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New Species of North American *Cystopteris* and *Polypodium*, with Comments on Their Reticulate Relationships

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Biosystematic studies of temperate species complexes in *Cystopteris* and *Polypodium* have helped to answer some of the seemingly intractable questions about patterns of variability among the diploid members of these genera. These studies have also resolved the origins of many polyploid species. By combining field observations, analyses of cultivated plants, studies of meiotic chromosome behavior, electrophoretic investigations of isozyme variants, as well as surveys of macro- and micro-morphological features using living and preserved specimens, we have found new species and worked out the reticulate patterns of hybridization and polyploidy. In developing contemporary treatments for the Flora North America project, we decided to assemble a separate report that would 1) recapitulate past systematic work in the two genera, 2) introduce some of the taxonomic complexities encountered in these groups, 3) discuss the characters analyzed, 4) describe new species, and 5) provide an overview of the remaining problems and future challenges facing systematists studying *Cystopteris* and *Polypodium*.

Hybridization, allopolyploid speciation, and the resulting reticulate patterns of evolution have been the primary impediments to developing a clear picture of species origins and interrelationships in *Cystopteris* and *Polypodium*. However, even when all polyploids are identified and only the remaining diploids are compared, obvious features for discriminating species can elude the casual observer. The morphological similarity of diploid species in these genera is in sharp contrast to the great differences among diploids in the Appalachian *Asplenium* complex. Ongoing studies of *Adiantum* (C. A. Paris), *Botrychium* (W. H. & F. S. Wagner), *Cryptogramma* (E. R. Alverson), *Dryopteris* (C. R. Werth), and *Gymnocarpium* (K. Pryer) are showing that the *Cystopteris*/*Polypodium* pattern of subtle morphological differentiation of species may be the rule rather than the exception in ferns. It is becoming clear that if our goal as systematic pteridologists is to recognize natural units and understand their evolutionary histories, we must be increasingly tolerant of treatments that emphasize cryptic characteristics. In this spirit, we offer the following taxonomic revisions.

BACKGROUND

Cystopteris.—Once Lellinger (1981) named *C. reevesiana*, systematic treatment

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of diploid taxa in North American *Cystopteris* seemed to reflect well the natural situation (Fig. 1). Thus, three North American diploids have been named: 1) *C. bulbifera* (L.) Bernh., a primarily cliff-dwelling species with elongate-triangular leaf blades that bear prominent laminar bulblets and unicellular glandular trichomes; 2) *C. protrusa* (Weath.) Blasdell, a species inhabiting forest floors in east-central North America and having distinctive rhizome pubescence and a prominent rhizome apex that protrudes beyond the current year's leaves; and 3) *C. reevesiana*, confined to mountains of the southwestern U.S. and having a creeping rhizome that lacks the peculiar pubescence and protruding apex of *C. protrusa* and commonly has more finely dissected leaves than either of the other diploids. During the present study, no additional diploids were encountered. Over parts of their ranges, *C. bulbifera* is sympatric with the other diploids, but populations of *C. protrusa* and *C. reevesiana* are separated by over a thousand miles.

In North American *Cystopteris*, the remaining systematic problems are at the polyploid level. Although one new tetraploid originating from extant diploids will be proposed, by far the most troublesome group centers on *C. fragilis* (L.) Bernh. This cosmopolitan polyploid contains considerable morphological variability, and in North America occurs at tetraploid and hexaploid levels. The origin of these polyploids is obscure (Fig. 1) and may involved an extinct diploid (Haufler, 1985). The cosmopolitan range of *C. fragilis* suggests that it is a relatively old species. Given its morphological variability, we may infer that evolution (and perhaps speciation) is actively taking place at the polyploid level. These complications confound attempts at developing a stable systematic treatment and argue for a conservative approach. Thus, except for the recognition of *C. tenuis* (Michx.) Desv., variants of *C. fragilis* will be discussed but not formally named.

Polypodium.—Members of the *P. vulgare* L. complex have probably received more attention from systematic pteriodologists over the years than any other group. This extraordinary attention can be attributed to biogeographical and morphological features. Members of this group are largely north temperate in distribution and thus are in the "backyards" of many pteridologists. In addition, *Polypodium* exhibits an array of ploidy levels that are accompanied by subtle but discrete variations in morphology. Our proposed systematic revisions in North American *Polypodium* are at the same time more complex and more straightforward than those in *Cystopteris*. We are suggesting more changes in *Polypodium* taxonomy, but the discovery of correlations between isozymic markers and stable, qualitative morphological characters (albeit somewhat cryptic) have made us quite confident about these systematic modifications.

Manton (1950) demonstrated that there were three ploidy levels among representatives of the *P. vulgare* complex in eastern North America. Until now, all three have been called cytotypes of *P. virginianum* L. Kott & Britton (1982) developed a careful analysis of the morphological characteristics that discriminate the three ploidy levels, showing discrete differences between diploids and tetraploids and the intermediacy of triploids. There has been

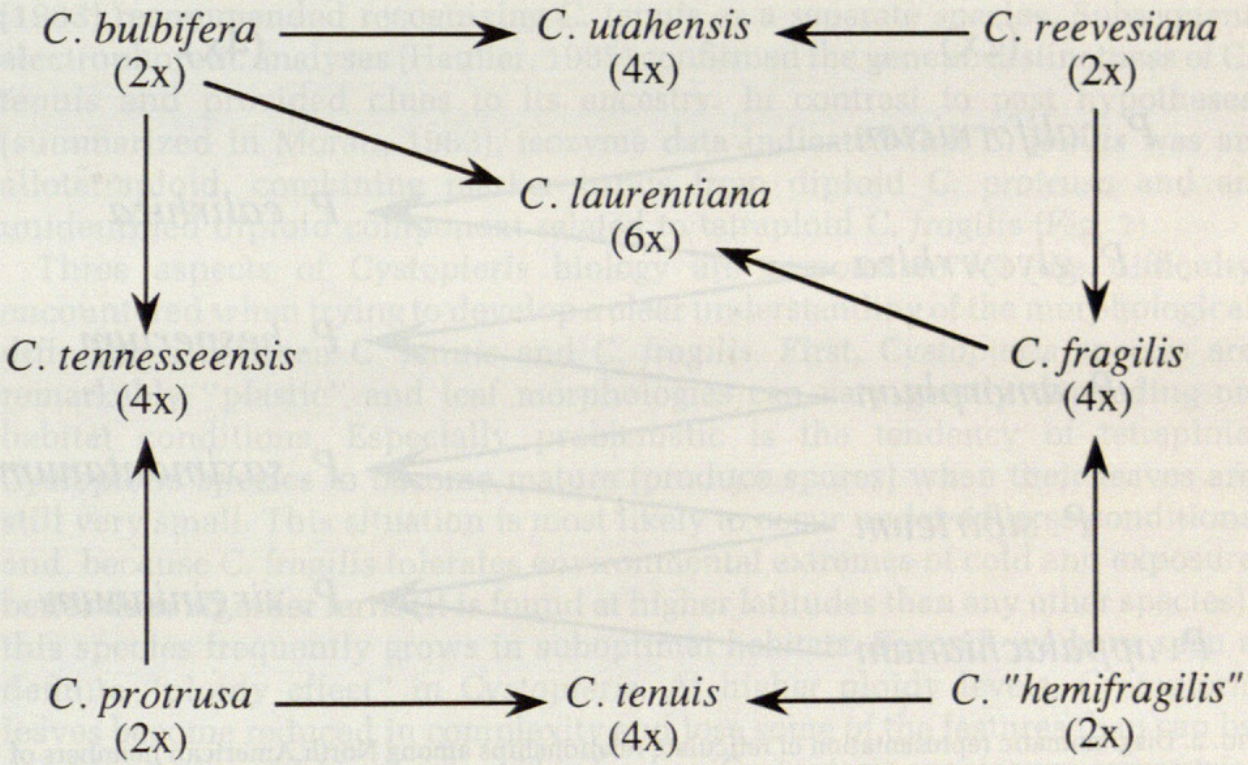

FIG. 1. Diagrammatic representation of reticulate relationships among North American members of the *Cystopteris fragilis* complex.

considerable debate over the origin of the tetraploid. Shivas (1961) showed abundant bivalent formation during meiosis in triploids and suggested that the diploid cytotype was one of the progenitors of the tetraploid. Evans (quoted in Lloyd & Lang, 1964) suggested that a diploid species from the Pacific Northwest of North America (now known as *P. amorphum* Suksdorf) appeared to be closely related to tetraploid *P. virginianum* and could represent the second progenitor genome. These hypotheses, therefore, were open for testing. This complex has even engendered nomenclatural debate. Although Löve & Löve (1977) argued that the type of *P. virginianum* was diploid, Cranfill & Britton's (1983) reexamination provided convincing evidence that the name *P. virginianum* belonged to the tetraploid cytotype. The diploid, therefore, had not been named.

In western North America, there are more *Polypodium* species than in the east. Lang's (1971) work helped to clarify the species in the Pacific Northwest and demonstrated that tetraploid *P. hesperium* Maxon originated from two extant diploids, *P. amorphum* and *P. glycyrrhiza* D. C. Eaton (Fig. 2). In northern California, allopolyploidy involving the diploids *P. glycyrrhiza* and *P. californicum* Kaulf. has generated a tetraploid that has been called "tetraploid *P. californicum*." Whitmore & Smith (1991) have been studying this group and have proposed the name *P. calirhiza* for the tetraploid (Fig. 2). As is true of the *P. virginianum* complex, sterile triploid backcrosses occur frequently when diploids and tetraploids are sympatric. The presence of these sterile hybrids has blurred the morphological distinctness of the sexual species and has

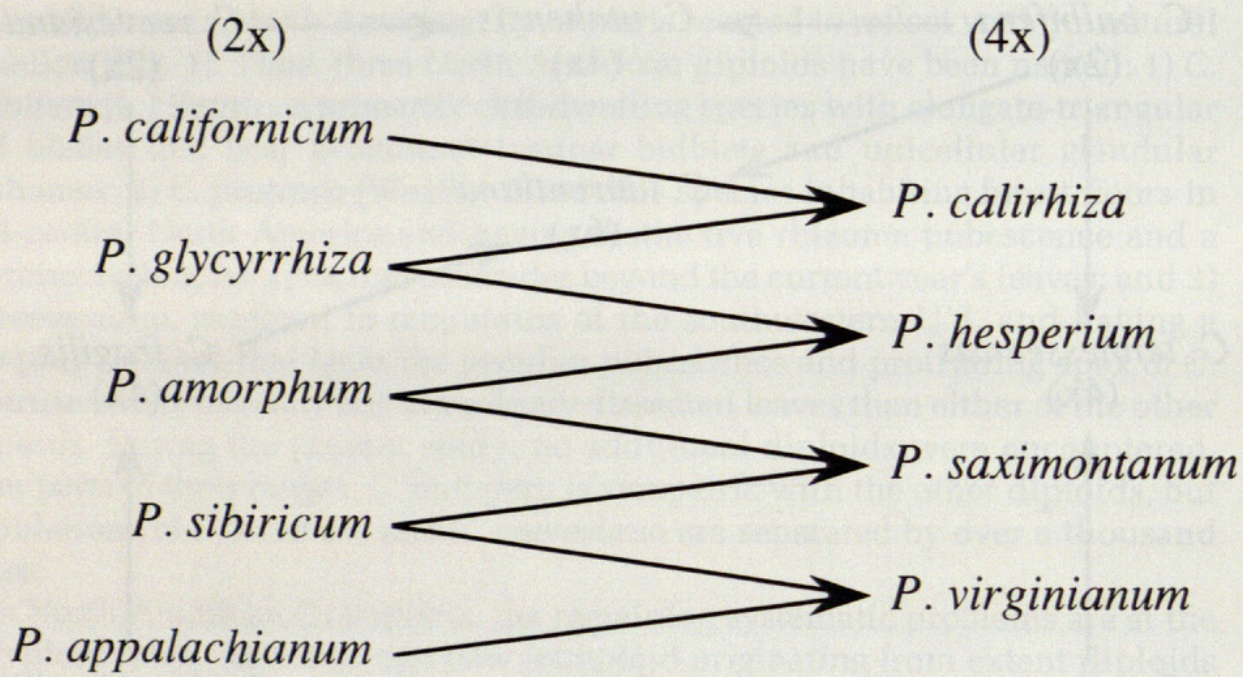

FIG. 2. Diagrammatic representation of reticulate relationships among North American members of the *Polypodium vulgare* complex.

contributed to the systematic controversies. Slight morphological differences between northern and southern California populations of the diploid *P. californicum* (Barrington et al., 1986) have also tended to confound the situation.

Windham (1991) has clarified relationships in *Polypodium* from the southwestern U.S. He described an additional tetraploid species, *P. saximontanum*, from the Rocky Mountains. This tetraploid had been confused with the more western tetraploid *P. hesperium*, and electrophoretic studies have demonstrated that the two tetraploids each contain a genome derived from *P. amorphum*. However, the two species are quite distinct genetically because the second genome was contributed by different species (Fig. 2) belonging to lineages that probably diverged millions of years ago. The fact that these distantly related tetraploids could be confused for so long emphasizes the complexities of this group and illustrates how reliance exclusively on aspects of gross leaf morphology can lead to inaccurate interpretations of species and their phylogenetic history.

NEW AND REVISED NAMES—*CYSTOPTERIS*

Cystopteris tenuis and *C. fragilis*.—In eastern North America, the most widely collected yet still confusing tetraploid element is *C. tenuis*. Long considered a variety of *C. fragilis* (*C. fragilis* var. *mackayi* Laws.), a detailed examination of critical morphological characteristics by Moran (1983) showed that there were consistent distinctive features for discriminating this taxon. Therefore, Moran

(1983) recommended recognizing *C. tenuis* as a separate species. Subsequent electrophoretic analyses (Haufler, 1985) confirmed the genetic distinctness of *C. tenuis* and provided clues to its ancestry. In contrast to past hypotheses (summarized in Moran, 1983), isozyme data indicated that *C. tenuis* was an allotetraploid, combining marker bands from diploid *C. protrusa* and an unidentified diploid component related to tetraploid *C. fragilis* (Fig. 1).

Three aspects of *Cystopteris* biology are responsible for the difficulty encountered when trying to develop a clear understanding of the morphological differences between *C. tenuis* and *C. fragilis*. First, *Cystopteris* species are remarkably "plastic" and leaf morphologies can vary greatly depending on habitat conditions. Especially problematic is the tendency of tetraploid *Cystopteris* species to become mature (produce spores) when their leaves are still very small. This situation is most likely to occur under adverse conditions and, because *C. fragilis* tolerates environmental extremes of cold and exposure better than all other ferns (it is found at higher latitudes than any other species), this species frequently grows in suboptimal habitats. Second, we have seen a definite "ploidy effect" in *Cystopteris*. At higher ploidy levels, *Cystopteris* leaves become reduced in complexity and lose some of the features than can be used in distinguishing species. Third, when *Cystopteris* species are sympatric, they are likely to hybridize, forming sterile morphological intermediates. This combination of features has made the systematics of *Cystopteris* especially challenging and has necessitated the application of techniques and characters not generally employed in recognizing fern species.

There are some geographical and ecological features differentiating *C. tenuis* from *C. fragilis*. While *C. tenuis* is common at lower latitudes and lower elevations in the northeastern U.S. and southeastern Canada, *C. fragilis* is commonly found further north (in Canada) and west (in the U.S. and Canada). In regions where *C. tenuis* is common, *C. fragilis* is confined to mountain tops. Ecologically, both species may be found on cliff faces, but *C. tenuis* also inhabits forest floors, perhaps owing to its *C. protrusa* parentage (Fig. 1). Morphologically, *C. tenuis* is difficult to distinguish from *C. fragilis*. It may be intermediate between its putative progenitors, but we do not have the *C. fragilis* diploid to make direct comparisons. Further, it is likely that the two tetraploids, *C. tenuis* and *C. fragilis* tend to resemble each other because the "polyploidy" effect leads to reduction in plant size and the complexity of leaf blade dissection in *Cystopteris*. In most cases, a combination of morphological features can be used to separate reliably the two tetraploids (Table 1). As pointed out by Moran (1983), the base of the proximal basiscopic pinnule of the proximal pinna in *C. tenuis* is cuneate while that in *C. fragilis* is nearly sessile and has a truncate base. This also provides evidence that *C. tenuis* is intermediate between *C. fragilis* and *C. protrusa* whose proximal basiscopic pinnule is stalked. Other features found in *C. tenuis* and distinguishing it from *C. fragilis* include 1) a more acute angle of pinna departure from the rachis, 2) a tendency for pinnae to curve towards the blade apex, and 3) narrower pinnae often having crenulate (vs. sharply toothed) margins. Admittedly there is considerable variability in these

TABLE 1. Comparison of *Cystopteris fragilis* and *C. tenuis*. Features represent those of "ideal" specimens. Most individuals fail to exemplify all of the characteristics.

	C. fragilis	*C. tenuis*
Base of proximal basiscopic pinnule of proximal pinna	Obtuse to truncate	Cuneate to obtuse
Leaf margin	Sharply toothed	Crenulate or with rounded teeth
Pinna axis of median pinnae	Straight	Curved apically
Angle of median pinnae axes with rachis	Perpendicular	Acute
Shape of pinnae along distal ⅓ of blade	Deltate to ovate	Ovate to narrowly elliptic

features, and there will be difficulty consistently separating these two closely related tetraploid *Cystopteris* species.

Cystopteris reevesiana and *C. utahensis*.—Tetraploid plants from Arizona and western Texas having glandular trichomes and mis-shapened bulblets have been called *C. tennesseensis* (Windham, 1983; Lellinger, 1985). However, these southwestern U.S. tetraploids are over 1,000 miles west of the nearest *C. tennesseensis* collection. With the report that a distinct diploid, *C. reevesiana*, occurs in the southwestern U.S. (Arizona, New Mexico, western Texas, Utah, Colorado), the identity of the western tetraploids referred to *C. tennesseensis* was called into question. Isozyme evidence established clearly that *C. tennesseensis* was an allotetraploid involving *C. protrusa* and *C. bulbifera* (Haufler et al., 1990), while tetraploid individuals from the southwestern U.S. lacked markers for *C. protrusa* and contained those of *C. reevesiana* (Fig. 1). Because *C. bulbifera* occurs in both regions, its common involvement as the second progenitor diploid of both tetraploids was not surprising. This new information, however, requires a reconsideration of the identity of specimens from the southwestern U.S. Given our knowledge of differing parentage, it is logically inconsistent and biologically meaningless to apply the same name to both eastern U.S. and southwestern U.S. tetraploids.

As usual in *Cystopteris*, problems arise in developing morphological criteria for distinguishing these two evolutionarily separate entities. This is not surprising because they share one diploid progenitor (*C. bulbifera*; Fig. 1). Although their other diploid progenitors may be clearly distinguished from each other, they share many morphological features (Table 2). Both *C. protrusa* and *C. reevesiana* have long-creeping rhizomes and ovate leaf blades that can be highly dissected. Rhizome characters provide the best means of differentiating the diploids. *Cystopteris protrusa* has golden pubescence on the rhizome and the rhizome apex usually protrudes beyond the current crop of leaf bases. *Cystopteris reevesiana*, on the other hand, lacks golden pubescence and usually

TABLE 2. Comparing morphologically similar diploids and tetraploids in the *Cystopteris utahensis/tennesseensis* complex. Features represent those of "ideal" specimens. Many specimens fail to exemplify all of the characteristics.

	C. reevesiana	C. utahensis	C. tennesseenis	C. protrusa
Blade shape	Ovate	Elongate deltate	Elongate deltate	Ovate
Rhizome internodes	Long	Short	Short	Long
Rhizome apex	Flush with leaf bases	Flush with leaf bases	Flush with leaf bases	Protruding beyond leaf bases
Rhizome trichomes	Absent	Absent	Absent	Present
Rhizome scales	Light brown	Dark brown, subclathrate	Light brown	Light brown
Multicellular gland-tipped trichomes	Common in pinna axils	Often abundant in pinna axils	Rare in pinna axils	Absent
Spore size averaging	33-41 μm	39-41 μm	38-42 μm	28-34 μm
Chromosome number (2*n*)	42II	84II	84II	42II
Distribution	Southwestern US, Mexico	Southwestern US	Eastern US	Eastern North America

has its current leaf bases flush with the rhizome apex. Although neither tetraploid has an obviously protruding rhizome apex, the rhizome scales of *C. utahensis* are dark brown and subclathrate with thick lateral walls whereas scales in *C. tennesseensis* are more uniform in color with tan to light brown lateral walls. In addition, multicellular, gland-tipped trichomes are frequent in the axils of pinnae in *C. utahensis* whereas such trichomes are rare in *C. tennesseensis*. These features may be considered cryptic, but isozyme characters provide clear markers to distinguish *C. reevesiana* from *C. protrusa* and demonstrate that only *C. reevesiana* markers occur in the southwestern U.S. tetraploid. Further, although frequently considered an inappropriate tool for diagnosing fern species (given the great vagility of their spores), geographic separation of the two tetraploids appears to be absolute. Thus, *C. utahensis* occurs only in the southwestern U.S. and *C. tennesseensis* is confined to the eastern U.S.

Cystopteris utahensis Windham & Haufler, sp. nov. (Fig. 3).—TYPE: United States, Utah: Grand Co., base of Morning Glory Arch in tributary of Negro Bill Canyon 3.93 km SE of its confluence with the Colorado River, 4300 ft, 2 July 1990, *Windham (90-282) & Windham* (UT; isotypes ASU, BRY, KANU, MO, UC, US, UTC).

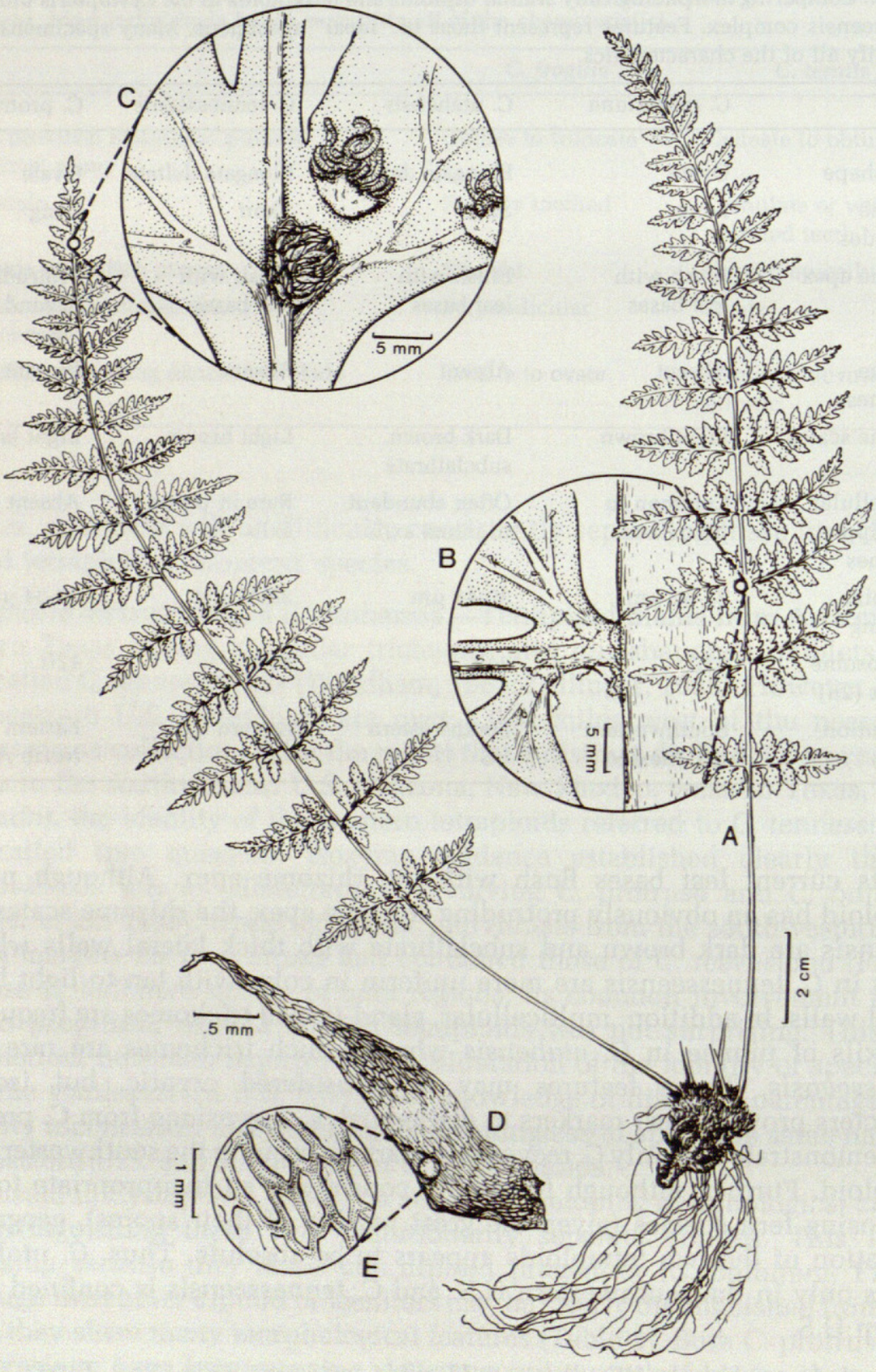

FIG. 3. Illustration of *Cystopteris utahensis*. A = Whole plant. B = Detail of pinna axil (located at open circle on whole leaf to right) showing multicellular glandular trichomes. C = Detail of abaxial blade surface (located at open circle on whole leaf to left) showing sori with fugacious, hood-shaped indusium, numerous unicellular glandular trichomes, and an abortive bulblet. D = Single rhizome scale. E = Detail of cellular structure of rhizome scale (from area enclosed by circle on whole scale to right) showing subclathrate nature of cells.

Cystopteri tennesseensi Shaver similis, a qua differt paleis rhizomatis atrobrunneis subclathratis, et trichomatibus numerosioribus multicellularibus gladulosis in axillis pinnarum.

Rhizome creeping, internodes short with leaves crowded near the apex, rhizome scales dark brown, lanceolate, subclathrate. Leaves up to 45 cm long. Petioles shorter than blade, variable in color but mostly dark brown at base, gradually becoming stramineous toward blade. Blade tripinnatifid, deltate to deltate-lanceolate, usually widest at or near the base; rachis with or without bulblets, with unicellular gland-tipped trichomes, pinna axils often with abundant multicellular, gland-tipped trichomes. Pinnae with short stalks toward blade base, broadly attached toward apex, pinnatifid, ovate to oblong, with serrate margins. Veins free, with veins directed into teeth and emarginations. Sori round, discrete, the indusium cup-shaped with truncate apex, broadly attached under receptacle, bearing unicellular, gland-tipped trichomes. Spores monolete, dark brown, echinate, averaging 39–41 μm long. *Chromosome number* $2n = 84$II (Windham, 1983 as *Cystopteris* cf. *tennesseensis*).

Sporulating summer to fall. Cracks and ledges on cliffs, rarely terrestrial, on calcareous substrates, 1700–2700 m. Arizona, Colorado, Texas, Utah.

Paratypes: **Arizona:** Apache Co., upper Canyon del Muerto, Canyon de Chelly National Monument, *R. Halse 329* (ARIZ); Coconino Co., cliffs on W slope of Elden Mountain, *Windham 93* (AC, ASU, UT), *Windham & Windham 319* (ASC, UNM); small canyon on N face of Munds Mountain, *Windham & Harbster 150* (ARIZ, UT); **Colorado:** Moffat Co., cliffs NW of Harding Hole, Dinosaur National Monument, *S. O'Kane 3170* (BRY, COLO); **Texas:** Culberson Co., South McKittrick Canyon, Guadalupe Mountains, *B. Warnock 23174* (SRSC); Hudspeth Co., Guadalupe Mountains National Park, Pine Canyon, *L. Higgins 8534* (ASU, BRY, UTC); **Utah:** Grand Co., Freshwater Canyon, Arches National Monument, *Welsh, Harrison, & Moore 2320* (BRY); Utah Co., S wall of American Fork Canyon, *Windham (89–07) & Windham* (UT).

Cystopteris fragilis reconsidered.—In addition to the puzzle of *C. tenuis* discussed above, *C. fragilis* poses additional problems in other parts of its range. *Cystopteris fragilis* is quite different from other polyploids in the genus. Other tetraploids have discrete distributions that generally overlap those of their presumed diploid parents. *Cystopteris fragilis* is the most geographically widespread member of the genus extending well beyond the ranges of all known diploids. Although this species is chromosomally a tetraploid, at many enzyme loci the population samples act like diploids. Such extensive gene silencing may be the hallmark of an ancient tetraploid (Werth & Windham, 1991). In some parts of the range of this polymorphic species, it is possible to identify distinctive morphological variants which, especially given the subtle criteria that have been employed in discriminating *C. tenuis* and *C. utahensis* above, could be used in describing additional species. However, these variants have proven to be genetically indistinguishable using isozymic data. The final problem is that, in some cases, characters such as distinctive spore surface features appear to correlate with geography and other morphological traits while in other cases they are mere populational polymorphisms. Thus, in most cases, it is not possible using either morphological or genetic features to resolve distinctive groups within this highly variable species. It would appear that *C.*

fragilis is one of the best examples we have of a species that is diversifying at the tetraploid level. It seems likely that reciprocal gene silencing has played a role in isolation of these different variants. Available techniques, however, are insufficient to elucidate fully what has occurred nor is it consistently possible to identify significant variants. Some of the more distinctive and/or problematic entities will be discussed below.

Hexaploid variants related to *C. fragilis*.—We first became aware of these odd entities through living collections sent to us by Dr. Donald Britton. To date, hexaploids in the North American *C. fragilis* alliance have been referred to *C. laurentiana* (Weath.) Blasdell, an allopolyploid involving tetraploid *C. fragilis* as one parent and diploid *C. bulbifera* as the other (Fig. 1). Dr. Britton's plants were obtained from limestone cliffs on Manitoulin Island in Ontario, Canada, and they contained a set of features distinguishing them from neighboring *Cystopteris* specimens. Besides being hexaploid, these plants have small leaves with reduced and oftentimes deltate pinnae, and large, rugose spores. Isozyme data indicated that these hexaploids probably originated via allopolyploidy involving *C. protrusa* and *C. fragilis*. Thus, there appeared to be ample evidence for describing a new taxon.

However, further exploration added complications. Hexaploids were obtained from other parts of the range of *C. fragilis* (Alaska, Montana, and Arizona) that had similar morphological and ecological features but did not originate from the same parents. Our isozyme work demonstrated, therefore, that these hexaploid variants are polyphyletic, having multiple origins from various elements within the *C. fragilis* complex. We believe that the variation in the hexaploids is another example of ploidy-related effects, not only in reduction of leaf cutting complexity, but also in the production of rugose spores and the preference for basic (limestone) substrates. Similar characteristics (rugose spores, calciphily) are seen in some hexaploid individuals reported from Europe (e.g., *C. regia* (L.) Desv. [Tutin et al., 1964]). Thus, although distinctive (especially for members of *Cystopteris*), these variants have not been formally recognized. They do, however, provide a dramatic demonstration of the difficulty confronting systematists in trying to resolve significant elements within the *C. fragilis* complex.

Cystopteris dickieana.—There is a long history involving the presumed distinctiveness of *C. dickieana* Sim, but all investigations suggest that this morphological variant is no more systematically meaningful than the hexaploids discussed above. *Cystopteris dickieana* is a tetraploid that was originally segregated from *C. fragilis* primarily on the basis of rugose spores as opposed to the echinate spores of other *Cystopteris* species. As described above for the hexaploids and by others (e.g., Jermy & Harper, 1971), there seems to be a variety of mechanisms by which rugose spores are generated. Furthermore, when surveying the morphology of plants bearing rugose spores, it is not possible to find a consistent set of sporophytic characteristics that correlates with rugose spores. Isozyme banding patterns of plants with rugose spores did not differ from those of *C. fragilis* having echinate spores. Particularly pertinent

information was obtained from a single locality in California (El Dorado Co., N. Fork Webber Creek, 3200 ft., rugose spores, 7 June 1944, *G. T. Robbins 1658* [UC]; same locality, spiny spores, 9 June 1945, *G. T. Robbins 1975* [UC]). Some individuals collected from this population had rugose spores but others did not. Those having rugose spores were not otherwise distinct from those with echinate spores. Thus, although this is a clear qualitative feature (something that is a rare event in *Cystopteris* systematics), it is only a single character. Following Blasdell's (1963) lead, we recommend placing *C. dickieana* in synonomy with *C. fragilis*.

Cystopteris fragilis in the Midwest and West.—In the western Great Plains (Saskatchewan and Manitoba south through the Dakotas and into Kansas and west into Utah and Nevada), there appear to be discrete variants of *C. fragilis*. Some plants resemble typical *C. fragilis* whereas others can be assigned to *C. tenuis*. Isozymic evidence indicates that nearly all collections fall within the bounds of *C. fragilis*. Only a few collections in Utah and Nevada carry both the morphological and isozymic features attributed to *C. tenuis*. In the western Great Plains, rugose spores are particularly frequent, suggesting that this single feature has become fixed among these populations. As discussed above, however, this characteristic fails to correlate with others and so has not been recognized taxonomically.

Cystopteris fragilis in the Northwest and in California pose special problems. Some plants collected in Idaho, Oregon, and Washington produced aborted spores. Assuming them to be of hybrid origin, populations were surveyed for distinctive characteristics that might represent genetically isolated taxa. Although plants growing on soil vs. rocky substrates had subtle morphological differences, they were not separable based on isozyme profiles. It seems likely that *C. fragilis* is diverging at the tetraploid level (perhaps via gene silencing mechanisms; see Werth & Windham, 1991). In the mountains of California, considerable variability exists in *Cystopteris* morphology. We wondered if perhaps *C. reevesiana* might occur there, but using a combination of rhizome habit, leaf morphology and spore size measurements, we could not identify individuals of this diploid species. As in the Northwest, it appears that diversification is taking place at the tetraploid level. Some specimens had rugose spores but, as discussed above for *C. dickieana*, one California locality contained plants having both rugose and spiny spores. Considering these data, the best treatment of *C. fragilis* at present seems to be as a single, highly variable species.

New and Revised Names—*Polypodium*

Polypodium virginianum.—With a clear understanding of the eastern U.S. elements in the *P. vulgare* complex and the formulation of new hypotheses about their ancestry (Fig. 2), some revision of names is necessary. Long ago, Manton & Shivas (1953) established that there were three cytological entities within the *P. virginianum* complex. Until now, all three have been considered cytological races of *P. virginianum*. Contrary to previous assumptions (Löve &

Löve, 1977), Cranfill & Britton (1983) showed that the type specimen belonged with the tetraploid cytotype and, therefore, that the diploid required a new name. Our recent studies have confirmed the distinctness of *P. virginianum* and established that it is an allotetraploid. As will be detailed elsewhere (Haufler, Windham, & Rabe, in prep.), we have been able to identify two diploid elements, both of which occur in eastern North America and have proven to be the progenitors of allotetraploid *P. virginianum*.

Polypodium appalachianum.—The common diploid element of the *P. virginianum* complex occurs from southeastern Canada, south along the Appalachian Mountains to Georgia and Alabama. Kott & Britton (1982) detailed subtle distinctions that separate the diploid and tetraploid elements and showed that the triploid hybrid between them generated much of the taxonomic confusion in this group. Once the triploid specimens having aborted spores are eliminated from consideration, aspects of lamina outline (the diploids are deltate and average 5.8 cm in width while the tetraploids are ovate-linear and average 4.5 cm in width) and pinna apex (diploids have pointed pinna apices and those of the tetraploid are rounded) can be used to discriminate the two species. Isozyme markers demonstrate that the diploid was one of the two progenitors of the tetraploid *P. virginianum*. Given the morphological, cytological, and isozymic characters that distinguish it (Table 3), we here describe the diploid as a new species.

Polypodium appalachianum Haufler & Windham, sp. nov. (Fig. 4).—TYPE: United States: New Hampshire: Eastman Lake, Grantham, 14 July 1990, *C. Haufler, P. Haufler, and H. Haufler s.n.* (KANU; isotypes GH, MO, UC, US, UT).

Ex affinitate *P. virginici* L. et *P. sibirici* Siplivinskij, ab utroque laminis elongatis deltatis latissimis basi vel prope basin, pinnis apice acutis vel anguste rotundatis, paleis rhizomatis aureobrunneis fere concoloris distinctum; insuper differt a *P. virginico* soris plus quam 40 sporangiasteris glandulosis instructis, sporis minus quam $\bar{x}$ = 52 μm longis metientibus, chromosomatum numero 2*n* = 37II; etiam differt a *P. sibirico* sporangiasteris trichomatibus abundantibus glandulosis praeditis.

Rhizome slender, up to 6 mm diam., acrid tasting, often whitish pruinose; rhizome scales lanceolate, contorted distally, denticulate, concolorous to weakly bicolorous, uniformly golden brown or slightly darker near the apex. Leaves up to 40 cm long. Petioles slender, up to 1.5 mm diam. Blades elongate-deltate, rarely oblong, usually widest at or near the base, up to 9 cm wide, subcoriaceous to herbaceous; rachises glabrous on adaxial surface, sparsely scaly to glabrescent on abaxial surface, the scales lanceolate-ovate, usually more than six cells wide. Segments linear to oblong with acute to narrowly rounded apices, less than 8 mm wide, midribs glabrous on adaxial surface, margins entire to crenulate. Veins free. Sori medial to submarginal, less than 3 mm in diam., circular when immature. Sporangiasters present, usually more than 40 per

TABLE 3. Comparison of Eastern North American members of the *Polypodium vulgare* complex.

	P. appalachianum	P. virginianum	P. sibiricum
Blade shape	Elongate-deltate to rarely oblong	Oblong to narrowly lanceolate	Oblong-linear
Rhizome scales	Mostly golden brown	Margins brown, dark central stripe	Uniformly dark brown
Sporangiasters	Usually more than 40/sorus; heads with glandular trichomes	Usually less than 40/sorus; heads with glandular trichomes	Usually less than 40/sorus; heads without glandular trichomes
Spore size averaging	46 μm long	54 μm long	44 μm long
Tuberculae on spore surfaces	Less than 3 μm tall	Less than 3 μm tall	More than 3 μm tall
Chromosome number (2*n*)	37II	74II	37II
Distribution	Eastern NA	Eastern to Central NA	Circumboreal

sorus, heads densely covered with glandular trichomes. Spores averaging 46 μm long, verrucate, with verrucae less than 3 μm high. $2n = 37II$ (Haufler & Wang, 1991).

Sporulating summer–fall. Cliffs and rocky slopes; found on a variety of substrates; 0–1800 m; New Brunswick, Newfoundland, Nova Scotia, Ontario, Quebec, Alabama, Connecticut, Georgia, Kentucky, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, West Virginia.

Paratypes: CANADA: **Quebec:** Kamouraska Co., wooded escarpment at agricultural station at Ste. Anne de la Pocatiere, *J. Calder 1406* (OKL, DUL); UNITED STATES; **Massachusetts:** Rockport, shaded granite ledge, *Smith & Gates 402* (CAN, CAS, DAO, DH, GH, LL, MICH, MIN, MO, MSC, NY, OKL, RM, RSA, SMU, TEX, UTC, WIS); **Virginia:** Amherst Co., Rte. 634 about 1 mi NE of Rte. 605, on Station's Creek, *A. Neas s.n.* (ASC).

Polypodium sibiricum and the importance of sporangiasters.—*Polypodium virginianum* overlaps the entire range of *P. appalachianum* and occurs to the west and north of this strictly Appalachian diploid. In Canada, at the northern limit of its range, *P. virginianum* is sympatric with a second species, *P. sibiricum*, named by Siplivinskij (1974) and recently confirmed as a diploid (Haufler & Wang, 1991). When originally described from northeastern Eurasian collections (Siplivinskij, 1974), this new species was diagnosed based on aspects of spore morphology and rhizome indument. *Polypodium sibiricum* has darker brown rhizome scales and spores with larger tubercles than those of other diploids in the *P. vulgare* complex. Our new studies have shown that *P. sibiricum* has a wide boreal distribution, throughout much of northern Canada and northern Asia (Japan, Mongolia, China, Siberia) and that it is isozymically

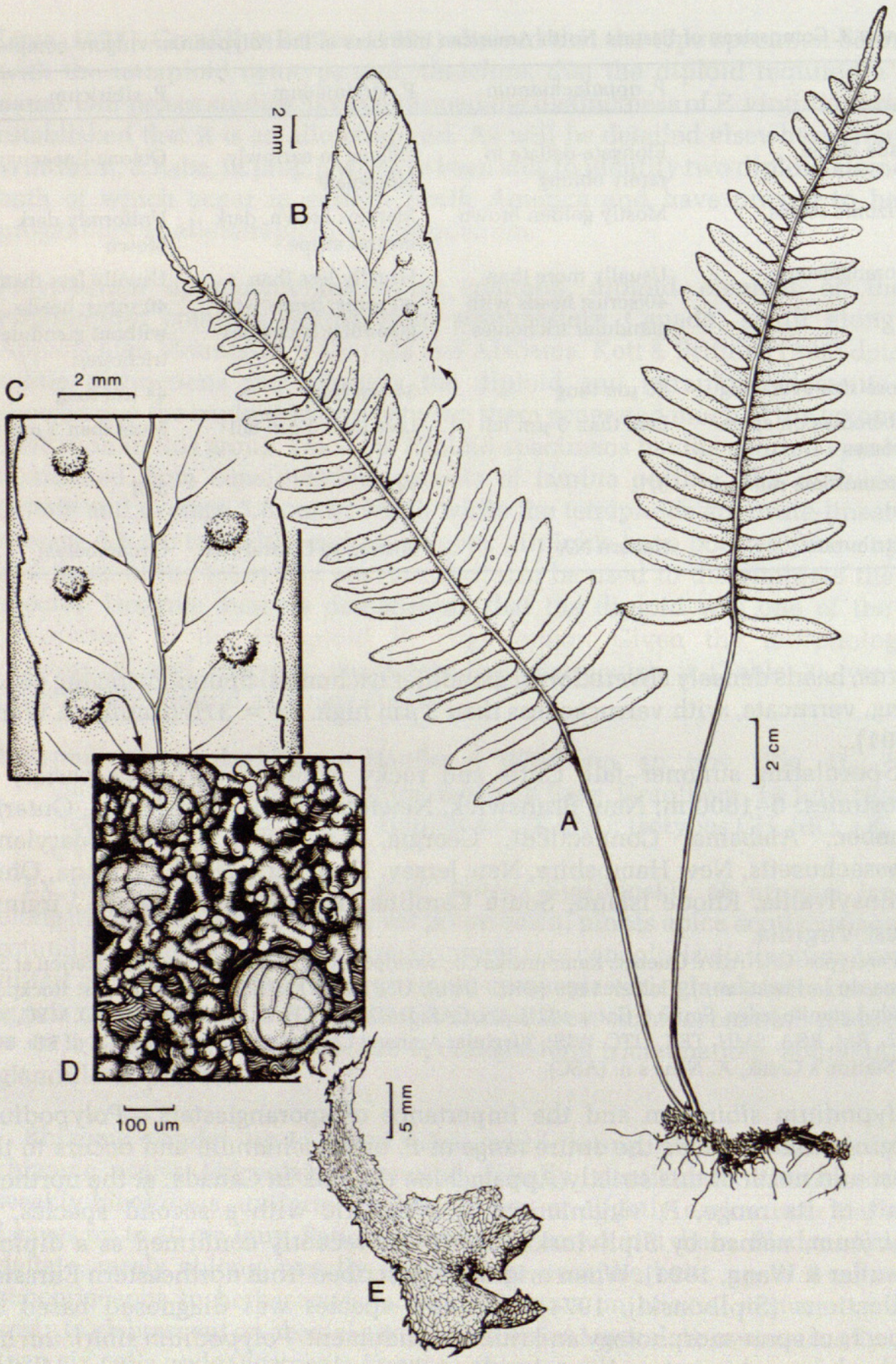

FIG. 4. Illustration of *Polypodium appalachianum*. A = Whole plant. B = Detail of pinna apex showing acute tip and crenulate margin. C = Detail of abaxial blade surface showing free venation and position of sori along veins. D = Detail of sorus showing several sporangia and numerous sporangiasters bearing distinctive unicellular, gland-tipped trichomes. E = Single rhizome scale.

distinct from its congeners. Further, we have shown that this species is remarkably important in understanding reticulate evolution in the *P. vulgare* complex (Fig. 2).

In contrast to the original description, we have found that the most easily recognizable, stable, morphological feature of the species concerns the sporangiasters (specialized paraphyses) found in the sori. In 1974, Peterson & Kott demonstrated that sporangiasters were developmentally linked to sporangia (perhaps representing neotenous sporangia) while the comparative study of Baayen & Hennipman (1987) indicated that sporangiasters are unique to the *P. vulgare* complex within the Polypodiaceae. Earlier than any of these studies, however, Martens (1943; 1950) described variability in the structure of the sporangiasters (the globular heads of the sporangiasters of some individuals bore glandular trichomes, but other individuals had "naked" sporangiasters). Further, Martens was able to demonstrate that the frequency of these glandular trichomes varied with geography. Sporangiasters having numerous glandular trichomes were found in most eastern North American collections, but the number of glandular trichomes became reduced in western North America, and disappeared entirely in northern Asia. Although this appeared to be a useful character among members of the *P. vulgare* complex, later workers (e.g., Morton & Neidorf, 1954) reported difficulty applying Martens' character in their systematic studies of North American *Polypodium* species.

With the accumulation of new information on the *P. vulgare* complex, we believe that the presence of sporangiasters constitutes a synapomorphy for the diploid species group consisting of *P. sibiricum*, *P. amorphum*, and *P. appalachianum*. The glandular trichomes associated with some sporangiasters provide further information. The sporangiasters of *P. appalachianum* are abundantly invested with trichomes (Fig. 4B), those of *P. amorphum* have a reduced number of trichomes, and those of *P. sibiricum* are nearly free of trichomes (Table 3). Inspection of the holotype and isotype of *P. sibiricum* confirmed that they have the "naked" sporangiasters seen on specimens from Japan and Canada.

Several factors forced Morton and Neidorf to consider sporangiaster characters inappropriate for species circumscription. First, in 1954 when they did their work, there had not yet been enough subdivision of natural units at the diploid level. A distinctive western North American species, *P. amorphum*, had not been recognized and the boreal *P. sibiricum* was thought to be conspecific with *P. appalachianum* (at that time called "diploid *P. virginianum*"). As discussed above, with the recognition of new diploids, the presence of sporangiasters and the frequency of glandular trichomes on them become species-defining features (Table 3). Second, when diploids having sporangiasters hybridized with those that did not, their derived allopolyploid species lacked sporangiasters and therefore resembled only one parent. This lack of character expression is odd because allopolyploids are typically intermediate in morphology between their diploid progenitors. However, if sporangiasters are derived through neoteny from sporangia, they may effectively represent sporangia whose developmental program has been interrupted. Thus,

if a species having sporangiasters hybridizes with one lacking them, the complete developmental program may be regenerated and none of the neotenous sporangiasters would be formed.

Finally, the glandular trichomes on sporangiasters add yet another complication. For example, some allopolyploids have one diploid progenitor whose sporangiasters bear glandular trichomes and a second progenitor with naked sprorangiasters. In this case, the polyploid species will have glandular sporangiasters (e.g., *P. virginianum* and *P. saximontanum*). However, when one of the diploid progenitors has sporangiasters with glandular trichomes and the other diploid lacks sporangiasters entirely, the sori of the allopolyploid will not have sporangiasters but may contain sporangia bearing glandular trichomes (e.g., *P. hesperium*). Finally, if one diploid progenitor had no sporangiasters and the other has eglandular sporangiasters, the sorus of the allopolyploid will have only sporangia (e.g., *P. vulgare*). Thus, using sporangiaster characters in assessing phylogeny or reticulate relationships is complex. However, it became obvious to us that this is an important and systematically significant character in delimiting species in temperate *Polypodium*.

Polypodium australe excluded.—In 1969, Lloyd & Hohn reported that a plant originally collected from San Clemente Island conformed to descriptions of European *P. australe*. This plant was growing in the University of California Botanical Garden at Berkeley but was originally brought into cultivation by P. H. Raven. However, Lloyd and Hohn refer to a specimen made from the Botanical Garden plant rather than an original Raven collection. In fact, the only Raven collection they do discuss is one of *P. californicum* obtained at the same time and place as the Botanical Garden plant. Because no plants collected from natural habitats have been identified as *P. australe*, and because others have been unable subsequently to find natural populations of *P. australe* (S. Whitmore, pers. comm.), we excluded this species from the flora North America treatment.

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Literature Cited

Baayen, R. P. and E. Hennipman. 1987. The paraphyses of the Polypodiaceae (Filicales). I. General part. Beitr. Biol. Pflanzen 62:251-316.

Barrington, D. S., C. A. Paris, and T. A. Ranker. 1986. Systematic inferences from spore and stomate size in the ferns. Amer. Fern J. 76:149–159.

Blasdell, R. F. 1963. A monographic study of the fern genus *Cystopteris*. Mem. Torrey Bot. Club 21:1–102.

Cranfill, R. and D. M. Britton. 1983. Typification within the *Polypodium virginianum* complex (Polypodiaceae). Taxon 32:557–560.

HAUFLER, C. H. 1985. Pteridophyte evolutionary biology: the electrophoretic approach. Proc. Roy. Soc. Edinburgh 86B:315–323.

HAUFLER, C. H., M. D. WINDHAM, and T. A. RANKER. 1990. Biosystematic analysis of the *Cystopteris tennesseensis* (Dryopteridaceae) complex. Ann. Missouri Bot. Gard. 77:314–329.

HAUFLER, C. H. and Z. R. WANG. 1991. Chromosomal analyses and the origin of allopolyploid *Polypodium virginianum*. Amer. J. Bot. 78:624–629.

KOTT, L. S. and D. M. BRITTON. 1982. A comparative study of sporophyte morphology of three cytotypes of *Polypodium virginianum* in Ontario. Canad. J. Bot. 60:1360–1370.

JERMY, A. C. and L. HARPER. 1971. Spore morphology of the *Cystopteris fragilis* complex. Brit. Fern Gaz. 10:211–213.

LANG, F. A. 1971. The *Polypodium vulgare* complex in the Pacific Northwest. Madroño 21:235–254.

LELLINGER, D. B. 1981. Notes on North American ferns. Amer. Fern J. 71:90–94.

LLOYD, R. M. and J. E. HOHN. 1969. Occurrence of the European *Polypodium australe* Fée on San Clemente Island, California. Amer. Fern J. 59:56–60.

LLOYD, R. M. and F. A. LANG. 1964. The *Polypodium vulgare* complex in North America. Brit. Fern Gaz. 9:168–177.

LÖVE, A. and D. LÖVE. 1977. New combinations in ferns. Taxon 26:324–326.

MANTON, I. 1950. Problems of Cytology and Evolution in the Pteridophyta. Cambridge University Press, London.

MANTON, I. and M. SHIVAS. 1953. Two cytological forms of *Polypodium virginianum* in eastern North America. Nature (London) 172:410.

MARTENS, P. 1943. Les organes glanduleux de *Polypodium virginianum:* I. Valeur systématique et réparition géographique. Jardin de L'État 17:1–14.

MARTENS, P. 1950. Les paraphyses de *Polypodium virginianum:* III, Nouvelles donnés géographiques, systématiques et histologiques. Cellule 53:187–212.

MORAN, R. C. 1983. *Cystopteris tenuis* (Michx.) Desv.: a poorly understood species. Castanea 48:218–223.

MORTON, C. V. and C. NEIDORF. 1954. *Polypodium vulgare* var. *virginianum*. Amer. Fern J. 44:111–114.

PETERSON, R. L. and L. S. KOTT. 1974. The sorus of *Polypodium virginianum:* some aspects of the development and structure of paraphyses and sporangia. Canad. J. Bot. 52:2283–2288.

SHIVAS, M. G. 1961. Contributions to the cytology and taxonomy of *Polypodium* in Europe and America. I. Cytology. Bot. J. Linn. Soc. 58:13–25.

SIPLIVINSKIJ, V. 1974. Notulae de Flora Baicalensi, II. Novosti Sist. Vyssh. Rast. 11:327–337.

TUTIN, T. G., V. H. HEYWOOD, N. A. GURGES, D. H. VALENTINE, S. M. WALTERS, and D. A. WEBB (eds.). 1964. Flora Europaea, Vol. 1. Cambridge University Press, London.

WERTH, C. R. and M. D. WINDHAM. 1991. A model for divergent, allopatric species of polyploid pteridophytes resulting from silencing of duplicate gene expression. Amer. Naturalist 137: (in press).

WHITMORE, S. and A. R. SMITH. 1991. Recognition of the tetraploid, *Polypodium calirhiza* (Polypodiaceae) in western North America. Madroño (in press).

WINDHAM, M. D. 1983. The ferns of Elden Mountain, Arizona. Amer. Fern J. 73:85–93.

WINDHAM, M. D. 1991. New species and combinations in the North American fern flora. Novon 1: (submitted).