HOST PREFERENCES OF BEECHDROPS (EPIFAGUS): EVIDENCE FROM CHLOROPLAST DNA SEQUENCE DATA

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ABSTRACT

In nearly two hundred years, botanists and the general public have assumed that Epifagus virginiana (beechdrops) parasitizes specifically on the roots of Fagus grandifolia (American beech tree). In this study we used sequences of the chloroplast gene rbcL from host roots of beechdrops to test the long-held theory. Host roots on which Epifagus grows were randomly collected from four localities in western Michigan. Our data show that although roots of maple and beech are intricately interwoven with the grappling roots of Epifagus, all of our root samples for which we verified the host-parasite direct connections under dissecting microscope were from American beech trees (Fagus grandifolia) except for one from Acer saccharum. The potential beechdrop-sugar maple relationship needs further verification from physiological investigations. Therefore, our DNA sequence data support the host preference of Epifagus on roots of Fagus and suggest that parasite-root interactions may be complex and DNA barcoding can be useful for studying the host preference of parasitic plants.

KEYWORDS: Epifagus, Fagus grandifolia, Acer saccharum, host specificity.

Holoparasitism has evolved more than 10 times in the evolutionary history of flowering plants (Barkman et al. 2007) and parasitic angiosperms belong to 22 families, 265 genera, and over 4000 species (Nickrent et al. 1998). Orobancheae are the largest parasitic family (Bennett and Mathews 2006) and consist of 65–87 genera (Wolfe et al. 2005; Judd et al. 2008), including Epifagus Nutt. (Beechdrops). Epifagus was first discovered in Virginia, U.S.A. and was given the name Orobanche virginiana by Linnaeus (Linnaeus 1753). It was Nuttall (1818) who pointed out the generic differences between Orobanche and the beechdrops, and first used the name Epifagus to refer to its parasitic habit on the roots of beech tree, Fagus grandifolia Ehrn. However, Barton (1818) was the first to publish the species name Epifagus virginiana.

Epifagus occurs mainly in eastern North America with some extension to Central America and has a general co-existence with Fagus grandifolia (Thieret 1969). People have dug and traced host roots to beech trees (Schrenk 1894). However, this is not always possible since host roots are generally fine ones and easily broken (Thieret 1969). Nevertheless, Epifagus has always been considered as growing solely on the roots of Fagus grandifolia since its original description (Barton 1818; Nuttall 1818; Schrenk 1894; Thieret 1969; Heide-Jørgensen 2008).

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In recent years DNA barcoding has been used to help identify plant species (Kress et al. 2005), and the technique is also found to be useful for ecological studies (Valentini et al. 2009). Zhang et al. (2009) used sequences of nrDNA internal transcribed spacer regions to help determine hosts of holoparasitic plant *Cynomorium*. The objective of the current study is to provide molecular test for the traditional view that *Epifagus* is an obligate parasite on the roots of *Fagus grandifolia* using nucleotide sequences of chloroplast gene *rbcL* because the gene has been widely used in phylogenetic studies (Chase et al. 1993) and recently recommended as a DNA barcoding marker for plants (Kress et al. 2005).

MATERIALS AND METHODS

Sixteen plants of *Epifagus* and their associated potential host roots were collected from four localities of western Michigan, each with 1–5 samples. The sampled individuals were widely spaced out to minimize the possibility of having hosts from the same beech tree. Because *Epifagus* uses its grappler roots to get hold of fine roots of a potential host and then forms haustoria to complete tissue connections with the host (Schrenk 1894), we brought the samples to lab to clean the roots and verify the parasite-host tissue fusion under a dissecting microscope (Fig. 1). Genomic DNAs of the potential host roots (with or without tissue connections with the parasite) were extracted using a Plant DNeasy Mini Kit (Qiagen, CA). Polymerase chain reactions (PCR) were conducted to amplify the chloroplast *rbcL* gene using primers rbcl5 and rbcl3 (Olmstead et al. 1992) and the thermocycler program of Jiao and Li (2007) in an Eppendorf MasterCycler Pro thermocycler. PCR products were purified using a Qiaquick PCR purification kit (Qiagen, CA, cat. #28106), and sequenced using the BigDye fluorescent chemistry (Applied Biosystems, CA). Sequences were obtained using a Genetic Analyzer 3130 (Applied Biosystems) at Hope College, and edited using Sequencher (version 4.1, GeneCode, Ann Arbor, MI).
We compared our new sequences with those in the GenBank using the BLAST function (www.ncbi.nlm.nih.gov). Because our BLAST analyses indicated that the sequences were most similar to those in the rosids, our phylogenetic analyses were limited to the rosids clade. An rbcL dataset (M3534.NX) of the rosids was obtained from TreeBASE (www.treebase.org) and to it we added our new sequences and additional rbcL sequences of Fagus and Acer from the GenBank. The sequences were readily aligned manually. Maximum parsimony analyses were performed on the dataset using PAUP* (version 4.0b10) (Swofford 2002), and the heuristic tree search options included 100 random sequence addition with 10 trees held each replicate, MaxTrees set to 5,000, TBR branch swapping, Multrees on, and steepest descent off. Bootstrap analyses (Felsenstein 1985) of 100 replicates were used to estimate relative support for individual clades and heuristic tree search options were as in parsimony analyses except for simple sequence addition.

RESULTS

Sequences of the rbcL gene were obtained from all sampled roots, and their lengths ranged from 1329–1371 bp. BLAST analyses showed that a majority of our sequences were most similar to Fagus, while a few new sequences had high affinity scores with Acer. Sequences were identical in several samples of the same species and only one sequence was submitted to the GenBank (Fagus grandifolia: GU434292, Acer saccharum: GU434290, and Acer rubrum: GU434291). In the MP trees (Fig. 2), all but one sequence from roots that were verified to have direct tissue connection with the haustoria of Epifagus formed a clade with Fagus grandifolia. In contrast, sequences from potential host roots that were tightly grabbed by Epifagus grapplers but had no direct connection with the haustoria of Epifagus were from either Fagus or Acer. One root sample with verified tissue connection with Epifagus, however, turned out to be from Acer saccharum.

DISCUSSION

Since the initial description of Epifagus virginiana, it has generally been accepted that Epifagus parasitizes only on the roots of Fagus grandifolia (Barton 1818; Nuttall 1818; Schrenk 1894; Thieret 1969; Heide-Jørgensen 2008). The long-held view has been based only on field observations tracing parasitized roots to host trees (Barton 1818; Schrenk 1894; Thieret 1969). Such observations have great accuracy but are not an easy task (Thieret 1969) and are limited in nature. So far there are no other lines of evidence to confirm the unique host-parasite relationship between Epifagus and Fagus grandifolia. In our random sampling of 16 potential host roots in four localities of western Michigan, we observe that all roots (with one exception) that have tissue connections with the haustoria of Epifagus are from beech trees, confirming the long-held view of the host-parasite relationship.

Epifagus is essentially coexistent with Fagus grandifolia (Thieret 1969), while Acer saccharum and Fagus grandifolia often grow in association with each other (Daubenmire 1978; Elias 1980). In our study sites Fagus grandifolia is accompanied by both Acer saccharum and Acer rubrum. Thus, it is not sur-
FIGURE 2. Strict consensus of 5,000 trees based on parsimony analyses of rbcL sequences of the rosids. All sequences are obtained from the GenBank except for the new sequences (E6175-E6190) and samples with verified tissue connections with *Epifagus* are in boldface. Numbers above branches show bootstrap percentages.
prising that some of the roots *Epifagus* holds on tightly using its grappler roots are from *Acer saccharum* or *A. rubrum*. However, only one of the maple roots had direct tissue connection with the haustoria of *Epifagus*. We have not observed any of the *Epifagus* samples having tissue connections with more than one host roots. Nevertheless, we can not rule out the possibility that there had been a beech-*Epifagus* tissue connection before the formation of the tissue fusion with the root of *Acer saccharum*. Therefore, our study suggests that *Epifagus* parasitizes nearly specifically on the roots of *Fagus grandifolia*. It is desirable to obtain additional samples from more populations to further test the hypothesis and to conduct histological studies to further verify the haustorial connections between *Epifagus* and *Acer saccharum*.

With cross contamination in mind, we did not sequence the *rbcL* gene from leaf samples of *Fagus grandifolia*, *Acer saccharum*, or *A. rubrum*. Instead, we used the existing *rbcL* sequences in the GenBank. Therefore, our DNA barcoding data for the first time provide alternate, unequivocal support for the high host preference of *Epifagus* on beech trees, and indicate a possible occasional association between *Epifagus* and *Acer saccharum*. However, the latter association needs further reevaluation with additional samples and histological verification of the host-parasite connection.

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**LITERATURE CITED**


