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Isozymic and Chromosomal Evidence for the Allotetraploid Origin of *Gymnocarpium dryopteris* (Dryopteridaceae)

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ABSTRACT. A critical examination of isozyme, chromosomal, and morphological characters in subspecies formerly included in *Gymnocarpium dryopteris* demonstrated that three sexual taxa can be distinguished. We recognize these taxa as distinct species: the widespread, fertile allotetraploid *G. dryopteris*, with one genome derived from the western diploid *G. disjunctum* and the other from **G. appalachianum** sp. nov., a previously undetected eastern North American diploid, which is described and illustrated here and for which we report a chromosome number of $2n = 80$. Population comparisons of allele frequencies between *G. disjunctum* and *G. appalachianum* yielded an average Nei's genetic identity value ($\bar{I}$) of 0.274. A wide-ranging assemblage of putatively triploid plants with both sterile, malformed spores and large, round spores capable of germination is believed to represent the backcrosses *G. disjunctum* × *dryopteris* and *G. appalachianum* × *dryopteris*. The name **G. × brittonianum** comb. nov. is applied here to *G. disjunctum* × *dryopteris*. A key to fertile species, species descriptions and illustrations, distribution maps, and habitat notes are included.

Gymnocarpium Newman (Dryopteridaceae) has been regarded as comprising six species native to the temperate regions of the northern hemisphere (Tryon and Tryon 1982). However, despite a recent series of systematic studies (Pryer 1981; Pryer and Britton 1983; Pryer et al. 1983; Sarvela 1978, 1980; Sarvela et al. 1981; P. Sorsa 1980), the taxonomy of this group has remained contentious. One of the more vexing problems has been the status of the taxa that have been included within *G. dryopteris* (L.) Newman. Plants of this species usually have been separated into two infraspecific taxa (Wagner 1966), most recently treated as subspecies (Sarvela 1978). *Gymnocarpium dryopteris* subsp. *dryopteris* has relatively small, bipinnate fronds, and is tetraploid with $2n = 160$ (Britton 1953; Manton 1950; Pryer 1981; Sorsa 1958; Vida 1963; Wagner 1963). This taxon is widely distributed in North America, Europe, and Asia (Hultén and Fries 1986; Jalas and Suominen 1972; Kato and Iwatsuki 1983). *Gymnocarpium dryopteris* subsp. *disjunctum* (Rupr.) Sarvela is restricted to the northwest coast of North America, southern Kamchatka, and Sakhalin Island (Kato and Iwatsuki 1983). It has larger, tripinnate fronds and is diploid with $2n = 80$ (V. Sorsa 1966; Taylor and Mulligan 1968; Wagner 1966). A putative triploid hybrid, *G. dryopteris* subsp. × *brittonianum* Sarvela, was described by Sarvela (1980) to include plants that are morphologically intermediate between these two subspecies and produce malformed spores.

It had been presumed that *G. dryopteris* subsp. *disjunctum* gave rise to subsp. *dryopteris* through autopolyploidy because of the very subtle differences in their vegetative morphology, and the close similarity of their perispore patterns (Pryer and Britton 1983) and chromatographic profiles (Pryer et al. 1983). However, if subsp. *dryopteris* were an autopolyploid, one would expect some multivalent associations during the first division of meiosis (Jackson and Casey 1982). All chromosome preparations of subsp. *dryopteris* consistently revealed 80 bivalents (pers. obs.), which raised two alternatives. Either subsp. *dryopteris* is an autopolyploid that has evolved a pairing control mechanism, thus

concealing its origin (Jackson 1982), or it is an allopolyploid and has arisen through interspecific hybridization.

The insights provided by data from enzyme electrophoresis in tracing the origins of polyploid ferns are well-known (Haufler 1985b, 1987; Werth 1989). The number of isozymes and their patterns of variability frequently allow one to distinguish between auto- and allopolyploids (Bryan and Soltis 1987; Crawford 1985; Haufler et al. 1985). The electrophoretic profile of an autopolyploid taxon should show a subset of the isozymes found in the diploid progenitor (Crawford and Smith 1984; Gastony 1988; Soltis and Rieseberg 1986). In contrast, an allopolyploid should manifest fixed heterozygous (nonsegregating) banding patterns for a majority of enzymes with the component bands corresponding to additivity of the isozymes derived from two diploid progenitor species (Haufler et al. 1990; Roose and Gottlieb 1976; Werth et al. 1985).

This paper reports on electrophoretic, cytogenetic, and morphological investigations of the taxa included in *G. dryopteris*. The primary objectives of this study were to determine whether subsp. *dryopteris* was derived through auto- or allopolyploidy and, if allopolyploidy is involved, to discover the identity of its second diploid progenitor. As detailed below, our results indicate that *G. dryopteris* subsp. *dryopteris* is an allotetraploid between *G. dryopteris* subsp. *disjunctum* and a previously undescribed eastern diploid taxon. We herein circumscribe *G. dryopteris* to include only the tetraploid plants, and recognize two diploid species, *G. disjunctum* (Rupr.) Ching (western North America) and *G. appalachianum*, newly described below (eastern North America). The putative triploid backcross between *G. dryopteris* and *G. disjunctum* is raised to specific status as *G.* × *brittonianum*.

Materials and Methods

Field Work. Plants of the *G. dryopteris* complex were collected in the summers of 1987 and 1988 from 42 localities over a broad range (Table 1); these collections provided the material for enzyme electrophoretic analysis. At each locality an average sample of 10 sporophytes was taken (range = 1–25). Due to the rhizomatous nature of *Gymnocarpium*, its populations are made up of clones. Leaf samples were taken several meters apart to increase the likelihood that different individuals would be collected. Because of the potential for clonal replication of individual genotypes within a population, Table 1 lists both the number of leaf samples (ramets) obtained and the number of different genotypes (genets) identified at each site. Fronds were stored in plastic bags and kept refrigerated until electrophoresis was conducted. A total of 374 individual sporophytes was examined.

Enzyme Electrophoresis. All sporophytes were surveyed for electrophoretically detectable enzyme variation using 12% starch gels. Small portions of fresh leaf material were ground in phosphate grinding buffer solution (Haufler 1985a) and the extract was absorbed into wicks of Whatman 3MM chromatography paper. The wicks were then frozen at −80°C (Ranker and Schnabel 1986) until they were inserted into gels. The following enzymes were resolved: aspartate aminotransferase (AAT), hexokinase (HK), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SkDH), and triosephosphate isomerase (TPI). Clear band patterns were expressed for PGI, PGM, and TPI using gel and electrode buffer system 6 (Soltis et al. 1983). The modified gel-electrode buffer system 8 (Haufler 1985a) was used to resolve LAP, HK, and AAT. The enzymes SkDH, IDH, 6PGD, and MDH were assayed using the morpholine gel-electrode buffer system (Werth 1985) at pH 7.0. The enzymes aconitase (ACON) and aldolase (ALD) were examined, but clear bands were not expressed consistently. Standard staining protocols were followed (Soltis et al. 1983). Nei's genetic identity values ($\bar{I}$) were calculated using LYNSPROG provided by M. D. Loveless (College of Wooster, Ohio).

Cytology. Leaf material with young sporangia undergoing meiosis was collected and fixed in Farmer's solution (absolute ethanol and glacial acetic acid, 3:1) and stored at about 4°C. Spore mother cells were stained and squashed following the technique of Haufler et al. (1985). Photographs of chromosome squashes were taken with Kodak Technical Pan film using a Nikon AFM camera on a Zeiss phase contrast microscope.

Spore Measurements. Spores were mount-

TABLE 1. Collection data for populations of the *Gymnocarpium dryopteris* complex sampled for electrophoretic analyses. All vouchers at CAN unless otherwise noted. APP = *G. appalachianum*, BRI = *G.* × *brittonianum*, DIS = *G. disjunctum*, DRY = *G. dryopteris*. Number of ramets = # of fronds obtained from each site; number of genets = # of different genotypes identified at each site.

Taxon	Number of ramets	Number of genets	Location
			U.S.A.:
APP	1	1	North Carolina: Ashe Co., Bluff Mtn. (no voucher)
APP	2	1	Pennsylvania: Bedford Co., Wolfsburg, 3 Jul 1988, *Pryer et al. 940*
APP	11	1	Virginia: Highland Co., Lantz Mtn., 4 Jul 1988, *Pryer et al. 942*
APP	9	2	Virginia: Madison/Page cos., Hawksbill Mtn., 6 Jul 1988, *Pryer et al. 945*
APP	11	2	Virginia: Page Co., Bush Mtn., 5 Jul 1988, *Pryer et al. 944*
APP	15	3	Virginia: Page Co., Stony Man Mtn., 6 Jul 1988, *Pryer et al. 948* (type locality)
APP	4	3	West Virginia: Hampshire Co., Ice Mtn., 11 Jun 1987, *Morse 9486*
APP	13	5	West Virginia: Hampshire Co., Ice Mtn., 7 Jul 1988, *Pryer et al. 949*
			CANADA:
BRI	14	1	Ontario: Prescott Co., Bourget (site 1), 6 Jun 1987, *Pryer & Jarvis 900*; 28 May 1988, *Pryer & Jarvis 935*
BRI	10	1	Ontario: Prescott Co., Bourget (site 2), 6 Jun 1987, *Pryer & Jarvis 901*
BRI	8	1	Ontario: Wellington Co., Belwood, 8 Jun 1987, *Pryer et al. 903* (type locality)
			U.S.A.:
DIS	1	1	Alaska: Juneau, West Glacier Trail, 6 Jun 1988, *Brodo et al. s.n.*
DIS	6	5	Alaska: Baranof Island (no voucher)
DIS	25	14	Oregon: Linn Co., Tombstone Prairie (no voucher)
DIS	21	8	Oregon: Yamhill Co., Carlton, 20 Jun 1987, *Alverson 905*
DIS	10	4	Washington: King Co., Deception Creek Trail, 7 Jun 1987, *Arnot et al. s.n.* (WTU)
DIS	12	4	Washington: King Co., Deception Creek Trail (no voucher)
DIS	12	7	Washington: Yakima Co., Mt. Aix, Sept 1987, *Alverson s.n.* (no voucher)
			CANADA:
DIS	8	3	British Columbia: Kootenay District, Rossland, 30 Aug 1987, *Cèska and Ogilvie 23074* (V)
DIS	10	2	British Columbia: New Westminster District, Mt. Seymour, 27 Jun 1987, *Mehrhoff s.n.*
DIS	23	12	British Columbia: Vancouver Island, Cathedral Grove, 22 Aug 1987, *Crins 7417* (UBC)
			U.S.A.:
DRY	15	4	Alaska: Fairbanks, 24 Jun 1987, *Batten 87-1* (ALA)
DRY	2	2	Arizona: Coconino Co., Dane Canyon, 17 Aug 1987, *Boucher 500* (ASC)
DRY	9	2	Michigan: Marquette Co., Ishpeming, 20 Aug 1986, *Windham & Ranker 878* (UT)
DRY	10	4	Minnesota: Lake Co., Gooseberry Falls, 13 Jun 1987, *Pryer & Klein 929*
DRY	3	1	New Hampshire: Grantham Co., along shores of Eastman Lake, 18 Jul 1988, *Haufler s.n.* (KANU)

TABLE 1. Continued.

Taxon	Number of ramets	Number of genets	Location
DRY	5	1	Pennsylvania: Lackawanna Co., Roaring Brook, 2 Jul 1988, *Pryer & Klein 939*
DRY	8	3	Vermont: Chittenden Co., Burlington (no voucher)
DRY	11	2	Wisconsin: Manitowoc Co., Point Beach Ridges, 21 Jun 1987, *Taylor s.n.*
DRY	3	2	Wisconsin: Polk Co., Interstate State Park, 21 Aug 1986, *Windham & Ranker 882* (UT)
			CANADA:
DRY	2	2	Ontario: Algoma District, Batchawanna Falls, 18 Aug 1986, *Windham & Ranker 867* (UT)
DRY	11	5	Ontario: Algoma District, Lafoe Creek, 9 Jun 1987, *Pryer & Klein 906*
DRY	9	5	Ontario: Algoma District, Magpie Falls, 10 Jun 1987, *Pryer & Klein 909*
DRY	3	2	Ontario: Frontenac Co., Ompah, 16 Aug 1986, *Pryer et al. s.n.*
DRY	10	2	Ontario: Ottawa-Carleton Regional Municipality, Albion Rd., 5 Jun 1988, *Pryer & Klein 937*
DRY	3	1	Ontario: Parry Sound District, Blackstone Lake Rd., 24 May 1987, *Britton 11310*
DRY	10	2	Ontario: Thunder Bay District, Kakabeka Falls, 12 Jun 1987, *Pryer & Klein 920*
DRY	6	4	Ontario: Thunder Bay District, Keemle Lake, 11 Jun 1987, *Pryer & Klein 910*
DRY	10	4	Ontario: Thunder Bay District, Mt. McKay, 13 Jun 1987, *Pryer & Klein 925*
DRY	9	3	Ontario: Thunder Bay District, Pass Lake, 12 Jun 1987, *Pryer & Klein 916*
DRY	5	3	Ontario: Thunder Bay District, Red Rock, 11 Jun 1987, *Pryer & Klein 913*
			JAPAN:
DRY	4	2	Niniu, Shimukappu Village, Ifutsu-gun, Kamifawa Pref. (no voucher)

ed in Hoyer's medium on glass slides and measured at 400× using a filar ocular micrometer on a Leitz Dialux 22 microscope. Between 20 and 30 mature spores per sporophyte were measured along their longest dimension to the outer exospore walls. Measurements did not include the irregular folds of the perispore.

Distributions. More than 3500 herbarium specimens were examined from A, CAN, CM, COLO, DAO, DUKE, FARM, GH, H, IA, ISTC, LE, LKHD, LSP, LYN, MICH, MIN, NEBC, NFLD, OAC, ORE, OS, QFA, QK, RM, SASK, SDU, SFS, SLU, TRT, UBC, UNM, US, V, VDB, VPI, VT, WAT, WILLI, WIN, WIS, WS, WTU, WVA. Data from these specimens were used to plot species distributions.

RESULTS AND DISCUSSION

***Origin of Tetraploid* G. dryopteris.** Ten enzyme systems encoded by 12 putative gene loci were resolved. Isozyme patterns were consistent with the hypothesis that *G. disjunctum* was one of the diploid progenitors of the tetraploid. However, *G. dryopteris* showed a large number of "orphan" isozymes (sensu Werth 1989) that could not be attributed to *G. disjunctum*. This pattern was evident at 7 of the 12 loci examined during this study. In PGI, for example, the faster migrating isozymes found in *G. dryopteris* had the same relative electrophoretic mobility as those found in *G. disjunctum*; however, bands with the same mobility as the slower migrating

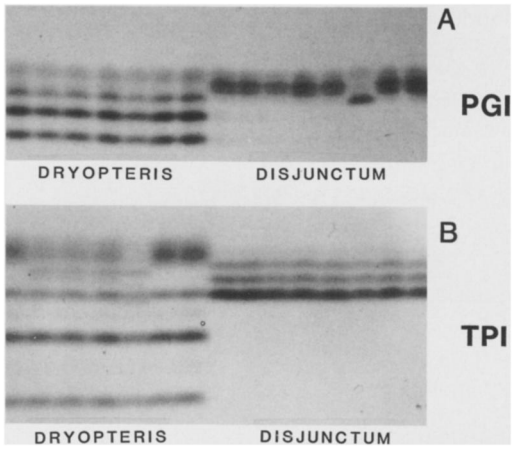


FIG. 1. Representative electrophoretic banding patterns of *Gymnocarpium dryopteris* and *G. disjunctum*. A. PGI variation at loci 1 and 2. B. TPI variation at loci 1 and 2. Note the "orphan" bands in *G. dryopteris* in both PGI and TPI.

bands of the tetraploid were absent in *G. disjunctum* (Fig. 1A). Similarly, in TPI, bands corresponding to the same mobility as the fastest and slowest migrating isozymes in *G. dryopteris* were not observed in *G. disjunctum* (Fig. 1B). These data suggest that *G. dryopteris* could be an allotetraploid resulting from hybridization between *G. disjunctum* and a previously undetected diploid. These results parallel those of a number of other recent studies, in which electrophoretic evidence for the allopolyploid origin of tetraploid ferns implicates a closely related and well-known diploid taxon and an unknown diploid parent (*Cryptogramma*, Alverson 1988; *Hemionitis*, Ranker 1988; *Polypodium*, Bryan and Soltis 1987).

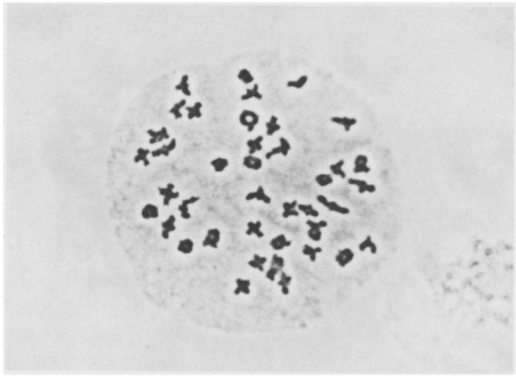


FIG. 2. Chromosomal preparation from spore mother cell of *Gymnocarpium appalachianum* at diakinesis with 40 bivalents. Voucher: West Virginia, Hampshire Co., Ice Mtn., 6 Jun 1988, *Morse 9578*, site 8 (CAN). ×2000.

The key to discovering the "missing" parental genome in *Gymnocarpium* was the correlation between ploidy level and spore size. Tetraploid *G. dryopteris* consistently exhibited a mean spore length of 36 μm, whereas diploid *G. disjunctum* had a mean spore length of 29.5 μm (Pryer and Britton 1983). The difference in mean spore lengths between plants of the two ploidy levels was statistically significant, and the range of means showed no overlap. This allowed us to use spores from herbarium specimens to identify possible diploid populations.

A survey of spore sizes revealed a concentration of small-spored populations in the Appalachian Mountains of Pennsylvania, West Virginia, and Virginia. Chromosomal preparations of spore mother cells at diakinesis of plants from Ice Mt., West Virginia, revealed $2n = 80$ (Fig. 2). Because this was the first report of a diploid count for the genus in eastern North America, it was reasonable to hypothesize that these plants could be a new taxon, and represent the missing parental genome of tetraploid *G. dryopteris*. According to the working hypothesis, the "orphan" isozymes observed in tetraploid *G. dryopteris* should be found in the newly discovered diploid, herein described as *G. appalachianum*. At the dimeric PGI-2 and TPI-2 loci, *G. dryopteris* exhibited a fixed heterozygous pattern composed of a fast and a slow band, each with the same relative mobility as the homozygous bands found in *G. disjunctum* and *G. appalachianum*, respectively (Figs. 3A and 3C). In the monomeric enzyme SkDH, three different allozymes were found in the diploid populations (Fig. 3B). The fixed heterozygous banding pattern observed in *G. dryopteris* at the SkDH locus was again additive of its putative diploid progenitors: the fast band had the same relative mobility as the homozygous band found in *G. appalachianum*, and the slow band had a mobility corresponding to the slow band in heterozygous *G. disjunctum*. This pattern was repeated at each of its seven fixed-heterozygous loci, strongly suggesting that *G. dryopteris* is an allotetraploid that originated following hybridization between the diploids *G. disjunctum* and *G. appalachianum*.

Various lines of evidence suggest that formation of the allotetraploid, *G. dryopteris*, was

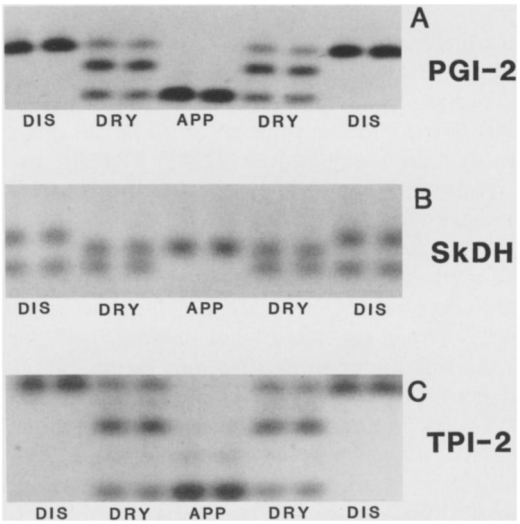


FIG. 3. Representative electrophoretic banding patterns of *Gymnocarpium disjunctum* (DIS), *G. dryopteris* (DRY), and *G. appalachianum* (APP). A. PGI-2. B. SkDH. C. TPI-2. Note here that the "orphan" bands shown in Figure 1 for PGI and TPI have the same relative mobility as the homozygous bands in *G. appalachianum*, indicating the allopolyploid origin of *G. dryopteris*.

not recent. Comparisons of allele frequencies (Table 2) between *G. disjunctum* and *G. appalachianum* yielded an average Nei's genetic identity value ($\bar{I}$) of 0.274, a figure that approximates the 0.33 average calculated for congeneric fern species (Soltis and Soltis 1989). This low value suggests that the diploids are distinct and probably old taxa, particularly when compared to the average value of 0.67 reported for congeneric angiosperm species (Crawford 1983; Gottlieb 1981). The allotetraploid, *G. dryopteris*, derived from these two diploids, currently has a broad circumboreal distribution well beyond the ranges of its diploid progenitors. It also undergoes normal chromosome pairing behavior at meiosis, without the formation of multivalents. The hybridization event leading to the origin of the allotetraploid possibly dates back to the Pleistocene, when the geographical ranges of northern species were contracted and displaced southward as a result of dramatic changes in climate (Davis 1983). Such distributional changes no doubt separated the previously contiguous ranges of many species, while forcing other formerly allopatric taxa into sympatry. This latter process may have occurred in the case of the diploid progenitors of *G. dryopteris*. Following hybridization and polyploidization, *G. dryopteris* achieved meiotic stability (i.e., consistent bivalent formation) and dispersed to oc-

TABLE 2. Allele frequencies for polymorphic loci in *Gymnocarpium disjunctum* and *G. appalachianum*. Presumed loci and alleles for each enzyme are numbered consecutively proceeding from anode to cathode. Numbers in parentheses indicate the sample sizes.

Locus	Allele	*G. disjunctum* (77)	*G. appalachianum* (16)
Pgi-2	1	0.4545	0.0000
	2	0.0649	0.0000
	3	0.4805	0.0000
	4	0.0000	0.1563
	5	0.0000	0.2500
	6	0.0000	0.2188
	7	0.0000	0.3750
Tpi-2	1	0.0065	0.0000
	2	0.9935	0.0000
	3	0.0000	0.5938
	4	0.0000	0.4063
Tpi-1	1	0.0000	1.0000
	2	0.2532	0.0000
	3	0.7468	0.0000
Lap	1	0.0000	1.0000
	2	0.5584	0.0000
	3	0.0065	0.0000
	4	0.0455	0.0000
	5	0.3896	0.0000
Hk	1	0.0000	0.4375
	2	0.9156	0.5000
	3	0.0000	0.0625
	4	0.0844	0.0000
Skdh	2	0.7338	0.0000
	3	0.0000	0.9688
	4	0.2662	0.0000
	6	0.0000	0.0313
Idh	1	0.0000	0.6875
	2	0.0779	0.0000
	3	0.0000	0.3125
	4	0.9156	0.0000
	5	0.0065	0.0000
6Pgd-2	1	0.1169	0.0000
	2	0.8636	1.0000
	3	0.0195	0.0000
Mdh-5	1	0.0130	0.0000
	2	0.0390	0.0000
	3	0.0000	1.0000
	4	0.0065	0.0000
	5	0.9416	0.0000
Mdh-2	1	0.0065	0.0000
	2	0.9740	1.0000
	3	0.0195	0.0000

cupy its present extensive range. As is characteristic of many allopolyploids (Ferris 1984; Haufler et al. 1990; Roose and Gottlieb 1976; Werth et al. 1985), *G. dryopteris* may have had a higher fitness in a wider range of habitats and thus have been able to exceed the distributional limits of either diploid parent. Populations of *G. disjunctum* and *G. appalachianum* are presently believed to be completely allopatric.

Sterile Triploid Backcrosses. In 1980, Sarvela described the hybrid *G. dryopteris* subsp. × *brittonianum* (new combination *G.* × *brittonianum* made herein), which he regarded as a sterile triploid backcross between *G. dryopteris* and *G. disjunctum* (then regarded as a subspecies). This hybrid was characterized by fronds morphologically intermediate between its two presumed parents, malformed spores, a peculiar distribution with plants in eastern and western North America but not in the center of the continent, and a putative triploid count from the type locality [count based on Pryer 1981; note that the caption to fig. 3 in Pryer (1981) was mislabelled and should read "*Pryer 375*, Wellington Co., West Garafraxa Township, Belwood, Ontario - type locality."].

In the context of the present understanding of the origin of *G. dryopteris* as presented herein, these putative triploids merit further investigation. There may have been ample opportunity for secondary contact between the wide-ranging allotetraploid, *G. dryopteris*, and each of its diploid parents, *G. appalachianum* and *G. disjunctum*. This may have resulted in two different triploid backcrosses, *G. appalachianum* × *dryopteris* and *G. disjunctum* × *dryopteris*, which would have the genome combinations AAD and ADD, respectively. Were the widespread, putative triploid plants derived from hybridization events between *G. disjunctum* and *G. dryopteris*, or between *G. appalachianum* and *G. dryopteris*, or both? Plants of *G.* × *brittonianum* from the type locality in southwestern Ontario, as well as plants from other localities that correspond morphologically to *G.* × *brittonianum*, have severe meiotic irregularities (Fig. 4). Numerous lagging chromosomes were observed at late anaphase I (Fig. 4a), many of which form micronuclei at teleophase II (Fig. 4b). These cells give rise to aneuploid spores that tend to be malformed and abortive (Pryer and Britton 1983). Exact chromosome counts from these hybrid plants were difficult to obtain due to the interpretation of and variability in the number of trivalents, bivalents, and univalents in a given preparation. In spite of these difficulties, interpretations of all squashes were consistent with a triploid compliment of $2n = 120$ (Figs. 4c, d; Pryer 1981). Unfortunately, we could not use chromosome squashes to identify the genomes involved.

At fixed heterozygous loci, enzyme profiles for triploid backcrosses between *G. disjunctum* and *G. dryopteris*, or between *G. appalachianum* and *G. dryopteris*, would not necessarily differ from the enzyme profiles for tetraploid *G. dryopteris*. The triploids would likely be "unbalanced," i.e., a single "dose" of one genome vs. two "doses" of the other alternative genome. However, because of numerous confounding possibilities, dosage effects as visualized through isozyme electrophoretic methods are not reliable for verification of the genomic constitution of individuals.

Two features of the isozyme profiles of plants identified here as *G.* × *brittonianum* are relevant to interpreting the origin of these triploid backcrosses. First, mechanisms of inheritance guarantee that when the triploid exhibits a three-banded pattern for a given monomeric enzyme, only two of the allozymes could have identical mobilities to bands found in the allotetraploid parent, *G. dryopteris*. Even if the allotetraploid had four different allozymes, two at each of its loci, after meiosis it could only pass on two to the triploid hybrid. One of these two allozymes in the tetraploid would correspond in mobility to a band found in its diploid progenitor *G. appalachianum*, whereas the other would correspond in mobility to a band found in the diploid *G. disjunctum*. The third allozyme in the triploid would have the same mobility as a band found in one or the other diploid progenitor, i.e., *G. disjunctum* or *G. appalachianum*. If each of the progenitor diploids of allotetraploid *G. dryopteris* had unique bands for such an enzyme, one could precisely determine the parentage of triploid backcrosses carrying three allozymes. For example, *G.* × *brittonianum* plants from the type locality exhibit a three-banded pattern in SkDH. Two of the three bands are identical in mobility to bands found in *G. disjunctum*, but not found in *G. appalachianum* (Fig. 5B). Using this knowledge, we can state with some certainty that these triploids resulted from hybridization between *G. dryopteris* and *G. disjunctum*.

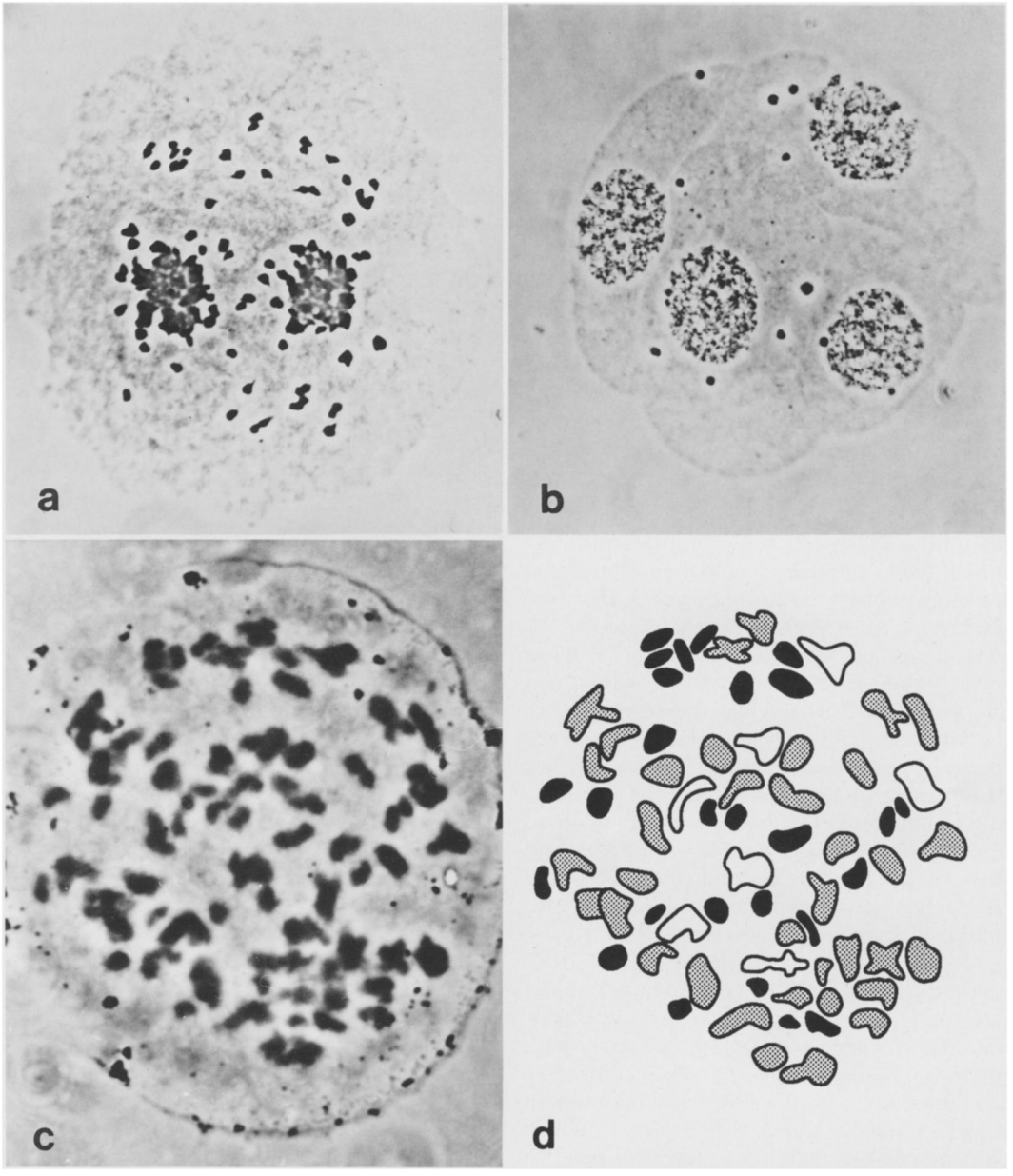


FIG. 4. Chromosomal preparations from spore mother cells of plants of *Gymnocarpium* × *brittonianum*. ×2000. a. Late anaphase I, showing numerous lagging univalents. b. Telophase II, showing micronuclei formed by fusion of those univalents that were not incorporated into functional nuclei. c. Diakinesis, showing a triploid compliment of 120 chromosomes. d. Interpretation of diakinesis preparation shown in c: solid shapes = univalents (27), stippled shapes = bivalents (36), open shapes = trivalents (7), for a total of 120 chromosomes. Voucher for 4a and 4b = Ontario: Wellington Co., West Garafraxa Township, Belwood (type locality), 8 Jun 1987, *Pryer et al. 902* (CAN). Voucher for 4C and 4D = Ontario: Prescott Co., Plantagenet Twp., Bourget, 30 May 1989, *Pryer & Bristow 951* (CAN).

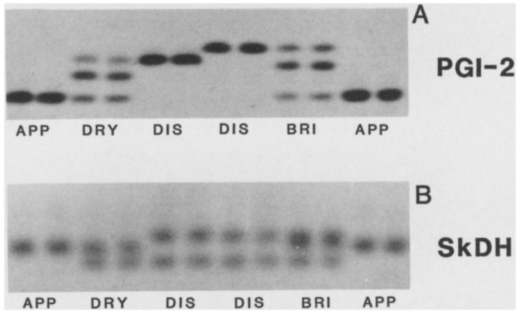


FIG. 5. Representative electrophoretic banding patterns of *Gymnocarpium appalachianum* (APP), *G. dryopteris* (DRY), *G. disjunctum* (DIS), and *G* × *brittonianum* (BRI). A. PGI-2. B. SkDH.

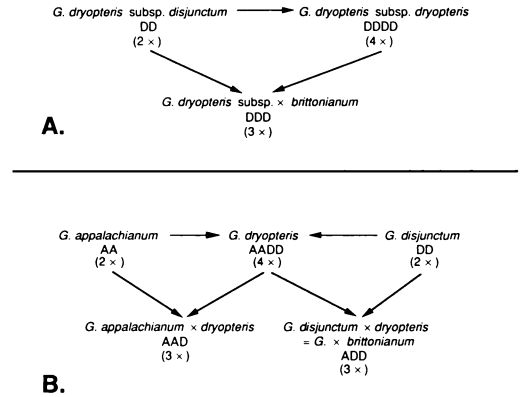


FIG. 6. Presumed relationships among members of the *Gymnocarpium dryopteris* complex. A. Based on Sarvela (1980), Pryer (1981) and Pryer et al. (1983). B. Based on present study.

A second observation strengthens this assertion. For most enzymes, the bands observed in *G. dryopteris* and *G.* × *brittonianum* had identical mobilities. However, some of the bands observed in *G.* × *brittonianum* had mobilities that were never observed in *G. dryopteris*, but were identical to those present in extant populations of *G. disjunctum*. For example, at PGI-2, the fast band present in *G.* × *brittonianum*, which was absent in *G. dryopteris*, had the same relative mobility as one of the two homozygous bands found in populations of *G. disjunctum*; the fast band present in *G. dryopteris* had the same mobility as the other homozygous band known in *G. disjunctum* (Fig. 5A). For SkDH, the slow and intermediate bands in *G.* × *brittonianum* were of the same relative mobility as the two bands found in *G. dryopteris*; however, the fast band in *G.* × *brittonianum*, which was absent in *G. dryopteris*, had a mobility corresponding to the fast band in heterozygous *G. disjunctum* (Fig. 5B). This fast band was common in extant populations of *G. disjunctum* (0.7338; see Table 2). On the other hand, the slow band observed in both *G. dryopteris* and *G.* × *brittonianum*, which corresponded in mobility to the slow band in heterozygous *G. disjunctum*, was observed at much lower frequencies in extant populations of *G. disjunctum* than the alternative allozyme (0.2662, see Table 2). The presence of bands in *G.* × *brittonianum* for PGI-2 and SkDH, with the same mobility as bands found in extant *G. disjunctum*, but not observed in *G. dryopteris*, supports the idea that the triploid plants from the type locality of *G.* × *brittonianum* involve a backcross between *G. dryopteris* and *G. disjunctum*.

There was no electrophoretic evidence in our sample to suggest the involvement of *G. appalachianum* in the *G.* × *brittonianum* backcross, as there were no triploid plants from the type locality with three-banded patterns in which two of the three allozymes were characteristic of *G. appalachianum*. Furthermore, there were no isozymes observed in extant plants of *G. appalachianum* that had mobilities that were unique to it and that were not also present in *G. dryopteris*. Based on preliminary morphological and geographical data, however, we believe that additional isozyme studies, using a wider sampling from throughout the range of abortive-spored plants, are likely to yield evidence for backcross hybrids between *G. appalachianum* and *G. dryopteris*.

Sterile triploid plants are not restricted to areas where the tetraploid overlaps with either diploid. The wide distribution of triploids could be explained if they resulted from several separate evolutionary events involving both long-lived triploid populations that originated when tetraploid and diploid species were sympatric and survived as rhizomes over long periods of time, and more recent hybridizations by long distance spore dispersal or "remote control" (to use the terminology of Wagner 1943). There is some limited evidence of fertility among the triploids. The spores produced by these plants are of two types: sterile, malformed, black spores with very exaggerated perispores and large, round spores with extensive reticulate perispores (Pryer and Britton 1983) that are capable of germination (Pryer 1981). If such large spores produce gametophytes capable of reproducing apomictically, this presumably would help to

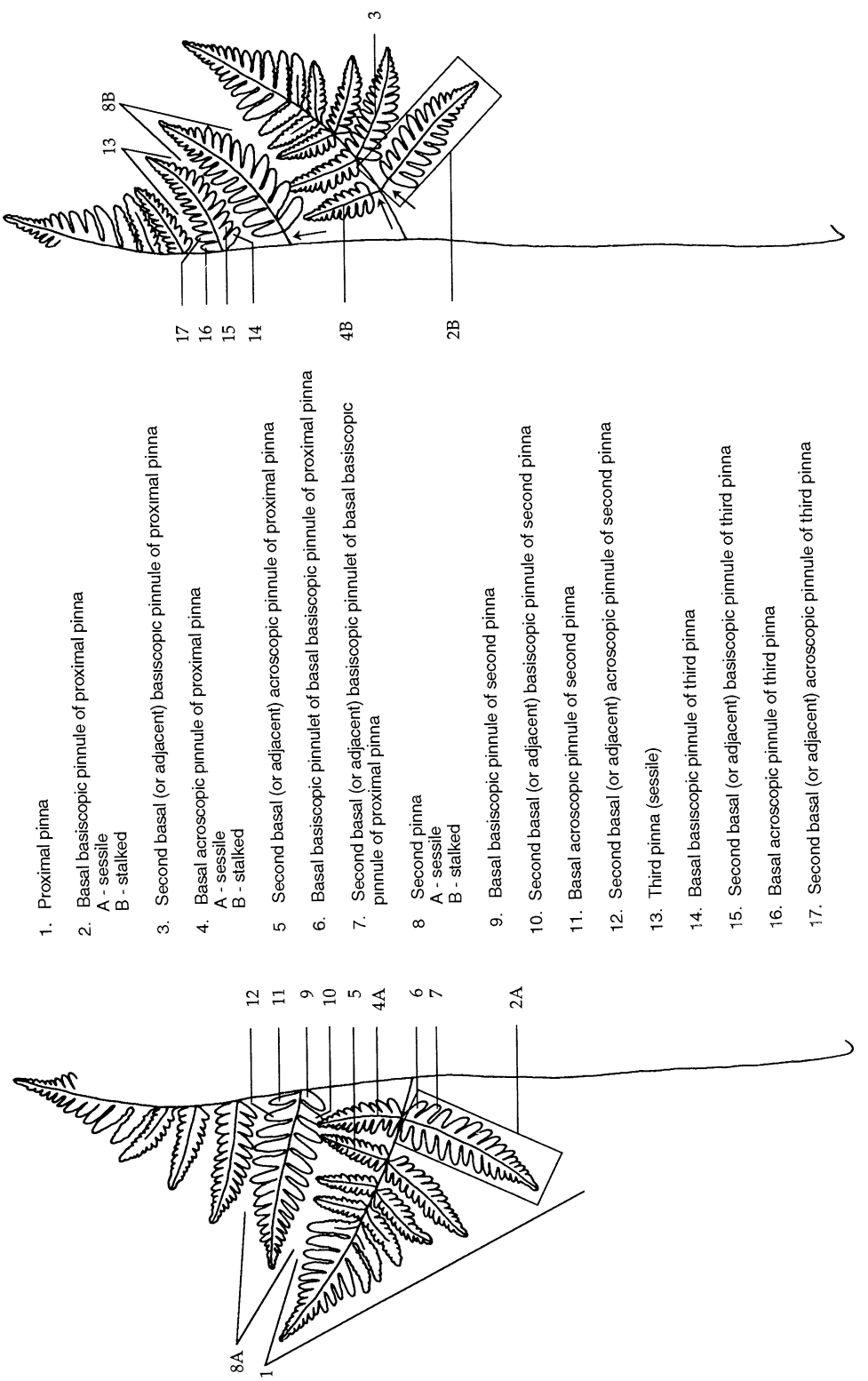
TABLE 3. Comparison of sexual members of the *Gymnocarpium dryopteris* complex. Superscript numbers and letters refer to frond terms depicted in Figure 7.

Characters	*G. appalachianum*	*G. disjunctum*	*G. dryopteris*
Condition of basal basiscopic pinnules[2] of proximal pinnae[1]	Pinnate-pinnatifid or pinnatifid and often stalked[2B], if sessile[2A], with basal basiscopic pinnulets[6] always shorter than adjacent basiscopic pinnulets[7]	Pinnate-pinnatifid and sessile[2A] with basal basiscopic pinnulets[6] usually longer than, though sometimes equaling or shorter than, adjacent basiscopic pinnulets[7]	Pinnatifid and sessile[2A] with basal basiscopic pinnulets[6] equaling or slightly longer than adjacent basiscopic pinnulets[7]
Condition of second pinnae[8]	Often stalked[8B], if sessile[8A], with basal basiscopic pinnules[9] shorter than adjacent basiscopic pinnules[10], and equaling basal acroscopic pinnules[11], the latter shorter than adjacent acroscopic pinnules[12]	Sessile[8A] with basal basiscopic pinnules[9] equaling or exceeding length of adjacent basiscopic pinnules[10] and markedly longer than basal acroscopic pinnules[11], the latter distinctly shorter than adjacent acroscopic pinnules[12] or absent	Sessile[8A] with basal basiscopic pinnules[9] about as long as adjacent basiscopic pinnules[10] and about equaling basal acroscopic pinnules[11], the latter nearly as long as adjacent acroscopic pinnules[12]
Condition of third pinnae[13]	Sessile with basal basiscopic pinnules[14] shorter than adjacent basiscopic pinnules[15] and equaling basal acroscopic pinnules[16], the latter shorter than adjacent acroscopic pinnules[17]	Sessile with basal basiscopic pinnules[14] equaling adjacent basiscopic pinnules[15] and longer than basal acroscopic pinnules[16], the latter distinctly shorter than adjacent acroscopic pinnules[17]	Sessile with basal basiscopic pinnules[14] about as long as adjacent basiscopic pinnules[15] and also about as long as basal acroscopic pinnules[16], the latter nearly as long as adjacent acroscopic pinnules[17]
Margins of ultimate segments of proximal pinnae	Crenate to entire, with entire, rounded tips	Slightly pinnatifid to crenate, often with crenulate, acute tips	Crenate to entire, with entire, rounded tips
Mean exposure length (μm)	27–*29*–31	27–*29.5*–31	34–*36.2*–39
Chromosome number	$2n = 80$	$2n = 80$	$2n = 160$

explain how otherwise sterile triploid hybrids could have such a broad range. The biology of these remarkable triploid plants merits further investigation.

TAXONOMIC CONCLUSIONS

We agree with Paris et al. (1989) that a system of classification should reflect as closely as possible the phylogenetic relationships of the taxa under study, and that cryptic species representing independent evolutionary lineages deserve species names, but only if morphological characters, however subtle, can be found to differentiate them. Based on morphology, the three sexual taxa in the *G. dryopteris* complex can be distinguished using a combination of features (see Table 3, as well as key and descriptions below). Because hybridization between the tetraploid and either diploid would produce sterile triploid backcrosses, and because the genetic identity between the two diploids is so low (av-



erage $\bar{I} = 0.274$), we feel that these taxa should be recognized as distinct species. Although isozymically distinct, members of the *G. dryopteris* complex are morphologically similar species differentiated by minor characters.

Figure 7 illustrates past and current hypotheses of relationships among members of the complex. Sarvela (1980) and Pryer et al. (1983) hypothesized that *G. disjunctum* gave rise to *G. dryopteris* through autopolyploidy, and that *G.* × *brittonianum* was a sterile triploid backcross between them (Fig. 6A). As currently defined, however, the *G. dryopteris* complex is thought to include two divergent diploids, *G. appalachianum* and *G. disjunctum*, a fertile allotetraploid, *G. dryopteris*, and an assemblage of two different, abortive-spored triploid backcrosses, *G. appalachianum* × *dryopteris* and *G. disjunctum* × *dryopteris* (Fig. 6B). The name *G.* × *brittonianum* is applied here to the latter hybrid combination (see below).

The following key will permit identification of most mature specimens, especially those possessing mature, fertile fronds (with sori). As is true for other members of the Dryopteridaceae, the best characters for distinguishing species include features of the basal pinnules of the proximal pinnae. The sterile hybrids are morphological intermediates between their parental species and are very difficult to identify using strictly vegetative features. The presence of mostly abortive spores is the most reliable character for their identification. In order to facilitate identification, important terms pertaining to the frond are schematically depicted in Figure 7 and are cross-referenced throughout the taxonomic treatment with small superscript numbers and letters.

Key to North American Members of the *Gymnocarpium dryopteris* Complex

(N.B. Superscript numbers and letters refer to frond terms depicted in Fig. 7)

1. Spores reniform and uniform in size and shape.
   2. Second pair of pinnae and basal basiscopic pinnules of proximal pinnae stalked[8B&2B] .. .. 1. *G. appalachianum*
   2. Second pair of pinnae sessile[8A] or stalked[8B] (rare); basal basiscopic pinnules of proximal pinnae sessile[2A].
      3. Second pair of pinnae sessile[8A] with basal pinnules[9&11] unequal in length (basiscopic[9] markedly longer); third pair of pinnae sessile[13] with basal pinnules[14&16] unequal in length (basiscopic[14] longer); blades large (8–24 cm long) ..... 2. *G. disjunctum*
      3. Second pair of pinnae rarely stalked[8B]; when sessile[8A], with basal pinnules[9&11] ± equal in length (basiscopic[9] ≈ acroscopic[11]); third pair of pinnae sessile[13] with basal pinnules[14&16] ± equal in length (basiscopic[14] ≈ acroscopic[16]); blades small (3–14 cm long).
         4. Sessile basal basiscopic pinnules of proximal pinnae[2A] with basal basiscopic pinnulets[6] ± equal in length to adjacent basiscopic pinnulets[7]; second pinnae almost always sessile[8A] with basal pinnules[9&11] ± equal in length to adjacent pinnules[10&12]; third pinnae sessile[13] with basal pinnules[14&16] ± equal in length to adjacent pinnules[15&17]; spores 34–39 μm long ..... 3. *G. dryopteris*
         4. Sessile basal basiscopic pinnules of proximal pinnae[2A] with basal basiscopic pinnulets[6] shorter than adjacent basiscopic pinnulets[7]; second pinnae sessile[8A] with basal pinnules[9&11] shorter than adjacent pinnules[10&12], or second pinnae stalked[8B] (rare); third pinnae sessile[13] with basal pinnules[14&16] shorter than adjacent pinnules[15&17]; spores 27–31 μm long ..... 1. *G. appalachianum*
1. Spores mostly malformed, irregular in shape, often with larger, round spores present .... 4. *G.* × *brittonianum* [or *G. appalachianum* × *dryopteris*; see comments under *G.* × *brittonianum*]

1. **Gymnocarpium appalachianum** Pryer & Haufler, sp. nov. (Fig. 8).—Type: U.S.A., Virginia, Page Co., Shenandoah Natl. Park, Stony Man Mt., growing among greenstone outcrops on NW-facing slope about

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Fig. 7. Schematic illustration explaining pertinent frond terminology used in the taxonomic treatment of *Gymnocarpium*. Arrows indicate stalked pinnules and pinna. The numbers and letters representing frond terms are cross-referenced as small superscripts throughout the taxonomic treatment.

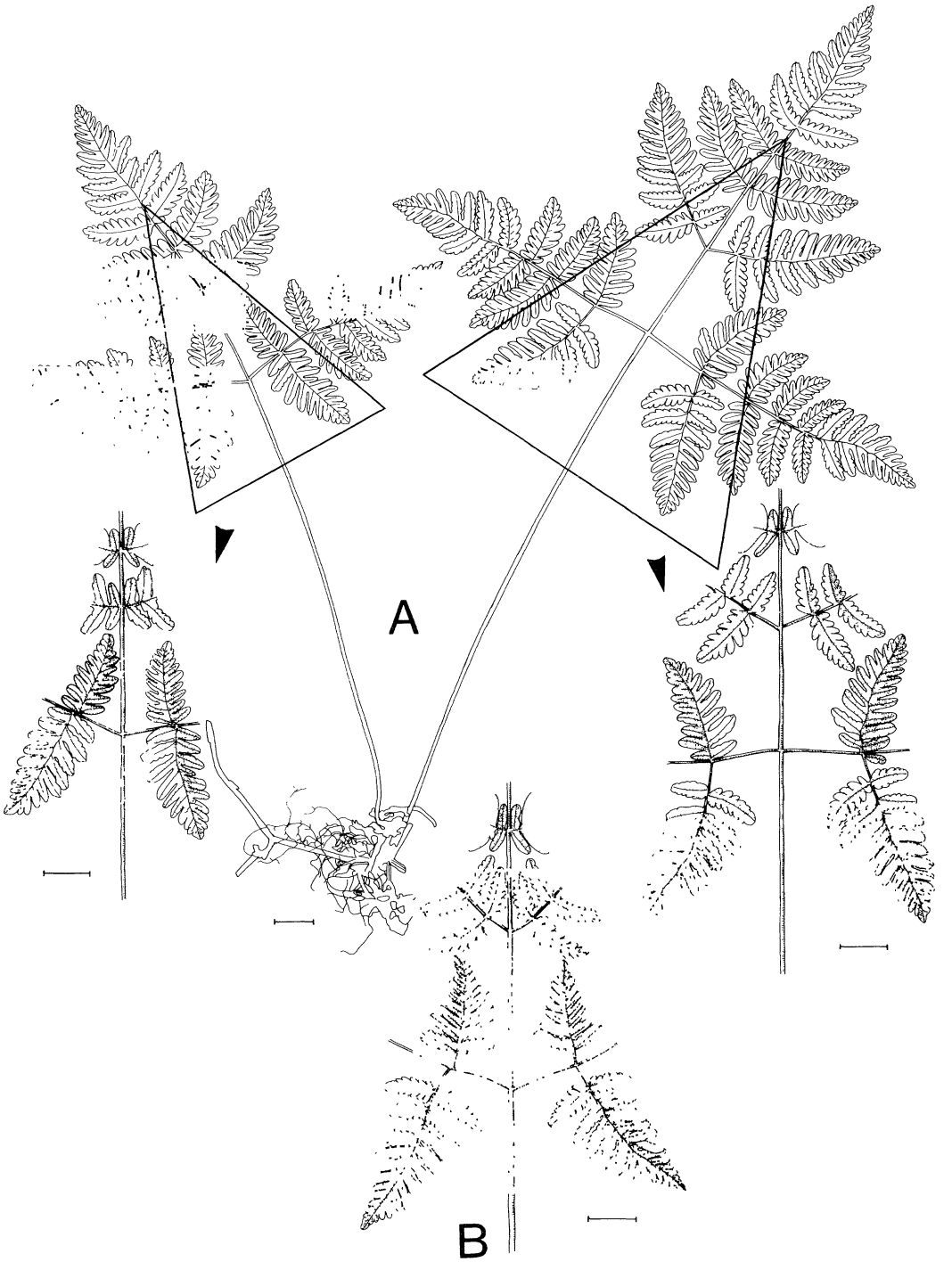

FIG. 8. *Gymnocarpium appalachianum.* A. Frond at right: i) second pinnae are stalked[8B], and ii) proximal pinnae[1] have stalked basal basiscopic pinnules[2B]. Frond at left: i) second pinnae are sessile[8A] with basal basiscopic pinnules[9] shorter than adjacent basiscopic pinnules[10] and equaling basal acroscopic pinnules[11], the latter shorter than adjacent acroscopic pinnules[12], and ii) proximal pinnae[1] have sessile basal basiscopic pinnules[2A] with basal basiscopic pinnulets[6] shorter than adjacent basiscopic pinnulets[7]. Based on holotype: *Pryer et al. 948* (US). B. Morphological "extreme" in plants of *G. appalachianum*: i) second pinnae are stalked[8B], and ii) proximal pinnae[1] have stalked basal basiscopic and acroscopic pinnules[2B&4B]. Based on *Windham 81-31* (UT). Bar lines = 1 cm.

30 yds from summit, 6 Jul 1988, *Pryer, Klein, and Morse 948* (holotype: US!, drawing in Fig. 8 of this paper; isotypes: CAN!, CM!, GH!, US!, VPI!, WVA!).—PARATYPES: North Carolina, Ashe Co., Bluff Mt., near summit on N slope, 9 Jul 1967, *Bozeman et al. 10678* (A, CM, DAO, FARM, LYN, MIN, VPI, WILLI, WVA). Ohio, Wayne Co., 25 Jun 1908, *Hopkins s.n.* (OS—4 sheets). Pennsylvania, Bedford Co., 2¾ mi SSW of Hyndman, 16 Jun 1948, *Berkheimer 9803* (CM); 5 mi S of Hyndman, 5 May 1951, *Buker s.n.* (CM); Wolfsburg, Raystown Branch of Juniata River, 3 Jul 1988, *Pryer et al. 940* (CAN). Virginia, Bath Co., Big Alleghany Mt., about 12 mi NE of Rimel, WVA, 6 Jun 1941, *Henry 2918* (VPI—2 sheets); on NW side of Warm Springs Mt., 0.5 mi SW of Sandy Gap, 29 Jun 1975, *Stevens 10886* (FARM, VPI); Greene Co., Bush Mt., 0.9 mi S of Bootens Gap on shady N-facing outcrop, 25 Jun 1972, *Wieboldt et al. 1033* (FARM); Highland Co., W side of Lantz Mt., Rt. 642, 25 Jun 1961, *Freer 2598* (GH, LYN—2 sheets, US, VPI, WILLI); Lantz Mt., along roadside on W side of summit, 4 Jul 1988, *Pryer et al. 942* (CAN); Madison/Page cos., Hawksbill Mt., westerly cliff top trail near summit, 6 Jul 1988, *Pryer et al. 945* (CAN); Nelson Co., Three Ridges, ¼ mi due N of summit on rocky N-facing slope, 22 Aug 1976, *Wieboldt 2645* (WILLI); Page Co., Bush Mt., 0.9 mi S of Bootens Gap along Skyline Drive, 5 Jul 1988, *Pryer et al. 944* (CAN); Hawksbill Mt., 23 Jun 1947, *Britton B215* (OAC); Hawksbill Mt., along side trail from the W, 15 Jul 1945, *Walker 3682* (US); Neighbor Mt., by ridgecrest trail 1 mi E of summit, 24 Aug 1975, *Stevens 11563* (VPI); Stony Man Mt., SE of Luray, 24 Aug 1927, *Wherry & Pennell s.n.* (VPI), *Windham 81-31* (UT); Rockingham Co., Tomahawk Mt., near Rawley Springs, on rocky N-facing slope, 3 Jun 1972, *Stevens 5006* (VPI); Slate Springs Mt., on N face near Flagpole Knob, 13 Jul 1969, *Stevens 1203* (FARM, VPI); Warren Co., Marshall Mt., N end near summit, 3 Jun 1953, *Hunnewell s.n.* (VPI). West Virginia, Hampshire Co., Ice Mt., 11 Jun 1987, *Morse 9486* (CAN, possibly mixed collection); 6 Jun 1988, *Morse 9578*, site 8 (CAN); 7 Jul 1988, *Pryer et al. 949* (CAN).

Pinnae basales pinnulis basalibus basiscopicis instructae, plerumque stipitatis aut si sessiles subpinnulis basiscopicis brevioribus quam subpinnulis basalibus basiscopicis secundis. Pinnae basales secundae plerumque stipitatae aut si sessiles pinnulis basalibus basiscopicis brevioribus quam pinnulis basalibus basiscopicis secundis et aequantibus pinnulas basales acroscopicas, pinnulae basales acroscopicae breviores quam pinnulis basalibus acroscopicis secundis. Sporae reniformes, 27–31 μm longae, testaceae. Chromosomatum numerus $2n = 80$.

Rhizomes 0.5–1.5 mm in diameter, with scales 1.5–3.0 mm long. Fertile fronds usually 10–32 cm tall. Stipes 6–20 cm long with scales up to 6 mm long. Blades 4–12 cm long, bipinnate-pinnatifid or tripinnate-pinnatifid. Pinnae with entire, rounded tips. Proximal pinnae[1] 3–10 cm long, with basal basiscopic pinnules either stalked[2B] and pinnate-pinnatifid or pinnatifid, or sessile[2A] and pinnate-pinnatifid or pinnatifid, if the latter, basal basiscopic pinnulets[6] always shorter than adjacent basiscopic pinnulets[7]; second basal basiscopic pinnules[3] sometimes stalked, if sessile, with basal basiscopic pinnulets shorter than adjacent basiscopic pinnulets; basal acroscopic pinnules sometimes stalked[4B], if sessile[4A], with basal basiscopic pinnulets shorter than adjacent basiscopic pinnulets. Second pinnae usually stalked[8B], if sessile[8A], with basal basiscopic pinnules[9] shorter than adjacent basiscopic pinnules[10], and equaling basal acroscopic pinnules[11], the latter shorter than adjacent acroscopic pinnules[12]; pinnules often with entire, rounded tips. Third pinnae sometimes stalked, if sessile[13], with basal basiscopic pinnules[14] shorter than adjacent basiscopic pinnules[15], and equaling or shorter than basal acroscopic pinnules[16], the latter equaling or shorter than adjacent acroscopic pinnules[17]. Ultimate segments of the lower pinnae oblong, entire to crenate, with entire, rounded tips. Spores reniform, 27–31 μm long. Diploid $2n = 80$ (Fig. 2).

Distribution. Restricted to the southern Appalachian region of the United States (Fig. 9): North Carolina, Ohio, Pennsylvania, Virginia, and West Virginia.

Habitat. Maple-birch-hemlock (*Acer–Betula–Tsuga*) woods on mountain slopes and summits, on moist sandstone talus and scree, talus slopes with cold air seepage (algific). 200–1400 m.

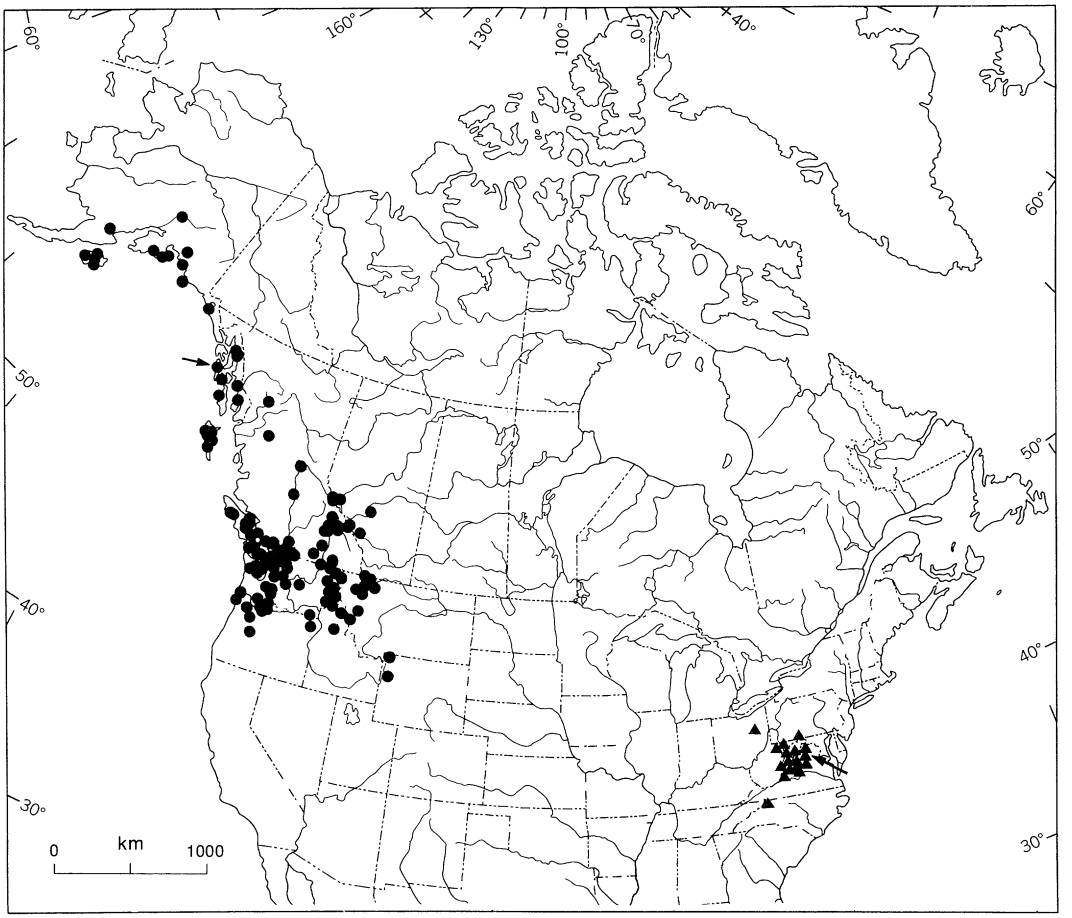

FIG. 9. North American distributions of *Gymnocarpium appalachianum* (triangles) and *G. disjunctum* (dots); the type localities of these species in Virginia and Alaska, respectively, are indicated by arrows.

Gymnocarpium × *heterosporum* W. H. Wagner Jr., a putative triploid hybrid known only from one locality in Pennsylvania that is now extirpated, is thought to have been the result of a unique hybridization event between *G. appalachianum* and the glandular, limestone oak fern *G. robertianum* (Hoffm.) Newm. (Pryer 1992).

2. GYMNOCARPIUM DISJUNCTUM (Rupr.) Ching, Acta Phytotax. Sin. 10: 304. 1965 (Fig. 10).—*Polypodium dryopteris* L. var. *disjunctum* Rupr., Distr. Crypt. Vasc. Ross. 52. 1845 ('γ *Polypodium disjunctum*').—*Polypodium disjunctum* (Rupr.) Schur, Oesterr. Bot. Z. 8: 193. 1858.—*Phegopteris dryopteris* (L.) Fée var.

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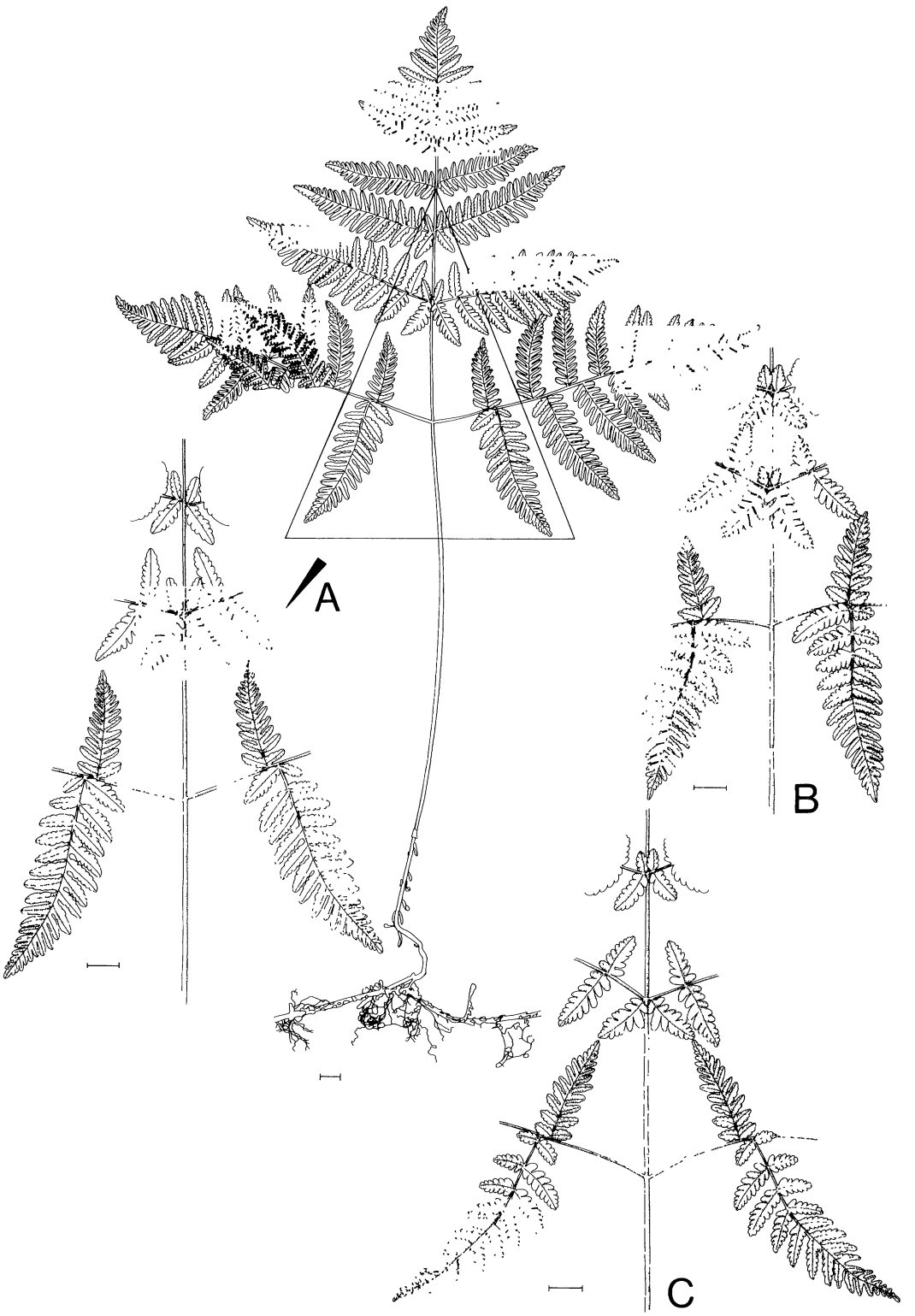
FIG. 10. *Gymnocarpium disjunctum*. A. Frond showing i) sessile second pinnae[8A] with basal basiscopic pinnules[9] equaling adjacent basiscopic pinnules[10] and markedly longer than basal acroscopic pinnules[11], the latter distinctly shorter than adjacent acroscopic pinnules[12], and ii) proximal pinnae[1] with basal basiscopic pinnules that are pinnate-pinnatifid and sessile[2A] with basal basiscopic pinnulets[6] equaling adjacent basiscopic pinnulets[7]. B–C: Morphological variation in plants of *G. disjunctum*. B. Proximal pinnae[1] with sessile basal basiscopic pinnules[2A] with basal basiscopic pinnulets[6] longer than adjacent basiscopic pinnulets[7]. C. i) Proximal pinnae[1] with sessile basal basiscopic pinnules[2A] with basal basiscopic pinnulets[6] shorter than adjacent basiscopic pinnulets[7], and ii) second pinnae sessile[8A] with basal acroscopic pinnules[11] absent. Based on *Alverson s.n.* (CAN 531499), *Calder & Savile 12265* (DAO), *Ulmer 614* (DUKE), *Jones s.n.* (US 855738). Bar lines = 1 cm.



disjuncta (Rupr.) Trel. in Harriman, Harriman Alaska Expedition 5: 382. 1904.—*Dryopteris linnaeana* C. Chr. var. *disjuncta* (Rupr.) Fomin in N. Busch, Fl. Sibir. Orient. Extr. 5: 79. 1930.—*Gymnocarpium dryopteris* (L.) Newman var. *disjunctum* (Rupr.) Ching, Contr. Biol. Lab. Chin. Assoc. Advancem. Sci., Sect. Bot. 9: 41. 1933.—*Dryopteris disjuncta* (Rupr.) C. Morton, Rhodora 43: 217. 1941.—*Carpogymnia disjuncta* (Rupr.) A. Löve & D. Löve, Taxon 16: 191. 1967.—*Gymnocarpium dryopteris* subsp. *disjunctum* (Rupr.) Sarvela, Ann. Bot. Fenn. 15: 103. 1978.—TYPE: U.S.A., Alaska, Sitka, "Sitcha. *Polypodium calcareum* Sm. Bongard Voy. Sitch. Dr. Mertens" [lectotype here designated: LE!, photo CAN!; isolectotype: "Sitcha. Dr. Mertens" LE!, photo CAN!; syntype: "Sitcha. Specimina minuta sterilia. *Polypodii calcarei.* Diff. a *P. Dryopteri.* Stipite paleacea. Dr. Fischer. 1840" (four small sterile fronds) LE!].

Rhizomes 1–3 mm in diameter, with scales 2–4 mm long. Fertile fronds usually 20–68 cm tall. Stipes 12–44 cm long with scales up to 6 mm long. Blades 8–24 cm long, tripinnate-pinnatifid. Pinnae with acuminate tips. Proximal pinnae[1] 5–18 cm long, with basal basiscopic pinnules sessile[2A], pinnate-pinnatifid (with basal pinnulets, and sometimes second and third basal pinnulets, not joined), and with basal basiscopic pinnulets[6] usually longer (sometimes equaling or shorter) than adjacent basiscopic pinnulets[7]; second basal basiscopic pinnules sessile[3] with basal basiscopic pinnulets usually longer than or equaling adjacent basiscopic pinnulets; basal acroscopic pinnules sessile[4A] with basal basiscopic pinnulets usually longer than or equaling adjacent basiscopic pinnulets. Second pinnae usually sessile[8A] with basal basiscopic pinnules[9] longer than or equaling adjacent basiscopic pinnules[10], and markedly longer than basal acroscopic pinnules[11], the latter absent (uncommon) or distinctly shorter than adjacent acroscopic pinnules[12]; pinnule tips often crenulate, obtuse. Third pinnae usually sessile[13] with basal basiscopic pinnules[14] longer than or equaling adjacent basiscopic pinnules[15] and also longer than basal acroscopic pinnules[16], the latter shorter than adjacent acroscopic pinnules[17]. Ultimate segments of the lower pinnae oblong, crenate to slightly pinnatifid, with crenulate, acute tips. Spores reniform, 27–31 μm long. Diploid $2n = 80$ (V. Sorsa 1966; Taylor and Mulligan 1968; Wagner 1966).

Distribution. Sakhalin Island, southern Kamchatka; western coast of North America from Alaska to Oregon and east to Montana and southwestern Alberta (Fig. 9).

Habitat. Shaded, rocky slopes and ravines, mixed coniferous woods, moist stream and creek banks. 0–2400 m.

3. GYMNOCARPIUM DRYOPTERIS (L.) Newman, Phytologist 4: 371. 1851 (Fig. 11A).—*Polypodium dryopteris* L., Sp. Pl. 2: 1093. 1753.—*Polypodium pulchellum* (Salisb., Prodr. Stirp. Chap. Allerton 404. 1796, nom. illeg., ICBN Art. 63.1.—*Polystichum dryopteris* (L.) Roth, Arch. Bot. (Leipzig) 2: 106. 1799.—*Nephrodium dryopteris* (L.) Michx., Fl. Bor.-Amer. 2: 270. 1803.—*Lastrea dryopteris* (L.) Bory, Dict. Class. Hist. Nat. 9: 233. 1826.—*Aspidium dryopteris* (L.) Baumg., Enum. Stirp. Transsilv. 4: 29. 1846.—*Polypodium dryopteris* var. *glabrum* Neilr., Fl. Wien 6. 1846 ('α').—*Phegopteris dryopteris* (L.) Fée, Mém. foug. 5. Gen. Filic. 243. 1852.—*Polypodium dryopteris* var. *genuinum* Ledeb., Fl. Ross. 4: 509. 1853 ('α'), *non rite publ.*, ICBN Art. 24.3.—*Polypodium triangulare* Dulac, Fl. Hautes-Pyrénées 31. 1867, nom. illeg., ICBN Art. 63.1, non L. (1774).—*Phegopteris triangularis* St. Lag. in Cariot, Étude Fl., 8th ed., 2: 964. 1889. nom. illeg., ICBN Art. 63.1.—*Dryopteris linnaeana* C. Chr., Index Filic. 275. 1905.—*Dryopteris pulchella* Hayek, Fl. Steiermark 1: 39. 1908, nom. illeg., ICBN

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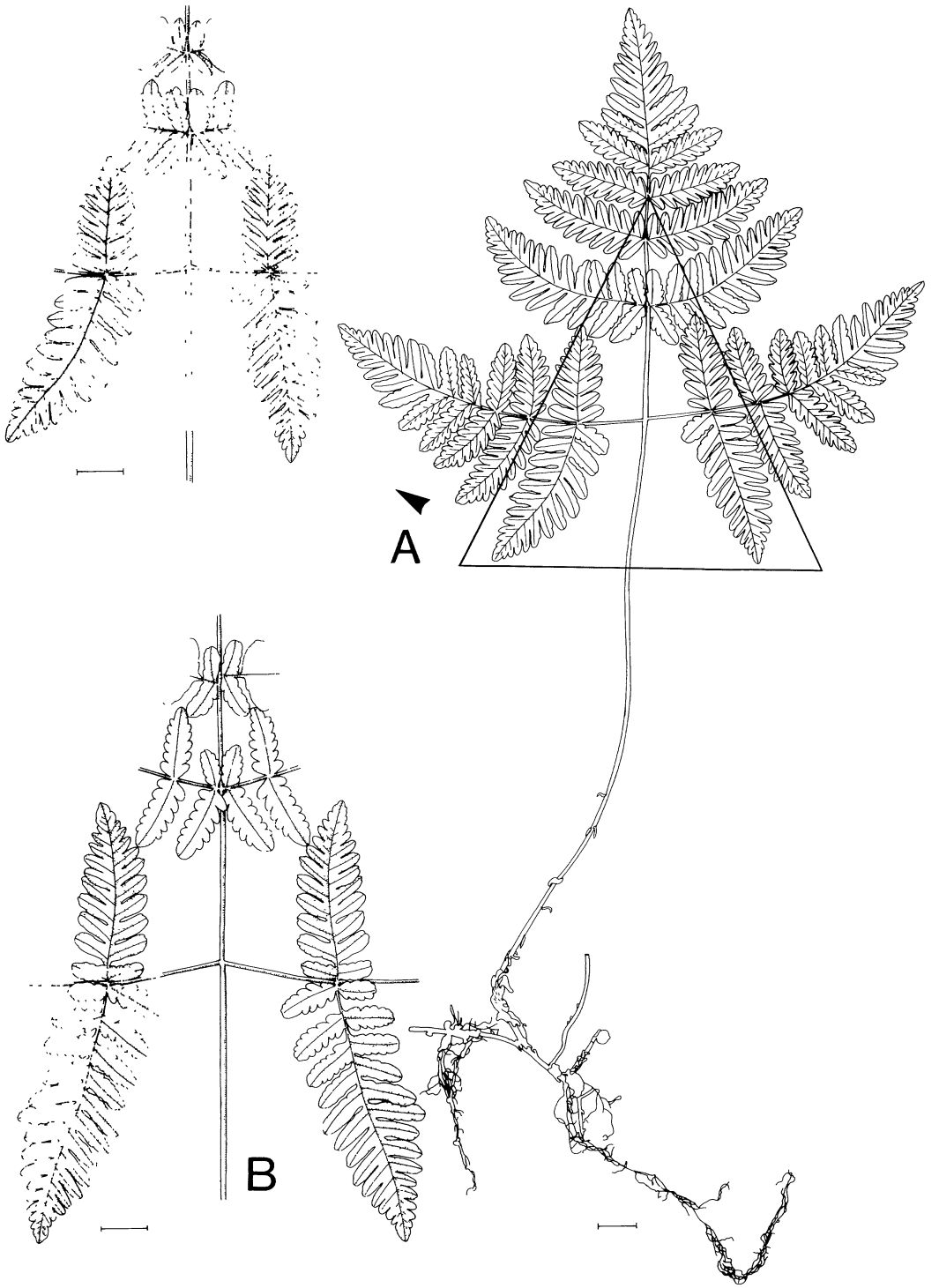
FIG. 11. *Gymnocarpium dryopteris* and *G.* × *brittonianum.* A. *G. dryopteris*: frond has i) sessile second pinnae[8A] with basal basiscopic pinnules[9] equaling adjacent basiscopic pinnules[10] and equaling basal acroscopic pinnules[11], the latter almost as long as adjacent acroscopic pinnules[12], and ii) proximal pinnae[1] with basal basiscopic pinnules that are pinnatifid and sessile[2A] with basal basiscopic pinnulets[6] equaling adjacent basiscopic pinnulets[7]. Based on *Pryer & Klein 920* (CAN), *Clausen & Trapido 2830* (MIN), *Deane s.n.* (MIN 486807). B. *G.* × *brittonianum*: i) sessile second pinnae[8A] with basal basiscopic pinnules[9] equaling adjacent basiscopic pinnules[10] and conspicuously longer than basal acroscopic pinnules[11], the latter shorter than adjacent acroscopic pinnules[12], and



ii) proximal pinnae[1] with basal basiscopic pinnules that are pinnate-pinnatifid and sessile[2A]. Mature, fertile fronds of *G.* × *brittonianum* have malformed, as well as large and round spores. Based on *Dickinson & Kan 363* (CAN). Bar lines = 1 cm.

Art. 63.1.—*Dryopteris triangularis* Herter, Bull. Herb. Boissier sér. 2, 8: 797. 1908, nom. illeg., ICBN Art. 63.1.—*Dryopteris dryopteris* (L.) Christ, Bull. Acad. Int. Géogr. Bot. 20[1]: 151. 1909, *non rite publ.*, ICBN Art. 23.4.—*Thelypteris dryopteris* (L.) Sloss. in Rydb., Fl. Rocky Mts. 1: 1044. 1917.—*Dryopteris pumila* Krecz. in Grossheim, Fl. Kavk., 2nd ed., 1: 19. 1939, nom. illeg., ICBN Art. 63.1.—*Currania dryopteris* (L.) Wherry, Bartonia 21: 15. 1942.—*Carpogymnia dryopteris* (L.) A. Löve & D. Löve, Univ. Colorado Stud., Ser. Biol. 24: 8. 1966.—TYPE: "Filix querna Bauh. Filix arborea Tragi. Eichelfarn. Baumfarn. In Lusatia, Bohemia, Dania." Burser specimen XX.32 (lectotype, designated in McNeill and Pryer 1985: UPS; microfiche!, photo in fig. 1 of McNeill and Pryer 1985).

Polypodium dryopteris var. *erectum* G. Laws., Edinburgh New Philos. J. 19: 109. 1864.—*Phegopteris dryopteris* f. *erecta* (G. Laws.) Broun, Index N. Amer. Ferns 134. 1938.—*Dryopteris disjuncta* (Rupr.) C. Morton f. *erecta* (G. Laws.) Roland, Proc. Nova Scotian Inst. Sci. 20: 92. 1941.—TYPE: Canada, Ontario, Kingston, Collins Bay, "Collins's Bay. *Polypodium dryopteris* var. *rigidium*," 18 Jun 1861, *G.W. Lawson s.n.* (lectotype here designated: E, photo CAN!; see comment below).

Phegopteris dryopteris f. *interrupta* Jewell, Fern Bull. 16: 86. 1908.—TYPE: none located; not at A, GH, or MAINE.

Rhizomes 0.5–1.5 mm in diameter, with scales 1–4 mm long. Fertile fronds usually 12–42 cm tall. Stipes 9–28 cm long with scales up to 6 mm long. Blades 3–14 cm long, bipinnate-pinnatifid. Pinnae with entire, rounded tips. Proximal pinnae[1] 2–12 cm long, with basal basiscopic pinnules usually sessile[2A], pinnatifid (with basal pinnulets confluent with adjacent pinnulets) or rarely pinnate-pinnatifid, and with basal basiscopic pinnulets[6] often equaling or longer than adjacent basiscopic pinnulets[7]; second basal basiscopic pinnules sessile[3] with basal basiscopic pinnulets equaling or longer than adjacent basiscopic pinnulets; basal acroscopic pinnules sessile[4A] with basal basiscopic pinnulets longer than or equaling adjacent basiscopic pinnulets. Second pinnae usually sessile[8A] with basal basiscopic pinnules[9] longer than or equaling adjacent basiscopic pinnules[10], and about equaling basal acroscopic pinnules[11], the latter equaling or slightly shorter than adjacent acroscopic pinnules[12]; pinnule tips often entire, rounded. Third pinnae sessile[13] with basal basiscopic pinnules[14] as long as adjacent basiscopic pinnules[15] and equaling basal acroscopic pinnules[16], the latter equaling or slightly shorter than adjacent acroscopic pinnules[17]. Ultimate segments of the lower pinnae oblong, entire to crenate, with entire, rounded tips. Spores reniform, 34–39 μm long. Tetraploid, $2n = 160$ (Britton 1953; Manton 1950; Pryer 1981; V. Sorsa 1958; Vida 1963; Wagner 1963).

Distribution. Circumboreal (Fig. 12). Throughout northern and central Europe; northern Asia to China and Japan; Greenland; temperate North America, Alaska to Newfoundland, southwards to Arizona and Pennsylvania.

Habitat. Commonly found in cool, coniferous and mixed woods, and at base of shale talus slopes. 0–3000 m.

Two syntypes for the name *Polypodium dryopteris* var. *erectum* were located at E. One was labeled "*Polypodium dryopteris* α" and the other "*Polypodium dryopteris* var. *rigidium.*" The first specimen is most likely what Lawson referred to as the "normal form," and the second what he had in mind for var. *erectum.* His change in varietal epithet was possibly due to his realization that Hooker had already published a *P. dryopteris* var. *rigidium* in 1832.

Other species included in *Gymnocarpium* in North America are *Gymnocarpium robertianum* and *G. jessoense* (Koidz.) Koidz. subsp. *parvulum* Sarvela, two morphologically distinct tetraploids (Sarvela et al. 1981) that differ most conspicuously from those species in the *G. dryopteris* complex by having an indument of minute (0.1 mm) glands on their leaves. Pryer (1990) presents a tabular comparison of the morphological and ecological attributes of *G. dryopteris* with those of *G. robertianum* and *G. jessoense* subsp. *parvulum.* Hybrids between the glabrous species, and also between the glabrous and glandular species, have played a significant role in obscuring species boundaries in *Gymnocarpium.*

4. **Gymnocarpium × brittonianum** (Sarvela) Pryer & Haufler, comb. et stat. nov. (Fig. 11B).—*Gymnocarpium dryopteris* subsp. ×

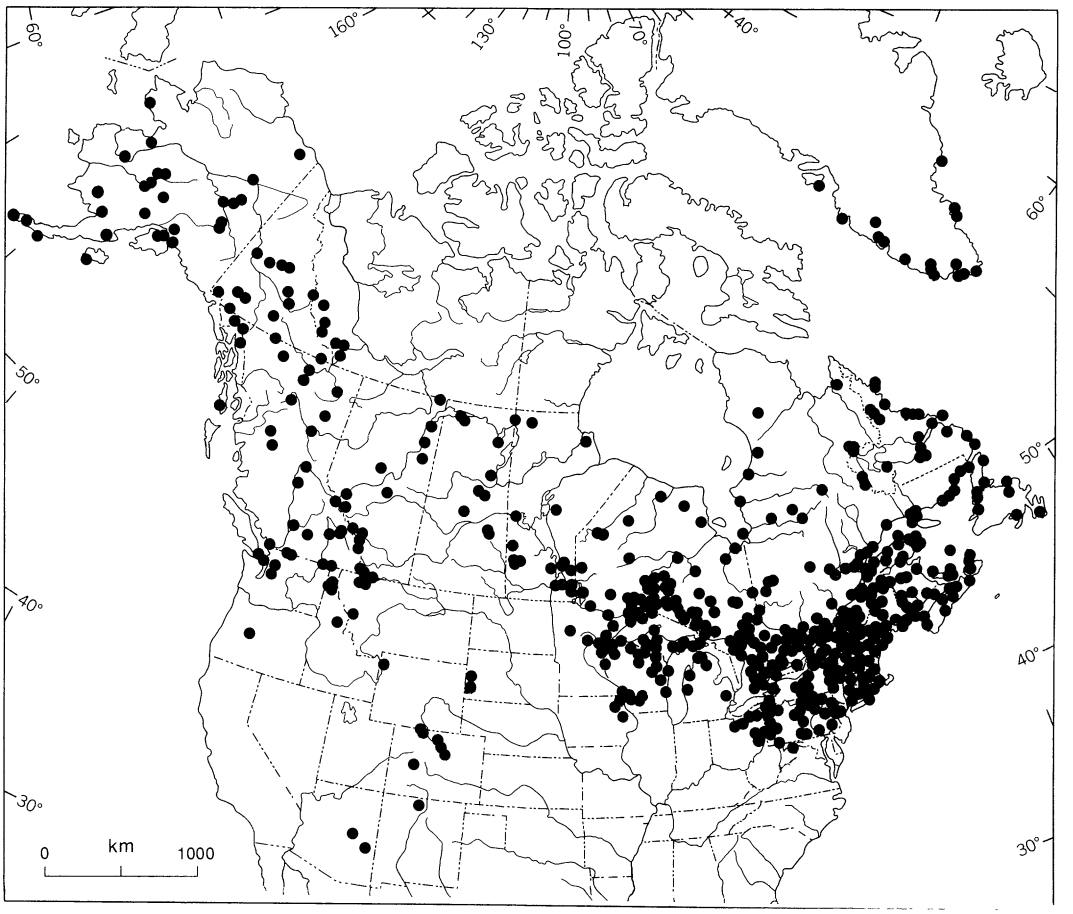


FIG. 12. North American distribution of *Gymnocarpium dryopteris*.

brittonianum Sarvela, Ann. Bot. Fenn. 17: 292. 1980.—TYPE: Canada, Ontario, Wellington Co., in moist cedar and yellow birch glade, 21 July 1978, *D.M. Britton and A. Anderson 6860* (holotype: H!; isotype: OAC!).

Rhizomes 1–2 mm in diameter, with scales 1–4 mm long. Fertile fronds usually 14–60 cm tall. Stipes 10–40 cm long with scales up to 6 mm long. Blades 4–20 cm long, usually tripinnate-pinnatifid, sometimes bipinnate-pinnatifid. Pinnae with acuminate tips. Proximal pinnae[1] 3–16 cm long, with basal basiscopic pinnules sessile[2A], pinnate-pinnatifid or sometimes pinnatifid, and with basal basiscopic pinnulets[6] usually equaling adjacent basiscopic pinnulets[7]; second basal basiscopic pinnules sessile[3] with basal basiscopic pinnulets usually equaling adjacent basiscopic pinnulets; basal acroscopic pinnules sessile[4A] with basal basiscopic pinnulets usually equaling adjacent basiscopic pinnulets. Second pinnae sessile[8A] with basal basiscopic pinnules[9] equaling adjacent basiscopic pinnules[10], and usually conspicuously longer than basal acroscopic pinnules[11], the latter distinctly shorter than adjacent acroscopic pinnules[12]; pinnule tips often crenulate, obtuse. Third pinnae usually sessile[13] with basal basiscopic pinnules[14] equaling adjacent basiscopic pinnules[15] and sometimes slightly longer than basal acroscopic pinnules[16], the latter usually slightly shorter than adjacent acroscopic pinnules[17]. Ultimate segments of the lower pinnae of large blades oblong, crenate to slightly pinnatifid, with crenulate, acute tips; those of small blades oblong, entire to crenate, with en-

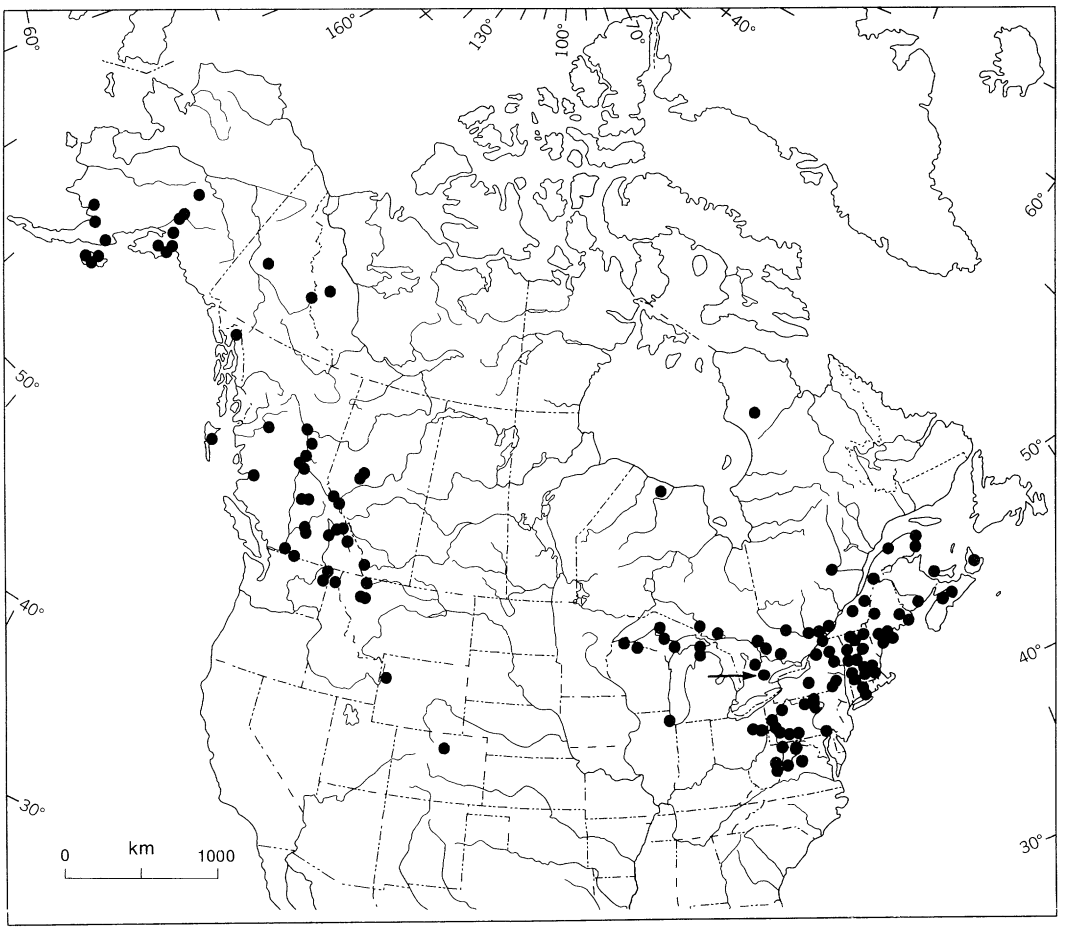


FIG. 13. North American distribution of plants with malformed spores in the *Gymnocarpium dryopteris* complex. The type locality of *G.* × *brittonianum* is indicated by an arrow in Ontario.

tire, rounded tips. Spores of two types: malformed, black spores with very exaggerated perispores and large, round spores with extensive reticulate perispores. Triploid, $2n = 120$ (Fig. 4; Pryer 1981).

Distribution. Western North America: Alaska to Colorado; eastern North America: Atlantic Provinces to Ontario and southward. Precise boundaries of range not well-understood (Fig. 13, in part).

Habitat. Rich, moist to wet mixed woods, wooded streambanks and creekbeds, montane forests. 0–2400 m.

The statement of putative parentage in Sarvela's (1980) diagnosis does not play a role in determining the application of the name here. Based on our morphological and electrophoretic observations of material from the type locality of *G.* × *brittonianum*, it is clear that *G. disjunctum* was the diploid involved in these triploid backcrosses. We cannot rule out a contribution from *G. appalachianum* in other parts of the range of triploid plants and, in fact, consider it likely. However, only one name can correctly apply to any particular hybrid formula; by implication, two different crosses cannot bear the same name (ICBN Article H.4.1). We therefore apply the name *G.* × *brittonianum* to the *G. disjunctum* × *dryopteris* combination (Fig. 6B). According to ICBN Article H.5.1, the appropriate rank of a hybrid is that of the postulated parent taxa. Consequently, the rank of this sterile hybrid is elevated here to that of species. The hybrid *G. appalachianum* × *dryopteris* is not assigned a binomial name, pending firm identification of hybrids with this parentage.

The fronds of the type specimen of *G.* × *brit-*

tonianum and of other collections examined from the type locality strongly resemble those of *G. disjunctum* (cf. Fig. 11B), having blades that are large, tripinnate and usually with the following morphological characteristics: a) proximal pinnae[1] with basal basiscopic pinnules that are sessile[2A] and pinnate-pinnatifid, b) second pinnae that are sessile[8A] with basal basiscopic pinnules[9] that are usually conspicuously longer than basal acroscopic pinnules[11], the latter being shorter than the adjacent acroscopic pinnules[12] and often with crenulate, acute tips, and c) the ultimate segments of the proximal pinnae of large blades tend toward crenate to slightly pinnatifid.

Hybrid plants that conform to this morphological description occur not only at the type locality, but throughout a substantial part of the North American range for abortive-spored plants in the *G. dryopteris* complex (Fig. 13). A detailed investigation of the morphology and distribution range of *G. appalachianum* × *dryopteris* will be the subject of future study.

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LITERATURE CITED

ALVERSON, E. R. 1988. Biosystematics of North American parsley ferns, *Cryptogramma* (Adiantaceae). American Journal of Botany 75: 136–137. Abstract.

BRITTON, D. M. 1953. Chromosome studies on ferns. American Journal of Botany 40: 575–583.

BRYAN, F. A. and D. E. SOLTIS. 1987. Electrophoretic evidence of allopolyploidy in the fern *Polypodium virginianum*. Systematic Botany 12: 553–561.

CRAWFORD, D. J. 1983. Phylogenetic and systematic inference from electrophoretic studies. Pp. 257–287 in *Isozymes in plant genetics and breeding*, Part A, eds. S. D. Tanksley and T. J. Orton. Amsterdam: Elsevier.

———. 1985. Electrophoretic data and plant speciation. Systematic Botany 10: 405–416.

——— and E. B. SMITH. 1984. Allozyme divergence and intraspecific variation in *Coreopsis grandiflora* (Compositae). Systematic Botany 9: 219–225.

DAVIS, M. B. 1983. Quaternary history of deciduous forests of eastern North America and Europe. Annals of the Missouri Botanical Garden 70: 550–563.

FERRIS, S. D. 1984. Tetraploidy and the evolution of the catostomid fishes. Pp. 55–93 in *Evolutionary genetics of fishes*, ed. B. J. Turner. New York: Plenum.

GASTONY, G. J. 1988. The *Pellaea glabella* complex: Electrophoretic evidence for the derivations of the agamosporous taxa and a revised taxonomy. American Fern Journal 78: 44–67.

GOTTLIEB, L. D. 1981. Electrophoretic evidence and plant populations. Progress in Phytochemistry 7: 1–46.

HAUFLER, C. H. 1985a. Enzyme variability and modes of evolution in *Bommeria*. Systematic Botany 10: 92–104.

———. 1985b. Pteridophyte evolutionary biology: The electrophoretic approach. Proceedings of the Royal Society of Edinburgh 86B: 315–323.

———. 1987. Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. American Journal of Botany 74: 953–966.

———, M. D. WINDHAM, D. M. BRITTON, and S. J. ROBINSON. 1985. Triploidy and its evolutionary significance in *Cystopteris protrusa*. Canadian Journal of Botany 63: 1855–1863.

———, ———, and T. A. RANKER. 1990. Biosystemat-

ic analysis of the *Cystopteris tennesseensis* (Dryopteridaceae) complex. Annals of the Missouri Botanical Garden 77: 314–329.

HULTÉN, E. and M. FRIES. 1986. *Atlas of North European vascular plants north of the Tropic of Cancer*. I. Königstein: Koeltz Scientific Books.

JACKSON, R. C. 1982. Polyploidy and diploidy: New perspectives on chromosome pairing and its evolutionary implications. American Journal of Botany 69: 1512–1523.

——— and J. CASEY. 1982. Cytogenetic analyses of autopolyploids: Models and methods for triploids to octoploids. American Journal of Botany 69: 487–501.

JALAS, J. and J. SUOMINEN, eds. 1972. *Atlas Florae Europaeae; Distribution of vascular plants in Europe. 1. Pteridophyta (Psilotaceae to Azollaceae)*. Helsinki: The Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo.

KATO, M. and K. IWATSUKI. 1983. Phytogeographic relationships of pteridophytes between temperate North America and Japan. Annals of the Missouri Botanical Garden 70: 724–733.

MANTON, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge Univ. Press.

MCNEILL, J. and K. M. PRYER. 1985. The status and typification of *Phegopteris* and *Gymnocarpium*. Taxon 34: 136–143.

PARIS, C. A., F. S. WAGNER, and W. H. WAGNER, JR. 1989. Cryptic species, species delimitation, and taxonomic practice in the homosporous ferns. American Fern Journal 79: 46–54.

PRYER, K. M. 1981. Systematic studies in the genus *Gymnocarpium* Newm. in North America. M.Sc. thesis, University of Guelph, Guelph, Ontario.

———. 1990. The limestone oak fern: New to the flora of Manitoba. Blue Jay 48: 192–195.

———. 1992. The status of *Gymnocarpium heterosporum* and *G. robertianum* in Pennsylvania. American Fern Journal 82: 34–39.

——— and D. M. BRITTON. 1983. Spore studies in the genus *Gymnocarpium*. Canadian Journal of Botany 61: 377–388.

———, ———, and J. MCNEILL. 1983. A numerical analysis of chromatographic profiles in North American taxa of the fern genus *Gymnocarpium*. Canadian Journal of Botany 61: 2592–2602.

RANKER, T. A. 1988. The origin of the tetraploid *Hemionitis pinnatifida*. American Journal of Botany 75: 142. Abstract.

——— and A. F. SCHNABEL. 1986. Allozymic and morphological evidence for a progenitor-derivative pair in *Camassia* (Liliaceae). Systematic Botany 11: 433–445.

ROOSE, M. L. and L. D. GOTTLIEB. 1976. Genetic and biochemical consequences of polyploidy in *Tragopogon*. Evolution 30: 818–830.

SARVELA, J. 1978. A synopsis of the fern genus *Gymnocarpium*. Annales Botanici Fennici 15: 101–106.

———. 1980. *Gymnocarpium* hybrids from Canada and Alaska. Annales Botanici Fennici 17: 292–295.

———, D. M. BRITTON, and K. M. PRYER. 1981. Studies on the *Gymnocarpium robertianum* complex in North America. Rhodora 83: 421–431.

SOLTIS, D. E. and L. H. RIESEBERG. 1986. Autopolyploidy in *Tolmiea menziesii* (Saxifragaceae): Genetic insights from enzyme electrophoresis. American Journal of Botany 73: 310–318.

——— and P. S. SOLTIS. 1989. Polyploidy, breeding systems, and genetic differentiation in homosporous pteridophytes. Pp. 241–258 in *Isozymes in plant biology*, eds. D. E. Soltis and P. S. Soltis. Portland: Dioscorides Press.

———, C. H. HAUFLER, D. C. DARROW, and G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal 73: 9–27.

SORSA, P. 1980. Spore morphology of the fern genus *Gymnocarpium* and its relations to the taxonomy. Annales Botanici Fennici 17: 86–90.

SORSA, V. 1958. Chromosome studies on Finnish Pteridophyta. I. Hereditas 44: 541–546.

———. 1966. A diploid *Gymnocarpium dryopteris* (L.) Newman in Alaska. Nature 210: 1375.

TAYLOR, R. L. and G. A. MULLIGAN. 1968. *Flora of the Queen Charlotte Islands. Part 2. Cytological aspects of the vascular plants*. Ottawa: Department of Agriculture Monograph 4.

TRYON, R. M. and A. F. TRYON. 1982. Ferns and allied plants, with special reference to tropical America. New York: Springer-Verlag.

VIDA, G. 1963. A *Dryopteris* nemzetség (sensu lato) szisztematikája. Botanikai Közlemények 50: 125–133.

WAGNER, W. H., JR. 1943. Hybridization by remote control. American Fern Journal 33: 71–73.

———. 1963. A biosystematic survey of United States ferns—Preliminary abstract. American Fern Journal 53: 1–16.

———. 1966. New data on North American oak ferns, *Gymocarpium*. Rhodora 68: 121–138.

WERTH, C. R. 1985. Implementing an isozyme laboratory at a field station. Virginia Journal of Science 36: 53–76.

———. 1989. The use of isozyme data for inferring ancestry of polyploid pteridophytes. Biochemical Systematics and Ecology 17: 117–130.

———, S. I. GUTTMAN, and W. H. ESHBAUGH. 1985. Electrophoretic evidence of reticulate evolution in the Appalachian *Asplenium* complex. Systematic Botany 10: 184-192.