BIogeographic disjunction between eastern Asia and north America in the Adiantum pedatum complex (Pteridaceae)\textsuperscript{1}

Jin-Mei Lu\textsuperscript{2,4}, De-Zhu Li\textsuperscript{2,3}, Sue Lutz\textsuperscript{4}, Akiko Soejima\textsuperscript{5}, Tingshuang Yi\textsuperscript{3}, and Jun Wen\textsuperscript{4,6}

\textsuperscript{2}Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China; \textsuperscript{3}Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species, Kunming, Yunnan 650204, China; \textsuperscript{4}Department of Botany, National Museum of Natural History, MRC 166, Smithsonian Institution, Washington, D.C. 20013-7012 USA; \textsuperscript{5}Biological Sciences, Graduate School of Science and Technology, Kumamoto University, Kurokami, Kumamoto 860-8555, Japan

Premise of the study: Biogeographic analyses of ferns with an eastern Asian–North American disjunction are few. The Adiantum pedatum complex has such a disjunct distribution. The monophyly of the complex needs to be tested and diversification history of the four species needs to be reconstructed.

Methods: Plastid (atpA, atpB, rbcL, trnL-F, and rps4-trnS) sequences of 100 accessions representing the biogeographic diversity of Adiantum were analyzed with parsimony and Bayesian inference. Biogeography of the Adiantum pedatum complex was inferred using programs DIVA and LAGRANGE. Divergence times of clades were estimated with the program BEAST.

Key results: The A. pedatum complex is monophyletic and sister to the eastern Asian A. edentulum. Accessions of A. pedatum do not form a clade; instead three subgroups are recognizable. The clade of A. aleuticum and A. viridimontanum is nested within A. pedatum. The Asian A. myriosorum is sister to the A. pedatum-A. aleuticum clade. Both DIVA and LAGRANGE analyses suggest an eastern Asian origin of the A. pedatum complex. The age of the crown A. pedatum complex is dated to be at 4.27 (2.24–6.57) million years ago.

Conclusions: The currently recognized eastern Asian–North American disjunct species A. pedatum needs to be segregated into three species, corresponding to populations in eastern North America, China, and Japan. The eastern Asian–North American disjunction in the complex is inferred to be the result of two intercontinental migrations, one from eastern Asia into North America in the late Tertiary and the other from North America back to eastern Asia in the Pleistocene.

Key words: Adiantum pedatum; biogeography; eastern Asia; eastern North America; North America; intercontinental disjunction; phylogeny; Pteridaceae.

The intercontinental disjunctions between eastern Asia (EA) and North America (NA) have attracted much attention from evolutionary biologists in the last two decades (Parks and Wendel, 1990; Wen, 1998, 1999; Xiang et al., 1998; Manchester, 1999; Manos and Donoghue, 2001; Donoghue and Smith, 2004; Wen et al., 2010). The EA-NA disjunction was suggested to have involved multiple historical events at different geologic times in different groups (Tiffney, 1985a, b; Manchester, 1999; Wen, 1998, 1999; Xiang et al., 1998). Many recent phylogenetic analyses were conducted to better understand this intercontinental disjunct pattern using phylogenetic analyses, fossil-calibrated molecular dating, and reconstruction of ancestral geographic ranges. Analyses so far have emphasized seed plants (reviewed in Wen et al., 2010).

Biogeographic analyses on ferns are relatively few in general in comparison with those on seed plants and animals. Some recent biogeographic studies were carried out on Australasian ferns (Perrie et al., 2003) and Hawaiian ferns (e.g., Geiger and Ranker, 2005; Driscoll and Barrington, 2007; Geiger et al., 2007). Most disjunctions between eastern Asia and North America in flowering plants are at the generic level or among species groups, whereas most fern taxa are closely related species or even the same species (Li, 1952; Wen, 1999). Iwatsuki and Ohba (1994) recorded five taxa distributed exclusively in eastern North America and Japan. Iwatsuki (1994) included 10 species occurring only in North America and Japan. Zang (1998) reported four genera disjunct between China and North America. Osmunda has a long evolutionary history with fossils founds nearly identical to those of the living O. claytoniana dating back to the Triassic (Phipps et al., 1998). Both Osmunda claytoniana and Osmundastrum cinnamomeum occur in North America and eastern Asia (Iwatsuki, 1994; Iwatsuki and Ohba, 1994). The phylogenetic analysis and classification of Osmundaceae by Metzgar et al. (2008) provided important insights into the biogeographic divergence within this ancient family.
Onoclea is distributed in eastern Asia and eastern North America, but fossils referable to Onoclea were widely distributed in the northern hemisphere during the Cretaceous and early Tertiary (Kato, 1993). Onoclea sensibilis is native to North America and eastern Asia and has become naturalized in western Europe, with two varieties recognized: var. interrupta in eastern Asia and var. sensibilis in eastern North America (Iwatsuki and Ohba, 1994). The walking ferns are another well-known example showing the disjunction between these two continents. The eastern North American Asplenium rhizophyllum and the eastern Asian A. ruprechtii (i.e., Camptosorus sibiricus Rupr.) inhabit similarly shaded cliffs and mossy boulders and possess the same “walking” habit (Kato, 1993; Judd et al., 2008). In the Adiantum pedatum complex (Pteridaceae), the eastern Asian populations are often inferred to be sister to all North American populations (e.g., Lee et al., 1996; Xiang et al., 1998; Wen, 1999). Kato (1993) suggested that the eastern Asian–eastern North American disjunct pattern in some fern taxa may have arisen multiple times via complex climatic and geologic events.

Adiantum comprises 150–200 species, with a wide distribution except in extremely cold or dry regions (Ching, 1957; Tryon and Tryon, 1982; Lin, 1990; Tryon et al., 1990; Paris, 1993; Hoshizaki and Moran, 2001). Most species of this genus occur in tropical to subtropical regions, with the greatest diversity in the neotropics (Huiet and Smith, 2004). Recent analyses suggest that Adiantum may be paraphyletic (Schuettpelz and Pryer, 2007; Bouma et al., 2010). The Adiantum pedatum complex includes four species distributed in North America and eastern Asia extending to the Himalayas (Fig. 1). This complex is characterized by its pedately divided frond in clusters from the clump-forming rhizome. The A. pedatum complex contains the northernmost taxa in Adiantum and can extend to Sitka (57° N), Alaska in North America [Adiantum aleuticum (Rupr.) C.A. Paris] and to Xiaoxinganling (50° N) of Heilongjiang province of China in eastern Asia (A. pedatum L.). There are two species (A. pedatum and A. myriosorum Baker) in eastern Asia, three species (A. pedatum, A. aleuticum, and A. viridimontanum C. A. Paris) in eastern North America, and only A. aleuticum in western North America (Lin, 1990; Paris, 1993). Adiantum viridimontanum has been proposed as an allotetraploid derivative of a sterile hybrid between A. pedatum and A. aleuticum (Paris and Windham, 1988), and it is restricted to north-central Vermont, USA and adjacent southern Quebec, Canada (Paris, 1991a).

The Adiantum pedatum complex has received much attention from botanists. Paris and Windham (1988) showed that the eastern serpentine maidenhair is a disjunct element of the western North American A. aleuticum, and A. pedatum in eastern Asia and eastern North America exhibits little morphological differentiation. Adiantum pedatum and A. myriosorum are morphologically highly distinct (Lin, 1990). Therefore, the A. pedatum complex may provide some unique insights into the rates of molecular and morphological evolution of disjunct ferns between eastern Asia and North America. Paris (1991b) reported three diploid lineages, a Japanese lineage, a western North American lineage, and an eastern North American lineage, in the A. pedatum complex; however, the relationships among the three lineages were not resolved. She proposed that A. aleuticum and the Japanese species shared a common ancestor. Paris (1991b) hypothesized that the A. pedatum complex arose as a component of the mixed mesophytic forest that was widespread throughout the northern hemisphere during much of the Tertiary, and the ancestor of the A. aleuticum-Japanese species was from eastern Asia and migrated to western North America through the Bering land bridge in the late Tertiary. Nakato and

![Fig. 1. Distribution of the Adiantum pedatum complex showing biogeographic disjunctions between eastern Asia and North America (modified from Paris, 1991b). Adiantum pedatum: red; A. myriosorum: green; A. aleuticum: blue; and A. viridimontanum: yellow.](image-url)
Kato (2005) proposed it is likely that the \( \chi = 29 \) diploid(s) originated within the \( \chi = 30 \) diploid populations of the Adiantum pedatum complex in Asia, then spread to North America via the Bering land bridge during the late Tertiary and the Quaternary.

Our objectives for this study were to (1) elucidate the phylogenetic relationships among the four species of the complex and (2) explore the biogeographic diversification of intercontinental disjunctions of this complex. We employed sequences of five chloroplast markers (atpA, atpB, rbcL, trnL-F, and rps4-trnS) and examined all four species of the A. pedatum complex and their close relatives.

**MATERIALS AND METHODS**

**Taxon sampling**—We sampled all four species of the A. pedatum complex from both eastern Asia and North America. To test the monophyly of the complex and provide a broader phylogenetic framework to estimate divergence times of clades, we sampled 100 accessions representing 41 species and varieties and 12 unidentified taxa covering the biogeographic diversity of Adiantum. All taxa included in this study, together with voucher information and collection sites, are listed in Appendix 1.

**DNA extraction, amplification, and sequencing**—Total DNAs were extracted from 15 mg of silica-gel-dried leaf material using DNeasy (QIAGEN, Santa Clara, California, USA) extraction kits. All amplifications were performed in a 25-µL reaction mixture using primers as shown in Table 1. The PCR reactions contained 1 U Taq DNA polymerase (Bioline, Taunton, Massachusetts, USA), 10X buffer, 0.25 mM/L dNTP, 1.5 mM/L Mg²⁺, 0.5 mM/L of each primer, 1 mg/mL BSA, and 25–60 ng sample DNA. For atpA and atpB, reactions were incubated at 95°C for 3 min, then cycled 35 times (95°C for 1 min, 50°C for 1 min, 72°C for 10 min) followed by a final extension for 10 min at 72°C. For rbcL and rtrnL-F, reactions were incubated at 95°C for 3 min, then cycled 35 times (95°C for 1 min, 51°C for 1 min, 72°C for 80 s), followed by a final extension for 10 min at 72°C. For rps4-trnS, reactions were incubated at 95°C for 3 min, then cycled 35 times (94°C for 30 s, 58°C for 45 s, 72°C for 80 s), followed by a final extension for 10 min at 72°C.

The PCR products were purified using the polyethylene glycol (PEG)–NaCl method of Kusukawa et al. (1990). Sequencing reactions were conducted with BigDye Terminator v3.1 Cycle Sequencing chemistry (Applied Biosystems, Foster City, California, USA), and the sequencing reactions were run on an ABI 3730 automated sequencer (Applied Biosystems). Total reaction volume was 10 µL (1 µL PCR template, 1.75 µL 5X Sequencing Buffer, 0.5 µL primer [5 µmol/L], 0.25 µL BigDye Terminator, and 6.5 µL ddH₂O).

The resulting sequences were assembled using the program Sequencher 4.8 (Gene Codes Corp., Ann Arbor, Michigan, USA). Sequences obtained in this study have been deposited in GenBank (Appendix 1). They were aligned using the program CLUSTAL_X version 1.83 (Thompson et al., 1997), followed by manual adjustments using the program Se-Al v.2.0a11 (Rambaut, 2007).

**Phylogenetic analyses**—Phylogenetic trees were constructed using maximum parsimony (MP) and Bayesian Markov chain Monte Carlo (MCMC) inference (BI; Yang and Rannala, 1997). The MP analyses was performed using the program PAUP⁺ version 4.0b10 (Swofford, 2003), treating gaps as missing data and using the heuristic search options with 1000 random replicates, stepwise data addition, tree-bisection-reconnection (TBR) swapping and MulTrees option on. Bootstrap analysis (Felsenstein, 1985) was performed with 1000 replicates to evaluate internal support, with 100 random taxon addition replicates saving all optimal trees at each step. It is difficult to code the indel in a matrix including many outgroups, and we conducted an additional analysis based on a small data set excluding Vittaria and most tropical Adiantum species and coded all informative indels as binary or multistate characters (Simmons and Ochoterena, 2000).

The optimal model of molecular evolution was determined by the Akaike information criterion (AIC) using the program MODELTEST version 3.7 (Posada and Crandall, 1998; Posada and Buckley, 2004). Bayesian analyses were implemented in the program MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) with the model GTR+I+G. We used four chains, with random initial trees. Trees were generated for 2000000 generations, sampling every 100 generations. Trees sampled before stable posterior probability (PP) values had been reached were excluded from consensus as a burn-in phase (initial 20% of the sampled trees). Nodes receiving bootstrap support (BS) of <70% in the MP analyses, or PP of <0.95 in the BI analyses, were not considered as well supported.

Congruence between plastid data sets was tested using the incongruence length difference (ILD) test (Farris et al., 1994), as implemented by the partition homogeneity test in PAUP⁺ for 100 replicates (heuristic search, simple addition, TBR branching swapping), each saving a maximum of 100 trees per replicate (Norup et al., 2006).

**Biogeographic analyses**—The dispersal–vicariance (DIVA) (Ronquist, 1996) and the maximum likelihood-based analyses with the program LAGRANGE (Ree and Smith, 2008) were performed to infer the biogeographic diversification history of the A. pedatum complex. Seven areas of endemism were defined to reflect our emphasis on intercontinental diversification of Adiantum: EA, NA, New Zealand and Australia, tropical America, tropical Asia, continental Africa, and Madagascar. The program DIVA reconstructs ancestral distributions in a given phylogeny without any prior assumptions about area relationships, and considers vicariance, dispersal and extinction as viable biogeographic events (Ronquist, 1996, 1997). The DIVA analyses were conducted using DIVA version 1.1 (Ronquist, 1996). Ancestral areas were inferred with the “maxareas” constrained to 2 because few species (e.g., A. capillus-veneris) occur in more than two areas (Donoghue et al., 2001). LAGRANGE version 2.0 (Ree and Smith, 2008) was employed to run the analysis with a simple model of one rate of dispersal and extinction constant over time and among lineages. The program cannot find the most likely ancestral areas at a node and the split of the areas in the two descendant lineages, it also calculates the probabilities of the most likely areas at each node (Ree et al., 2005; Ree and Smith, 2008). Analyses were conducted on an ultrametric tree estimated using the log-normal relaxed clock model (Drummond et al., 2006) implemented in an MCMC with the program BEAST version 1.4.8 (Drummond and Rambaut, 2007), using the ML tree as the start tree and a GTR+I+G model of nucleotide substitution, with a total run of 10 million generations. This tree was imported.

**Table 1. Primers used in this study, including sequences, target regions, and references.**

<table>
<thead>
<tr>
<th>Primer names</th>
<th>Primer sequences (5′-3′)</th>
<th>Target region</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESATPF412F</td>
<td>GARCARGTTCGACACGAAAT</td>
<td>atpA</td>
<td>Schuettpeäl et al. (2006)</td>
</tr>
<tr>
<td>ESATPA535F</td>
<td>AACGAGTACTGACAGTACAT</td>
<td>atpA</td>
<td>Schuettpeäl et al. (2006)</td>
</tr>
<tr>
<td>ESATPA877R</td>
<td>CATGGCCGAGATGCTTCTCG</td>
<td>atpA</td>
<td>Schuettpeäl et al. (2006)</td>
</tr>
<tr>
<td>ESTKNR46F</td>
<td>AAACGTTTGGTCAACTTTCAG</td>
<td>rbcL</td>
<td>Little and Barrington (2003)</td>
</tr>
<tr>
<td>ESATB172F</td>
<td>AAACGTTTGGTCAACTTTCAG</td>
<td>trnL-F</td>
<td>Little and Barrington (2003)</td>
</tr>
<tr>
<td>ESATPE454R</td>
<td>ATTAGGAGACACACACACAGGA</td>
<td>rbcL</td>
<td>Little and Barrington (2003)</td>
</tr>
<tr>
<td>RbcL-1F</td>
<td>ATTAGGAGACACACACACAGGA</td>
<td>rbcL</td>
<td>Little and Barrington (2003)</td>
</tr>
<tr>
<td>RbcL-1379R</td>
<td>TTCAAGGGAGGAGGACTTGGAGG</td>
<td>trnL-F</td>
<td>Taberlet et al. (1991)</td>
</tr>
<tr>
<td>TmlLFc</td>
<td>CGCAAAATGGTACGACGACGCG</td>
<td>trnL-F</td>
<td>Taberlet et al. (1991)</td>
</tr>
<tr>
<td>TmlLF-f</td>
<td>ATAGGAGACACACACACAGGA</td>
<td>trnL-F</td>
<td>Taberlet et al. (1991)</td>
</tr>
<tr>
<td>TmlLF-p1</td>
<td>TTCCGGAGGCTGCGACAGTCT</td>
<td>trnL-F</td>
<td>Taberlet et al. (1991)</td>
</tr>
<tr>
<td>trnS</td>
<td>ATAGGAGACACACACACAGGA</td>
<td>trnS</td>
<td>Taberlet et al. (1991)</td>
</tr>
<tr>
<td>rps4.5</td>
<td>ATAGGAGACACACACACAGGA</td>
<td>rps4-trnS</td>
<td>Taberlet et al. (1991)</td>
</tr>
</tbody>
</table>

Souza-Chies et al. (1997)
Fig. 2. Strict consensus tree of four maximally parsimonious trees derived from the analysis of atpA, atpB, rbcL, trnL-F, and rps4-trnS sequences (tree length = 6028 steps, CI = 0.543, and RI = 0.909). The bootstrap values for 1000 replicates are shown above the branches, the Bayesian posterior probabilities are shown below the branches. ENA, eastern North America.
Molecular dating—The combined data set of rbcL-IntronAlnApB was used to
date the divergence times of the Adiantum pedatum complex. Sequences of 25
species were downloaded from GenBank (Appendix 2). Time estimates were
made based on a relaxed molecular clock and fossil data. Bayesian dating ap-
based, on a relaxed clock model, were used in the time estimates (Drummond et al.,
2006).

The Bayesian coalescent approach to estimating the divergence times of
each clade in Adiantum is Peris sp. from the mid-Cenomanian fish beds of Nammoura,
Lebanon (Krasilov and Bacchia, 2000). The Cenomanian is dated to about
99.6–93.5 Ma (million years ago) (De Bodt et al., 2005). Although the pinnule
morphology and the presence of pseudoidusia allow the fossil to be assigned
to the extant genus Peris, such forked leaves can occasionally occur in the vit-
tarioids. Schneider et al. (2004) used additional calibration points and obtained
the molecular age 97.41(100.75±6.72) Myr for the pteridoids (PT) (node 16 in
fig. 1 of Schneider et al., 2004, i.e., the crown of Pteridaceae), thus we used 93.5
Myr to calibrate the crown of Pteridaceae. The oldest fossil record of Acrosti-
chum was discovered in the Deccan Interglacial beds of India from the Maa-
strichtian (Bonde and Kumaran, 2002). The Maastrichtian is the last stage of the
Cretaceous period, which spanned from 70.6 to 65.5 Ma (Hickey, 1971).

Fossil calibration—The earliest and the most reliable fossil assigned to
Pteridaceae is Peris sp. from the mid-Cenomanian fish beds of Nammoura,
Lebanon (Krasilov and Bacchia, 2000). The Cenomanian is dated to about
99.6–93.5 Ma (million years ago) (De Bodt et al., 2005). Although the pinnule
morphology and the presence of pseudoidusia allow the fossil to be assigned
to the extant genus Peris, such forked leaves can occasionally occur in the vit-
tarioids. Schneider et al. (2004) used additional calibration points and obtained
the molecular age 97.41(100.75±6.72) Myr for the pteridoids (PT) (node 16 in
fig. 1 of Schneider et al., 2004, i.e., the crown of Pteridaceae), thus we used 93.5
Myr to calibrate the crown of Pteridaceae. The oldest fossil record of Acrosti-
chum was discovered in the Deccan Interglacial beds of India from the Maa-
strichtian (Bonde and Kumaran, 2002). The Maastrichtian is the last stage of the
Cretaceous period, which spanned from 70.6 to 65.5 Ma (Hickey, 1971).

The small five-marker data set had 5900 nucleotides and 34
indels and included 74 accessions. This data set contained 1743
variable sites (29.4%), of which 1394 were phylogenetically
informative (23.5%). The MP analysis on the small five-marker
data set yielded two maximally parsimonious trees of 3238
steps, a consistency index (CI) of 0.655, and a retention index
(RI) of 0.937.

The overall topology of the five-marker trees based on small
data set was similar to Fig. 2 concerning major clades; thus,
the figure was not presented here. The analysis also strongly
supported the monophyly of the A. pedatum complex (BS, 100;
PP, 1.00). The close relationship between the A. prince-
cep-A. tenerum clade and the aggregate of the temperate
clad (CE) was strongly supported (BS, 98; PP, 0.99). The sister
relationship between the Chinese A. pedatum group and the A. aleuticum
group was also supported by posterior probability (PP, 0.96).

Biogeographic analyses—The DIVA and the maximum
likelihood analyses using LAGRANGE suggested that the an-
cestral area of the A. pedatum complex was eastern Asia and
that it subsequently migrated into North America (Fig. 3). Then
the ancestor of the Chinese A. pedatum-A. aleuticum clade dis-
persed from North America back to eastern Asia.

Divergence times of the Adiantum pedatum complex—The
divergence of the AD clade (including Adiantum, Vittaria,
Rheopteris, and their allied groups) from the cheilanthoids was estimated to be 73.73 (60.79–85.19) Ma. The divergence of Adiantum from the vittarioids was estimated to be 61.74 (48.15–74.15) Ma. The crown Adiantum was dated to be 50.53 (36.88–64.02) Ma (Fig. 4).

The divergence of the A. pedatum complex from A. edentulum was estimated to be 6.7 (3.59–10.26) Ma, and the crown of the A. pedatum complex was dated to be 4.27 (2.24–6.57) Ma (Fig. 4, Table 2). The divergence between the Japanese A. pedatum and the A. aleuticum-Chinese A. pedatum-eastern North American A. pedatum clade was 2.87 (1.39–4.64) Ma. The divergence of the eastern North American A. pedatum and the Chinese A. pedatum–North American A. aleuticum clade was estimated to be 1.25 (0.52–2.11) Ma. The divergence between the Chinese A. pedatum and the North American A. aleuticum clade was 0.81 (0.28–1.41) Ma.

The divergence of the A. pedatum complex was estimated to be 5.76 (3.07–8.95) Ma, and the crown of the A. pedatum complex was dated to be 3.69 (1.9–5.68) Ma (Table 2) when the second calibration scheme (93.5 Ma for the divergence time between pteridoid and eupolypod ferns and 65.2 Ma for the divergence between the CE clade and the PT clade) was used.

**DISCUSSION**

**Phylogenetic position and relationships of the Adiantum pedatum complex**—The Adiantum pedatum complex is supported as a monophyletic group (BS, 99; PP, 1.00, Fig. 2). The monophyly of the clade is also defined by morphological synapomorphies of fan-shaped blade and glabrous stipes and raclises, and one indel CCAGC. Within the A. pedatum complex, the eastern Asian A. myriosorum is sister to the A. pedatum-A. aleuticum (including A. viridimontanum) clade with strong support (BS, 99; PP, 1.00; Fig. 2). Morphologically, A. pedatum and A. myriosorum can be distinguished easily. The lower leaf surface of A. myriosorum is glaucous (vs. green in A. pedatum). The lobes at segment apices have sharply triangular serrate teeth in A. myriosorum (vs. obtuse in A. pedatum). The sinuses at acrosopic margin of segment are shallow in A. myriosorum, whereas they are deep (1/3–1/2) in A. pedatum (Lin, 1990). Furthermore, there is a significant ecological difference in the two Asian maidenhair species. Adiantum myriosorum is an understory component in thermophilic forests in lower latitudes in Central and southwestern China, Taiwan, and northern Burma and may extend to Bhutan, Nepal, and Pakistan; whereas Asian A. pedatum is widely distributed in deciduous woodland forests of eastern Asia in Far East Russia, Japan, Korea, and northern and northeastern China.

Adiantum pedatum is not monophyletic because the clade of A. aleuticum and the hybrid A. viridimontanum is nested within it (Fig. 2). Three subgroups of A. pedatum are recognizable: the Chinese group, the eastern North American group, and the Japanese group (Fig. 2). With the type of A. pedatum from eastern North America (Linnaeus, 1753), the Chinese A. pedatum and the Japanese A. pedatum should each be recognized at the species level, if A. aleuticum is to be maintained as a distinct species. Our examination of the herbarium specimens suggests that there is a low level of morphological differentiation among the three geographic subclades of A. pedatum. The overall morphological similarities in the eastern Asian and the eastern North American A. pedatum may be attributable to convergence from adapting to similar deciduous forest habitat in both eastern Asia and eastern North America. Similar habitats have been proposed to explain the maintenance of morphological similarities of some EA-ENA disjunct seed plants (Parks and Wendel, 1990; Wen, 1999, 2001; Nie et al., 2006).

The Japanese A. pedatum clade is sister to the clade composed of other A. pedatum accessions and A. aleuticum (including A. viridimontanum) with strong support (BS, 99; PP, 1.00; Fig. 2). Taxonomic and phylogenetic diversity of A. pedatum in Japan may be more complicated than presently revealed in our sampling. Cytogeographic study indicated that there may be two taxa in the Japanese A. pedatum complex, one with x = 29, and the other with x = 30 (Paris, 1991b; Nakato and Kato, 2005). The diploids with 2n = 60 are widely distributed in Japan, whereas those with 2n = 58 are known only from two lowland sites in eastern Hokkaido (Nakato and Kato, 2005). Although chromosome counts have not been carried out in the current study, we assume that the chromosome base number of the Japanese samples we analyzed was x = 30 because they are from Osaka Prefecture, Shizuoka Prefecture, and Nagano Prefecture of Honshu. In the UPGMA tree based on isozyme data (Paris, 1991b), the Japanese A. pedatum grouped with A. aleuticum. Even the two populations FKM and KAM from Hokkaido were nested within the A. aleuticum group (see fig. 3.2 of Paris, 1991b). The chromosome count showed that the chromosome base number of KAM was x = 29 (Paris, 1991b), which implied that the Japanese populations with x = 29 are more closely related to A. aleuticum than those with x = 30. The present phylogenetic results imply that the Chinese A. pedatum, the Japanese A. pedatum (x = 30), and the eastern North American A. pedatum are not the same taxon. The ancestral chromosome base number in Adiantum was suggested to be x = 30, from which x = 29 or other aneuploid numbers diverged (Tryon and Tryon, 1982; Paris, 1991b; Nakato and Kato, 2005). The occurrence of x = 29 or its multiples in several species indicated a second series that may have derived from loss or fusion of individual chromosomes (Tryon and Tryon, 1982; Nakato and Kato, 2005). Japanese members of the A. pedatum complex have the chromosome base numbers of both x = 29 and 30, whereas North American plants are known to have only x = 29 (Paris, 1991b; Nakato and Kato, 2005). Adiantum edentulum, the closest relative of the A. pedatum complex, has x = 30 (Wang et al., 1984; Kato et al., 1992). Nakato and Kato (2005) inferred that the cytotype of x = 29 in the A. pedatum complex originated in Asia and plants with x = 29 subsequently migrated to North America.

Accessions of A. aleuticum from eastern and western North America form a clade, with eastern North American accession (J. Wen 10452) nested within A. aleuticum from western North America (Fig. 2), supporting the treatment of the eastern North American serpentine maidenhair as conspecific with the western North American A. aleuticum by Paris (1991a, b). Ruprecht (1845) published A. pedatum var. aleuticum based on the material from Unalaska and Kodiak Island, Alaska. Fernald (1905) compared the materials from the Gaspé Peninsula, Canada and the typical A. pedatum and reported the differences between the two concerning their stature, texture, venation, teeth of pinnules, and indusium morphology. He designated the Gaspé plant as A. pedatum var. aleuticum Ruprecht. Calder and Taylor (1965) published A. pedatum subsp. aleuticum (Rupr.) Calder & Roy L. Taylor in their studies of the flora of the Queen Charlotte Islands, British Columbia. Cody (1983) thought that the plants of serpentine and dolomitic rocks are even more distinct
Fig. 4. Chronogram of *Adiantum* inferred from BEAST with *atpA*, *atpB*, and *rbcL* sequences. Clade constraints are indicated with black asterisks (the first calibration scheme) and black hexagons (the second calibration scheme). Node 1: A. aleuticum and A. viridimontanum; node 2: A. aleuticum-Chinese *A. pedatum*; node 3: eastern North American *A. pedatum-A. aleuticum* and Chinese *A. pedatum*; node 4: Japanese *A. pedatum*-eastern North American and Chinese *A. pedatum*, and *A. aleuticum*; node 5: the *A. pedatum* complex; node 6: A. edentulum-*A. pedatum* complex.

---

November 2011

**LU ET AL.—BIogeOGRAPHIC DISSUNCTION IN THE ADIANTUM PEDATUM COMPLEX**

---

October 2011

**LU ET AL.—BIogeOGRAPHIC DISSUNCTION IN THE ADIANTUM PEDATUM COMPLEX**
Table 2. Divergence times (Ma) of disjunct clades in the *Adiantum pedatum* complex.

<table>
<thead>
<tr>
<th>Calibration points</th>
<th>Node</th>
<th>Clades</th>
<th>Node age</th>
<th>Height_95%-HPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>93.5 Ma: crown of Pteridaceae</td>
<td>A. aleuticum and A. viridimontanum</td>
<td>0.43</td>
<td>0.08–0.85</td>
<td></td>
</tr>
<tr>
<td>65.2 Ma: crown of CE clade</td>
<td>A. aleuticum-Chinese A. pedatum</td>
<td>0.81</td>
<td>0.28–1.41</td>
<td></td>
</tr>
<tr>
<td>5 eastern North American A. pedatum-A. aleuticum</td>
<td>1.25</td>
<td>0.52–2.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 A. pedatum</td>
<td>Japanese A. pedatum- eastern North American and Chinese A. pedatum, and A. aleuticum</td>
<td>2.87</td>
<td>1.39–4.64</td>
<td></td>
</tr>
<tr>
<td>5 A. pedatum complex</td>
<td>4.27</td>
<td>2.24–6.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 A. edentulum-A. pedatum complex</td>
<td>5.76</td>
<td>3.07–8.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>93.5 Ma: divergence time between pteroid and eupolypod ferns 65.2 Ma: divergence time between CE clade and PT clade</td>
<td>A. aleuticum and A. viridimontanum</td>
<td>0.38</td>
<td>0.08–0.75</td>
<td></td>
</tr>
<tr>
<td>2 A. aleuticum-Chinese A. pedatum</td>
<td>0.7</td>
<td>0.26–1.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 eastern North American A. pedatum-A. aleuticum and Chinese A. pedatum</td>
<td>1.09</td>
<td>0.46–1.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 A. pedatum complex</td>
<td>3.69</td>
<td>1.9–5.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 A. edentulum-A. pedatum complex</td>
<td>5.76</td>
<td>3.07–8.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

than *A. pedatum* subsp. *aleuticum* is from *A. pedatum* subsp. *pedatum* after he examined 400 sheets of specimens of the *A. pedatum* complex. He proposed a new name *A. pedatum* subsp. *calderi* for the plants of the serpentine and dolomitic rocks from southeastern Quebec, northern Vermont, western Newfoundland, California, and Washington. Paris and Windham (1988) showed that the eastern North American serpentine populations (*A. pedatum* subsp. *calderi*) were nested within the western North American *A. pedatum var. aleuticum* and treated the eastern serpentine maidenhair fern as a disjunct element of the western North American *A. aleuticum*. Paris (1991b) published the new combination *A. aleuticum* and treated five infraspecific taxa as synonyms. These were *A. boreale*, *A. pedatum var. aleuticum*, *A. pedatum subsp. aleuticum*, *A. pedatum subsp. calderi*, and *A. pedatum var. subpulvinum*, a dwarf coastal variety proposed by Wagner and Boydstun (1978). Paris and Windham (1988) demonstrated that there is an allotetraploid derivative (= *A. viridimontanum*) of a sterile hybrid between *A. pedatum* and *A. aleuticum*. Paris (1993) noted that the morphological differences between the eastern North American *A. pedatum* and the rocky-wood western North American *A. aleuticum* are obscured by the serpentine *A. aleuticum* and the hybrid *A. viridimontanum*. Paris (1991a) also noted that *A. viridimontanum* generally bears greater resemblance to *A. aleuticum* on the serpines (e.g., at the type location in Vermont, USA), whereas it shows greater resemblance to the woodland *A. pedatum* in shady conditions. Our analysis shows that *A. viridimontanum* is nested within the *A. aleuticum* clade, which supports the hypothesis of a close relationship between *A. viridimontanum* and *A. aleuticum* by Paris (1991b). With the maternal inheritance of chloroplast DNA in ferns (Yatskievych et al., 1988), the present result with *A. viridimontanum* nested within the *A. aleuticum* clade suggests that the maternal parent of *A. viridimontanum* may be *A. aleuticum*, supporting Paris’ (1991b) conclusions based on chloroplast DNA restriction fragment data (also see Paris and Windham, 1988).

Superficially the *A. pedatum* complex is more similar to *A. hispidulum* and *A. flabellulatum* in the frond architecture, sharing a dichotomous branching flabellate lamina. Nayar (1961) included *A. hispidulum*, *A. flabellulatum*, and *A. pedatum* in his pedatum group. In the present study, the *A. pedatum* complex was nested within the temperate clade I, whereas *A. hispidulum* was nested within the pantropical grade and *A. flabellulatum* was nested in the temperate clade II (Fig. 2). In the *A. pedatum* complex, the blade is fan-shaped; frond architecture is relatively simpler in the other *Adiantum* species. Furthermore, the stipes, rachises, petiolules, and pinnales are all glabrous in the *A. pedatum* complex; the stipes, rachises, and petiolules are densely covered with hispid hairs in *A. hispidulum*; and the stipes are glabrous, but upper regions of rachises and their branches are covered with hairs in *A. flabellulatum*. False indusia of the *A. pedatum* complex are oblong to crescent-shaped, whereas they are round, covered with reddish brown, stiff, and needle-like bristles in *A. hispidulum*; and those of *A. flabellulatum* are semirounded to oblong. The *A. pedatum* complex is distributed in the North Temperate zone; *A. myriosorum*, the earliest diverged member in the *A. pedatum* complex, is distributed in southwestern China, overlapping with *A. edentulum*; and *A. hispidulum* and *A. flabellulatum* are in tropical and subtropical Asia (Lin, 1990; Paris, 1993). The eastern Asian *Adiantum edentulum* is shown to be sister to the *A. pedatum* complex although this relationship does not seem to be well defined by any morphological synapomorphies.

**Evolution of intercontinental disjunctions between eastern Asia and North America in the *A. pedatum* complex**—The molecular dating result suggests an ancient origin of the *Adiantum* group, with the *Adiantum* crown group dated to 50.53 (36.88–64.02) Ma in the early Eocene (Fig. 4). Our results support a relatively recent origin of the *A. pedatum* complex, with the crown *A. pedatum* complex dated to 4.27 (2.24–6.57) Ma in the Pliocene. The biogeographic analyses and the Bayesian dating suggest an eastern Asian origin of the *A. pedatum* complex and its spread into North America in the Pliocene to early Pleistocene (Table 2; Figs. 3, 4).

Biogeographic relationships have been established by various factors, of which dispersal and vicariance are two major events (Briggs, 1991). In comparison with the seed plants, long-distance dispersal is more common in ferns (Barrington, 1993; Smith, 1993). Ferns are dispersed by small, wind-dispersed spores that are produced in very large numbers and capable of dispersing thousands of kilometers (Wolf et al., 2001). Homosporous ferns have independently living, sometimes bisexual gametophytes, which can undergo intragametophytic breeding to give rise to sporophytes singly, and can thus establish new populations in distant localities even by dispersal of single spores (Kato, 1993). Thus, distributions in most ferns are assumed to be a function of dispersal rather than vicariance (Wolf...
et al., 2001). Paris (1991b) demonstrated that gametophytes of Adiantum aleuticum are usually unisexual, and Peck et al. (1990) showed that A. pedatum carries higher levels of genetic load, which restricts the intragametophytic selfing of gametophytes. Paris (1991b) pointed out that long-distance dispersal is unlikely in the A. pedatum lineage because intergametophytic mating is probably the rule for this complex. Paris (1991b) postulated that speciation in the A. pedatum complex was a consequence of the fragmentation of the Tertiary mixed mesophytic forest, in which the complex presumably originated widespread throughout the northern hemisphere during much of the Tertiary period. The estimated recent disjunct distribution in the A. pedatum complex may be explained by the dispersal and migration of the complex across the Bering land bridge and adjacent areas. The Bering land bridge was available for exchanges of temperate deciduous plants between eastern Asia and North America almost throughout the Tertiary, at least until 3.5 Ma (Hopkins, 1967), which is similar to the estimated divergence time of the entry of the A. pedatum clade from Asia to North America (node 4 in Fig. 4, Table 2).

The biogeographic analyses and the Bayesian dating suggest that there was a migration of the ancestor of the Chinese A. aleuticum and the North American A. aleuticum clade from North America back to Asia (China) dated around 0.8 (0.28–1.41) Ma. Adiantum aleuticum is currently distributed in western North America, including the Aleutian islands of the Northern Pacific Ocean. The migration of the ancestor of A. aleuticum and the Chinese A. pedatum from North America to Asia was most likely via the Aleutian islands south of the Bering Sea.

Adiantum aleuticum is disjunct in wet rock fissures in western North America and serpentine in eastern North America (Newfoundland, Quebec, Maine, Maryland, Pennsylvania, and Vermont) (Paris, 1993). The Bayesian dating suggests that the disjunction of A. aleuticum between western and northeastern North America was very recent, in the late Pleistocene. The phylogenetic result suggests a western North American origin of A. aleuticum and its spread into eastern North America (Fig. 2). Fernald (1925) proposed that some plants in the Gaspé Peninsula in Quebec were once widespread and represented disjunct populations of western Cordilleran species and that these species survived the Pleistocene glaciations in the Gaspé Peninsula on ice-free mountaintops. Paris (1989) suggested that the western A. aleuticum had an incremental migration (a series of more local dispersals) along the highly distributed terrain exposed with the retreat of the Wisconsin ice, followed by its restriction to serpentinite substrates in northwestern North America. Barrington (1993) and Wolf et al. (2001) accepted the vicariance hypothesis for the A. pedatum complex in North America. Barrington and Paris (2007) further presumed that A. aleuticum migrated eastward as the glacial retreat proceeded in the Holocene. A detailed phylogeographic analysis of A. aleuticum with an ENA-WNA disjunction is needed to test its diversification history in North America.

The EA-NA biogeographic disjunctions have involved multiple historical events at different geologic times in different groups (Tiffney, 1985a, b; Kato, 1993; Manchester, 1999; Wen, 1998, 1999; Xiang et al., 1998; Wen et al., 2010). The Bering and the North Atlantic land bridges are inferred to be the most likely migration route for floristic exchanges between Asian and North American in Tertiary (Tiffney, 1985a, b; Xiang et al., 1998; Nie et al., 2006, 2008) in seed plants. The phylogenetic and biogeographic results, in combination with the divergence time of the complex, suggest that the current intercontinental disjunction of the A. pedatum complex can be explained by two events of dispersal/migration between eastern Asia and North America. First, the ancestor of this complex migrated from eastern Asia (Japan) into North America in the late Pliocene or the early Pleistocene via the Bering land bridge. Second, the ancestor of the Chinese A. pedatum-A. aleuticum clade migrated from North America back to eastern Asia in the mid Pleistocene, most likely via the Aleutian islands near the Bering Sea.

**LITERATURE CITED**


Appendix 1. Voucher information and GenBank accession numbers for taxa used in this study.

Taxon: Voucher specimen (Herbarium), Collection locality; GenBank accessions: rbcl, atpB, atpA, trnL–F, and rps4–trnS.
Appendix 2. Samples examined in the study to estimate divergence times.

**Taxon**: GenBank accessions, *rbcL, atpB, atpA*.

**Acrostichum danaeifolium**: EF452129, EF452008, EF452065. *Adiantopsis radiata*: EF452131, EF452010, EF452067.

**Adiantum tetraphyllum**: EF452135, EF452015, EF452073. *Anetium citrifolium*: U21284, EF452017, EF452075.


**Blechnum occidentale**: U05909, U93838, EF452080.

**Ceratopteris richardi**: AB059585, AY612691, EF452082. *Coniogramme fraxinea*: AM177359, AY612693, AM176470; *Cryptogramma crispa*: EF452148, EF452027, EF452087.

**Dennstaedtia punctilobula**: U05918, U93836, EF452090.


**Jamesonia verticalis**: EF452155, EF452038, EF452099.

**Llavea cordifolia**: U27726, EF452039, EF452100.

**Monogramma graminea**: EF452157, EF452040, EF452102.


**Onychium japonicum**: U05641, EF452045, EF452107.


**Vittaria graminifolia**: U21295, EF452064, EF452128.