Chapter 15
Genomic Evolution in Orobanchaceae

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15.1 Introduction

The broomrape family, Orobanchaceae, is widely recognized as *the* model group to study genomic evolution in parasitic plants, especially because it is the only parasitic family to include the entire range of evolutionary transitional stages, from a fully autotrophic via semi-heterotrophic to completely holo-heterotrophic lifestyle (Westwood et al. 2010). Orobanchaceae, encompassing an estimated number of 2,000 species,[1] are confidently placed in the large and diverse group of Lamiales, which contains a great number of species with highly specialized life forms including desiccation tolerance, carnivory, and parasitism (Schäferhoff et al. 2010). The parasitic lifestyle has brought about numerous morphological and developmental changes. Substantial progress has been made during the past few years in uncovering basic genetic reconfigurations and signalling pathways necessary in establishing a haustorial connection to another plant. Nevertheless, little is known about the evolution of nuclear and mitochondrial genes and genomes in Orobanchaceae, even 20 years after the first plastid genome of a non-photosynthetic member of the family has been sequenced. This is especially astonishing given the great advance in molecular biological methods and sequencing technologies over the past 5–10 years. Reasons therefore are manifold—as usual. Owing to the great diversity of the family, evolutionary studies based upon molecular data are restricted to only a few members of either species-rich and commonly distributed genera or to members of considerable ecological importance such as *Orobanche*,

[1] This chapter uses the most recent taxonomic changes outlined in Chap. 14.

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Phelipanche, and *Striga*. Additionally, *in vitro* cultivation of obligate parasites is often difficult or requires permits in some countries, hampering genetic and reverse genetic approaches. Finally, the trend for rather large genomes renders Orobanchaceae challenging objects for genomic surveys.

This chapter summarizes the current knowledge of the genomic evolution in Orobanchaceae. The following sections provide overviews about nuclear, mitochondrial, and plastid genomics and about horizontal DNA transfer. Subsequently, a short concluding paragraph outlines some prospects on where genomics in the broomrape family may be headed in the next few years (see also Sect. 4.5 for the evolution of parasite-specific functions).

15.2 The Nuclear Genome

15.2.1 Nuclear Genes

Nothing is known about the evolution of nuclear coding regions in the Orobanchaceae, and thus very little is known about the molecular basis of parasite-specific life stages. Recently, a hydroxylase (PRCYP707A1) functioning in the abscisic acid (ABA) catabolic pathway in *Phelipanche ramosa* has been identified as playing a major role during germination of seeds after exposure to germination stimulants (Lechat et al. 2012). The same study also identified two heat shock proteins and a few more transcripts associated with ABA cascades. Even though those transcripts have not been characterized in detail, preliminary lines of evidence link those transcripts to proteins active during seed germination in *Arabidopsis thaliana*. Experiments with the holoparasitic *Orobanche minor* have shown that the involvement of phytochromes (PHY) during germination, shoot elongation, and anthocyanin content differs from that observed in photosynthetic plants, suggesting reconfigured regulatory cascades involving at least PHY proteins A and B (Takagi et al. 2009). Relative to *Arabidopsis*, 26 amino acids are substituted in PHYA of *O. minor* (Trakulnaleamsai et al. 2005). Of these, some substitutions perhaps hold the potential to alter protein function, thereby contributing to unusual light responses in the holoparasite compared to autotrophic plants (Trakulnaleamsai et al. 2005; Takagi et al. 2009). Given that expression patterns as well as cellular localizations of PHYA in *O. minor* are comparable to autotrophic plants and that the chromatophore-binding site in PHYA is highly conserved, the reported amino acid changes may also represent results of coevolution with rapidly evolving PHYA-interacting photosynthesis genes.

In *Striga hermonthica*, Yoshida et al. (2010) found 589 assembled fragments of expressed genes (unigenes) that are not similar to known plant genes, implying that at least some of these may be specific to parasitism. Sequencing of cDNAs from the facultative hemiparasite *Triphysaria versicolor* revealed an up-regulation of more than a hundred unigenes during early haustorium initiation. These fragments were

assigned similarity to proteins functioning in quinone detoxification, transcription and regulatory processes, membrane transport, and the citric acid cycle (Matvienko et al. 2001). Two of these transcripts, a quinone oxidoreductase (QR1) and a protein associated to plant signalling pathways (*TvPirin*), have been further characterized as essential for haustorium initiation after contact to host roots or exposure to haustorium-inducing chemicals (Bandaranayake et al. 2010; see also Sects. 4.4 and 4.5). However, not much is known about the molecular evolution of those genes, and future studies will have to show whether genes relevant for the development of lateral haustoria of *Triphysaria* are also essential for the induction of terminal haustoria (see Sect. 4.4). Deep sequencing of ultrathin slices of host-parasite interface tissue of *T. versicolor* furthermore revealed the differential expression of a β-expansin gene (TvEXPB1) when the parasite was grown in the presence of different hosts (Honaas et al. 2013). However, it still needs to be elucidated whether cell wall modifying proteins and their differential expression are common among other Orobanchaceae.

Using three Orobanchaceae species differing in their extent of heterotrophy, the ongoing large-scale transcriptome-sequencing approach of the *Parasitic Plant Genome Project* (PPGP, Westwood et al. 2010, 2012) aims, among other aspects, at discovering and studying genes that are exclusive to specific ontogenetic stages. In a first study, Wickett et al. (2011) found that expression of nuclear-encoded photosynthesis subunits in aboveground tissue is considerably reduced in the obligate hemiparasite *S. hermonthica* compared to the facultative hemiparasite *T. versicolor*. No expression of nuclear-encoded photosynthesis genes was detected in *P. aegyptiaca*, where these genes might have become pseudogenes or have already been deleted from the genome. In contrast, genes for chlorophyll synthesis were still expressed in *Phelipanche* (Wickett et al. 2011), corroborating results of the detection of trace amounts of chlorophyll a in some holoparasites of Orobanchaceae (*Epifagus*, *Myzorrhiza cooperi* [syn. *Orobanche cooperi*], *Aphyllon uniflora* [syn. *O. uniflora*]) and other families (Cummings and Welschmeyer 1998).

A survey of the evolution of the small ribosomal RNA subunit (SSU) found that parasitic plants possess significantly elevated nucleotide substitution rates (Nickrent and Duff 1996). However, comparative studies across parasitic and myco-heterotrophic plants (see Sect. 1.8) did not show a significant acceleration in the SSU evolution in several Orobanchaceae holoparasites, and the pattern of rate acceleration across lineages remains widely elusive (Lemaire et al. 2011).

15.2.2 Chromosome Numbers

Chromosome numbers have been the focus of several studies on hemiparasitic Orobanchaceae, although the vast majority of these reports lacked an explicit evolutionary context. Chromosome numbers and ploidy are highly variable in the family and apparently do not correlate with genome size. The current knowledge of chromosome numbers and genome sizes in Orobanchaceae is graphically

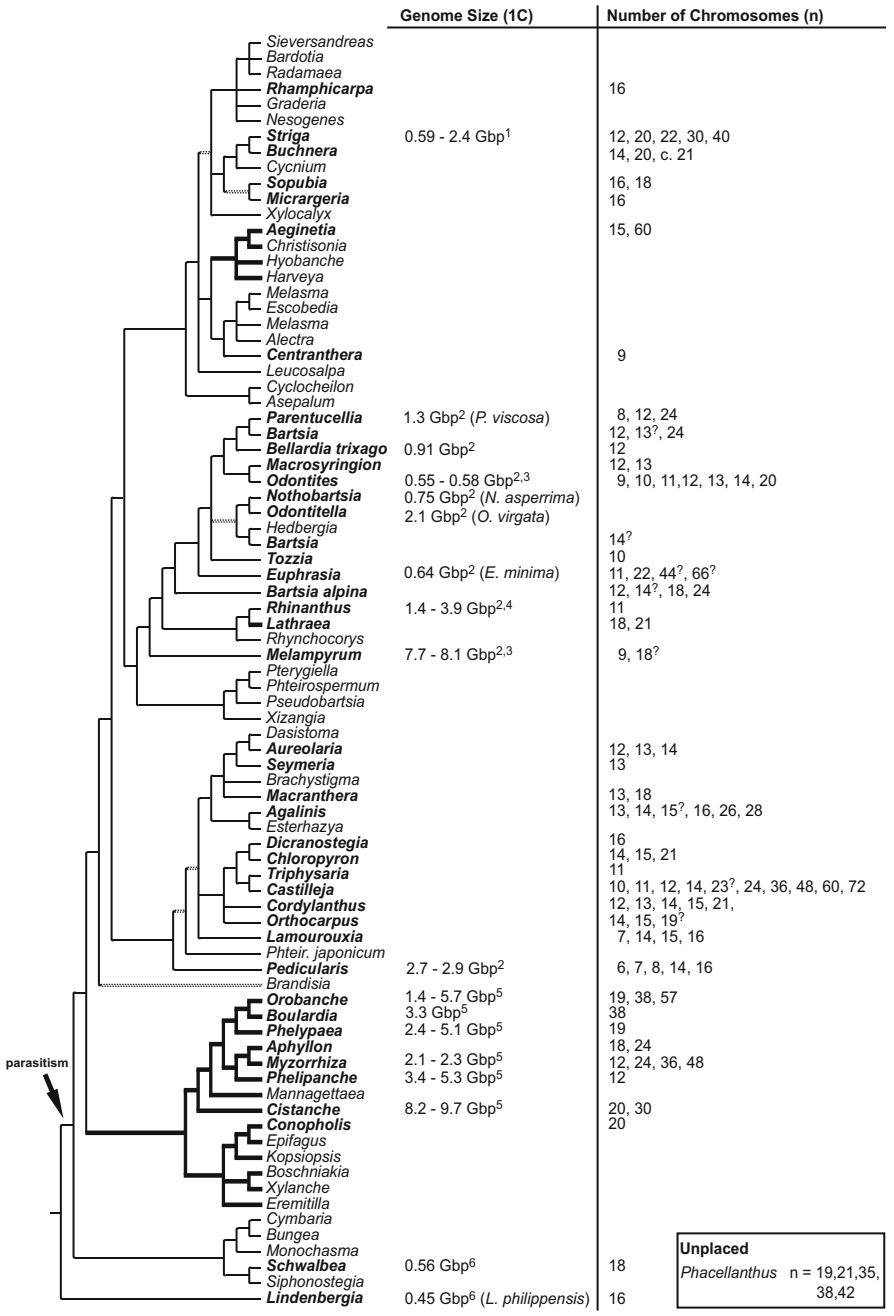

Fig. 15.1 Evolution of chromosome number and genome size in Orobanchaceae. *Arrow*, the origin of parasitism. *Thin branches*, autotrophic and photosynthetic heterotrophs; *thick branches*, non-photosynthetic heterotrophs; *dashed branches*, uncertain placement. Tree topology after

summarized in Fig. 15.1. *Lindenbergia* and *Schwalbea*, members of the first branching lineages, have $n = 16$ (Hjertson 1995) and $n = 18$ (Kondo et al. 1981), respectively. Being mostly diploid with the exception of one tetraploid species, the closely related sister group of Orobanchaceae, *Rehmannia*, harbours $n = 14$ chromosomes (Albach et al. 2007). In the light of current data, there seems to be a slight trend towards higher chromosome numbers in the exclusively holoparasitic *Orobanche* clade (see Sect. 14.2.2.3), although polyploidy appears to be common in some of the hemiparasites as well (e.g. *Castilleja*, *Striga*, *Euphrasia*; Tank et al. 2009; Kondo et al. 1981; Barker et al. 1988; Iwo et al. 1993). Except for *Phelipanche* with $n = 12$, most genera of the holoparasite clade have $n = 19$ or more chromosomes (Fig. 15.1; Schneeweiss et al. 2004). Plots of synonymous substitutions of selected expressed genes revealed unambiguously that *Phelipanche* must have undergone at least one whole-genome duplication after the split from hemiparasitic ancestors (Wickett et al. 2011), corroborating previous hypotheses of ancient duplication events in the *Orobanche* clade (Schneeweiss et al. 2004). Differences in chromosome number indicate that lineages within this clade might have undergone at least one or more rounds of polyploidization. Inconsistent chromosome morphologies imply that these events might have even occurred independently (Schneeweiss et al. 2004). It will be interesting to see whether independent changes and ancient polyploidization also occurred in other holoparasitic groups, especially in the light of the severe ecological changes accompanying the transition to the non-photosynthetic lifestyle. A change of ploidy level in *O. transcaucasica* apparently coincides with a shifted host range, suggesting that, at least in this particular case, genome duplication favours ecological differentiation from its progenitors (Schneeweiss et al. 2004).

It is unclear whether several independent rounds of polyploidization and dysploidization (i.e. reduction of ploidy/chromosome numbers) in the hemiparasites of the Old and New World led to the great diversity of chromosome number, which ranges from $n = 8$ to $n = 20$ (Fig. 15.1). Changes of ploidy level are a substantial basis for speciation among angiosperms, allowing offsprings to settle into new niches (Wood et al. 2009). Niching due to host range shifts as a result of polyploidization or dysploidization may thus be a more significant aspect for speciation in parasitic lineages. At this point, such putative correlations between the degrees of parasitism and chromosomal evolution including ploidy are, however, hypothetical at best. Caryological studies in most of the tropical and/or Asian hemi- and holoparasitic lineages (i.e. nearly 50 % of all Orobanchaceae genera) are still lacking.

Fig. 15.1 (continued) Schneeweiss (see Chap. 14). *Question mark* indicates uncertain chromosome counts. Chromosome data: IPCN (Goldblatt and Johnson 1979), Fedorov (1969), and Moore (1982). Genome size data: [1]Yoshida et al. (2010); Estep et al. (2012); [2]Castro et al. 2012; [3]Hanson et al. (2002); [4]Zonneveld et al. (2005); Nagl and Fusenig (1979); [5]Weiss-Schneeweiss et al. (2006); [6]Piednoël et al. (2012).

15.2.3 Genome Size

In line with the rampant, even though uncorrelated occurrence of polyploidy, genome sizes vary greatly within Orobanchaceae. *Lindenbergia* (1C = 0.45 Gbp), *Schwalbea* (1C = 0.56 Gbp; Piednoël et al. 2012), and *Odontites* (1C = 0.55–0.56 Gbp; Hanson et al. 2002) possess small genomes, the sizes of which are comparable to those of poplar and rice. Other photosynthetic Orobanchaceae such as *Rhinanthus* and *Melampyrum* have considerably larger genomes, with that of *Melampyrum* reaching almost three times the size of the human genome (Fig. 15.1; Hanson et al. 2002). The smallest genome of the holoparasitic *Orobanche* clade is found in *O. cumana* (Weiss-Schneeweiss et al. 2006). The *Orobanche* genus contains some polyploids (e.g. *O. transcaucasica*, *O. gracilis*) that may exceed 1C = 5.5 Gbp. Among diploids, *O. crenata* with 1C = 2.8 Gbp ranks among the biggest according to currently available measurements (Fig. 15.1; Weiss-Schneeweiss et al. 2006). In contrast to *Orobanche*, *Phelipanche* has larger genomes on average (Weiss-Schneeweiss et al. 2005), but the largest genomes in Orobanchaceae have been described so far for species of *Cistanche* with 1C = 8.7 Gbp in *C. phelypaea* (Weiss-Schneeweiss et al. 2006). Even larger ones may occur in other *Cistanche* species (N. Ataei, D. Quandt, and H. Weiss-Schneeweiss, unpublished data). Nevertheless, compared to the (cryptically) photosynthetic heterotrophs of other parasitic angiosperm families such as *Cuscuta* (1C = 0.57–32.1 Gbp, McNeal et al. 2007a) or members of Santalales (1C = 0.3–80.2 Gbp, Martin 1983; Hanson et al. 2001; Zonneveld 2010), the range of genome sizes is rather moderate in Orobanchaceae, a fact that contributes to its status as the 'model family' among parasitic plants.

As in most plant genomes, the abundance of repetitive DNA contributes substantially to genome size differences in Orobanchaceae. The five economically most important species of *Striga* show considerable genomic variation with respect to the 14 largest genus-specific repeat families residing in the genomes with more than a few hundred copies (Estep et al. 2012). Those repetitive DNAs account for 10–19 % of the nuclear genomes of *Striga* species, but they are not strictly correlated with genome size. They belong to classes commonly found in angiosperm genomes with transposable elements being the most abundant. The analyses of repeat classes point towards a ploidy series in the genus *Striga* (Estep et al. 2012). Interestingly, the variability among different populations of single species of *Striga*, e.g. *S. asiatica* or *S. gesnerioides*, is moderate or even low, respectively (Botanga et al. 2002; Botanga and Timko 2005, 2006; see Sect. 19.2).

The genomes of seven holoparasitic broomrapes and two photosynthetic Orobanchaceae were characterized employing a whole-genome shotgun pyrosequencing approach (Piednoël et al. 2012). The proportion of repeat DNA sequences is low in the small-sized genomes of the nonparasite *Lindenbergia* and the hemiparasite *Schwalbea* with repetitive elements accounting for no more than 30 % of the genomes. As implied by chromosomal and genome size data, divergent dynamics of genome evolution exist in the sister groups *Orobanche* and *Phelipanche*.

This hypothesis is corroborated by differing quantities of genus-specific clusters of transposable elements (Piednoël et al. 2012). The proportion of long and short interspersed nuclear elements (LINE and SINE, respectively) seems to be generally lower in *Phelipanche* than in *Orobanche*. LINEs contribute to the increase in genome size (as do many retrotransposons) in that they autonomously copy themselves. *Phelipanche* spp. may have evolved a more sophisticated machinery for silencing transposable elements, which results in a more stable genomic and chromosomal evolution. Control and regulatory mechanisms for transposable elements are lineage-specific and contribute widely to genome stability (e.g. He et al. 2012). It will be interesting to see whether genome size evolution is related to host range and/or to the degree of parasitism. For instance, in some plants, nutrient limitation leaves behind genomic signatures (Acquisti et al. 2009a, b), but obligate parasites may not be affected by those limitations in the same way because of the host-provided nutrient supply.

Polyploid Orobanchaceae tend to a reduction of the monoploid genome size (1Cx value) after events of polyploidization, which is in congruence with several nonparasitic polyploid angiosperm lineages (Leitch and Bennett 2004). In most cases, 1Cx values from polyploids are smaller than those of diploid relatives (Weiss-Schneeweiss et al. 2005). Although the genetic mechanisms are still poorly understood, genome-size reduction may be selected for because of, e.g. biophysical (e.g. chromosome pairing in meiosis and mitosis) and biochemical reasons ('biochemical economy') (Leitch and Bennett 2004; Leitch and Leitch 2012). Perhaps there is a trade-off between genomic plasticity that comes with genome size and nutritional constraints. An obligate parasitic way of life might favour moderately to large-sized genomes irrespective of the ability to carry out photosynthesis, enhancing chances of sub- or neofunctionalization of duplicated genes that contribute to host specificity and host adaptation, leading eventually to speciation within parasite lineages.

Several other Orobanchaceae groups may have had comparable scenarios of frequent increase and decrease of chromosome number and genome size like those observed in the *Orobanche* clade. Independent events of polyploidization have also been hypothesized for some other lineages (e.g. *Euphrasia, Lathraea*) based upon duplications of the phytochrome A gene (Bennett and Mathews 2006).

15.3 The Plastid Genome

The plastid chromosome (plastome) is the best understood cellular genome in angiosperms. The plastome normally has a highly conserved structure with a large and a small single-copy region (LSC and SSC, respectively) that are separated from each other by two large and virtually identical inverted repeats. Plastomes encode a large set of subunits for the photosynthesis apparatus including genes for photosystems I and II, the cytochrome complex, an ATP synthase, and an NAD(P)H complex as well as few genes involved in photosynthetic energy gain (*rbcL*, *ccsA*,

cemA) or lipid synthesis (*accD*). Several proteins for the genetic apparatus are solely plastid encoded including several ribosomal protein genes, a plastid-encoded polymerase complex, as well as few others involved in either transcript maturation (*matK*) or protein turnover (*infA*, *clpP*, photosystem assembly factors *ycf3*, *ycf4*). The essential function of the two largest plastid genes (*ycf1*, *ycf2*) is as yet unknown, but both reading frames are conserved among photosynthetic and non-photosynthetic land plants (Wicke et al. 2011). Based on protein-domain comparisons, both proteins probably function in housekeeping processes rather than having a metabolic function (Wolfe 1994; Boudreau et al. 1997; Drescher et al. 2000). The plastid genome normally also harbours two sets of four ribosomal RNA genes as well as 30 tRNA genes, the latter of which enable the delivery of all codons due to (extended) wobbling and superwobbling (Lagerkvist 1978; Rogalski et al. 2008; Alkatib et al. 2012).

Due to its compact nature and its prime role in photosynthesis, the evolution of the plastid genome of non-photosynthetic plants has received attention early on. Already in 1990, dePamphilis and Palmer reported the loss of all genes for the plastid NAD(P)H dehydrogenase complex from the plastome of the holoparasite *Epifagus virginiana*. Soon the complete plastid genome sequence of *E. virginiana* was described (Wolfe et al. 1992b). Massive gene loss led to an extraordinary structure of the plastome which is reduced to less than half the size of that of photosynthetic relatives. Nevertheless, the relative order of genes in LSC, SSC, and the inverted repeats remains largely colinear to photosynthetic plants (Fig. 15.2). Besides *ndh* genes, most genes involved in light and dark reaction of photosynthesis are completely absent from the plastome; only a few photosynthesis-related genes reside in the plastome as pseudogenes (e.g. Ψ*rbcL*, Ψ*atpA*). Furthermore, several genes encoding proteins of the genetic apparatus are (functionally) lost including tRNA genes, the plastid-encoded polymerase complex, and some ribosomal protein genes (Morden et al. 1991; Wolfe et al. 1992b). Comparable dramatic reductions of plastid DNAs occur in a variety of parasitic plants, including *Cuscuta* species (Funk et al. 2007; McNeal et al. 2007b), mistletoes (Nickrent and García 2009), green algae (Knauf and Hachtel 2002; de Koning and Keeling 2006) as well as myco-heterotrophic plant lineages, including non-photosynthetic orchids (Logacheva et al. 2011; Delannoy et al. 2011) and achlorophyllous Ericaceae (Braukmann and Stefanović 2012). As in some of the other parasites, residing plastid genes of the translation apparatus in *Epifagus* evolved significantly faster than those of nonparasitic relatives (Wolfe et al. 1992a). Nevertheless, the retained plastid genes of *E. virginiana* are transcribed, mature, and are translated into functional RNAs and proteins (Morden et al. 1991; Wolfe et al. 1992a; Ems et al. 1995; Lohan and Wolfe 1998; also see Wimpee et al. 1991, 1992).

In terms of gene losses, other holoparasitic Orobanchaceae lineages possess considerably different plastomes than *E. virginiana*, indicating that reductive evolution of plastid DNA is a highly lineage-specific process within Orobanchaceae (and presumably within other parasitic plant lineages as well). Extensive restriction-mapping experiments suggested that *Conopholis americana* has an even smaller plastid genome (ca. 42kb) than the closely related *E. virginiana*,

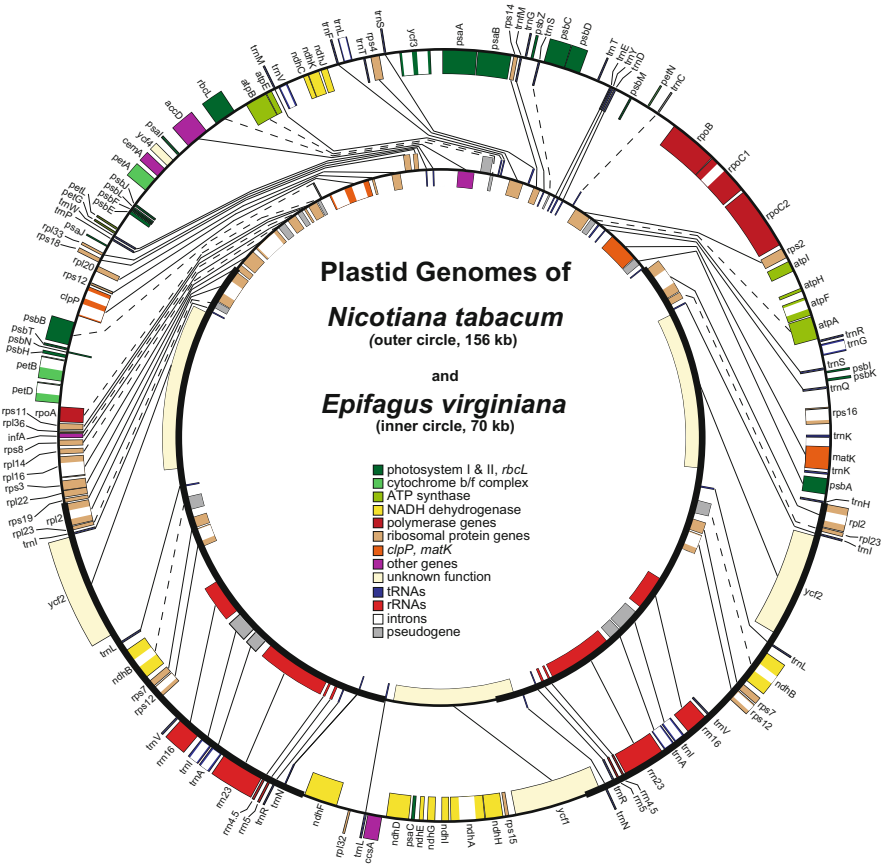

Fig. 15.2 Comparison of plastid genome structure of *Epifagus virginiana* (*inner circle*) and the nonparasitic plant *Nicotiana tabacum* (*outer circle*). The large inverted repeats are indicated as thickened chromosomal segments relative to the large and the small single-copy regions. Genes are coloured according to their function with the name of genes depicted on the *outer circle*. Pseudogenes are coloured in *grey*. *Thin lines* from the *Nicotiana* to the *Epifagus* genome indicate structural reorganizations due to massive gene loss. genes No line between *Nicotiana* and *Epifagus* indicates that the region has been lost in the latter; a *dashed* connection indicates pseudogenization in *Epifagus*. Genome maps were drawn using OGDRAW (Lohse et al. 2007) based upon Shinozaki et al. (1986) and Wolfe et al. (1992b)

mainly due to the loss of one large inverted repeat (Downie and Palmer 1992; Colwell 1994). Other large deletions are comparable to those of *Epifagus*, implying that functional reduction is similar in both species (Colwell 1994). Conversely, restriction mapping and PCR screens suggest that the plastid genome of the holoparasite *Lathraea clandestina* is ca. 100–110 kb in size with gene synteny mostly colinear to *Epifagus* and most photosynthetic plants (Delavault et al. 1996). Reductive evolution of the plastid genome in *Lathraea* has obviously not proceeded as far as in *E. virginiana*. Still, some losses have also affected the plastid genome of

Lathraea: most prominently all SSC-located *ndh* genes have been deleted (Delavault et al. 1996). This is in line with data from other holoparasitic and myco-heterotrophic lineages with minimally reduced plastomes (Funk et al. 2007; McNeal et al. 2007b; Wickett et al. 2008; Delannoy et al. 2011; Logacheva et al. 2011).

Further support for the hypothesis of a highly lineage-specific reductive plastome evolution comes from studies using a broad taxon sampling, but focusing only on a few plastome regions. Because of its prime function during photosynthetic carbon fixation, most data is available for the plastid gene *rbcL*, which encodes the large subunit of RuBisCO. Some non-photosynthetic lineages (e.g. *Lathraea*, *Harveya*, *Myzorrhiza* [syn. *Orobanche sect. M.*]) preserve an intact reading frame for *rbcL* (Delavault et al. 1995, 1996; Wolfe and dePamphilis 1997, 1998; Randle and Wolfe 2005). In *Lathraea*, however, *rbcL* is transcribed by a nuclear-encoded polymerase rather than by the normally used plastome-encoded polymerase, which also transcribes most of the other photosynthesis genes (Lusson et al. 1998). Accordingly, it is not surprising that plastome-encoded polymerase-specific promoter regions are lost in *Epifagus* (Morden et al. 1991) and also in some other parasites (Krause et al. 2003; Berg et al. 2004). Several non-photosynthetic species harbour only a pseudogene copy (e.g. *Aphyllon* [syn. *Orobanche sect. Gymnocaulis*], *Hyobanche*, most *Orobanche* s. str.; Wolfe and dePamphilis 1997; Delavault and Thalouarn 2002; Manen et al. 2004; Young and dePamphilis 2005), and several lines of evidence indicate that the *rbcL*-gene region is deleted from the plastomes of *Phelipanche* (Manen et al. 2004; Park et al. 2007a; Wicke et al. in prep.). Low levels of *rbcL* expression have been detected in holoparasitic *Harveya* and *Lathraea* (Lusson et al. 1998; Randle and Wolfe 2005). *Myzorrhiza corymbosa* maintains functional upstream and downstream untranslated regulatory elements, which is indicative of maintained transcription activity (Wolfe and dePamphilis 1997); expression data is, however, lacking. The function of the translated polypeptide transcribed from *rbcL* has not been investigated in holoparasites. A function of RuBisCO that is unrelated to photosynthesis has been speculated (e.g. Wolfe and dePamphilis 1997; Leebens-Mack and dePamphilis 2002), corroborated by findings that link RuBisCO to amino acid synthesis and a glycolysis-bypassing pathway (Tolbert 1997; Schwender et al. 2004). The transcript of an *rbcL* pseudogene has been detected in *Hyobanche* (Randle and Wolfe 2005), implying that regulation of the largely nuclear-encoded transcription (and transcript processing) machineries lacks behind plastid DNA evolution, at least in this particular case.

Evolutionary analyses of *rps2* and *matK* show low rates of nucleotide substitution of these genes in *rbcL*-preserving lineages (dePamphilis et al. 1997; Young and dePamphilis 2005), suggesting that *rps2* and *matK* are under purifying selection and, thus, still functional. Furthermore, *Orobanche minor* retains most plastid tRNAs, although some only as pseudogenes (Lohan and Wolfe 1998), and it retains several DNA fragments that are deleted from the plastome of *Epifagus* and *Conopholis*. Taken together, this and the previously mentioned studies point towards less-reduced plastid genomes in *Harveya*, *Hyobanche*, *Myzorrhiza*, and

Orobanche. The reasons for these lineage-specific reductions are not yet understood, but the *time* since transition to holoparasitism seems to play a most relevant role. In general, older holoparasitic Orobanchaceae lineages, such as *Epifagus*, have greater reductions than younger ones, such as *Lathraea* and the *Harveya*/*Hyobanche* lineages (Leebens-Mack and dePamphilis 2002; also consider phylogenetic relationships depicted in Fig. 15.1).

Besides time, factors influencing the rate of gene loss and physical reduction of the plastome are still widely elusive. However, there seems to be a strong correlation between the deletion of a plastomic gene region and its physical proximity to indispensable genes of housekeeping or metabolic function (Wicke et al. submitted). The localization of a gene that has become dispensable after the loss of photosynthesis is apparently also protected by its location within an operon that encodes genes of various functional complexes (Wicke et al. submitted). Given the high gene density of plastid chromosomes, both effects are not mutually exclusive for the protection of physical gene deletion. A complex interaction of species-specific repair and recombination rates may further be elucidated as important factors in "regulating" how plastid genome reduction proceeds in holoparasites (Wicke et al. in prep.).

Structural reorganization of the plastid DNAs (e.g. inversions) in parasites occurs to a considerably smaller extent (if at all), compared with the amount of segmental DNA losses. The only reports of large-scale structural changes come from Colwell (1994) and Downie and Palmer (1992), who revealed the independent loss of one of the inverted repeats in and its *Conopholis* and *S. asiatica*, respectively. Outside Orobanchaceae, the highly reduced plastome of the underground orchid *Rhizanthella* has severe alterations around the inverted repeats (Delannoy et al. 2011), whereas the less dramatically reduced genome of the closely related bird's-nest orchid *Neottia nidus-avis* shows no genomic rearrangements (Logacheva et al. 2011). Small inversions were reported in plastomes of some species of *Cuscuta*, but no large-scale plastomic reconfigurations were found (Krause 2011). Thus, the generally high degree of structural conservation reported for the majority of angiosperm plastomes (Wicke et al. 2011) appears to be upheld in Orobanchaceae holoparasites for a long duration after the loss of photosynthesis. In contrast, ongoing research suggests that the relaxation of functional constraints and subsequent gene loss rapidly commence after the transition to a (obligate) heterotrophic way of life (Wicke et al. submitted).

15.4 The Mitochondrial Genome

Unlike plastomes, mitochondrial genomes (chondriomes) are highly susceptible to the incorporation of both horizontally transferred DNA and DNA from other cellular genomes (Won and Renner 2003; Bergthorsson et al. 2004; Davis et al. 2005; Knoop et al. 1996, 2011). Additionally, the high variability of size and gene content of plant chondriomes makes them difficult targets for phylogenetic and comparative evolutionary studies (Knoop et al. 2011).

As of this writing, no complete sequence of a chondriome of a parasitic flowering plant is available. Only few studies focused on the molecular evolution of mitochondrial genes, although several genes (e.g. *matR*, *atp1*, and *coxI*) have been employed to infer the placement of parasitic plant lineages in the tree of flowering plants. Despite the fact that DNA evolves normally more slowly than nuclear and plastid DNAs (Wolfe et al. 1987), some holoparasitic lineages (e.g. Apodanthaceae, Rafflesiaceae) exhibit elevated nucleotide substitution rates in the mitochondrial small ribosomal RNA (mtSSU) as well as in the mitochondrial genes *coxI*, *atp1*, *matR*, and in exons B and C of *nad1* (Duff and Nickrent 1997; Nickrent et al. 2004; Barkman et al. 2004, 2007; Filipowicz and Renner 2010). However, no rate acceleration has been found in holoparasitic Orobanchaceae genera such as *Epifagus*, *Orobanche*, and *Boschniakia* (supposedly *Kopsiopsis*; Mower et al. 2004; Barkman et al. 2007). Hemiparasitic Orobanchaceae (e.g. *Lamourouxia*, *Agalinis*, *Pedicularis*, *Hedbergia*, *Parentucellia*, *Bartsia*, *Buchnera*) also appear to evolve at similar evolutionary rates as *Lindenbergia* (Mower et al. 2004).

Little is known about the evolution of macro- and microstructural changes, such as (small) insertions, deletions, and inversions in coding and non-coding mitochondrial DNAs of parasitic plants. Duff and Nickrent (1997) reported a slight increase of indel events in the mtSSU of non-asterid parasite lineages. In contrast, mtSSU in *Epifagus* shows only an insignificant length variation (2 nt) compared to photosynthetic relatives. Remarkably, parasitic plants frequently possess an intron in the *coxI* gene. The *coxI* intron is found in ten of the at least 12 independently evolved angiosperm lineages with a parasitic lifestyle (Barkman et al. 2007). The source of the intron remains largely unclear. While an initial acquisition of the *coxI* intron via horizontal homing from a fungus seems likely for some angiosperms (Vaughn et al. 1995; Adams et al. 1998; Cho and Palmer 1999; Seif et al. 2005, cp. Cusimano et al. 2008), most gains in parasites seem to have occurred by horizontal plant-to-plant transfers (Sanchez-Puerta et al. 2008; see below). The close interaction between a parasitic plant and its host further supports the hypothesis of a plant donor of parasite *coxI* introns.

15.5 Horizontal DNA Transfer

The identification of true horizontal DNA transfers from one plant to another and its origin can be very problematic (critically reviewed in Renner and Bellot 2012). Parasite–host systems appear to be especially prone to horizontal gene/DNA transfer (HGT) (see Sect. 6.5.2). In plants, the uptake and incorporation of DNA from another species occur more frequently in mitochondrial DNA, although comparative data of functional and non-functional HGT is still widely lacking for the nuclear genome. Prominent cases of HGT involving mitochondrial genes concern the *atp1* region of parasitic plants of the Rafflesiaceae and Apodanthaceae (Davis and Wurdack 2004; Nickrent et al. 2004). A chimeric copy consisting of parasite-specific and horizontally acquired genic parts of *atp1* was found in the mitochondria

in *Pilostyles thurberi* (Apodanthaceae; Barkman et al. 2007). Copies of host *atp1* appear to have also been independently transferred to species of the *Bartsia* clade of Orobanchaceae, and to *Cuscuta* (Mower et al. 2004, 2010). Other mitochondrial genes involved in HGT have not yet been identified in Orobanchaceae.

The transfer of macromolecules such as RNAs predominantly from the host into the parasite was reported for *Triphysaria versicolor* (Tomilov et al. 2008) and *Phelipanche aegyptiaca* (Aly et al. 2009; see Sect. 6.5.1). *Phelipanche* seems to also take up a host protein (Aly et al. 2011). A similar phenomenon was found in *Cuscuta* (reviewed by LeBlanc et al. 2012); comparable data from other parasitic plant families are currently lacking. Although the cellular components involved in RNA trafficking in host–parasite systems supposedly differ according to haustorial anatomy (see Sect. 3.9.3), current data suggest that parasites may have access to a great variety of host RNAs, including those encoding proteins that are functionally located in host plastids (e.g. *rbcS*, LeBlanc et al. 2012). Mobile RNAs may eventually also be incorporated into the nuclear genome of the parasite. Such cases have already been reported for an expressed nuclear gene of unknown function in *Striga hermonthica* (Yoshida et al. 2011) and also for *Rafflesia* (Xi et al. 2012).

Another case of HGT involves fragments of the plastid regions *rbcL*, *rps2,* and *trnL-F*, which appear to have been transmitted from *Orobanche* into some species of *Phelipanche* (Park et al. 2007a). Current data on plastid genome evolution in both genera suggest, however, that the horizontally acquired fragments from at least two out of the five originally studied *Phelipanche* species do not reside in the plastid genome (Wicke et al. submitted). Those fragments may be located in either the nuclear or the mitochondrial genome, as hypothesized earlier (Park et al. 2007a). Regardless of the location of the horizontally acquired copies, putative HGT remains highly interesting as it might involve transmission via a host plant as the vector, even though a vertical transmission may, however, also be considered (Park et al. 2007b).

15.6 Conclusions

Orobanchaceae possess highly dynamic genomes, which is in part due to the rampant occurrence of polyploidy as implied by genome-size data and chromosomal evolution. Transcript-profiling and transcriptome-sequencing projects have already identified several genes that are candidates for being newly recruited in parasite-specific developmental pathways, and large-scale sequencing in combination with basic genetic work has revealed complex patterns of macromolecule trafficking and signalling in selected host–parasite systems. Studies of plastid genes and genomes of members of Orobanchaceae have additionally brought to light the first insights into the complexity and differential dynamics in the process of plastid genome reduction after the loss of photosynthesis.

Ongoing and future research and research networks will allow the stepwise elucidation of the physiological evolution of the Orobanchaceae from the autotrophic to holo-heterotrophic lifestyle.

Despite the great progress that has already been achieved, our understanding of genomic evolution in Orobanchaceae is still hampered by a substantial lack of data on, for instance, chromosome numbers, genome sizes, gene content and organization, gene expression, and epigenetic variation (see Chap. 13). Further basic and comparative research are needed, including large-scale transcriptome and genome sequencing, to determine basic parasite-specific genetics and to elucidate the complex (co-)evolution of the parasites and their hosts.

Orobanchaceae, the largest and most diverse family of parasitic angiosperms has already proven to be highly suitable for studying the functional basis of a parasitic lifestyle in higher plants. Several projects are currently underway that will shed further light on genomic evolution as well as on the extent of horizontal gene transfer. Besides crucial physiological and ultrastructural works, genome surveys utilizing the rapidly developing sequencing technologies and large-scale proteomic approaches should be a key element in understanding the evolution of parasitism and all adaptations that come with the parasitic way of life, e.g. haustorium formation, host recognition, and nutrient acquisition. The sequencing of reference genomes of Orobanchaceae species is inevitable (though challenged by its genome size) and will eventually be an important step towards finding effective ways for control of the weedy species (see Chaps. 21 and 24).

The availability of reverse genetic approaches is another key point that will bring parasitic plant research to another level. *Agrobacterium*-mediated transformation systems have already been established successfully for the hemiparasites *Triphysaria versicolor* (Tomilov et al. 2007), *Phtheirospermum japonicum* (Ishida et al. (2011), and for the holoparasite *Phelipanche aegyptiaca* (Fernandez-Aparicio et al. 2011). Thus, the broomrape family provides a unique framework to experimentally test the function of putative parasite-specific genetic elements and to study physiological evolution across close relatives with differing degrees of heterotrophy.

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