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A REEXAMINATION OF THE APOGAMOUS TETRAPLOID *PHEGOPTERIS* (THELYPTERIDACEAE) FROM NORTHEASTERN NORTH AMERICA

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ABSTRACT. Populations of apogamous, tetraploid plants of the beech fern genus, *Phegopteris*, have been discovered in northern New England, prompting an investigation of their putative origin as a hybrid between apogamous, triploid *P. connectilis* and sexual, diploid *P. hexagonoptera*. The present study combined evidence from morphometric analysis and isozyme electrophoresis to determine if *P. hexagonoptera* and *P. connectilis* both contribute to the tetraploid genome. Fifteen morphological characters and six gene loci representing five enzyme systems were evaluated. While our results reveal an affinity between the genomes of *P. connectilis* and the tetraploid *Phegopteris*, they do not support *P. hexagonoptera* as a progenitor of the apogamous tetraploid lineage.

Key Words: fern, apogamy, hybrid, isozyme, *Phegopteris*, polyploidy, evolutionary origin

The beech fern genus, *Phegopteris* Fée, comprises three species widely distributed in the Northern Hemisphere. *Phegopteris hexagonoptera* (Michx.) Fée is a sexual diploid ($n = 30$, $2n = 60$) of temperate eastern North America. *Phegopteris decursivepinnata* (van Hall) Fée includes three cytotypes: a sexual diploid ($n = 30$, $2n = 60$), a sexual tetraploid ($n = 60$, $2n = 120$) and a triploid hybrid ($n = 90$), all in temperate Asia (Mitui 1970, as cited in Masuyama 1979). *Phegopteris connectilis* (Michx.) Watt includes two cytotypes: an apogamous triploid ($n = 90$, $2n = 90$) and a sexual diploid ($n = 30$, $2n = 60$). Triploid *P. connectilis* has a circumboreal distribution with extensions into the Appalachian Mountains at high altitudes, but the diploid race has been reported only from the Japanese Alps of central Honshu, Japan (Matsumoto 1982).

Mulligan et al. (1972) described an apogamous tetraploid *Phegopteris* ($n = 120$, $2n = 120$) from Rougemont, Québec. They speculated that the tetraploid arose via a fertilization event involving an unreduced triploid

sperm from apogamous *P. connectilis* and a haploid egg from *P. hexagonoptera*. Their conclusion about the hybrid nature of the tetraploid was supported by a lack of chromosome pairing at meiotic metaphase, chromosome numbers and breeding behavior of the putative progenitors, and by a perceived morphological similarity between the tetraploid, *P. connectilis*, and *P. hexagonoptera*.

Later, Mulligan and Cody (1979) reported additional populations of the apogamous tetraploid from New Brunswick and Nova Scotia. Since *Phegopteris hexagonoptera* does not now occur in that area, Mulligan and Cody (1979) surmised that multiple populations of the tetraploid arose when *P. hexagonoptera* was more widely distributed than today. Recently, one of us (A.V.G.) has discovered additional populations of this plant in northern Vermont (Figure 1; Tables 1 and 2). Contrary to Mulligan et al. (1972), Gilman observed, upon close examination of these plants, a lack of morphological intermediacy between *P. connectilis* and *P. hexagonoptera*. This prompted us to further investigate the hybrid hypothesis in the current study.

Although morphological intermediacy is common in pteridophyte hybrids (Wagner 1983), several authors (e.g., Barrington et al. 1989; Rieseberg and Carney 1998) warn that hybrids are not restricted to intermediate character expression and commonly exhibit parental and transgressive (falling outside the parental range) morphological character states. Since postulation of a hybrid origin based on morphological characters may be unreliable, other approaches, including isozyme analysis, may be used to test hypotheses of hybridization. Isozyme electrophoresis is a valuable technique to investigate plant hybrids and polyploid taxa, as co-dominant inheritance of discrete marker bands (allozymes) allows the detection of additive profiles in hybrid taxa where parental taxa are fixed for different alleles or where allele frequencies differ significantly (Roose and Gottlieb 1976; Werth 1989).

To test the hybrid hypothesis of Mulligan et al. (1972), we reexamined their data, evaluated additional morphological characters, and surveyed isozymes to assess a genetic component of variation. Specifically we sought to determine whether morphological characters and isozyme banding patterns were consistent with a hybrid origin of the tetraploid from *Phegopteris hexagonoptera* and *P. connectilis*.

MATERIALS AND METHODS

Field work. Whole leaves from natural populations of *Phegopteris connectilis* and the putative interspecific hybrid (hereafter *the unknown*)

Figure 1. Sporophyte of apogamous tetraploid *Phegopteris* from Cabot, Vermont (*A. V. Gilman 01141*, VT).

Table 1. Locality data for collections providing material for electrophoretic analyses. Vouchers of all collections deposited in VT.

Collection Details
Phegopteris hexagonoptera
Maine
Washington Co., 5 Jul 2000, *A. V. Gilman 97127*
Vermont
Chittenden Co., Burlington, Ethan Allen Park, 5 Jul 2000, *A. V. Gilman s.n.*
Chittenden Co., Charlotte, Lost Forest, 9 Oct 2001, *H. E. Driscoll 61, 62*
Chittenden Co., Charlotte, Thompson's Point, 5 Jul 2000, *A. V. Gilman 2K082*, with *D. S. Barrington, C. A. Paris & P. Hope*
Chittenden Co., Shelburne, Wake Robin, 5 Jul 2000, *D. S. Barrington s.n.*
Phegopteris connectilis
Vermont
Caledonia Co., St. Johnsbury, 18 Aug 2001, *A. V. Gilman & H. E. Driscoll 2, 3, 5*
Caledonia Co., Waterford, 18 Aug 2001, *A. V. Gilman & H. E. Driscoll 7, 9*
Washington Co., Cabot, 1 Oct 2001, *H. E. Driscoll 24, 25, 28, 30–39, 43–51, 54, 55*
Washington Co., Northfield, 29 Aug 2001, *A. V. Gilman & H. E. Driscoll 11, 13–15*
Unknown species
Vermont
Caledonia Co., St. Johnsbury, 18 Aug 2001, *A. V. Gilman & H. E. Driscoll 1, 4, 10.*
Caledonia Co., Waterford, 18 Aug 2001, *A. V. Gilman & H. E. Driscoll 6, 8*
Washington Co., Cabot, 1 Oct 2001, *H. E. Driscoll 23, 27, 29, 40, 41–42*
Washington Co., Montpelier, 29 Aug 2001, *A. V. Gilman & H. E. Driscoll 18–22, 56, 57*
Washington Co., Northfield, 29 Aug 2001, *A. V. Gilman & H. E. Driscoll 12, 16, 17*

were collected from five localities in northern Vermont between 1997 and 2001 for inclusion in the isozyme analysis (Table 1). Samples of *P. hexagonoptera* were also collected from four Vermont localities and one site in Maine for use in isozyme investigations. Material for the morphological analysis was collected from some of these localities as well as some unique areas (Table 2). Herbarium vouchers are deposited at the University of Vermont (VT). Leaves used in the isozyme analysis were kept in individual plastic bags and refrigerated for no more than two weeks, after which the samples were fixed with liquid nitrogen and stored in an ultra-cold freezer (–80°C). Material stored in this way is useful for at least three years.

Table 2. Locality data for collections providing material for morphological analyses. Vouchers of all collections deposited in VT.

Collection Details
Phegopteris hexagonoptera
Maine
Knox Co., Washington Twp., Patrick Mtn., 11 Jul 1997, *A. V. Gilman 97127*
Vermont
Chittenden Co., Charlotte, Mt. Philo, 14 Jul 1993, *A. V. Gilman 93145 & Briggs*
Chittenden Co., Charlotte, Thompson's Point, 5 Jul 2000, *A. V. Gilman 2K082* with *D. S. Barrington, C. A. Paris & P. Hope*
Chittenden Co., Charlotte, Thompson's Point, 5 Jul 2000, *A. V. Gilman 2K083* with *D. S. Barrington, C. A. Paris & P. Hope*
Phegopteris connectilis
Vermont
Caledonia Co., Danville, near Keiser Pond, 15 Jul 1997, *A. V. Gilman 97237*
Caledonia Co., Peacham, Peacham Corner, 23 Jul 2000, *A. V. Gilman 2K124*
Orange Co., Brookfield, Northfield Gulf, 17 Jul 1996, *A. V. Gilman 96132*
Washington Co., Cabot, Hooker Mtn., 23 Jul 2000, *A. V. Gilman 2K126*
Unknown
Vermont
Caledonia Co., St. Johnsbury, near Sleeper River, 21 Jun 1996, *A. V. Gilman 96061*
Caledonia Co., Waterford, Passumpsic, 17 Aug 1996, *A. V. Gilman 96248*
Washington Co., Cabot, Hooker Mtn., 3 Sep 2001, *A. V. Gilman 01141*
Washington Co., Northfield, Paine Mtn., 2 Sep 1993, *A. V. Gilman 93257*

Morphological analysis. The unknown was identified with reference to the description and Figure 3 in Mulligan et al. (1972); one clone (*A. V. Gilman 96248*) was cytologically determined as a tetraploid using standard techniques. Frond-shape measurements were taken from pressed, dried fronds of four clones of each taxon. Three fronds of each clone were scored.

We scored five quantitative frond-shape characters: 1) width to length ratio of lowest pinna, 2) ratio of length of lowest pinna to adjacent pinna, 3) which (xth from rachis) basiscopic pinnule on the lowest pinna is the longest, 4) which (xth from base) pinna is equivalent in length to the longest basiscopic pinnule, and 5) average width of abaxial costal scales in number of cells at the widest point (for this analysis, a total of five scales from five separate fronds of one clone of each taxon was scored). To test the hypothesis that the ratio of the genomic contribution (3*n* *Phegopteris connectilis*: 1*n* *P. hexagonoptera*) skews the morphology of the putative hybrid towards *P. connectilis*, quantitatively scored

Table 3. Morphological analysis of *Phegopteris connectilis*, *P. hexagonoptera*, and the tetraploid plant, for comparison with predicted intermediate quantitative scores of a putative hybrid assuming 3:1 genomic contributions of possible parents, *P. connectilis* and *P. hexagonoptera*. We determined (fully explained in text) characters 1–5.

Character	*P. hexagonoptera*	*P. connectilis*	Unknown	Predicted
1. Width:length of basal pinnae	0.303	0.241	0.218	0.256
2. Basal and adjacent pinna ratio	1.071	1.093	1.096	1.087
3. Longest basiscopic pinnule is xth from rachis	5.33	6.25	6.33	6.02
4. Pinna equal to longest basiscopic pinnule	8.91	13.25	14.17	12.17
5. Width of abaxial scales in cells	6.1	22.2	19.8	18.2

characters of the tetraploid were compared to values interpolated from the putative parents using the 3:1 ratio (Table 3).

We also reexamined data presented by Mulligan et al. (1972; Table 4), in which they compared nine qualitative morphological characters: 1) surface ornamentation of spores, 2) shape of fronds, 3) size of fronds, 4) position of lowest pair of pinnae, 5) shape of lower pinnae, 6) presence or absence of wing along rachis connecting two lowest pinna pairs, 7) shape and color of scales on lower leaf surface, 8) nature of hairs on surface of pinnae, and 9) branching of hairs in notch between pinnae.

Isozyme electrophoresis. Samples were surveyed for electrophoretically detectable isozyme variation using 12% pH-buffered hydrolyzed starch gels. Because isozymes had not previously been used in systematic studies of *Phegopteris*, we tested a variety of extraction, gel, and electrode buffer systems for optimum band resolution on all three taxa. Small portions of fresh leaves were ground in cold vegetative-extraction buffer II (Cheliak and Pitel 1984) and the homogenate was absorbed into Whatman 3mm chromotography paper wicks. Saturated wicks were stored at –80°C until inserted into gels. The following eight enzymes were resolved: isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), shikimate dehydrogenase (SkDH), and triosephosphate isomerase (TPI). The enzymes G6PDH and 6-PGD were resolved on gel and electrode buffer system 5 of Soltis et al.

(1983). The enzymes IDH, MDH, PGM, and SkDH gave clearest band expression with gel and electrode buffer system 11 of Soltis et al. (1983). The enzymes TPI and PGI were resolved most clearly using gel and electrode buffer systems 6 and 8, respectively, of Soltis et al. (1983). Standard staining schedules were followed (Soltis et al. 1983). Although eight enzymes were resolved, only the five (PGM, PGI, TPI, MDH, and SkDH) that provided taxon-specific genetic markers for *P. connectilis*, *P. hexagonoptera*, and the unknown are considered here.

Genetic control of isozyme banding patterns was inferred based on the typical subunit structure and subcellular compartmentalization of the enzymes (Gottlieb 1981). All enzymes migrated anodally. When more than one isozyme was present for an enzyme, they were numbered sequentially with the most anodally migrating isozyme designated 1. Alleles were designated using relative mobilities of the allozymes they encode. The most common allele across all taxa for each isozyme was assigned a mobility value of 100, and all other alleles were assigned numbers representing the percent anodal migration relative to the 100 allele.

RESULTS

Morphological analysis. Morphological differences between the unknown and *Phegopteris hexagonoptera* were pronounced, but the unknown showed a morphological similarity to *P. connectilis* (Tables 3 and 4). For four of five quantitative characters (all except width of abaxial scales in number of cells), the scores for the tetraploid actually fell outside the range determined by the two other taxa on the *P. connectilis* end of the scale. Width of abaxial scales in number of cells was not transgressive, but was closer to *P. connectilis* than would be predicted by interpolation; it actually fell about halfway between *P. connectilis* and the predicted score.

We reevaluated and confirmed most of the nine qualitative scores given by Mulligan et al. (1972). Of these, only one (size of fronds, "large") was shared with *Phegopteris hexagonoptera*. Five characters (surface ornamentation of spores, absence of wing along rachis connecting two lowest pinna pairs, shape and color of scales on lower leaf surface, nature of hairs on surface of pinnae, and branching of hairs in notch between pinnae) scored with *P. connectilis*. Only two qualitative characters (shape of fronds and position of lowest pair of pinnae) had intermediate scores. We did not confirm one character: width:length ratio of basal pinnae was actually transgressive (Table 3, No. 1); the basal

Table 4. Qualitative morphological analysis for *Phegopteris* adopted from Mulligan et al. (1972) as confirmed by our observations. The fourth row is our assessment of the unknown's relation to the two progenitors proposed by Mulligan et al. (1972).

Character	Qualitative Description
1. Surface of fertile spores	
P. hexagonoptera	Blunt verrucate granules
P. connectilis	Irregular thread-shaped fibers
Unknown	Irregular thread-shaped fibers
Unknown relationship	Similar to *P. connectilis*
2. Shape of fronds	
P. hexagonoptera	Usually broader than long
P. connectilis	Usually longer than broad
Unknown	Usually slightly longer than broad
Unknown relationship	Intermediate
3. Size of fronds	
P. hexagonoptera	"Large"
P. connectilis	"Small"
Unknown	"Large"
Unknown relationship	Similar to *P. hexagonoptera*
4. Position of lowest pinnae	
P. hexagonoptera	Slightly or not at all projected downward
P. connectilis	Usual prominently projected downward
Unknown	Slightly projected downward
Unknown relationship	Intermediate
5. Shape of lower pinnae	
P. hexagonoptera	Broadly lanceolate to narrowly rhombic, abruptly contracted towards base
P. connectilis	Lanceolate, gradually narrowed from just above the middle to the base
Unknown	Lanceolate, gradually narrowed from just below the middle to the base
Unknown relationship	Narrower than either *P. connectilis* or *P. hexagonoptera*
6. Wings of rachis	
P. hexagonoptera	Present
P. connectilis	Absent
Unknown	Absent
Unknown relationship	Similar to *P. connectilis*
7. Scales on lower leaf surface	
P. hexagonoptera	Narrowly lanceolate, nearly colorless
P. connectilis	Lanceolate, brownish
Unknown	Lanceolate, brownish
Unknown relationship	Similar to *P. connectilis*

Table 4. Continued.

Character	Qualitative Description
8. Hairs on surface of pinnae	
P. hexagonoptera	Copiously glandular-puberulent beneath, scattered strigose-pubescent
P. connectilis	Sometimes slightly glandular-puberulent beneath, copiously strigose-pubescent
Unknown	Sometimes slightly glandular-puberulent beneath, copiously strigose-pubescent
Unknown relationship	Similar to *P. connectilis*
9. Branching of hairs in notch between pinnae	
P. hexagonoptera	Unbranched
P. connectilis	Branched
Unknown	Branched
Unknown relationship	Similar to *P. connectilis*

pinnae were in fact relatively narrower than those of the other two taxa, not intermediate as scored by Mulligan et al. (1972).

Isozyme electrophoresis. Ten presumed gene loci representing five enzyme systems were resolved. Six of the ten loci were consistently interpretable across all species studied and could be used for interspecific comparison: *Pgm-1*, *Pgm-2*, *Skdh*, *Tpi-1*, *Pgi-2*, and *Mdh-2*. Table 5 reports inferred isozyme genotypes interpreted from isozyme banding patterns.

The monomeric enzyme PGM was represented in all species by two well-separated sets of bands that we interpret as encoded by two loci, *Pgm-1* and *Pgm-2*. Both loci were monomorphic in *Phegopteris connectilis*, for alleles $Pgm\text{-}1^{100}$ and $Pgm\text{-}2^{100}$. The unknown had a phenotype identical to *P. connectilis* for *Pgm-1*, but at *Pgm-2* it possessed a two-banded phenotype for alleles $Pgm\text{-}2^{100/115}$, interpreted as heterozygous. In *P. hexagonoptera*, *Pgm-1* was represented by a different single invariant band, $Pgm\text{-}1^{86}$, while *Pgm-2* had two phenotypes. Individuals with a single-banded phenotype for allele $Pgm\text{-}2^{76}$ and those with a two-banded phenotype for alleles $Pgm\text{-}2^{76/115}$ are interpreted as homozygotes and heterozygotes, respectively.

The monomeric enzyme SkDH was represented by a single locus. All taxa exhibited a phenotype with one band, interpretable as homozygotes.

Table 5. Inferred genotype frequencies for informative isozyme loci in *Phegopteris* (not all plants expressed for all isozymes).

	P. hexagonoptera (n = 8)		*P. connectilis* (n = 34)		Unknown (n = 21)	
Locus	Mobility	Frequency	Mobility	Frequency	Mobility	Frequency
Pgm-1	86:86	1.00	100:100:100	1.00	100:100:100:100	1.00
Pgm-2	76:76	0.67	100:100:100	1.00	100:100:115:115	1.00
	76:115	0.33				
Skdh	71:71	1.00	100:100:100	1.00	100:100:100:100	1.00
Tpi-1	84:84	1.00	100:100:100	1.00	100:100:100:100	1.00
Pgi-2	114:114	0.60	56:77:100	1.00	56:56:100:100	1.00
	91:114	0.20				
	73:95	0.20				
Mdh-2	45:45	1.00	100:100:100	1.00	100:100:100:100	1.00

Phegopteris connectilis and the unknown shared the allele $Skdh^{100}$, whereas *P. hexagonoptera* was fixed for the allele $Skdh^{71}$.

Expression of TPI, a dimeric enzyme, was segregated into two regions. Locus *Tpi-2*, represented by a single invariant band, $Tpi\text{-}2^{100}$, exhibited identical mobility in all taxa. A single band, $Tpi\text{-}1^{100}$, characterized that locus for both *Phegopteris connectilis* and the unknown, while *P. hexagonoptera* was fixed for a different allele, $Tpi\text{-}1^{84}$.

There were two loci for PGI, each with complex banding patterns. Resolution of *Pgi-1* was inferior to *Pgi-2*, and so that locus was not included in the analysis. Individuals of the unknown possessed a phenotype with three well-marked bands, $Pgi\text{-}2^{56}$, $Pgi\text{-}2^{77}$, and $Pgi\text{-}2^{100}$, interpreted as two-allele heterozygotes for this dimeric enzyme. All individuals of *Phegopteris connectilis* had a five-banded phenotype comprising bands $Pgi\text{-}2^{56}$, $Pgi\text{-}2^{68}$, $Pgi\text{-}2^{77}$, $Pgi\text{-}2^{91}$, and $Pgi\text{-}2^{100}$. These triploid individuals were interpreted as three-allele heterozygotes. *Phegopteris hexagonoptera* was polymorphic at *Pgi-2*. One phenotype was a broad single band at $Pgi\text{-}2^{114}$. Another had three bands, $Pgi\text{-}2^{91}$, $Pgi\text{-}2^{100}$, and $Pgi\text{-}2^{114}$. These two phenotypes for *P. hexagonoptera* were interpreted as homozygous and heterozygous, respectively. A third banding pattern in *P. hexagonoptera* was a tight triplet comprising alleles $Pgi\text{-}2^{73}$, $Pgi\text{-}2^{82}$, and $Pgi\text{-}2^{95}$. None of the *P. hexagonoptera* alleles was shared with either of the apogamous taxa.

In our sample of MDH, a dimeric enzyme, all plants that resolved shared the same three most anodal bands; *Phegopteris hexagonoptera* was unique in expressing an additional, most cathodal band. We inferred from the observed phenotypes that there are two MDH isozymes expressed in the taxa in this study. All individuals had a non-segregating, asymmetrically stained, three-banded phenotype at anodally migrating locus *Mdh-1* and a one-banded pattern at cathodally migrating locus *Mdh-2*. We followed Gastony (1988) in positing a fixed three-banded pattern at *Mdh-1* attributed to post-translational modification. Under this interpretation, all taxa were inferred to be homozygotes at *Mdh-1*: allele $Mdh\text{-}1^{100}$ produced both unmodified allozyme $Mdh\text{-}1^{100}$ and modified allozyme $Mdh\text{-}1^{50}$; the two interacted to produce a heterodimeric third band $Mdh\text{-}1^{73}$. Allozyme $Mdh\text{-}2^{100}$ co-migrated with the modified variant $Mdh\text{-}1^{50}$ in *P. connectilis* and the unknown, but migrated more slowly in *P. hexagonoptera*, as $Mdh\text{-}2^{45}$.

An isozyme probe of two cultivated individuals of *Phegopteris decursivepinnata* revealed little overlap in bands with our study plants.

DISCUSSION

This study has tested the hybrid hypothesis of Mulligan et al. (1972), which proposed that the apogamous triploid *Phegopteris connectilis* had crossed with the sexual diploid *P. hexagonoptera* to give rise to the tetraploid unknown. We have found that the weight of morphological and electrophoretic evidence precludes involvement of *P. hexagonoptera* in the origin of the apogamous tetraploid plants included in the present study. Results from an isozyme probe of *P. decursivepinnata* suggest that this taxon is also unlikely to have been involved in the history of the apogamous lineage.

Our morphological analysis indicates that the tetraploid plants resemble *Phegopteris connectilis* quite closely and that resemblance to *P. hexagonoptera* is superficial. Quantitative scores were in all cases close to *P. connectilis* and in four of five instances (all except scale width) were closer to *P. connectilis* than to predicted interpolated values. Indeed, four of five metrics were not only closer to *P. connectilis* but were actually transgressive. Qualitative characters showed a similar pattern, with six of nine scoring with *P. connectilis*, only one with *P. hexagonoptera*, and two intermediate. Although mindful that hybrids are not morphologically intermediate in every character, we nevertheless

found no convincing morphological evidence that *P. hexagonoptera* was a progenitor of the unknown.

The isozyme data provide six independent tests of the hybrid hypothesis of Mulligan et al. (1972). At *Pgm-1*, *Skdh*, *Tpi-1*, *Pgi-2*, and *Mdh-2*, the allozymes fixed in *Phegopteris hexagonoptera* were absent from the unknown. At *Pgm-2*, the more common allele in *P. hexagonoptera*, $Pgm\text{-}2^{76}$ was absent in the unknown, although the rarer allele was present. Thus considering the six loci (*Pgm-1*, *Pgm-2*, *Skdh*, *Tpi-1*, *Pgi-2*, and *Mdh-2*) that are relevant to judging the ancestry of the unknown, five argue against the involvement of *P. hexagonoptera* and one is consistent with *P. hexagonoptera* not being involved; no locus argues for its inclusion. Therefore, the isozyme evidence leads us to reject the hypothesis that apogamous *P. connectilis* had crossed with sexual *P. hexagonoptera* to give rise to the tetraploid unknown.

The isozyme data also offer insight about the relationship of the apogamous taxa. Of the six variable loci in our sample, four were fixed for the same allele in *Phegopteris connectilis* and the unknown. *Phegopteris connectilis* could be distinguished from the unknown at two loci, *Pgm-2* and *Pgi-2*. Locus *Pgm-2* was fixed for an allele in *P. connectilis* that was one member of the fixed heterozygote band pattern in the unknown. At *Pgi-2*, the two apomicts shared two out of three alleles; *P. connectilis* had an additional unique allele. Thus, each apomict had distinct allozymes that the other did not. Despite similarities between the genomes of *P. connectilis* and the unknown, the combination of obligately apogamous lifestyles and the presence of unique alleles at *Pgm-2* and *Pgi-2* suggest that the apomicts have different histories.

The progenitors of the apogamous unknown and apogamous *Phegopteris connectilis* remain to be discovered. An isozyme analysis that includes the diploid and presumably sexual cytotype of *P. connectilis* from Honshu, Japan would help bring to light whether this diploid race was involved in the origins of the apogamous *Phegopteris* lineages. The possibility also exists that sexual cytotypes of *P. connectilis* remain undiscovered in North America.

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