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GLOBAL CHLOROPLAST PHYLOGENY AND BIOGEOGRAPHY OF BRACKEN (PETRIDIUM; DENNSTAEDIACEAE)¹

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Bracken ferns (genus Pteridium) represent an ancient species complex with a natural worldwide distribution. Pteridium has historically been treated as comprising a single species, but recent treatments have recognized several related species. Phenotypic plasticity, geographically structured morphological variation, and geographically biased sampling have all contributed to taxonomic confusion within the genus. We sampled bracken specimens worldwide and used various regions of the chloroplast genome to investigate phylogenetics and reticulate evolution within the genus. Our results distinguish two major clades within Pteridium, a primarily northern hemisphere Laurasian/African clade, which includes all taxa currently assigned to P. aquilinum, and a primarily southern hemisphere Austral/South American clade, which includes P. esculentum and P. arachnoideum. All European accesses of P. aquilinum subsp. aquilinum appear in a monophyletic group and are nested within a clade containing the African P. aquilinum taxa (P. aquilinum subsp. capense and P. aquilinum subsp. centralis-africanum). Our results allow us to hypothesize the maternal progenitors of two allotetraploid bracken species, P. caudatum and P. semihastatum. We also discuss the biogeography of bracken in the context of the chloroplast phylogeny. Our study is one of the first to take a worldwide perspective in addressing variation in a broadly distributed species complex.

Key words: allopolyplody; Dennstaedtiaceae; fern; hybridization; phylogeography; population; Pteridium; worldwide.

Describing any biological characteristic of organisms requires adequate sampling so that statements are universally valid. Thus, one should consider the range of variation within the taxon (be it taxonomic, morphological, physiological, ecological, genetic, or geographic) to accurately describe diversity within the group (Hillis, 1998). To capture such variation, one must sample sufficiently to understand both the limits and typical ranges of variation. Such sampling means collecting and examining representatives across the distribution of the taxon. In general, sampling across a taxon's range is easier to do for lower taxonomic levels because successively smaller clades encompass increasingly narrow ranges. However, sampling across geographic ranges becomes logistically challenging for species or species complexes with worldwide (or nearly so) distributions.

Examples of widespread species for which a global perspective has been taken include highly mobile birds (Burg and Croxall, 2004), invertebrates (Lee, 2000; Boyer et al., 2007), and fungi (Hibbett, 2001; Banke and McDonald, 2005). Within green plants, worldwide distributions are common among “invasive” species, i.e., those with recent (less than 500 years), human-mediated, widespread distributions. Examples include aquatic weeds (Barrett, 1989) among others (Holm et al., 1997). At least one study has examined evolutionary history in a widespread invasive species, Cardamine flexuosa (Lihová et al., 2006), but few examples, if any, have examined the phylogenetic structure of a species complex with an ancient (preagricultural; 10,000 years) worldwide distribution (but see Bakker et al., 1995). For a species to occupy such a broad range, it must have a wide ecological amplitude (i.e., ecological valence) or be able to adapt quickly to a wide range of local environmental conditions without speciation. Additionally, to achieve a wide distribution, a species must have a high dispersal and colonization ability, or be an old taxon with a broad ancestral distribution, or both. The genus Pteridium Gled. ex Scop. (bracken fern, Dennstaedtiaceae) represents one such taxon, having achieved a natural worldwide distribution and occupying diverse habitats (Page, 1976, 1986; Holm et al., 1997). Fossil evidence indicates that bracken had achieved a worldwide distribution by the Oligocene, ~23.8 mya (reviewed by Page, 1976), and several lines of evidence indicate that bracken can disperse and establish following long distance dispersal by spores (Punetha, 1991; Rumsey et al., 1991).

Pteridium is often treated as a monotypic genus after Tryon (1941), but most contemporary systematists recognize the genus as a species complex in need of taxonomic revision (Page, 1976; Brownsey, 1989; Page and Mill, 1995; Thomson, 2000b). Bracken ferns are easily recognized and well differentiated from other genera in Dennstaedtiaceae, and many infrageneric taxa within Pteridium have high levels of geographically based morphological structure. However, great confusion in defining infrageneric taxa has resulted from the fact that bracken has high levels of phenotypic plasticity, few diagnostic morphological characters, and the presence of intermediate phenotypes where different morphological forms come into contact, demonstrating that reproductive barriers are incomplete (Page, 1976). These factors, coupled with local taxonomic judgments

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based on geographically biased sampling, has led to a large number of local forms being described as new species, subspecies, or varieties, resulting in a multiplicity of names (Tryon, 1941; Page, 1976; Thomson, 2000a). The genus is distributed worldwide and is notorious as a weed because of its exceptional ability to grow rhizomatously in dense patches, overgrowing open fields and pasture (Tryon, 1941; Holm et al., 1997).

Bracken ferns have a long and complex taxonomic history. The first bracken species were described by Linnaeus in the genus \textit{Pteris} L. (Linnaeus, 1753). Later authors followed this generic circumscription, but Agardh (1839) was the first to examine specimens worldwide and set the brackens apart as section \textit{Orrnthopteris} J. Agardh (Tryon, 1941; Brownsey, 1989). Later, Hooker (1858), in a comprehensive treatment of \textit{Pteris}, subsumed all brackens as varieties of \textit{Pteris aquilina} L. Various authors segregated the brackens from \textit{Pteris}, but it was not until Kuhn (1879) defined \textit{Pteridium aquilinum} (L.) Kuhn that the brackens were widely accepted as a distinct genus (Tryon, 1941).

The last global revision of the genus was Tryon’s (1941) monograph, in which he reduced more than 135 previously named variants into a single species, with two subspecies containing 12 varieties. Subsequent authors have continued to modify the taxonomy of bracken, but most works reflect a geographically limited perspective (Brownsey, 1989; Page and Mill, 1995; Wolf et al., 1995; Speer, 2000; Thomson and Alonso-Amelot, 2002; Gureyeva and Page, 2005; Thomson et al., 2005, 2008). Recent evidence suggests that two \textit{Pteridium} taxa have allopolyploid hybrid origins, and these two tetraploids are currently recognized as segregate species (Tan and Thomson, 1990b; Thomson, 2000a, b; Thomson and Alonso-Amelot, 2002).

Recent work has reexamined the systematic and morphological characters (Thomson and Martin, 1996), characterized the structure of the chloroplast genome (Tan and Thomson, 1990a; Tan, 1991), and used genetic fingerprinting to examine evolutionary history in \textit{Pteridium} (Thomson, 2000a, b). Much of this work contributes toward a taxonomic revision of the genus. The typification of \textit{Pteridium aquilinum} (L.) Kuhn has been revisited, and nomenclature within ‘latiusculum’ morphotypes (N. America, Europe and northeast Asia) has been clarified (Thomson, 2004; Thomson et al., 2008).

Global perspectives on phyleogeography can be inferred using chloroplast DNA sequence variation to detect and reconstruct historical evolutionary events, including population demographics, migration, colonization, and both ancient and contemporary hybridization events (Rieseberg and Soltis, 1991; McCauley, 1995; Rieseberg et al., 1996; Ennos et al., 1999). Variation in chloroplast sequence data has been used to reconstruct phylogenetic relationships among plants as diverse and ancient as land plants to variation among recently diverged populations within a single species. \textit{Pteridium} is a well-defined and evolutionarily isolated genus comprising several closely related taxa. Examining patterns of chloroplast variation presents an excellent opportunity to infer patterns of widespread dispersal, colonization, and divergent evolutionary history on a global scale. This study represents one of the only studies of this work contributes toward a taxonomic revision of the genus. The typification of \textit{Pteridium aquilinum} (L.) Kuhn has been revisited, and nomenclature within ‘latiusculum’ morphotypes (N. America, Europe and northeast Asia) has been clarified (Thomson, 2004; Thomson et al., 2008).

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The objectives of this present study are threefold. First, we examine chloroplast DNA variation within \textit{Pteridium} in a global phylogenetic context to ascertain evolutionary patterns of divergence, dispersal and colonization. Second, we examine maternal ancestry in the two tetraploid taxa hypothesized to be of hybrid origin. Third, we establish a clear framework for developing hypotheses of evolutionary history that can be tested with comparative nuclear sequence data. These results also provide important information necessary for taxonomic revision of infragenetic taxa in \textit{Pteridium}.

**MATERIALS AND METHODS**

**Taxonomic sampling**—Sampling was designed to cover the range of morphological and geographical diversity within \textit{Pteridium}, representing nearly all currently recognized species, and most infraspecific taxa, with the exception of \textit{P. aquilinum} subs. \textit{feei} (W. Schaffn. ex Féé) J. A. Thomson, Mickel & Mehltreter, endemic to central Mexico. Determination of specimens used in this study follow Thomson and Alonso-Amelot (2002), Thomson (2004), and Thomson et al. (2005, 2008). In addition to the materials used here, a large series of specimens from major herbaria has been examined in the course of the taxonomic revisions listed. We sampled 77 bracken specimens, most of which were included previously in the taxonomic studies described. Tissue samples were collected from either wild sources or from sporophytes grown in a common garden derived from known wild sources and propagated from rhizome segments or mass sporangia sowings (Thomson, 2000a). Complete voucher information with geographic sources and GenBank accession numbers is provided in Appendix 1.

**DNA extraction, PCR amplification, and sequencing**—Total genomic DNA was extracted from tissue that had been silica-dried, freeze-dried, or pickled in CTAB/NaCl/ascorbate (Thomson, 2002). The chloroplast markers trnS\textit{GAC}-\textit{tpS4} spacer+gene and \textit{rpl}16 intron were amplified in 25-μL polymerase chain reactions (PCR) using the fern-specific primers published in Small et al. (2005). Each PCR reaction contained 25–50 ng of template DNA, 1× Promega PCR buffer (Promega, Madison, Wisconsin, USA), 1.5 mM MgCl₂, 0.20 mM of each dNTP, 0.25 μM each of the forward and reverse primers, and ~12.5 U Taq polymerase. Most PCR reactions were completed under a standard temperature cycle procedure beginning with a 2 min denaturation step at 94°C, followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C. Problematic samples were amplified using a slow ramp thermal cycle protocol that began with a 2 min denaturation step at 95°C, followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C. Problematic samples were amplified using a slow ramp thermal cycle protocol that began with a 2 min denaturation step at 95°C, followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C. Problematic samples were amplified using a slow ramp thermal cycle protocol that began with a 2 min denaturation step at 95°C, followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C.

PCR products were directly cycle-sequenced in CTAB/NaCl/ascorbate (Thomson, 2002). The chloroplast markers trnS\textit{GAC}-\textit{tpS4} spacer+gene and \textit{rpl}16 intron were amplified in 25-μL polymerase chain reactions (PCR) using the fern-specific primers published in Small et al. (2005). Each PCR reaction contained 25–50 ng of template DNA, 1× Promega PCR buffer (Promega, Madison, Wisconsin, USA), 1.5 mM MgCl₂, 0.20 mM of each dNTP, 0.25 μM each of the forward and reverse primers, and ~12.5 U Taq polymerase. Most PCR reactions were completed under a standard temperature cycle procedure beginning with a 2 min denaturation step at 94°C, followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C. Problematic samples were amplified using a slow ramp thermal cycle protocol that began with a 2 min denaturation step at 95°C, followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C. Problematic samples were amplified using a slow ramp thermal cycle protocol that began with a 2 min denaturation step at 95°C, followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C. Problematic samples were amplified using a slow ramp thermal cycle protocol that began with a 2 min denaturation step at 95°C, followed by 30 cycles of 94°C, 48°C and 72°C each for 1 min; finishing with a 7 min elongation step at 72°C.
lengths across partitions. Two independent runs, with six chains each, were conducted simultaneously for 100 000 000 generations. Parameter estimates were sampled every 1000 generations. The average standard deviation of split frequencies and the potential scale reduction factor (PSRF) were calculated after discarding the first 25% of the generations (2.5 million generations) as burn-in to assess topological and parameter convergence (respectively) between the two runs. Bayesian inference clade credibility values (i.e., posterior probabilities for cladades) and tree posterior probabilities were also calculated after the first 2.5 million generations were discarded as burn-in.

Alignment of two outgroup taxa [Dennstaedthia davalliodae (R. Br.) T. Moore and Hypolepis muelleri N. A. Wakef.] with the Pteridium sequences was not possible due to a high level of divergence (both sequence substitution saturation and ambiguous indels). Inclusion of these taxa in preliminary phylogenetic analyses contributed to the destabilization of clades within Pteridium. Because of this, the midpoint rooting method implemented in PAUP* was used to root the Pteridium phylogeny in both MP and BI analyses. While this approach is not ideal, it has yielded acceptable results in other taxa where appropriate data are not available (Schuettpelz and Hoot, 2006). The monophyly of Pteridium and the midpoint root was confirmed by outgroup phylogenetic analysis of rbcL sequences from a subset of our sampled Pteridium taxa aligned with additional outgroup taxa from Dennstaedtiaceae (data not shown).

**Biogeographic analysis**—Broad geographic regions were mapped onto the phylogeny using the parsimony criterion to elucidate major biogeographic events. Geographic areas were coded as six unordered character states

<table>
<thead>
<tr>
<th>Geographic Area</th>
<th>Character State</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central &amp; South America</td>
<td>1</td>
</tr>
<tr>
<td>Europe</td>
<td>2</td>
</tr>
<tr>
<td>Asia</td>
<td>3</td>
</tr>
<tr>
<td>Africa</td>
<td>4</td>
</tr>
<tr>
<td>Australia</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
</tbody>
</table>

with additional outgroup taxa from Dennstaedtiaceae (data not shown).

**Sequence characteristics**—The aligned trnS–rps4 spacer+gene and rpl16 intron data matrices included 1018 and 753 nucleotide sites with 29 and 25 variable sites, respectively. Of those variable sites, 22 were parsimony-informative in the trnS–rps4 spacer+gene, and 21 sites were parsimony-informative in the rpl16 intron. There were two 5–bp repeat sequence indels located 32 nucleotides apart in the trnS–rps4 spacer. Individuals either lacked one or both of the repeat sequences, resulting in three observed indel haplotypes. These haplotypes have been reported previously (Thomson et al., 2008), and our study is consistent with those findings, so we adopt their nomenclature here (haplotypes A, B, and C). This study newly establishes the haplotypes for Pteridium aquilinum subsp. decompositum (Gaudich.) Lamoureux ex J. A. Thomson, P. caudatum (L.) Maxon, and P. semihastatum (Wall. ex J. Agardh) S. B. Andrews, and extends coverage of P. aquilinum subsp. pinetorum (C. N. Page & R. R. Mill) J. A. Thomson to eastern North America. Haplotype B (the presence of the first repeat sequence, GTTTTT) was observed in P. aquilinum subsp. aquilinum from Europe and both P. aquilinum subsp. centrali-africanum Hieron. and P. aquilinum subsp. capense (Thunb.) C. Chr. in Africa, while haplotype C (the presence of the second repeat sequence, AGTCT) was observed in P. arachnoideum (Kaulf.) Maxon in Central and South America, P. esculentum (G. Forst.) Cockayne in Australia, New Zealand, and New Caledonia, P. semihastatum in Australia and Malaysia, and a single accession of P. caudatum from Costa Rica. Haplotype A (the absence of both repeat sequences) was observed in the remaining taxa (i.e., P. aquilinum from Asia and North America and the remaining P. caudatum accessions). Comparison of bracken haplotypes with those found in Dennstaedthia davalliodae and Hypolepis muelleri reveals that haplotype C is likely to be plesiomorphic. Parsimony character reconstruction of these indels on our phylogeny indicates that the second repeat was lost in the common ancestor of P. aquilinum, and the first repeat was gained in the common ancestor of the African and European P. aquilinum subspecies. The evolution of these indels is mapped on the Bayesian phylogeny (Fig. 1).

**Phylogenetic analyses**—Maximum parsimony (MP) analysis of the concatenated data set resulted in 12 most parsimonious trees with a length of 59 (consistency index, CI = 0.9153; retention index, RI = 0.9892). The best fit model used in Bayesian inference (BI) was GTR+Γ and HKY+Γ for trnS–rps4 spacer+gene and rpl16 intron, respectively. The AICc weight of the best fit model was 1.4 times greater than the next-best model for trnS–rps4 spacer+gene and 1.3 times greater for rpl16 intron. The topology of the MP strict consensus tree (Fig. 2A) is congruent with the BI phylogeny (Fig. 1). MP bootstrap support (BS) and BI posterior probabilities of clades (PP, i.e., clade credibility values) are reported on the tree for supported nodes (Fig. 1). Two fully supported clades are resolved at the base of the phylogeny, separating P. arachnoideum, P. esculentum, and P. semihastatum from P. aquilinum. There is a basal polytomy within the P. aquilinum clade. A single accession of the tetraploid species P. caudatum is grouped with P. semihastatum in the former clade, while the remaining P. caudatum accessions are grouped with subspecies pseudo-caudatum (Clute) Hultén in the P. aquilinum clade.

**Biogeographic analysis**—Mapping geographic areas onto the phylogeny revealed a number of biogeographic patterns (Fig. 2A). The Austral taxon P. esculentum is found in a clade with P. arachnoideum from South and Central America and P. semihastatum from Southeast Asia and Australia, forming a group with a post-Gondwanan (after Africa split away) southern continental distribution referred to here as the Austral/South American clade. Within the P. aquilinum clade, basal biogeographic patterns are unresolved, but the European P. aquilinum subsp. aquilinum accessions emerge from within the African brackens and P. aquilinum subsp. decompositum in Hawaii is in a clade with all of the sampled accessions of P. aquilinum subsp. pubescens (Underw.) J. A. Thomson, Mickel & Mehltreter from western North America. The locations of specimens included in this study are indicated on a global map (Fig. 2B).

**Phylogenetic relationships among bracken taxa**—Pteridium species form two distinct and fully supported basal clades (100% maximum parsimony bootstrap—BS; 1.0 Bayesian posterior probability—PP). The first clade contains P. esculentum from Australia, New Caledonia, and New Zealand and P. arachnoideum from the neotropics, but neither of these species form distinct monophyletic groups. The tetraploid species P. semihastatum and a single accession of the tetraploid species P. caudatum from Costa Rica are included in this first main clade and together are monophyletic (85% BS, 1.0 PP). This tetraploid group is derived from a clade containing an accession of P. esculentum from New Caledonia (55% BS, 0.99 PP). The second main clade in Pteridium includes all of the P. aquilinum accessions we sampled and three of the four P. caudatum accessions we included in our analysis (Costa Rica and northern South America). This second main Pteridium clade (the P. aquilinum clade) is fully supported as monophyletic (100% BS, 1.0 PP), but phylogenetic relationships of lineages within this
Fig. 1. Bayesian phylogram inferred from the concatenated trnS-rpS4 spacer-gene and rpL16 intron alignment. Branch lengths are proportional to the number of nucleotide substitutions/site. Support values from maximum parsimony bootstrap analysis (BS) and Bayesian posterior probabilities for clades (PP) are given above branches (BS/PP). Clades receiving less than 50% BS or 0.5 PP are indicated with a dash (—).
Fig. 2. Biogeography of bracken. (A) Broad geographic distribution of bracken specimens traced onto the maximum parsimony phylogeny using parsimony-based character reconstruction. Geographic areas were coded as six unordered character states: Hawaii, North/South America, Europe, Asia/India, Africa, Austral. (B) Global map of sampled bracken specimens, color-coded consistently with the geographic area character states in (A).
group are not resolved. The three \textit{P. caudatum} accessions in the \textit{P. aquilinum} clade are supported as monophyletic with 63\% BS and 0.99 PP and are included in a polytomy with all of the \textit{P. aquilinum} subsp. \textit{pseudocaudatum} accessions we sampled (Florida, USA) and a \textit{P. aquilinum} subsp. \textit{latusculus} (Desv.) Hultén accession from Michigan, USA (64\% BS, 0.98 PP). \textit{Pteridium aquilinum} subsp. \textit{latusculus} accessions are scattered throughout the \textit{P. aquilinum} clade and therefore is not monophyletic in our analyses. This phylogenetic pattern in \textit{P. aquilinum} subsp. \textit{latusculus} may be explained if this taxon is primarily defined by plesiomorphies (morphologically) and/or if it is susceptible to widespread hybridization (Speer et al., 1998). Together, \textit{P. aquilinum} subsp. \textit{japonicum} (Nakai) A. Löve & D. Löve from coastal Asia and \textit{P. aquilinum} subsp. \textit{pinetorum} from the boreal Asian interior form a clade supported by 63\% BS and 0.98 PP, but neither subspecies forms a monophyletic group. \textit{Pteridium aquilinum} subsp. \textit{decompositum} (Gaudich.) Lamoureux ex J. A. Thomson from Hawaii and \textit{P. aquilinum} subsp. \textit{pubescens} from western North America and a single \textit{P. aquilinum} subsp. \textit{latusculus} accession from Massachusetts, USA, form a polytomy, supported by 86\% BS and 1.0 PP. This pattern suggests a trade wind dispersal route from North America to Hawaii consistent with other wind-dispersed fern taxa (Geiger et al., 2007). \textit{Pteridium aquilinum} subsp. \textit{wightianum} (J. Agardh) W. C. Shieh from the Himalayas of northern India (isolate YGIN) emerges from the \textit{P. aquilinum} clade polytomy, while the remaining \textit{P. aquilinum} subsp. \textit{wightianum} accessions from Sri Lanka and Southeast Asia form a monophyletic group supported by 87\% BS and 1.0 PP. Isolate YGIN was also distinguished from other \textit{P. aquilinum} subsp. \textit{wightianum} by both a distinctive nuclear genome marker and morphology in an earlier study (Thomson, 2000a). All the \textit{P. aquilinum} subsp. \textit{aquilinum} accessions we sampled (Europe) are monophyletic (62\% BS, 1.0 PP), but emerge from a paraphyletic grade of African \textit{P. aquilinum} subsp. \textit{capense}. Also emerging from \textit{P. aquilinum} subsp. \textit{capense} is \textit{P. aquilinum} subsp. \textit{centrali-africanaum}, which is supported as monophyletic with 87\% BS and 1.0 PP.

We emphasize here that apparent paraphyly at the level of species or below in our data set should not be used to recircumscribe taxa for two reasons. First, we have examined only variation in chloroplast DNA. Because of lineage sorting and recombination, we have no reason to expect exactly the same patterns for nuclear genes (Soltis et al., 1992). Second, some models of speciation predict a high frequency of paraphyly among recently diverged species (Rieseberg and Brouillet, 1994; Funk and Omland, 2003).

**Hybridization and the origin of the tetraploid species**—Although the majority of \textit{Pteridium} taxa are at the same ploidy level (2\(N\) = 104; Page, 1976), recent evidence suggests that \textit{P. semihastatum} and \textit{P. caudatum} are tetraploid taxa (4\(N\) = 208; Tan and Thomson, 1990b; Thomson, 2000a, b; Thomson and Alonso-Amelot, 2002). Allopolyploidy appears to be a common speciation process in plants, and it often involves a triploid intermediate (Ramsey and Schemske, 1998). Two main types of evidence for allopolyploid speciation can be gathered. Nuclear markers, preferably fixed for different alleles in the different parents, can show additive effects in allotetraploids, and simultaneously reveal both parents, whereas uniparentally inherited markers will reveal one parent and whether there have been multiple origins (Soltis and Soltis, 1993). Although we have no evidence for the mechanism of inheritance of chloroplast DNA in \textit{Pteridium}, several studies have demonstrated maternal inheritance in other species of ferns (Gastony and Yatskievych, 1992; Vogel et al., 1998).

\textit{Pteridium caudatum} and \textit{P. semihastatum} have been shown to be tetraploid bracken species based on DNA c-values, spore size, guard cell length, and morphology of the cells in the false induc- sium (Thomson, 2000a, b; Thomson and Alonso-Amelot, 2002). Hypothesized allopolyploid origins have been inferred from intermediate morphology and additive AP-PCR DNA genotypes of these two species (Thomson, 2000a; Thomson and Alonso-Amelot, 2002). These studies have suggested putative progenitors of \textit{P. caudatum} to be \textit{P. aquilinum} subsp. \textit{pubescens} in the north and \textit{P. arachnoideum} in the south (Thomson and Alonso-Amelot, 2002). These data have similarly been used to infer the putative progenitors of \textit{P. semihastatum} in Southeast Asia and northern Australasia as \textit{P. aquilinum} subsp. \textit{wightianum} (=\textit{P. revolutum} sensu Brownsey, 1989) and \textit{P. esculentum} (Thomson, 2000a; Thomson and Alonso-Amelot, 2002). Our data based on the phylogeny of chloroplast sequences support the maternal parentage of \textit{P. semihastatum} to be \textit{P. esculentum}, while \textit{P. cau- datum} emerges from two parts of the phylogeny (with North American \textit{P. aquilinum} subsp. \textit{semicaudatum} \textit{latiusculum} and embedded in the Austral/South American clade, which includes \textit{P. arachnoideum}, \textit{P. esculentum}, and \textit{P. semihastatum}). Two possible explanations for the phylogenetic placement of our \textit{P. caudatum} accessions seem plausible: (1) reciprocal and multiple hybrid origins of this species or (2) a single hybrid origin of \textit{P. caudatum} with the maternal parent as \textit{P. aquilinum} subsp. \textit{pseudocaudatum} \textit{latiusculum} with subsequent introgression of the tetraploid \textit{P. semihastatum} chloroplast genomes. Additionally, the Austral/South American parent of \textit{P. caudatum} may be an unsampled haplotype of the co-occurring \textit{P. arachnoideum} from South America or long-distance hybridization from an Austral member of that clade. Long-distance hybridization in bracken has been documented between eastern North America and Scotland (Rumsey et al., 1991). To evaluate these hypotheses, we need nuclear genetic data to determine parentage and additivity of genomic elements in the allotetraploid species. Nuclear sequence data promise to be especially useful in elucidating specific phylogenetic relationships among the various bracken taxa (Schuettpezl et al., 2008).

**Phylogeography of bracken**—\textit{Pteridium} occurs throughout the world, except in hot and cold desert regions. Range-wide sampling is necessary to adequately assess variation within the genus and delineate taxa. Only one such study has been accomplished in \textit{Pteridium}: that of Tryon (1941). While the varieties in Tryon’s treatment are often geographically based, he only briefly discussed the geographic ranges of each taxon and instead focused on the morphological differences between the varieties. Page (1976) expanded and updated the ecological and phytogeographic information known for each of Tryon’s varieties, but emphasized that the adoption of Tryon’s nomenclature was done for practical reasons rather than validation of these taxa and that an updated worldwide revision of the genus was needed. While our sampling strategy attempts to cover the range of variation in \textit{Pteridium} worldwide, our study would benefit from additional sampling from Madagascar, South and Central America, the Caribbean, and parts of North America.

Recent phylogenetic analyses of ferns, which have included extensive taxonomic sampling and have used fossils for molecular date calibrations, have estimated divergence dates be-}
et al., 2004) and 92 mya (Prayer et al., 2004). These estimates represent upper ages for the evolution of Pteridium and correspond to the Cretaceous period. Fragments of fossils attributable to Pteridium are known from the Tertiary period as far back as the Oligocene (33.7–23.8 mya) from Europe to Australia, suggesting that even by this time, bracken may have achieved a widespread distribution (Page, 1976). During the time when bracken likely originated and the earliest diversifications may have occurred (from 100 to 30 mya), Africa and India separated from a southern landmass that included Australia, Antarctica, and South America (Scotese, 2001). This separation may correspond to the basal divergence among brackens, splitting the Austral/South American clade from the P. aquilinum clade of African and Laurasian affinity. The polytomy in the P. aquilinum clade may represent a rapid radiation of African and Indian bracken as they moved north, colliding with a warm temperate and paratropical Laurasia by the end of the Paleocene (about 50 mya). Alternatively, the polytomy in the P. aquilinum clade may result from insufficient data to distinguish phyllogenetic relationships among lineages in the clade.

As continents have come to their modern global locations, and with the onset of the current ice age, the Quaternary glaciation beginning 2.5 mya, there have been periods of extensive ice sheets and cold, dry steppe coming into Europe and North America and likely driving bracken into southern refugia. These repeated glacial periods may be invoked to explain the phylogeographic pattern whereby the European brackens are derived from within the African taxa if Africa served as a source for colonization of Europe during recent geological times. The strong geographic structure observed at large (continental) scales among bracken taxa is consistent with patterns expected under a vicariance model, with range expansion across land bridges and only occasional long-distance dispersal resulting in hybrid forms. This evidence contradicts the common assumption in ferns that repeated long-distance dispersal of spores will erode biogeographic patterns resulting from vicariance in widespread species via constant gene flow (Tryon, 1985; Wolf et al., 2001). Recent empirical evidence in Ceratopteris Brongn. shows that genetic reproductive barriers can accumulate quickly in allopatry, thereby restricting gene flow and potentially maintaining the signature of vicariance (Nakazato et al., 2007).

Conclusions—This study highlights the importance of range-wide perspectives when undertaking evolutionary and monographic studies. Any taxonomic revision of Pteridium must reflect a global perspective and should include information from diverse areas of research, including morphology, cytology, genetics, and reproductive biology. To evaluate the biogeographic and hybrid origin hypotheses presented here, we will need a better-resolved phylogenetic tree — and correspond to additional reticulation and species phylogeny with increased population-level sampling that might also allow us to examine additional reticulation and lineage sorting in the evolutionary history of Pteridium as well as evaluate contemporary and historical gene flow.

LITERATURE CITED


