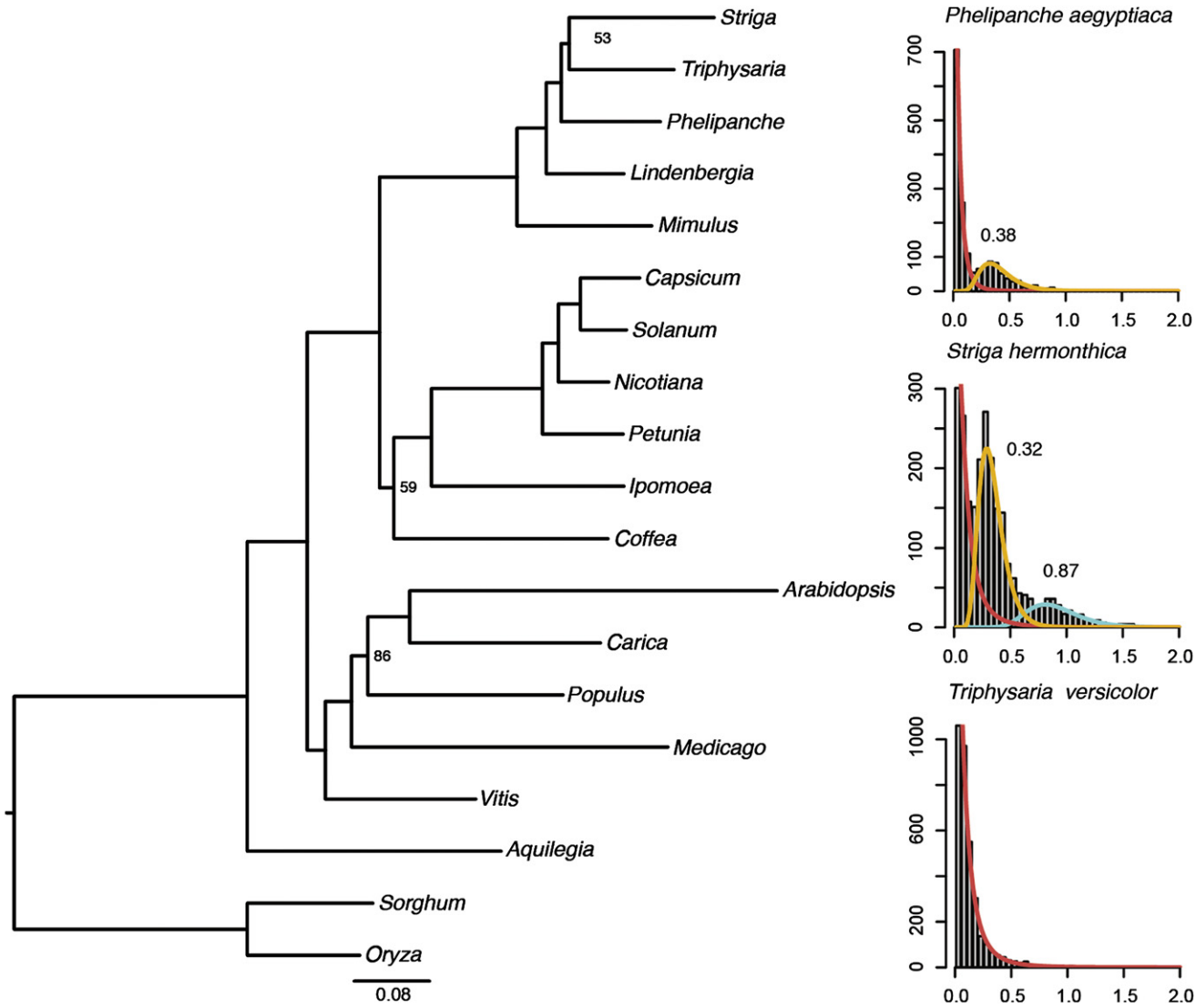

Figure 3. Phylogenetic Relationships Inferred from 339 Nuclear Genes and Ancient Genome Duplication Events Inferred from the Distribution of K_s for Paralogs

Hypothesis of relationships of the three parasitic plants included in this study (*Triphysaria versicolor*, *Striga hermonthica*, and *Phelipanche aegyptiaca*) and outgroup taxa based on 100 maximum-likelihood bootstrap replicates (left) and distributions of K_s values for duplicate gene pairs (right). All branches are supported at 100% unless noted otherwise. The plots on the right indicate the number of gene pairs (potential paralogs) assigned to each K_s (synonymous substitutions per synonymous site) bin. The curves overlaid on the K_s distributions indicate the number of significant components that best describe the distribution (minimize the Bayesian information criterion). For all three plots, a shared fourth component describing a steeper background plot was removed to better visualize the component curves. The red curve describes the "background" distribution of paralogs due to normal gene births and deaths in a genome. Components that describe a peak in gene pairs of K_s other than the background curve (orange and blue curves) may indicate a potential ancient whole-genome duplication event.

species have not been clearly resolved [28]. To this end, we used a concatenated alignment of 339 putative single-copy nuclear genes, comprising 510,875 characters, to reconstruct the relationships between our study species, a preliminary assembly of the nonparasitic *Lindenbergia philippensis* (data stored and available at http://ppgp.huck.psu.edu), representing the basal lineage of Orobanchaceae, and the sequenced genome of the closely related nonparasitic species *Mimulus guttatus* (http://www.phytozome.net). Although supermatrix approaches to phylogenetic reconstruction such as this can suffer from missing data, the percent coverage of these species in the supermatrix (between 84% and 96%) is excellent and likely mitigates the effect of missing sequence data [29, 30].

Here, the resolution of *Lindenbergia* sister to parasitic Orobanchaceae is consistent with other analyses [3, 6, 31, 32], as is the inability to resolve, with strong support, the relationship between *Triphysaria*, *Striga*, and *Phelipanche* [28]. This unresolved topology may reflect a rapid radiation of ancestral Orobanchaceae following the shift to parasitism. Geographic shifts, rather than morphological innovations, may disproportionately increase diversification rates [33]; for parasites, this may be true if one considers the range of potential hosts as unfilled geographical niches. Sampling cDNAs from a more exhaustive set of Orobanchaceae may allow us to more accurately resolve the timing of the origin and diversification of parasitism in Orobanchaceae, and the potential role and timing of genome duplications.

Genome duplication has been implicated in the evolution of several major groups of plants [34–38]. Here, analyses of the rate of synonymous substitutions (K_S) for paralogous gene pairs, calibrated by lineage-specific K_s from 339 single-copy genes, resulted in distributions consistent with the expected gene birth-death process ("background" distribution; red lines in Figure 3) [37, 39] and several older concentrations of gene duplications that might be indicative of ancient polyploidy events. *S. hermonthica* and *P. aegyptiaca* exhibit a strongly distinguishable peak with a mean K_s of 0.32 and 0.38, respectively (Figure 3). Additionally, *S. hermonthica* is characterized by an older, diffuse peak with a mean K_s of 0.87. *T. versicolor*, the facultative parasite, exhibits only the background distribution, suggesting the absence of ancient polyploidy. Because of the preliminary nature of the available data, it is difficult to reconstruct ancient genome duplication events in the nonparasitic genus of Orobanchaceae, *Lindenbergia*. However, the suggestion that the obligate parasites here may share an ancient genome duplication event that is not shared with the facultative parasite could reflect Searcy's hypothesis [40] that proposed a marked increase in genetic information as new, autotrophic functions are acquired.

Conclusion

The results of the present study add a new layer of molecular evolutionary and gene expression analysis to emerging evidence pointing to a complicated set of constraints on the photosynthetic apparatus. For example, recent studies of mycoheterotrophs, plants that obtain fixed atmospheric carbon from fungi associated with another, autotrophic plant [41], suggest that the loss of observable chlorophyll may lag behind the loss of photosynthetic capacity as a plant evolves further toward a completely heterotrophic life history [42], or that chlorophyll and photosynthesis can be simultaneously reduced in achlorophyllous forms of an otherwise chlorophyllous species [43]. The evidence presented here for constraints on the chlorophyll synthesis genes in the absence of detectable gene expression of crucial components of the photosynthetic apparatus suggests that the evolution of plants from autotrophic to partially autotrophic to fully heterotrophic is not a simple, linear trajectory. It also highlights the valuable contribution of genomic and transcriptomic data to our understanding of complex physiological processes.

Accession Numbers

The NCBI Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/sra) accession numbers for the sequences reported in this paper are SRX040930, SRX041138, SRX008134, and SRX008135 (*Triphysaria versicolor*); SRX040928, SRX040929, SRX008132, and SRX008133 (*Striga hermonthica*); and SRX040924, SRX040925, SRX008130, and SRX008131 (*Phelipanche aegyptiaca*).

Supplemental Information

Supplemental Information includes one figure, four tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2011.11.011.

Acknowledgments

Support by the National Science Foundation Plant Genome Research Program (IOS #0701748 to J.H.W., C.W.d., M.P.T., and J.Y.) for our work on the Parasitic Plant Genome Project, with additional support from the US Department of Agriculture (Hatch project number 135657 to J.H.W.), is gratefully acknowledged. We thank Danny Joel for *Phelipanche* seeds, Paula Ralph and Verlyn Stromberg for laboratory assistance, Lynn Tomsho and Stephan Schuster for 454 sequencing, and Yongde Bao for Illumina sequencing. The *Mimulus guttatus* sequence data were produced by the US Department of Energy Joint Genome Institute (http://www.jgi.doe.gov/) in collaboration with the user community.

Received: February 12, 2011
Revised: August 17, 2011
Accepted: November 4, 2011
Published online: December 8, 2011

References

1. Barkman, T.J., McNeal, J.R., Lim, S.H., Coat, G., Croom, H.B., Young, N.D., and dePamphilis, C.W. (2007). Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. BMC Evol. Biol. *7*, 248.
2. Westwood, J.H., Yoder, J.I., Timko, M.P., and dePamphilis, C.W. (2010). The evolution of parasitism in plants. Trends Plant Sci. *15*, 227–235.
3. Young, N.D., Steiner, K.E., and dePamphilis, C.W. (1999). The evolution of parasitism in Scrophulariaceae/Orobanchaceae: Plastid gene sequences refute an evolutionary transition series. Ann. Mo. Bot. Gard. *86*, 876–893.
4. Olmstead, R.G., dePamphilis, C.W., Wolfe, A.D., Young, N.D., Elisons, W.J., and Reeves, P.A. (2001). Disintegration of the scrophulariaceae. Am. J. Bot. *88*, 348–361.
5. Young, N.D., and dePamphilis, C.W. (2005). Rate variation in parasitic plants: correlated and uncorrelated patterns among plastid genes of different function. BMC Evol. Biol. *5*, 16.
6. Bennett, J.R., and Mathews, S. (2006). Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. Am. J. Bot. *93*, 1039–1051.
7. Parker, C. (2009). Observations on the current status of *Orobanche* and *Striga* problems worldwide. Pest Manag. Sci. *65*, 453–459.
8. Wolfe, K.H., Morden, C.W., and Palmer, J.D. (1992). Function and evolution of a minimal plastid genome from a nonphotosynthetic parasitic plant. Proc. Natl. Acad. Sci. USA *89*, 10648–10652.
9. dePamphilis, C.W., and Palmer, J.D. (1990). Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. Nature *348*, 337–339.
10. McNeal, J.R., Kuehl, J.V., Boore, J.L., and dePamphilis, C.W. (2007). Complete plastid genome sequences suggest strong selection for retention of photosynthetic genes in the parasitic plant genus *Cuscuta*. BMC Plant Biol. *7*, 57.
11. Funk, H.T., Berg, S., Krupinska, K., Maier, U.G., and Krause, K. (2007). Complete DNA sequences of the plastid genomes of two parasitic flowering plant species, *Cuscuta reflexa* and *Cuscuta gronovii*. BMC Plant Biol. *7*, 45.
12. Joel, D.M. (2009). The new nomenclature of *Orobanche* and *Phelipanche*. Weed Res. *49*, 6–7.
13. Torres, M.J., Tomilov, A.A., Tomilova, N., Reagan, R.L., and Yoder, J.I. (2005). Pscroph, a parasitic plant EST database enriched for parasite associated transcripts. BMC Plant Biol. *5*, 24.
14. Yoshida, S., Ishida, J.K., Kamal, N.M., Ali, A.M., Namba, S., and Shirasu, K. (2010). A full-length enriched cDNA library and expressed sequence tag analysis of the parasitic weed, *Striga hermonthica*. BMC Plant Biol. *10*, 55.
15. Wall, P.K., Leebens-Mack, J., Müller, K.F., Field, D., Altman, N.S., and dePamphilis, C.W. (2008). PlantTribes: a gene and gene family resource for comparative genomics in plants. Nucleic Acids Res. *36* (Database issue), D970–D976.
16. Wickett, N.J., Zhang, Y., Hansen, S.K., Roper, J.M., Kuehl, J.V., Plock, S.A., Wolf, P.G., dePamphilis, C.W., Boore, J.L., and Goffinet, B. (2008). Functional gene losses occur with minimal size reduction in the plastid genome of the parasitic liverwort *Aneura mirabilis*. Mol. Biol. Evol. *25*, 393–401.
17. Delannoy, E., Fujii, S., Colas des Francs-Small, C., Brundrett, M., and Small, I. (2011). Rampant gene loss in the underground orchid *Rhizanthella gardneri* highlights evolutionary constraints on plastid genomes. Mol. Biol. Evol. *28*, 2077–2086.
18. Cummings, M.P., and Welschmeyer, N.A. (1998). Pigment composition of putatively achlorophyllous angiosperms. Plant Syst. Evol. *210*, 105–111.

19. Sakuraba, Y., Yokono, M., Akimoto, S., Tanaka, R., and Tanaka, A. (2010). Deregulated chlorophyll b synthesis reduces the energy transfer rate between photosynthetic pigments and induces photodamage in *Arabidopsis thaliana*. Plant Cell Physiol. *51*, 1055–1065.
20. Bollivar, D.W. (2006). Recent advances in chlorophyll biosynthesis. Photosynth. Res. *90*, 173–194.
21. Solymosi, K., and Schoefs, B. (2010). Etioplast and etio-chloroplast formation under natural conditions: the dark side of chlorophyll biosynthesis in angiosperms. Photosynth. Res. *105*, 143–166.
22. Tanaka, R., and Tanaka, A. (2007). Tetrapyrrole biosynthesis in higher plants. Annu. Rev. Plant Biol. *58*, 321–346.
23. Kleine, T., Voigt, C., and Leister, D. (2009). Plastid signalling to the nucleus: messengers still lost in the mists? Trends Genet. *25*, 185–192.
24. Wolfe, A.D., and dePamphilis, C.W. (1997). Alternate paths of evolution for the photosynthetic gene rbcL in four nonphotosynthetic species of *Orobanche*. Plant Mol. Biol. *33*, 965–977.
25. Wolfe, A.D., and dePamphilis, C.W. (1998). The effect of relaxed functional constraints on the photosynthetic gene *rbcL* in photosynthetic and nonphotosynthetic parasitic plants. Mol. Biol. Evol. *15*, 1243–1258.
26. Randle, C.P., and Wolfe, A.D. (2005). The evolution and expression of *rbcL* in holoparasitic sister-genera *Harveya* and *Hyobanche* (Orobanchaceae). Am. J. Bot. *92*, 1575–1585.
27. Leebens-Mack, J., and dePamphilis, C. (2002). Power analysis of tests for loss of selective constraint in cave crayfish and nonphotosynthetic plant lineages. Mol. Biol. Evol. *19*, 1292–1302.
28. Tank, D.C., Beardsley, P.M., Kelchner, S.A., and Olmstead, R.G. (2006). Review of the systematics of Scrophulariaceae s.l. and their current disposition. Aust. Syst. Bot. *19*, 289–307.
29. de Queiroz, A., and Gatesy, J. (2007). The supermatrix approach to systematics. Trends Ecol. Evol. (Amst.) *22*, 34–41.
30. Driskell, A.C., Ané, C., Burleigh, J.G., McMahon, M.M., O'meara, B.C., and Sanderson, M.J. (2004). Prospects for building the tree of life from large sequence databases. Science *306*, 1172–1174.
31. Park, J.M., Manen, J.F., Colwell, A.E., and Schneeweiss, G.M. (2008). A plastid gene phylogeny of the non-photosynthetic parasitic *Orobanche* (Orobanchaceae) and related genera. J. Plant Res. *121*, 365–376.
32. Wolfe, A.D., Randle, C.P., Liu, L., and Steiner, K.E. (2005). Phylogeny and biogeography of Orobanchaceae. Folia Geobot. *40*, 115–134.
33. Moore, B.R., and Donoghue, M.J. (2007). Correlates of diversification in the plant clade Dipsacales: geographic movement and evolutionary innovations. Am. Nat. *170* (*Suppl 2*), S28–S55.
34. Lynch, M., and Conery, J.S. (2003). The origins of genome complexity. Science *302*, 1401–1404.
35. Lynch, M., and Conery, J.S. (2000). The evolutionary fate and consequences of duplicate genes. Science *290*, 1151–1155.
36. Barker, M.S., Kane, N.C., Matvienko, M., Kozik, A., Michelmore, R.W., Knapp, S.J., and Rieseberg, L.H. (2008). Multiple paleopolyploidizations during the evolution of the Compositae reveal parallel patterns of duplicate gene retention after millions of years. Mol. Biol. Evol. *25*, 2445–2455.
37. Cui, L.Y., Wall, P.K., Leebens-Mack, J.H., Lindsay, B.G., Soltis, D.E., Doyle, J.J., Soltis, P.S., Carlson, J.E., Arumuganathan, K., Barakat, A., et al. (2006). Widespread genome duplications throughout the history of flowering plants. Genome Res. *16*, 738–749.
38. Soltis, D.E., Albert, V.A., Leebens-Mack, J., Bell, C.D., Paterson, A.H., Zheng, C.F., Sankoff, D., dePamphilis, C.W., Wall, P.K., and Soltis, P.S. (2009). Polyploidy and angiosperm diversification. Am. J. Bot. *96*, 336–348.
39. Blanc, G., and Wolfe, K.H. (2004). Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. Plant Cell *16*, 1667–1678.
40. Searcy, D.G. (1970). Measurements by DNA hybridization in vitro of the genetic basis of parasitic reduction. Evolution *24*, 207–219.
41. Leake, J.R. (1994). The biology of myco-heterotrophic (saprotrophic) plants. New Phytol. *127*, 171–216.
42. Cameron, D.D., Preiss, K., Gebauer, G., and Read, D.J. (2009). The chlorophyll-containing orchid *Corallorhiza trifida* derives little carbon through photosynthesis. New Phytol. *183*, 358–364.
43. Julou, T., Burghardt, B., Gebauer, G., Berveiller, D., Damesin, C., and Selosse, M.A. (2005). Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. New Phytol. *166*, 639–653.