

Low-copy nuclear data confirm rampant allopolyploidy in the Cystopteridaceae (Polypodiales)

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Abstract Here we present the first nuclear phylogeny for Cystopteridaceae (Polypodiales), using the single-copy locus *gapCp* “short”. This phylogeny corroborates broad results from plastid data in demonstrating strong support for the monophyly of the family’s three genera—*Cystopteris*, *Acystopteris*, and *Gymnocarpium*—and of the major groups within *Cystopteris* (*C. montana*, the sudetica and bulbifera clades, and the *C. fragilis* complex). In addition, it confirms the rampant hybridization (allopolyploidy) that has long been suspected within both *Cystopteris* and *Gymnocarpium*. In some cases, these data provide the first DNA-sequence-based evidence for previous hypotheses of polyploid species origins (such as the cosmopolitan *G. dryopteris* being an allotetraploid derivative of the diploids *G. appalachianum* and *G. disjunctum*). Most of the allopolyploids, however, have no formal taxonomic names. This pattern is particularly strong within the *C. fragilis* complex, where our results imply that the eight included accessions of “*C. fragilis*” represent at least six distinct allopolyploid taxa.

Keywords amplicon cloning; *Cystopteris fragilis*; fern phylogeny; *Gymnocarpium*; hybridization; reticulate evolution; species complex

Supplementary Material The Electronic Supplement (Fig. S1) is available in the Supplementary Data section of the online version of this article at <http://www.ingentaconnect.com/content/iapt/tax>

■ INTRODUCTION

Polyploidy and reticulate evolution are unusually common among ferns (Lovis, 1978; Otto & Whitton, 2000) and, in certain genera, hybrid polyploids (allopolyploids) have become so abundant that members of the diploid progenitor lineages are rarely encountered (e.g., Beck & al., 2010; Li & al., 2012). *Cystopteris* (L.) Bernh. is a particular case in point, with rampant hybridization and polyploidy making the *C. fragilis* (L.) Bernh. complex “perhaps the most formidable biosystematic problem in the ferns” (Lovis, 1978). Based on recent phylogenetic analyses, Rothfels & al. (2012b) challenged previous assignments of *Cystopteris* to the Dryopteridaceae (Smith, 1993) and Woodsiaceae (Smith & al., 2006), and placed the genus in the Cystopteridaceae, together with *Acystopteris* Nakai, *Gymnocarpium* Newman, and the enigmatic *Cystoathyrium* Ching. Further investigations have clarified the relationships of Cystopteridaceae to other eupolypod ferns (Rothfels & al., 2012a), elucidated patterns of morphological evolution within the family (Sundue & Rothfels, 2014), and established the major phylogenetic relationships among and within genera (Rothfels & al., 2013b), including demonstrating that *Cystoathyrium* is embedded within *Cystopteris* (Wei & Zhang, 2014). In addition, over the past forty years, various

regional efforts have made great inroads into unraveling particular reticulate complexes within Cystopteridaceae (Vida, 1974; Sarvela, 1980; Vida & Mohay, 1980; Sarvela & al., 1981; Moran, 1982, 1983a, b; Pryer & al., 1983, 1984; Haufler & al., 1985, 1990, 1993; Haufler & Windham, 1991; Pryer, 1992, 1993; Pryer & Haufler, 1993; Paler & Barrington, 1995). However, we are still far from an adequate understanding of the extent of reticulation in Cystopteridaceae (Rothfels, 2012).

With the recent availability of data from the nuclear genome (Schuettpelz & al., 2008; Rousseau-Gueutin & al., 2009; Duarte & al., 2010; Lo & al., 2010; Chen & al., 2012; Zimmer & Wen, 2012; Cacho & Strauss, 2013; Rothfels & al., 2013a), plant systematists have novel opportunities to elucidate complex cases of reticulate evolution. Phylogenetic data from low-copy nuclear genes is proving particularly useful in groups where morphological discontinuities have been obscured by past reticulation events (e.g., Adjie & al., 2007; Brysting & al., 2007; Kim & al., 2008; Schuettpelz & al., 2008; Shepherd & al., 2008; Grusz & al., 2009; Guggisberg & al., 2009; Ishikawa & al., 2009; Beck & al., 2010; Juslén & al., 2011; Nitta & al., 2011; Chao & al., 2012; Dyer & al., 2012; Li & al., 2012; Schneider & al., 2012; Sessa & al., 2012; Lee & Park, 2013; Zhang & al., 2013; Chen & al., 2014; Hori & al., 2014). Here we seek to gain insight into the extent of reticulation in the

Cystopteridaceae, using a family-wide taxon sample, the plastid *trnG-trnR* spacer (henceforth “*trnGR*”), and the single-copy nuclear locus *gapCp* “*short*” (sensu Schuettpelz & al., 2008 and Rothfels & al., 2013a), henceforth simply *gapCp*.

■ MATERIALS AND METHODS

We obtained plastid *trnGR* and nuclear *gapCp* sequences from a sample of 28 Cystopteridaceae accessions (Appendix 1). Our sampling includes representatives from all three accepted genera—*Gymnocarpium*, *Cystopteris*, *Acystopteris* (see Wei & Zhang, 2014)—and captures much of the phylogenetic diversity of each (Rothfels & al., 2013b). Species delimitations are poorly understood in this family, especially within the polyploids, but following the tentative (and conservative) species list in appendix E of Rothfels (2012), our sample includes six of the eight species of *Gymnocarpium*, two of the three species of *Acystopteris*, and seven of the twenty-five species of *Cystopteris*.

Genomic DNA was extracted either from herbarium specimens or silica-dried material (Appendix 1) using a 96-well modification (Beck & al., 2011a, b) of a standard CTAB protocol (Doyle & Dickson, 1987), or with a DNeasy kit (Qiagen, Valencia, California, U.S.A.). Amplifications were performed in 21 μ L reactions following established protocols (Rothfels & al., 2013b). Plastid *trnGR* was amplified in two overlapping pieces with primers *trnGIF*+*CRcysTRNGrl* and *CRcysTRNGfl*+*trnR22R* (Nagalingum & al., 2007; Rothfels & al., 2013b) and *gapCp* in one contiguous piece with primers *ESGAPCP8F1* and *ESGAPCPIIR1* (Schuettpelz & al., 2008). The *gapCp* PCR products were cloned following established protocols (Schuettpelz & al., 2008), and the colony PCR products were visualized on agarose gels prior to sequencing with the M13 forward and M13 reverse primers supplied by Invitrogen (Carlsbad, California, U.S.A.). Sequencing was done on an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, California, U.S.A.) at the Duke University Genome Sequencing and Analysis Core Resource, again using established protocols (Schuettpelz & Pryer, 2007).

The *trnGR* sequences were manually aligned in Mesquite v2.75 (Maddison & Maddison, 2011), and areas of ambiguous alignment (28 out of 1165 sites) were excluded prior to subsequent analysis. Building the nuclear dataset was more complex. Following the identification and removal of PCR recombinant sequences (see Cronn & al., 2002), the remaining pool of 253 *gapCp* sequences was manually aligned in Mesquite v2.75 (Maddison & Maddison, 2011); unambiguous indels were recoded by simple gap recoding (Simmons & Ochoterena, 2000), using the Python script *gapcode.py* (Ree, 2008). Identical sequences from individual accessions were reduced to a single representative, resulting in a 157-sequence “all unique” alignment (available in TreeBASE, <http://www.treebase.org>, study number 15964). These data included variants that differed from one another by a small number of substitutions that almost certainly represent PCR errors (see Grusz & al., 2009; Beck & al., 2011c; Li & al., 2012; Rothfels & Schuettpelz, 2014). To remove these errors, we first estimated a maximum parsimony

tree from the full 157-sequence dataset using PAUP* v.4.0a133 (Swofford, 2002). On that tree, we identified the maximum exclusive clades—the largest clades that included sequences from only one accession—and reduced each of those clades to a single sequence (see below), discarding the others. For this pruning step, we treated as “exclusive” any single-accession pool of sequences that could form a clade without having to break any branches—polytomies involving multiple accessions thus did not interfere with those sequences being pruned. When selecting the sequence to be retained from a group, we picked the one that had the fewest apomorphies, or, if there were multiple such sequences, we selected one at random (see Rothfels & Schuettpelz, 2014). This final *gapCp* alignment is available in TreeBASE, <http://www.treebase.org>, study number 15964.

Each dataset was analyzed using the optimal partitioning scheme as determined by an exhaustive search in PartitionFinder (Guindon & al., 2010; Lanfear & al., 2012), applying the corrected Akaike information criterion (AICc). Because we encountered computational difficulties with PartitionFinder when model parameters were optimized on trees containing zero-length branches, we conducted model selection using a version of each dataset that contained no redundant sequences. After model selection, the full dataset was used for subsequent analyses. For *trnGR* the AICc favoured a three-partition model, and for *gapCp* a five-partition model (Table 1); indels were optimized under a MkV model (Lewis, 2001). For each dataset, a maximum likelihood (ML) tree search was performed with GARLI v.2.0 (Zwickl, 2006) from each of 10 random-addition starting trees. To assess support, we performed 1000 ML bootstrap pseudoreplicates, again with GARLI, under the same settings, but with each search performed from only two random-addition starting trees (Fig. 1). In addition, for each locus we performed 1000 maximum parsimony bootstrap searches in PAUP* v.4.0a133 (Swofford, 2002), again with each search undertaken from two different random-addition starting trees.

■ RESULTS

The final *trnGR* dataset comprises 1137 aligned base pairs for 28 taxa, and has 6.2% missing data (including gaps). The final *gapCp* dataset comprises 745 aligned base pairs plus 28 recoded indel characters for 48 sequences (alleles), from the same 28 taxa as the *trnGR* dataset, with 14.9% missing data (including gaps; Appendix 1). The main structure of the inferred phylogenies (*Gymnocarpium*, *Cystopteris*, and *Acystopteris* each monophyletic; *C. montana* (Lam.) Bernh. ex Desv. sister to the rest of *Cystopteris*; the *sudetica* clade, *bulbifera* clade [s.c. and b.c., respectively, Fig. 1B], and *C. fragilis* complex deeply diverged from one another; and *C. protrusa* (Weath.) Blasdell sister to the remainder of the *C. fragilis* complex) are congruent between the plastid *trnGR* data (Fig. 1A) and the nuclear *gapCp* data (Fig. 1B). Both gene phylogenies are additionally congruent with previously published multi-locus plastid DNA analyses (Rothfels & al., 2013b).

The nuclear data give the first sequence-based indication as to the extent of reticulation within the family (Fig. 1B). Despite

our relatively sparse sampling, and the stringent sequence pruning protocol we followed, over half of the taxa in our sample harbour multiple deeply divergent alleles, suggesting that they are allopolyploids.

DISCUSSION

The congruence of the main relationships inferred from the plastid and nuclear data (Fig. 1) is a welcome result, especially given the strong historic reliance of fern phylogenetics on plastid data alone (Rothfels & al., 2013a). The most striking pattern in these data, however, is the extensive reticulation within the family (Fig. 1B). As hypothesized based on morphology and cytogenetics (e.g., Vida, 1972, 1974; Lovis, 1978; Vida & Mohay, 1980), reticulate evolution within the Cystopteridaceae is comparable to or exceeds that observed in other well-studied polyploid plant groups (e.g., Brysting & al., 2007, 2011; Kim & al., 2008; Chao & al., 2012; Marcussen & al., 2012; Chen & al., 2014; Hori & al., 2014; Triplett & al., 2014). This pattern is most apparent in *Gymnocarpium* and in the *C. fragilis* complex, but may also be occurring in *Acystopteris* and in other clades of *Cystopteris* that were only sparsely sampled in this study. These sparsely sampled clades were pruned more heavily by our sequence-thinning strategy (because the sequences from a given accession were more likely to form an exclusive clade, even if they were considerably divergent from each other), and our results thus likely underestimate the extent of reticulation involving those lineages.

Within *Gymnocarpium*, our results (Fig. 1B) corroborate the hypothesized parentage of the cosmopolitan allotetraploid *G. dryopteris* (L.) Newman. As inferred by Pryer & Haufler (1993), its progenitors are the southeastern North American endemic diploid *G. appalachianum* Pryer & Haufler and the western North American/East Asian diploid *G. disjunctum* (Rupr.) Ching. In all individuals of *G. dryopteris* thus far sampled, *G. disjunctum* is the maternal parent (Rothfels & al., 2013b). Our *G. dryopteris* accession from Japan is somewhat anomalous in that it has a *G. appalachianum*-type allele that is otherwise found only in our samples of tetraploid

G. jessoense subsp. *parvulum* Sarvela (Fig. 1B). This is a particularly interesting result in the context of *G. dryopteris* var. *aokigarharaense* Nakaike, a taxon described from Japan with the notation that it appears intermediate between *G. dryopteris* and *G. jessoense* (Koidz.) Koidz. (Nakaike, 1969). The intermediate morphology of this taxon may indicate a genetic contribution from *G. jessoense*, which could explain the presence of the unusual *G. appalachianum*-type allele in our Japanese accession. Additional molecular and morphological data will be necessary to confirm or refute this hypothesis.

Among the glandular species of *Gymnocarpium*, *G. remotepinnatum* (Hayata) Ching and *G. robertianum* (Hoffm.) Newman each harbor two widely divergent alleles, suggesting that both are allotetraploids. While chromosome counts have previously demonstrated that *G. robertianum* is tetraploid (Manton, 1950; Löve & Löve, 1967; Sarvela & al., 1981; Pellinen & al., 1998; Ivanova & Piekos-Mirkowa, 2003), this is the first indication that it may have a hybrid ancestry from diploid progenitors that remain unidentified. *Gymnocarpium remotepinnatum* is even less well understood; both the taxonomy and nomenclature of this group of *Gymnocarpium* are unclear (Fraser-Jenkins, 1992). The likely allotetraploid nature of our accession provides some evidence against Fraser-Jenkins's (1992, 2008) hypothesis that *G. remotepinnatum* is conspecific with *G. jessoense* subsp. *jessoense*, which is reportedly diploid (Mitui, 1970). Further investigations of Asian *Gymnocarpium* will be necessary before we can attain a reliable understanding of the entities involved, and their evolutionary histories.

Gymnocarpium remotepinnatum and *G. robertianum* each have one deeply divergent allele that is unique, and another allele from a well-supported clade encompassing all glandular taxa included in our sampling (*G. remotepinnatum*, *G. robertianum*, *G. jessoense* subsp. *parvulum*). The presence of this “glandular clade” (Fig. 1) in the sequence data reaffirms results from chromatographic data showing a deep split between the glandular and glabrous *Gymnocarpium* species (Pryer & al., 1983), and suggests that these species may share a glandular common ancestor. To our surprise, *G. jessoense* subsp. *parvulum* also contains two divergent alleles, with the

Table 1. Best-fit partitioning scheme and models of evolution for the final *trnGR* and *gapCp* datasets.

Partition	Model	Exchangeability parameters ^a	State frequencies	Rate heterogeneity	Proportion invariant
<i>trnGR</i>					
tRNAs	JC	One rate	Equal	None	None
<i>trnG</i> intron	K81uf+G	0 1 2 2 1 0	Estimated	Gamma	None
<i>trnG-trnR</i> spacer	K81+I	0 1 2 2 1 0	Equal	None	Estimated
<i>gapCp</i>					
1st position	HKY+I	0 1 0 0 1 0	Estimated	None	Estimated
2nd position	TrN+I+G	0 1 0 0 2 0	Estimated	Gamma	Estimated
3rd position	SYM+G	0 1 2 3 4 5	Equal	Gamma	None
Noncoding	K81uf+G	0 1 2 2 1 0	Estimated	Gamma	None
Indels	Mkv	One rate	Equal	None	None

^aIn the order: A-C, A-G, A-T, C-G, C-T, G-T.

non-“glandular clade” allele being closely related to *G. appalachianum* (Fig. 1B). Although *G. jessoense* subsp. *parvulum* has been considered a tetraploid cytotype of the Asian diploid *G. jessoense* (Sarvela, 1978; Sarvela & al., 1981), our data suggest that the two subspecies likely have very different evolutionary histories and thus each warrants species status. The most appropriate name for the allotetraploid widely known as *G. jessoense* subsp. *parvulum* would thus appear to be *G. continentalis* (Petrov) Pojark. (Pryer, 1993; Jäkäläniemi, 1994).

Reticulation in *Cystopteris* is even more intricate, particularly within the *C. fragilis* complex sensu Rothfels & al. (2013b). In Fig. 1B, we labeled major divergent allele-types within the *C. fragilis* complex with the letters A–F. Despite our very sparse sampling within this cosmopolitan complex (it occurs on every continent except Antarctica; Blasdell, 1963; Rothfels & al., 2013b), our sample includes nine phylogenetically distinct taxa. First, sister to the remainder of the complex is the eastern North American *C. protrusa* (Fig. 1), a predominantly diploid

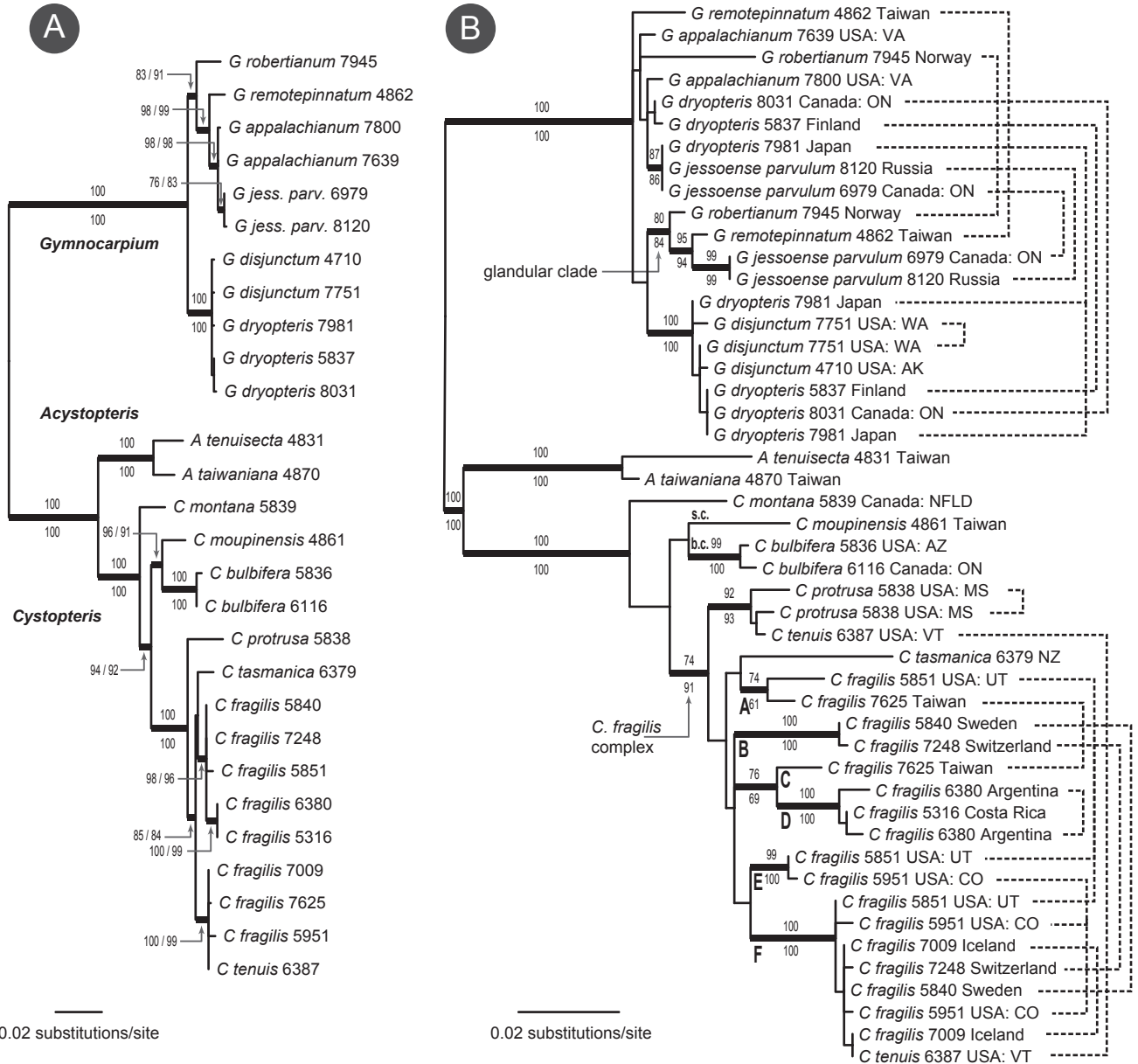


Fig. 1. Maximum likelihood (ML) phylogram of plastid *trnGR* sequences (A) and nuclear *gapCp* alleles (B). Thickened branches have $\geq 70\%$ ML bootstrap support (ML bootstrap support shown above the branch, and maximum parsimony support shown below). Dotted lines on the right-hand side of Fig. 1B track the phylogenetic position of different *gapCp* alleles obtained from individual accessions. Numbers following the specific epithets are accession numbers from the Fern Lab Database (<http://fernlab.biology.duke.edu/>). The labels “s.c.” and “b.c.” indicate the sudetica clade (represented here by *C. moupinensis*) and the bulbifera clade, respectively. Within the *C. fragilis* complex, six divergent sequence types are marked with the letters A–F, for ease of discussion.

species with scattered autotriploid populations (Haufler & al., 1985). Our single *C. tenuis* sample contains one allele from the *C. protrusa* clade and a second allele from clade F (Fig. 1B). This result provides the first sequence-based corroboration of the hypothesized parentage of this primarily eastern North American tetraploid as a hybrid between *C. protrusa* and an unidentified diploid member of the core *C. fragilis* complex (often referred to as *C. “hemifragilis”*; Haufler & Windham, 1991; Haufler & al., 1993). Within the core *C. fragilis* complex only one of our samples corresponds unambiguously to a named segregate of *C. fragilis* s.l.: *C. tasmanica* Hook. This is an alpine tetraploid of southeastern Australia and New Zealand (Tindale & Roy, 2002). Because we obtained only a single cloned sequence from this specimen (Appendix 1), we are unable to ascertain whether it is an allo- or autopolyploid—no related diploids are known, and the relationships of *C. tasmanica* within the *C. fragilis* complex remain obscure (Rothfels & al., 2013b).

The remaining six phylogenetically distinct taxa are generally classified under *C. fragilis* (Haufler & al., 1993; Jermy, 1993; Moran, 1995; Mickel & Smith, 2004; Knapp, 2011). Using the labeled allele-types from Fig. 1B, they consist of an AEF taxon from Utah (5851), an AC taxon from Taiwan (7625), two BF accessions from Europe (5840 and 7248), a D accession from Costa Rica (5316), a DD taxon from Argentina (6380), an FF accession from Iceland (7009), and an EFF taxon from Colorado (5951). Most of these unique genomic combinations would require new names if recognized as species. The likely exceptions are “BF”, which may correspond to *C. fragilis* s.str. (a Linnaean name, typified on European material), and accessions containing only “D” alleles, which may correspond to *C. diaphana* (Bory) Blasdell (Blasdell, 1963; Murphy & Rumsey, 2005); see appendix E in Rothfels (2012). Furthermore, a maximum of four of these genome types correspond to known or previously inferred diploid taxa: genome C may correspond to an unnamed diploid cytotype from Asia (Knapp, 2011); D to a diploid cytotype of “*C. diaphana*” inferred from spore measurements (Blasdell, 1963; see also accession 6380 in Appendix 1); E to diploid *C. reevesiana* Lellinger of southwestern North America (Lellinger, 1981); and F to the hypothesized diploid *C. “hemifragilis”* (Haufler & Windham, 1991; Haufler & al., 1985). These results strongly mirror those of the landmark studies of Vida and colleagues (Vida, 1974; Vida & Mohay, 1980), who used hybridization and DNA content to infer the genomic complement of some European accessions of the *C. fragilis* complex. They concluded that there were at least two distinct tetraploids in Europe (with “XXYY” and “YYZZ” genomic complements, respectively) and three distinct hexaploids (“PPXXYY”, “RRXXYY”, “QQYYZZ”); of the six putative diploid progenitors, only one (“PP”; *C. protrusa*) is known (Vida, 1974; Vida & Mohay, 1980).

This work on European *Cystopteris* (Vida, 1974; Vida & Mohay, 1980) together with the geographically widespread sampling of Cystopteridaceae presented here provides a very rudimentary picture of lineage diversity in this cosmopolitan fern family. Particularly conspicuous gaps in our sampling include Asian *Gymnocarpium* (especially *G. jessoense* subsp.

jessoense), *Acystopteris japonica* (Luerss.) Nakai, additional *A. tenuisecta* (Blume) Tagawa cytotypes (e.g., Bir, 1971), members of the *Cystopteris sudetica* A. Braun & Milde clade, and putative allopolyploids involving crosses between *C. bulbifera* (L.) Bernh. and members of the *C. fragilis* complex (tetraploid *C. tennesseensis* Shaver and *C. utahensis* Windham & Haufler, and hexaploid *C. laurentiana* (Weath.) Blasdell; Weatherby, 1926; Shaver, 1950; Haufler & al., 1990; Haufler & Windham, 1991). Incorporating these taxa, and a much denser sampling of cryptic lineages within the *C. fragilis* complex, will be essential to a full understanding of reticulation and polyploid evolution in the Cystopteridaceae, and is a focus of our ongoing research. A top priority is the identification and analysis of the “missing” diploid lineages that underlie the rampant reticulation observed in our largely polyploid sampling.

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■ LITERATURE CITED

- Adjie, B., Masuyama, S., Ishikawa, H. & Watano, Y. 2007. Independent origins of tetraploid cryptic species in the fern *Ceratopteris thalictroides*. *J. Pl. Res* 120: 129–138.
- Beck, J.B., Windham, M.D., Yatskievych, G. & Pryer, K.M. 2010. A diploids-first approach to species delimitation and interpreting polyploid evolution in the fern genus *Astrolepis* (Pteridaceae). *Syst. Bot.* 35: 223–234. <http://dx.doi.org/10.1600/036364410791638388>
- Beck, J.B., Alexander, P.J., Allphin, L., Al-Shehbaz, I.A., Rushworth, C.A., Bailey, C.D. & Windham, M.D. 2011a. Does hybridization drive the transition to asexuality in diploid *Boechea*? *Evolution* 66: 985–995. <http://dx.doi.org/10.1111/j.1558-5646.2011.01507.x>
- Beck, J.B., Alexander, P.J., Allphin, L., Al-Shehbaz, I.A., Rushworth, C., Bailey, C.D. & Windham, M.D. 2011b. Data from: Does hybridization drive the transition to asexuality in diploid *Boechea*? Dryad Digital Repository. <http://dx.doi.org/10.5061/dryad.11p757m0>
- Beck, J.B., Windham, M.D. & Pryer, K.M. 2011c. Do asexual polyploid lineages lead short evolutionary lives? A case study from the fern genus *Astrolepis*. *Evolution* 65: 3217–3229. <http://dx.doi.org/10.1111/j.1558-5646.2011.01362.x>
- Bir, S.S. 1971. Cytological observations on some Indian species of *Cystopteris* Bernh. *Nucleus* 14: 56–62.
- Blasdell, R.F. 1963. A monographic study of the fern genus *Cystopteris*. *Mem. Torrey Bot. Club* 21: 1–102.

- Britton, D.M.** 1953. Chromosome studies on ferns. *Amer. J. Bot.* 40: 575–583.
- Britton, D.M.** 1964. Chromosome numbers of ferns in Ontario. *Canad. J. Bot.* 64: 1149–1154.
- Britton, D.M., Stewart, W.G. & Cody, W.J.** 1984. *Cystopteris protrusa*, creeping fragile fern, an addition to the flora of Canada. *Canad. Field-Naturalist* 99: 380–382.
- Brysting, A.K., Oxelman, B., Huber, K.T., Moulton, V. & Brochmann, C.** 2007. Untangling complex histories of genome mergings in high polyploids. *Syst. Biol.* 56: 467–476. <http://dx.doi.org/10.1080/10635150701424553>
- Brysting, A.K., Mathiesen, C. & Marcussen, T.** 2011. Challenges in polyploid phylogenetic reconstruction: A case story from the arctic-alpine *Cerastium alpinum* complex. *Taxon* 60: 33–347.
- Cacho, N.I. & Strauss, S.Y.** 2013. Single-copy nuclear gene primers for *Streptanthus* and other Brassicaceae from genomic scans, published data, and ESTs. *Appl. Pl. Sci.* 1: 1200002. <http://dx.doi.org/10.3732/apps.1200002>
- Chao, Y.-S., Dong, S.-Y., Chiang, Y.-C., Liu, H.-Y. & Chiou, W.-L.** 2012. Extreme multiple reticulate origins of the *Pteris cadieri* complex (Pteridaceae). *Int. J. Molec. Sci.* 13: 4523–4544. <http://dx.doi.org/10.3390/ijms13044523>
- Chen, C.-W., Kuo, L.-Y., Wang, C.-N. & Chiou, W.-L.** 2012. Development of a PCR primer set for intron 1 of the low-copy gene *LEAFY* in Davalliaceae. *Amer. J. Bot.*: e223–e225. <http://dx.doi.org/10.3732/ajb.1100498>
- Chen, C.-W., Ngan, L.T., Hidayat, A., Evangelista, L., Nootboom, H.P. & Chiou, W.-L.** 2014. First insights into the evolutionary history of the *Davallia repens* complex. *Blumea* 59: 49–58.
- Cronn, R., Cedroni, M., Haselkorn, T., Grover, C. & Wendel, J.F.** 2002. PCR-mediated recombination in amplification products derived from polyploid cotton. *Theor. Appl. Genet.* 104: 482–489. <http://dx.doi.org/10.1007/s001220100741>
- Dalgaard, V.** 1989. Additional chromosome numbers in vascular plants from the Disko Bugt area (west Greenland). *Willdenowia* 19: 199–213.
- Doyle, J.D. & Dickson, E.E.** 1987. Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon* 36: 715–722. <http://dx.doi.org/10.2307/1221122>
- Duarte, J.M., Wall, P.K., Edger, P.P., Landherr, L.L., Ma, H., Pires, J.C., Leebens-Mack, J.H. & dePamphilis, C.W.** 2010. Identification of shared single copy nuclear genes in *Arabidopsis*, *Populus*, *Vitis* and *Oryza* and their phylogenetic utility across various taxonomic levels. *B. M. C. Evol. Biol.* 10: 61. <http://dx.doi.org/10.1186/1471-2148-10-61>
- Dyer, R.J., Savolainen, V. & Schneider, H.** 2012. Apomixis and reticulate evolution in the *Asplenium monanthes* fern complex. *Ann. Bot. (Oxford)* 110: 1515–1529. <http://dx.doi.org/10.1093/aob/mcs202>
- Ebihara, A., Nakato, N., Matsumoto, S., Chao, Y.-S. & Kuo, L.-Y.** 2014. Cytotaxonomic studies on thirteen ferns of Taiwan. *Bull. Natl. Sci. Mus. Tokyo B* 40: 19–28.
- Fraser-Jenkins, C.R.** 1992. The ferns and allies of the far west Himalaya – Some additions and corrections. *Bot. Helv.* 102: 143–157.
- Fraser-Jenkins, C.R.** 2008. *Taxonomic revision of three hundred Indian subcontinental pteridophytes with a revised census-list: A new picture of fern-taxonomy and nomenclature in the Indian subcontinent.* Dehra Dun: Bishen Singh Mahendra Pal Singh.
- Gämperle, E. & Schneller, J.J.** 2002. Phenotypic and isozyme variation in *Cystopteris fragilis* (Pteridophyta) along an altitudinal gradient in Switzerland. *Flora* 197: 203–213. <http://dx.doi.org/10.1078/0367-2530-00031>
- Grusz, A.L., Windham, M.D. & Pryer, K.M.** 2009. Deciphering the origins of apomictic polyploids in the *Cheilanthes yavapensis* complex (Pteridaceae). *Amer. J. Bot.* 96: 1636–1645. <http://dx.doi.org/10.3732/ajb.0900019>
- Guggisberg, A., Mansion, G. & Conti, E.** 2009. Disentangling reticulate evolution in an arctic-alpine polyploid complex. *Syst. Biol.* 58: 55–73. <http://dx.doi.org/10.1093/sysbio/syp010>
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O.** 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol.* 59: 307–321. <http://dx.doi.org/10.1093/sysbio/syq010>
- Haufler, C.H.** 1985. Pteridophyte evolutionary biology: The electrophoretic approach. *Proc. Roy. Soc. Edinburgh, B, Biol. Sci.* 86: 315–323.
- Haufler, C.H. & Windham, M.D.** 1991. New species of North American *Cystopteris* and *Polypodium*, with comments on their reticulate relationships. *Amer. Fern J.* 81: 7–23. <http://dx.doi.org/10.2307/1547129>
- Haufler, C.H., Windham, M.D., Britton, D.M. & Robinson, S.J.** 1985. Triploidy and its evolutionary significance in *Cystopteris protrusa*. *Canad. J. Bot.* 63: 1855–1863.
- Haufler, C.H., Windham, M.D. & Ranker, T.A.** 1990. Biosystematic analysis of the *Cystopteris tennesseensis* (Dryopteridaceae) complex. *Ann. Missouri Bot. Gard.* 77: 314–329. <http://dx.doi.org/10.2307/2399546>
- Haufler, C.H., Moran, R.C. & Windham, M.D.** 1993. *Cystopteris*. Pp. 263–270 in: Flora of North America Editorial Committee (ed.), *Flora of North America north of Mexico*, vol. 2. New York: Oxford University Press.
- Hori, K., Tono, A., Fujimoto, K., Kato, J., Ebihara, A., Watano, Y. & Murakami, N.** 2014. Reticulate evolution in the apogamous *Dryopteris varia* complex (Dryopteridaceae, subg. *Erythrovariae*, sect. *Variae*) and its related sexual species in Japan. *J. Pl. Res.* <http://dx.doi.org/10.1007/s10265-014-0652-0>
- Ishikawa, N., Yokoyama, J. & Tsukaya, H.** 2009. Molecular evidence of reticulate evolution in the subgenus *Plantago* (Plantaginaceae). *Amer. J. Bot.* 96: 1627–1635.
- Ivanova, D. & Piekos-Mirkowa, H.** 2003. Chromosome numbers of Polish ferns. *Acta Biol. Cracov., Ser. Bot.* 45: 93–99.
- Jäkäläniemi, A.** 1994. A review of the fern, *Gymnocarpium continentalis* (V. Petrov) Pojark. in northeastern Finland. *Aquilo, Ser. Bot.* 33: 47–53.
- Jermey, A.C.** 1993. *Cystopteris* Bernh. Pp. 24–25 in: Tutin, T.G., Burges, N.A., Chater, A.O., Edmondson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walter, S.M. & Webb, D.A. (eds.), *Flora Europaea*, ed. 2, vol. 1. Cambridge: Cambridge University Press.
- Juslén, A., Väre, H. & Wikström, N.** 2011. Relationships and evolutionary origins of polyploid *Dryopteris* (Dryopteridaceae) from Europe inferred using nuclear *pgiC* and plastid *trnL-F* sequence data. *Taxon* 60: 1284–1294.
- Kim, S.-T., Sultan, S.E. & Donoghue, M.J.** 2008. Allopolyploid speciation in *Persicaria* (Polygonaceae): Insights from a low-copy nuclear region. *Proc. Natl. Acad. Sci. U.S.A.* 105: 12370–12375. <http://dx.doi.org/10.1073/pnas.0805141105>
- Knapp, R.** 2011. *Ferns and fern allies of Taiwan.* Taiwan: KBCC Press.
- Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S.** 2012. Partition-Finder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molec. Biol. Evol.* 29: 1695–1701. <http://dx.doi.org/10.1093/molbev/mss020>
- Lee, S.-J. & Park, C.-W.** 2013. Relationships and origins of the *Dryopteris varia* (L.) Kuntze species complex (Dryopteridaceae) in Korea inferred from nuclear and chloroplast DNA sequences. *Biochem. Syst. Ecol.* 50: 371–382. <http://dx.doi.org/10.1016/j.bse.2013.05.008>
- Lellinger, D.B.** 1981. Notes on North American ferns. *Amer. Fern J.* 71: 90–94. <http://dx.doi.org/10.2307/1546734>
- Lewis, P.O.** 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* 50: 913–925. <http://dx.doi.org/10.1080/106351501753462876>
- Li, F.-W., Pryer, K.M. & Windham, M.D.** 2012. *Gaga*, a new fern genus segregated from *Cheilanthes* (Pteridaceae). *Syst. Bot.* 37: 845–860. <http://dx.doi.org/10.1600/036364412X656626>

- Lo, E.Y.Y., Stefanovic, S. & Dickinson, T.A. 2010. Reconstructing reticulation history in a phylogenetic framework and the potential of allopatric speciation driven by polyploidy in an agamic complex in *Crataegus* (Rosaceae). *Evolution* 64: 3593–3608. <http://dx.doi.org/10.1111/j.1558-5646.2010.01063.x>
- Löve, A. & Löve, D. 1967. New combinations in *Carpogymnia*. *Taxon* 16: 191–192. <http://dx.doi.org/10.2307/1216988>
- Löve, A., Löve, D. & Pichi Sermolli, R.E.G. 1977. *Cytotaxonomical Atlas of the Pteridophyta*. Vaduz: Strauss & Cramer.
- Lovis, J. 1978. Evolutionary patterns and processes in ferns. *Advances Bot. Res.* 4: 229–415. [http://dx.doi.org/10.1016/S0065-2296\(08\)60371-7](http://dx.doi.org/10.1016/S0065-2296(08)60371-7)
- Maddison, W.P. & Maddison, D.R. 2011. Mesquite: A modular system for evolutionary analysis, version 2.75. <http://mesquiteproject.org>
- Manton, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge University Press.
- Marcussen, T., Jakobsen, K.S., Danihelka, J., Ballard, H.E., Blaxland, K., Brysting, A.K. & Oxelman, B. 2012. Inferring species networks from gene trees in high-polyploid North American and Hawaiian violets (*Viola*, Violaceae). *Syst. Biol.* 61: 107–126. <http://dx.doi.org/10.1093/sysbio/syr096>
- Mickel, J.T. & Smith, A.R. 2004. *The pteridophytes of Mexico*. New York: The New York Botanical Garden Press.
- Mitui, K. 1970. Chromosome studies on Japanese ferns (4). *J. Jap. Bot.* 45: 84–90.
- Moran, R.C. 1982. *Cystopteris* × *illinoensis*: A new natural hybrid fern. *Amer. Fern J.* 72: 41–44. <http://dx.doi.org/10.2307/1547054>
- Moran, R.C. 1983a. *Cystopteris* × *wagneri*: A new naturally occurring hybrid between *C. ×tennesseensis* and *C. tenuis*. *Castanea* 48: 224–229.
- Moran, R.C. 1983b. *Cystopteris tenuis* (Michx.) Desv.: A poorly understood species. *Castanea* 48: 218–223.
- Moran, R.C. 1995. *Cystopteris*. P. 228 in: Moran, R.C. & Riba, R. (eds.), *Flora Mesoamericana*, vol. 1. Mexico: Universidad Nacional Autónoma de México.
- Murphy, R.J. & Rumsey, F.J. 2005. *Cystopteris diaphana* (Bory) Blasdel (Woodsiaceae) – An overlooked native new to the British Isles? *Watsonia* 25: 255–263.
- Nagalingum, N.S., Schneider, H. & Pryer, K.M. 2007. Molecular phylogenetic relationships and morphological evolution in the heterosporous fern genus *Marsilea*. *Syst. Bot.* 32: 16–25. <http://dx.doi.org/10.1600/036364407780360256>
- Nakaike, T. 1969. A new variety of *Gymnocarpium dryopteris* from Kai-prov., Central Japan. *J. Geobot.* 17: 96–97.
- Nitta, J.H., Ebihara, A. & Ito, M. 2011. Reticulate evolution in the *Crepidomanes minutum* species complex (Hymenophyllaceae). *Amer. J. Bot.* 98: 1782–1800. <http://dx.doi.org/10.3732/ajb.1000484>
- Otto, S.P. & Whitton, J. 2000. Polyploid incidence and evolution. *Annual Rev. Genet.* 34: 401–437. <http://dx.doi.org/10.1146/annurev.genet.34.1.401>
- Paler, M.H. & Barrington, D.S. 1995. The hybrid *Cystopteris fragilis* × *C. tenuis* (Dryopteridaceae) and the relationship between its tetraploid progenitors. *Syst. Bot.* 20: 528–545. <http://dx.doi.org/10.2307/2419807>
- Pellinen, K., Sarvela, J. & Uotila, P. 1998. Chromosome counts on the fern genus *Gymnocarpium* (Dryopteridaceae) from Finland. *Ann. Bot. Fenn.* 35: 265–266.
- Pryer, K.M. 1992. The status of *Gymnocarpium heterosporum* and *G. robertianum* in Pennsylvania. *Amer. Fern J.* 82: 34–39. <http://dx.doi.org/10.2307/1547760>
- Pryer, K.M. 1993. *Gymnocarpium*. Pp. 258–262 in: Flora of North America Editorial Committee (ed.), *Flora of North America north of Mexico*, vol. 2. New York: Oxford University Press.
- Pryer, K.M. & Hafler, C.H. 1993. Isozymic and chromosomal evidence for the allotetraploid origin of *Gymnocarpium dryopteris* (Dryopteridaceae). *Syst. Bot.* 18: 150–172. <http://dx.doi.org/10.2307/2419795>
- Pryer, K.M., Britton, D.M. & McNeill, J. 1983. A numerical analysis of chromatographic profiles in North American taxa of the fern genus *Gymnocarpium*. *Canad. J. Bot.* 61: 2592–2602.
- Pryer, K.M., Britton, D.M. & McNeill, J. 1984. Hybridization in the fern genus *Gymnocarpium* Newm. (Aspleniaceae) in North America. *Amer. J. Bot.* [abstract] 71: 142. <http://dx.doi.org/10.1139/b83-285>
- Ree, R. 2008. gapcode.py, version 2.1. <http://www.reelab.net/home/software>
- Reeves, T. 1976. *Cystopteris bulbifera* in the southwestern United States. *Amer. Fern J.* 66: 60–62. <http://dx.doi.org/10.2307/1546520>
- Rothfels, C.J. 2012. *Phylogenetics of Cystopteridaceae: Reticulation and divergence in a cosmopolitan fern family*. Ph.D. Dissertation. Duke University, Durham, North Carolina, U.S.A.
- Rothfels, C.J. & Schuettpelz, E. 2014. Accelerated rate of molecular evolution for vittarioid ferns is strong and not driven by selection. *Syst. Biol.* 63: 31–54. <http://dx.doi.org/10.1093/sysbio/syt058>
- Rothfels, C.J., Larsson, A., Kuo, L.-Y., Korall, P., Chiou, W.-L. & Pryer, K.M. 2012a. Overcoming deep roots, fast rates, and short internodes to resolve the ancient rapid radiation of eupolypod II ferns. *Syst. Biol.* 61: 490–509. <http://dx.doi.org/10.1093/sysbio/sys001>
- Rothfels, C.J., Sundue, M.A., Kuo, L.-Y., Larsson, A., Kato, M., Schuettpelz, E. & Pryer, K.M. 2012b. A revised family-level classification for eupolypod II ferns (Polypodiidae: Polypodiales). *Taxon* 61: 515–533.
- Rothfels, C.J., Larsson, A., Li, F.-W., Sigel, E.M., Huiet, L., Burge, D.O., Ruhsam, M., Graham, S.W., Stevenson, D.W., Wong, G.K.-S., Korall, P. & Pryer, K.M. 2013a. Transcriptome-mining for single-copy nuclear markers in ferns. *PLoS ONE* 8: e76957. <http://dx.doi.org/10.1371/journal.pone.0076957>
- Rothfels, C.J., Windham, M.D. & Pryer, K.M. 2013b. A plastid phylogeny of the cosmopolitan fern family Cystopteridaceae (Polypodiopsida). *Syst. Bot.* 38: 295–306. <http://dx.doi.org/10.1600/036364413X666787>
- Rousseau-Gueutin, M., Gaston, A., Aïnouche, A., Aïnouche, M.L., Olbricht, K., Staudt, G., Richard, L. & Denoyes-Rothan, B. 2009. Tracking the evolutionary history of polyploidy in *Fragaria* L. (strawberry): New insights from phylogenetic analyses of low-copy nuclear genes. *Molec. Phylog. Evol.* 51: 515–530. <http://dx.doi.org/10.1016/j.ympev.2008.12.024>
- Sarvela, J. 1978. A synopsis of the fern genus *Gymnocarpium*. *Ann. Bot. Fenn.* 15: 101–106.
- Sarvela, J. 1980. *Gymnocarpium* hybrids from Canada and Alaska. *Ann. Bot. Fenn.* 17: 292–295.
- Sarvela, J., Britton, D.M. & Pryer, K.M. 1981. Studies on the *Gymnocarpium robertianum* complex in North America. *Rhodora* 83: 421–431.
- Schneider, H., Navarro-Gomez, A., Russell, S.J., Ansell, S.W., Grundmann, M. & Vogel, J.C. 2012. Exploring the utility of three nuclear regions to reconstruct reticulate evolution in the fern genus *Asplenium*. *J. Syst. Evol.* 51: 142–153. <http://dx.doi.org/10.1111/j.1759-6831.2012.00226.x>
- Schuettpelz, E. & Pryer, K.M. 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56: 1037–1050. <http://dx.doi.org/10.2307/25065903>
- Schuettpelz, E., Grusz, A.L., Windham, M.D. & Pryer, K.M. 2008. The utility of nuclear *gapCp* in resolving polyploid fern origins. *Syst. Bot.* 33: 621–629. <http://dx.doi.org/10.1600/036364408786500127>
- Sessa, E.B., Zimmer, E.A. & Givnish, T.J. 2012. Unraveling reticulate evolution in North American *Dryopteris* (Dryopteridaceae). *B. M. C. Evol. Biol.* 12: 104. <http://dx.doi.org/10.1186/1471-2148-12-104>
- Shaver, J.M. 1950. A new fern, *Cystopteris tennesseensis* sp. nov., from Tennessee. *J. Tennessee Acad. Sci.* 25: 106–113.
- Shepherd, L.D., Perrie, L.R. & Brownsey, P.J. 2008. Low-copy

- nuclear DNA sequences reveal a predominance of allopolyploids in a New Zealand *Asplenium* fern complex. *Molec. Phylogen. Evol.* 49: 240–248. <http://dx.doi.org/10.1016/j.ympev.2008.06.015>
- Simmons, M.P. & Ochoterena, H.** 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol.* 49: 369–381. <http://dx.doi.org/10.1093/sysbio/49.2.369>
- Smith, A.R.** 1993. Dryopteridaceae. Pp. 246–308 in: Flora of North America Editorial Committee (ed.), *Flora of North America north of Mexico*, vol. 2. New York: Oxford University Press.
- Smith, A.R., Pryer, K.M., Schuettpelz, E., Korall, P., Schneider, H. & Wolf, P.G.** 2006. A classification for extant ferns. *Taxon* 55: 705–731. <http://dx.doi.org/10.2307/25065646>
- Sorsa, V.** 1958. Chromosome studies on Finnish pteridophyta I. *Hereditas* 44: 541–546. <http://dx.doi.org/10.1111/j.1601-5223.1958.tb03499.x>
- Sorsa, V.** 1961. Chromosome studies on Finnish Pteridophyta II. *Hereditas* 47: 480–488. <http://dx.doi.org/10.1111/j.1601-5223.1961.tb01784.x>
- Sorsa, V.** 1966. A diploid *Gymnocarpium dryopteris* (L.) Newman in Alaska. *Nature* 5043: 1375. <http://dx.doi.org/10.1038/2101375a0>
- Sundue, M.A. & Rothfels, C.J.** 2014. Stasis and convergence characterize morphological evolution in eupolypod II ferns. *Ann. Bot. (Oxford)* 113: 35–54. <http://dx.doi.org/10.1093/aob/mct247>
- Swofford, D.L.** 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0. Sunderland, Massachusetts: Sinauer.
- Taylor, R.L. & Mulligan, G.A.** 1968. *Flora of the Queen Charlotte Islands*, part 2, *Cytological aspects of the vascular plants*. Ottawa: Department of Agriculture.
- Tindale, M.D. & Roy, S.K.** 2002. A cytotoxic survey of the Pteridophyta of Australia. *Austral. Syst. Bot.* 15: 839–937. <http://dx.doi.org/10.1071/SB00034>
- Triplett, J.K., Clark, L.G., Fisher, A.E. & Wen, J.** 2014. Independent allopolyploidization events preceded speciation in the temperate and tropical woody bamboos. *New Phytologist* 204: 66–73. <http://dx.doi.org/10.1111/nph.12988>
- Tsai, J.-L.** 1992. Cytotoxic studies on the pteridophytes of Taiwan. Pp. 121–145 in: Tsai, J.-L. & Shieh, W.-C. (eds.), *Proceedings of the Second Seminar on Asian Pteridology: Taiwan, 17–22 March 1992*. Taipei: National Chung Hsing University and National Science Council.
- Tsai, J.L. & Shieh, W.C.** 1985. A cytotoxic survey of the fern family Aspidiaceae (sensu Copeland) in Taiwan. *J. Sci. Engin.* 40: 121–144.
- Vida, G.** 1972. Cytotoxic and genome analysis of the European ferns. Pp. 51–60 in: Vida, G. (ed.), *Evolution in plants*. Budapest: Akadémiai Kiadó.
- Vida, G.** 1974. Genome analysis of the European *Cystopteris fragilis* complex. I: Tetraploid taxa. *Acta. Bot. Acad. Sci. Hung.* 20: 181–192.
- Vida, G. & Mohay, J.** 1980. Cytophotometric DNA studies in polyploid series of the fern genus *Cystopteris* Bernh. *Acta. Bot. Acad. Sci. Hung.* 26: 455–461.
- Wagner, W.H., Jr.** 1955. Cytotoxic observations on North American ferns. *Rhodora* 57: 219–240.
- Wagner, W.H., Jr.** 1966. New data on North American oak ferns, *Gymnocarpium*. *Rhodora* 68: 121–138.
- Wagner, W.H., Jr. & Hagenah, D.J.** 1956. A diploid variety in the *Cystopteris fragilis* complex. *Rhodora* 58: 78–87.
- Weatherby, C.A.** 1926. A new North American variety of *Cystopteris fragilis*. *Rhodora* 28: 129–131.
- Wei, R. & Zhang, X.-C.** 2014. Rediscovery of *Cystoathyrium chinense* Ching (Cystopteridaceae): Phylogenetic placement of the critically endangered fern species endemic to China. *J. Syst. Evol.* 52: 450–457. <http://dx.doi.org/10.1111/jse.12075>
- Zhang, R., Liu, T., Wu, W., Li, Y., Chao, L., Huang, L., Huang, Y., Shi, S. & Zhou, R.** 2013. Molecular evidence for natural hybridization in the mangrove fern genus *Acrostichum*. *B. M. C. Pl. Biol.* 13: 74. <http://dx.doi.org/10.1186/1471-2229-13-74>
- Zimmer, E.A. & Wen, J.** 2012. Using nuclear gene data for plant phylogenetics: Progress and prospects. *Molec. Phylogen. Evol.* 65: 774–785. <http://dx.doi.org/10.1016/j.ympev.2012.07.015>
- Zwickl, D.J.** 2006. *GARLI: Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. Dissertation. The University of Texas, Austin, U.S.A.

Appendix 1. Voucher table for the sequence datasets, with GenBank numbers and ploidy information. DB#: Fern Lab Database accession number (<http://fernlab.biology.duke.edu/>). Numerals following GenBank numbers in the “total unique sequences” column indicate the number of times that particular sequence was obtained. The second row for each accession summarizes the known ploidy data available for that accession; the *Cytotaxonomical Atlas of the Pteridophyta* (Löve & al., 1977) was invaluable in gathering these data. Spore size measurements refer to the long axis of the spore. The measurement protocol of Tzu-Tong Kao (used for DB#7009 and 7625) includes the perispore and models the spore as an ellipse; it produces size estimates that are approximately 20% larger than the others presented here (T.-T. Kao, unpub.). Otherwise, the spore measurements exclude the perispore.

DB#	Accession	gapCp		Alleles	trnGR
		Total clones	Total unique sequences		
4870	<i>Acystopteris taiwaniana</i> (Tagawa) A.Löve & D.Löve. <i>Schuettpelz 1127A</i> (DUKE). Taiwan: Nantou Co. Probable diploid. Inference from chromosome counts of conspecific individuals (Tsai, 1992).	5	2 (KJ946938 ×4; KJ946939)	1 (KJ946938)	JF832188 (Rothfels & al., 2012a)
4831	<i>A. tenuisecta</i> (Blume) Tagawa. <i>Schuettpelz 1088A</i> (DUKE). Taiwan: Nantou Co. Diploid. Chromosome count ($n = 42$; Electr. Suppl.: Fig. S1). See also Ebihara & al. (2014).	4	2 (KJ946940 ×3; KJ946941)	1 (KJ946940)	JF832189 (Rothfels & al., 2012a)
5836	<i>Cystopteris bulbifera</i> (L.) Bernh. <i>Windham 94-189</i> (DUKE). U.S.A.: Arizona. Coconino Co. Diploid. Chromosome count ($n = 42$; no photo available).	3	1 (KJ946942 ×3)	1 (KJ946942)	JX874076 (Rothfels & al., 2013b)

Appendix 1. Continued.

DB#	Accession	Total clones	gapCp		trnGR
			Total unique sequences	Alleles	
6116	<i>C. bulbifera</i> . <i>Almack s.n.</i> (DUKE). Canada: Ontario. Durham Reg.	13	8 (KJ946950 ×3; KJ946944; KJ946943 ×2; KJ946946; KJ946948; KJ946947 ×2; KJ946945 ×2; KJ946949)	1 (KJ946944)	KJ947095
Probable diploid. Inference from chromosome counts of conspecific individuals (Britton, 1953; Wagner, 1955; Wagner & Hagenah, 1956; Reeves, 1976; Haufler & al., 1990).					
5316	<i>C. fragilis</i> (L.) Bernh. <i>Matos 08-147</i> (DUKE). Costa Rica: San Jose.	13	7 (KJ946951 ×3; KJ946952 ×5; KJ946953; KJ946954; KJ946955; KJ946956; KJ946957)	1 (KJ946951)	JX874078 (Rothfels & al., 2013b)
Tetraploid. Chromosome count ($n = 84$; no photo available), and spore size of this specimen falls within range of documented tetraploids (length = $38.35 \pm 2.73 \mu\text{m}$; 24 spores measured).					
6380	<i>C. fragilis</i> . <i>Arana 889</i> (DUKE). Argentina: Prov. San Luis.	5	5 (KJ946985; KJ946986; KJ946987; KJ946988; KJ946989)	2 (KJ946985; KJ946987)	JX874081 (Rothfels & al., 2013b)
Diploid. Spore size of this specimen falls within range of documented diploids (length = $33.80 \pm 1.09 \mu\text{m}$; 10 spores measured).					
5840	<i>C. fragilis</i> . <i>Christenhusz 2931</i> (DUKE). Sweden: Uppsala.	10	7 (KJ946959 ×2; KJ946962 ×3; KJ946958; KJ946960; KJ946961; KJ946963; KJ946964)	2 (KJ946959; KJ946962)	KJ947096
Tetraploid. Spore size of this specimen falls within range of documented tetraploids (length = $41.18 \pm 3.21 \mu\text{m}$; 24 spores measured).					
5851	<i>C. fragilis</i> . <i>Kelsey s.n.</i> (DUKE). U.S.A.: Utah. Salt Lake Co.	15	9 (KJ946965 ×4; KJ946970 ×4; KJ946966; KJ946967; KJ946968; KJ946969; KJ946971; KJ946972; KJ946973)	3 (KJ946965; KJ946970; KJ946973)	JX874087 (Rothfels & al., 2013b)
Probable tetraploid. Inference from chromosome counts of this species from this area. M.D. Windham, unpub.					
7009	<i>C. fragilis</i> . <i>Heidmarsson cf03</i> (DUKE). Iceland: Vesturland Reg.	17	10 (KJ946990 ×2; KJ946992 ×2; KJ946997 ×2; KJ946994 ×5; KJ946993; KJ946991; KJ946995; KJ946996; KJ946998; KJ946999)	2 (KJ946997; KJ946994)	KJ947098
Probable tetraploid. Spore size of this specimen falls within range of documented tetraploids (length = $51.51 \pm 3.97 \mu\text{m}$; 61 spores measured; Tzu-Tong Kao, pers. comm.).					
7248	<i>C. fragilis</i> . <i>Sigel 2010-59</i> (DUKE). Switzerland: Bern Canton.	7	5 (KJ947003 ×3; KJ947000; KJ947001; KJ947002; KJ947004)	2 (KJ947001; KJ947003)	KJ947099
Probable polyploid. Tetraploid and hexaploid plants are common in Europe whereas diploids are unknown (L. Ekrt, pers. comm.; Manton, 1950; Sorsa, 1961; Vida, 1974; Dalgaard, 1989; Gämperle & Schneller, 2002).					
7625	<i>C. fragilis</i> . <i>Li 1056</i> (DUKE). Taiwan: Taichung Co.	6	4 (KJ947006 ×2; KJ947007 ×2; KJ947005; KJ947008)	2 (KJ947005; KJ947006)	KJ947100
Probable tetraploid. Spore size of this specimen falls within range of documented tetraploids (length = $50.18 \pm 3.37 \mu\text{m}$; 62 spores measured; Tzu-Tong Kao, pers. comm.). Diploids and tetraploids are reported from Taiwan (Tsai & Shieh, 1985; M.D. Windham, pers. obs.).					
5951	<i>C. fragilis</i> . <i>S.F. Smith 3</i> (DUKE). U.S.A.: Colorado. Park Co.	21	11 (KJ946975 ×2; KJ946976 ×10; KJ946974; KJ946977; KJ946978; KJ946979; KJ946980; KJ946981; KJ946982; KJ946983; KJ946984)	3 (KJ946976; KJ946977; KJ946980)	KJ947097
Probable tetraploid. Inference from chromosome counts of this species from this area (M.D. Windham, unpub.).					
5839	<i>C. montana</i> (Lam.) Bernh. ex Desv. <i>LeBlond 6448</i> (DUKE). Canada: NFLD. St. Barbe N Dist.	4	3 (KJ947009; KJ947010 ×2; KJ947011)	1 (KJ947009)	JF832205 (Rothfels & al., 2012a)
Probable tetraploid. Inference from chromosome counts of this species (Manton, 1950; Britton, 1964).					

Appendix 1. Continued.

DB#	Accession	<i>gapCp</i>		Alleles	<i>trnGR</i>
		Total clones	Total unique sequences		
4861	<i>C. moupinensis</i> Franch. <i>Schuettpeiz 1118A</i> (DUKE). Taiwan: Nantou Co.	5	5 (KJ947012; KJ947013; KJ947014; KJ947015; KJ947016)	1 (KJ947015)	JF832206 (Rothfels & al., 2012a)
	Diploid. Spore size of this specimen falls within range of documented diploids (length = 29.95 ± 3.60 µm; 18 spores measured).				
5838	<i>C. protrusa</i> (Weath.) Blasdell. <i>Alford 2088</i> (DUKE). U.S.A.: Mississippi. Wilkinson Co.	5	4 (KJ947018 ×2; KJ947017; KJ947019; KJ947020)	2 (KJ947017; KJ947018)	JX874101 (Rothfels & al., 2013b)
	Probable diploid. Inference from chromosome counts of this species (Electr. Suppl.: Fig. S1). See also Wagner & Hagenah (1956), Haufler & al. (1985, 1990).				
6379	<i>C. tasmanica</i> Hook. <i>Thorsen 192/07</i> (DUKE). New Zealand: Otago.	1	1 (KJ947021)	1 (KJ947021)	JX874110 (Rothfels & al., 2013b)
	Probable tetraploid. Inference from chromosome counts of this species (Tindale & Roy, 2002).				
6387	<i>C. tenuis</i> (Michx.) Desv. <i>Barrington 2373</i> (DUKE). U.S.A.: Vermont. Chittenden Co.	10	7 (KJ947022 ×4; KJ947023; KJ947024; KJ947025; KJ947026; KJ947027; KJ947028)	2 (KJ947022; KJ947026)	JX874114 (Rothfels & al., 2013b)
	Probable tetraploid. Inference from chromosome counts of this species (Britton & al., 1984; Haufler, 1985; Paler & Barrington, 1995).				
7639	<i>Gymnocarpium appalachianum</i> Pryer & Haufler. <i>Rothfels 3914</i> (DUKE). U.S.A.: Virginia. Highland Co.	8	5 (KJ947038 ×4; KJ947037; KJ947039; KJ947040; KJ947041)	1 (KJ947038)	JX874119 (Rothfels & al., 2013b)
	Probable diploid. Inference from chromosome counts and spore measurements from this species from this area (<i>CJR3896</i> , length = 25.27 ± 1.99 µm, 12 spores measured; <i>CJR3900</i> , length = 25.28 ± 0.89 µm, 10 spores measured) (Pryer & Haufler, 1993).				
7800	<i>G. appalachianum</i> . <i>Rothfels 3897</i> (DUKE). U.S.A.: Virginia. Page Co.	11	8 (KJ947031 ×2; KJ947032 ×2; KJ947033 ×2; KJ947029; KJ947030; KJ947034; KJ947035; KJ947036)	1 (KJ947032)	JX874120 (Rothfels & al., 2013b)
	Probable diploid. Inference from chromosome counts and spore measurements from this species from this area (<i>CJR3896</i> , length = 25.27 ± 1.99 µm, 12 spores measured; <i>CJR3900</i> , length = 25.28 ± 0.89 µm, 10 spores measured) (Pryer & Haufler, 1993).				
4710	<i>G. disjunctum</i> (Rupr.) Ching. <i>Metzgar 224</i> (DUKE). U.S.A.: Alaska. Kenai Pen. Borough.	4	4 (KJ947042; KJ947043; KJ947044; KJ947045)	1 (KJ947043)	JX874121 (Rothfels & al., 2013b)
	Probable diploid. Inference from chromosome counts and spore measurements from this species (Sorsa, 1966; Wagner, 1966; Taylor & Mulligan, 1968; Pryer & Haufler, 1993).				
7751	<i>G. disjunctum</i> . <i>Sigel 2010-82</i> (DUKE). U.S.A.: Washington. Snohomish Co.	11	7 (KJ947050 ×2; KJ947047 ×4; KJ947046; KJ947048; KJ947049; KJ947051; KJ947052)	2 (KJ947047; KJ947048)	JX874122 (Rothfels & al., 2013b)
	Probable diploid. Inference from counts and spore measurements from this species (Sorsa, 1966; Wagner, 1966; Taylor & Mulligan, 1968; Pryer & Haufler, 1993).				
5837	<i>G. dryopteris</i> (L.) Newman. <i>Christenhusz 3758</i> (DUKE). Finland: Varsinais-Suomi Arch.	7	3 (KJ947053 ×2; KJ947054; KJ947055 ×4)	2 (KJ947053; KJ947055)	JX874124 (Rothfels & al., 2013b)
	Probable tetraploid. Inference from chromosome counts and spore measurements from this species (Manton, 1950; Sorsa, 1958; Wagner, 1966; Pryer & Haufler, 1993; Pellinen & al., 1998).				
7981	<i>G. dryopteris</i> . <i>Ebihara & Kadota HK2007-815</i> . Japan: Hokkaido Pref.	18	11 (KJ947057 ×4; KJ947058 ×2; KJ947061 ×2; KJ947064 ×2; KJ947065 ×2; KJ947056; KJ947059; KJ947060; KJ947062; KJ947063; KJ947066)	3 (KJ947057; KJ947058; KJ947065)	JX874125 (Rothfels & al., 2013b)
	Probable tetraploid. Inference from chromosome counts and spore measurements from this species (Manton, 1950; Sorsa, 1958; Wagner, 1966; Pryer & Haufler, 1993; Pellinen & al., 1998).				

Appendix 1. Continued.

DB#	Accession	<i>gapCp</i>			<i>trnGR</i>
		Total clones	Total unique sequences	Alleles	
8031	<i>G. dryopteris. Oldham 38191</i> (DUKE). Canada: Ontario. Thunder Bay Dist.	15	9 (KJ947067 ×5; KJ947071 ×2; KJ947069 ×2; KJ947070; KJ947068; KJ947072; KJ947073; KJ947074; KJ947075)	2 (KJ947067; KJ947070)	KJ947101
Probable tetraploid. Inference from chromosome counts and spore measurements from this species (Manton, 1950; Sorsa, 1958; Wagner, 1966; Pryer & Haufler, 1993; Pellinen & al., 1998).					
6979	<i>G. jessoense</i> subsp. <i>parvulum</i> Sarvela. <i>Harris 08-131</i> (DUKE). Canada: Ontario. Thunder Bay Dist.	3	2 (KJ947076; KJ947077 ×2)	2 (KJ947076; KJ947077)	KJ947102
Probable tetraploid. Inference from chromosome counts from this species (Sarvela & al., 1981; Pellinen & al., 1998).					
8120	<i>G. jessoense</i> subsp. <i>parvulum. Legler 877</i> (NY). Russia: Sakhalin Reg.	12	5 (KJ947078 ×6; KJ947081 ×3; KJ947079; KJ947080; KJ947082)	2 (KJ947078; KJ947081)	JX874131 (Rothfels & al., 2013b)
Probable tetraploid. Inference from chromosome counts from this species (Sarvela & al., 1981; Pellinen & al., 1998).					
4862	<i>G. remotepinnatum</i> (Hayata) Ching. <i>Schuettpelz 1119A</i> (DUKE). Taiwan: Nantou Co.	8	5 (KJ947086 ×3; KJ947083 ×2; KJ947084; KJ947085; KJ947087)	2 (KJ947085; KJ947086)	JX874133 (Rothfels & al., 2013b)
Probable tetraploid. Inference from spore size (length = 38.3 μm).					
7945	<i>G. robertianum</i> (Hoffm.) Newman. <i>Larsson 282</i> (DUKE). Norway: Nordland. Fauske Co.	12	7 (KJ947090 ×4; KJ947093 ×2; KJ947091 ×2; KJ947089; KJ947092; KJ947088; KJ947094)	2 (KJ947090; KJ947091)	JX874137 (Rothfels & al., 2013b)
Probable tetraploid. Inference from chromosome counts from this species (Wagner, 1966; Sarvela & al., 1981; Pellinen & al., 1998).					

TAXON

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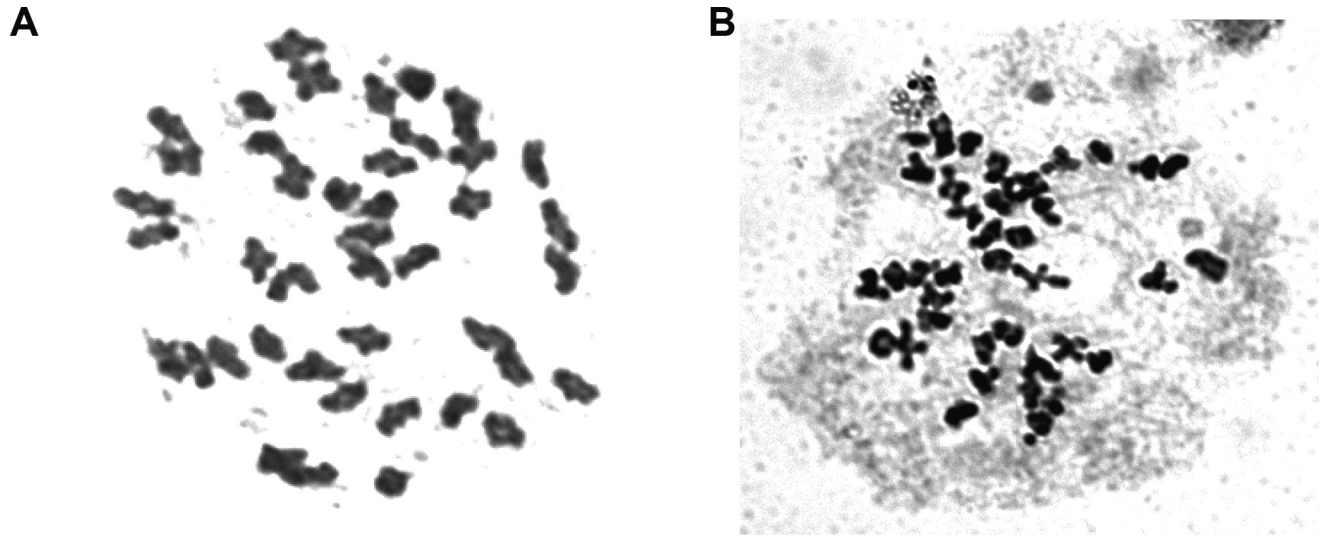


Fig. S1. Meiotic chromosome squashes. **A**, *Acystopteris tenuisecta*, *E. Schuettpelz 1088A* (DUKE), Nantou County, Taiwan, $n = 42$; **B**, *Cystopteris protrusa*, *C.J. Rothfels 2793* (DUKE), Sevier County, Tennessee, U.S.A., $n = \sim 42$.