# A numerical analysis of chromatographic profiles in North American taxa of the fern genus *Gymnocarpium*

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As part of a systematic investigation of the genus *Gymnocarpium* in North America, a survey of chromatographic profiles in species and hybrids of the genus was initiated. It was established through cluster analysis and ordination of the phenolic data that morphologically distinguishable taxa of *Gymnocarpium* can be recognized by their chromatographic profiles alone. These data provide supportive evidence for the recognition of *G. robertianum* and *G. jessoense* ssp. *parvulum* as distinct taxa and for the hybrid status of *G.* × *intermedium*. They also suggest that, as currently circumscribed, *G. jessoense* ssp. *jessoense* is a heterogeneous taxon.

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Les profils chromatographiques des espèces et des hybrides du genre *Gymnocarpium* ont été étudiés dans le cadre d'une recherche systématique sur ce genre en Amérique du Nord. Une analyse de groupement et une ordination des données phénoliques montrent que les taxons morphologiquement distincts dans le genre *Gymnocarpium* peuvent être reconnus par leurs seuls profils chromatographiques. Ces données confirment que le *G. robertianum* et le *G. jessoense* ssp. *parvulum* peuvent être considérés comme deux taxons distincts et appuient le statut hybride du *G.* × *intermedium*. Elles indiquent aussi que le *G. jessoense* spp. *jessoense*, tel que délimité actuellement, est un taxon hétérogène.

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## Introduction

In recent decades, there has been an increasing interest in the application of chemical evidence to taxonomic problems. The rationale of biochemical systematics has been discussed in such comprehensive works as Alston and Turner (1963), Swain (1966), and Harborne and Swain (1969).

Phenolic compounds are natural products that have been used extensively in chemotaxonomic studies. These secondary metabolites have provided useful information on problems at the specific and generic levels, supporting cases of suspected interspecific hybridization and providing clues to the origin of polyploid taxa (Smith and Levin 1963; Alston and Turner 1963; Giannasi 1978).

Prior to the reviews of Bohm and Tryon (1967), Swain and Cooper-Driver (1973), and Giannasi (1974), relatively little was known concerning the distribution of phenolic compounds in the pteridophytes. The classic chromatographic study of *Asplenium* L. by Smith and Levin (1963), and similar pattern work by Scora and Wagner (1964) on *Dryopteris* Adans., indicated the potential of biochemical studies in ferns, although structural identification of the chemical constituents was not carried out until a later time. Increased knowledge of the identity and structural complexity of the fern flavonoids and related compounds in the past few years has provided further insights into fern phylogeny (Cooper-Driver 1980; Giannasi 1980; Smith 1980).

Chromatographic profiles, without the identification of phenolic compounds, continue to represent the initial step in a number of systematic surveys. Apparent differences in chromatographic profiles among taxa commonly correlate with similar distinctions based on morphological and (or) other characters (Alston 1967).

A preliminary chromatographic investigation of the genus *Gymnocarpium* Newm. was carried out by Oliver (1972). Chromatograms and electrophoretograms of extracts from *Gymnocarpium* were compared with those of representatives of *Phegopteris* (Presl) Fée, *Thelypteris* Schmidel, and *Dryopteris*. Oliver attempted to determine the generic status of *Gymnocarpium* because it had been placed in all three of these genera at various times; however, no significant affinities were indicated in the chromatographic profiles among the different genera. The results of that particular study are of limited value, however, and cannot be compared with those detailed below, because only a one-dimensional analysis was utilized.

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By using paper chromatography, a survey of phenolic profiles in species and hybrids of *Gymnocarpium* from North America was initiated here. Some material from Europe and Asia was also investigated for comparative purposes. Although no spectral analysis of the compounds was attempted, the chromatographic profiles were subjected to a numerical analysis with a view to determining whether morphologically recognizable taxa of *Gymnocarpium* could be distinguished by their phenolic constituents alone and, if so, if the phenolic profiles would aid in resolving taxonomic problems in the group.

## Materials and methods

Specimens of *Gymnocarpium* used for the phenolic profile analyses were selected from a broad geographic range (Table 1). Most of the analyses were carried out using herbarium specimens, although some fresh fronds from field collections were also used. Replicate chromatograms were run as a check for several specimens and 109 chromatograms were analysed in all, representing 63 separate specimens.

Each chromatogram was prepared from a single frond. The age and condition of the fronds were noted in each case, as these varied from fronds with young sporangia to others with mature spores.

Extracts were prepared by powdering the whole frond and soaking 0.1 g of material in 1 mL of absolute methanol for 48 h. Approximately 200 μL of extract was then pipetted onto Whatman 3MM chromatographic paper. Separation was achieved in the ascending fashion in two solvent systems: first in *n*-butanol – acetic acid – water (12:3:5) for 36 h, followed by a 2% formic acid solution for 6 h in the second dimension.

The dried chromatograms were examined in ultraviolet light before and after "fuming" with concentrated ammonia. $R_f$ values, color reactions, and intensity and frequency of occurrence were noted for each spot. Spots on separate chromatograms, presumed initially to be identical on the basis of color reaction and position, were assigned the same code. To provide some test of the validity of this presumption, adjusted $R_f$ values were plotted on a two-dimensional scatter diagram for each color group. The $R_f$ values were adjusted to minimize differences between chromatograms in the rate of movement of the compounds. This was done separately for each dimension by calculating the overall mean $R_f$ value for each spot on the basis of the provisional assignments. The adjusting factor for a particular chromatogram was the mean of the deviations of its $R_f$ values from these means.

In the vast majority of cases, the spots were clearly defined (Fig. 1). In the few cases (less than 3%) where there was doubt as to the identity of the spot, it was discounted, that is, it was removed from the group to which it had been assigned and the record for that spot (and any other spot to which it might be assigned) was treated as "missing" in the subsequent numerical analyses.

Pair-wise similarities between chromatograms were calculated on a basis that combined a score for the joint presence of a particular spot with a measure of the similarity in spot intensity, recorded on a scale of 1 (very faint) to 4 (strong). Mutual absence of a spot did not contribute to the similarity assessment. The formula used was

$$[1] \quad S_{AB} = (SJ_{AB} + (1 - D_{AB}^2)^{\frac{1}{2}})/2,$$

where $S_{AB}$ is the similarity between the chromatograms A and B, $SJ_{AB}$ is a Jaccard coefficient (Sneath and Sokal 1973) calculated from the mutual occurrence of spots in chromatograms A and B, and $D_{AB}$ is the Euclidean distance between the spot intensity values calculated only over those spots present in both chromatogram A and chromatogram B and divided by the range of intensity values (in this case, 3). The values of $S_{AB}$ were the input data for clustering and principal-coordinates analysis using the S045 program of the Statistics Research Section, Engineering and Statistics Research Institute, Agriculture Canada, Ottawa. In this program the similarities ($S$) are converted, where necessary, to dissimilarities (distances) ($D$) as $D = (1 - S^2)^{\frac{1}{2}}$.

Clustering was carried out using the group average (UPGMA) and flexible sorting methods (Sneath and Sokal 1973). For a discussion of the effects of the parameters α and β used in the flexible sorting method see McNeill (1975).

## Results and discussion

The dendrogram in Fig. 2 depicts the results of a cluster analysis using the phenolic spot presence and intensity data. In this dendrogram (Fig. 2) derived by the flexible sorting method (Lance and Williams 1967; McNeill 1975), each of the taxa recognized on morphological grounds (Pryer 1981) is clearly demarcated. The initial most striking feature of the dendrogram is the separation of two large groups: the nonglandular *G. dryopteris* (L.) Newm., comprising three subspecies, forms almost all of the first group and the glandular taxa *G.* × *intermedium* Sarvela, *G. jessoense* (Koidz.) Koidz., and *G. robertianum* (Hoffm.) Newm. make up, for the most part, the second group.

Three subgroups are well-defined within the large *G. dryopteris* group (Fig. 2). These subgroups correspond to the subspecific taxa *G. dryopteris* ssp. × *brittonianum* Sarvela, *G. dryopteris* ssp. *dryopteris*, and *G. dryopteris* ssp. *disjunctum* (Rupr.) Sarvela. The single anomalous member of these subspecies was "DD11" which clustered with the *G. dryopteris* ssp. × *brittonianum* subgroup. The two samples, "DE20" and "DE21" represent *G. dryopteris* ssp. *dryopteris* material from France which clusters with the North American representatives of this taxon.

An interesting result of the cluster analysis in the *G. dryopteris* group is that fronds from Japan determined by K. Mitsui (*in litt.*) as diploid ($n = 40$) and identifiable as *G. jessoense* ssp. *jessoense* by using Sarvela's *Gymnocarpium* key (1978) clustered with the western North American diploid taxon *G. dryopteris* ssp. *disjunctum* (Fig. 2). Sarvela (1978) recognizes *G. jessoense* ssp. *jessoense* as being either glabrous or densely glandular, although *G. jessoense*, when originally described from Japan, was said to have fronds "fere glaberrimae" (Koidzumi 1924). The Japanese speci-

TABLE 1. Sources of *Gymnocarpium* material used in chromatography study

| Taxon | Province or country | Chromatogram code [a] | Voucher (OAC) |
|---|---|---|---|
| *G. dryopteris* ssp. *dryopteris* | B.C. | (DD23) | Alaska Hwy., Liard Hot Springs Prov. Park, *Grenville s.n.* |
| | Ont. | (DD07,DD05) | Algoma Distr., Magpie High Falls, *Britton 7155* |
| | | (DD16,DD13,DD14,DD15) | Algoma Distr., Magpie High Falls, *Pryer 400* |
| | | (DD06,DD08) | Thunder Bay Distr., Crooks Twp., *Garton 19097* |
| | | (DD18) | Thunder Bay Distr., Ravine Lake, *Pryer 463* |
| | | (DD02,DD01) | Wellington Co., Guelph, *Pryer 373* |
| | | (DD19) | Wellington Co., Guelph, *Pryer 558* |
| | | (DD12,DD11) | Wellington Co., Guelph, *Britton 6794* |
| | | (DD10,DD09) | Wellington Co., Irish Creek, *Britton 6990* |
| | | (DD17) | Wellington Co., Irish Creek, *Pryer 373* |
| | P.Q. | (DD22,DD03)(DD04) | Nouveau Québec, Schefferville, *Pryer 490* |
| | France | (DE20,DE21) | Isère, Grenoble, *Fraser-Jenkins 7357* |
| *G. dryopteris* ssp. × *brittonianum* | Ont. | (DB04,DB07) | Algoma Distr., Lafoe Creek, *Pryer 394* |
| | | (DB06,DB01,DB03) | Prescott Co., Plantagenet Twp., *Britton 6908* |
| | | (DB13,DB11)(DB14,DB15) | Prescott Co., Plantagenet Twp., *Pryer 380* |
| | | (DB09,DB12) | Prescott Co., Plantagenet Twp., *Pryer 548* |
| | | (DB10) | Prescott Co., Plantagenet Twp., *Pryer 553* |
| | | (DB05,DB02) | Wellington Co., West Garafraxa Twp., *Britton 6879* |
| | | (DB08) | Wellington Co., West Garafraxa Twp., *Pryer 612* |
| *G. dryopteris* ssp. *disjunctum* | B.C. | (DJ03) | Queen Charlotte Islands, Moresby Island, *Marchant s.n.* |
| | | (DJ05,DJ06) | Vancouver City, Cypress Bowl, *Ceska and Ceska s.n.* |
| | | (DJ08)(DJ02,DJ01,DJ04) | Vancouver Island, MacMillan Memorial Grove, *Britton 7204* |
| | | (DJ07) | Vancouver Island, MacMillan Memorial Grove, *Britton 8092* |
| *G.* × *intermedium* | Ont. | (IN03,IN02) | Thunder Bay Distr., Current River, *Britton 6800* |
| | | (IN12) | Thunder Bay Distr., Kakabeka Falls, *Britton 5868* |
| | | (IN08) | Thunder Bay Distr., Mt. McRae, *Pryer 589* |
| | | (IN07) | Thunder Bay Distr., Nipigon, *Pryer 576* |
| | | (IN05,IN04,IN06) | Thunder Bay Distr., Sibley Twp., *Garton 18960* |
| | | (IN11,IN10)(IN01,IN09) | Thunder Bay Distr., Sibley Twp., *Pryer 595* |
| | Finland | (IE01) | Kuusamo, Juuma, Jäkälävuoma, *Sarvela s.n.* |

TABLE 1 (*concluded*)

| Taxon | Province or country | Chromatogram code [a] | Voucher (OAC) |
|---|---|---|---|
| *G. jessoense* ssp. *parvulum* | Ont. | (JP10,JP11) | Thunder Bay Distr., Dorion Twp., *Garton 18906* |
| | | (JP01,JP13)(JP02,JP03) | Thunder Bay Distr., Kaministiquia River, *Garton 19075; 19076* |
| | | (JP18) | Thunder Bay Distr., Kaministiquia River, *Pryer 450* |
| | | (JP14,JP15) | Thunder Bay Distr., Kilkenny Twp., *Pryer 415* |
| | | (JP04) | Thunder Bay Distr., McKirdy, *Pryer 571* |
| | | (JP12) | Thunder Bay Distr., Mt. McRae, *Pryer 591* |
| | | (JP08,JP20) | Thunder Bay Distr., Nipigon Twp., *Britton 7401* |
| | | (JP19,JP09) | Thunder Bay Distr., Nipigon Twp., *Pryer 422* |
| | | (JP16) | Thunder Bay Distr., Sibley Twp., *Pryer 460* |
| | | (JP07,JP06)(JP17,JP05) | Thunder Bay Distr., Sibley Twp., *Pryer 593* |
| *G. robertianum* | Ont. | (RB02,RB07) | Bruce Co., Bruce Peninsula, *Britton 7128* |
| | | (RB08,RB09) | Bruce Co., Bruce Peninsula, *Pryer 390* |
| | | (RB12,RB11) | Bruce Co., Bruce Peninsula, *Pryer 483* |
| | | (RB10) | Bruce Co., Bruce Peninsula, *Pryer 557* |
| | | (RB05)(RB03,RB04,RB01) | Frontenac Co., Palmerston Twp., *Pryer 386* |
| | | (RB13) | Manitoulin Island, Fossil Hill, *Kott s.n.* |
| | | (RB06) | Timiskaming Distr., New Liskeard, *Pryer 614* |
| | Finland | (RE01) | Kuusamo, Oulankajoki, Purkuputaanoja, *Sarvela s.n.* |
| | France | (RE03,RE02) | Isère, Grenoble, *Fraser-Jenkins 7360* |
| *G. jessoense* ssp. *jessoense* | India | (JJ03,JJ05) | Baltistan, Kargil, *Fraser-Jenkins 7463* |
| | | (JJ10,JJ02) | Jammu-Kashmir, Srinagar, *Fraser-Jenkins 7416* |
| | Japan | (JJ09,JJ08)(JJ07,JJ06) | Nagano Pref., Mt. Toyoguchi, *Mitsui s.n.* |
| | Pakistan | (JJ04,JJ01) | Kalam, Upper Swat Valley, *Fraser-Jenkins 7981* |
| *G. remote-pinnatum* | Taiwan | (RM01,RM04)(RM02,RM03) | Chiayi Co., Mt. Morrison, *Kuo 11920* |

[a]Each set of parentheses represents an individual frond; the codes within the parentheses represent different chromatograms run for that frond. These codes are used in Fig. 2.

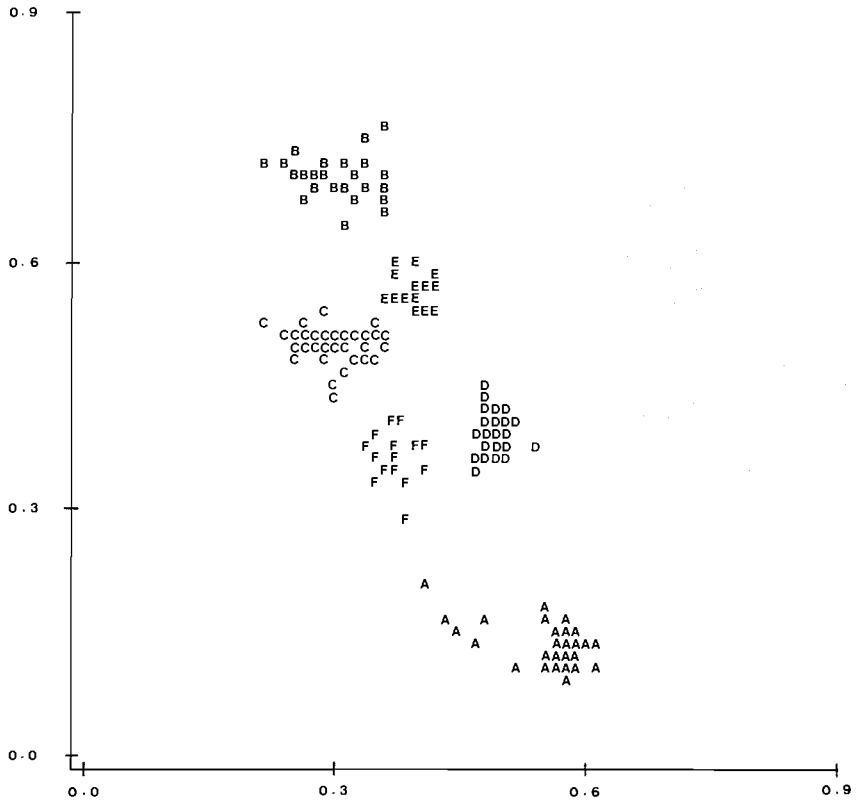


FIG. 1. Plot of the $R_f$ values of all the yellow–green spots on chromatograms of *Gymnocarpium* taxa, after adjustment for the differential mobility of each chromatogram (for explanation see text). The letters A–F represent the phenolic constituents 8–13, respectively, that were distinguished on this basis (see Table 2).

mens used in this study were glabrous, but by using morphological criteria they could not be mistaken for specimens of *G. dryopteris* ssp. *disjunctum*. From the phenolic data, it seems that the glabrous and diploid *G. jessoense* ssp. *jessoense* material from Japan has more in common with *G. dryopteris* ssp. *disjunctum* in western North America, which is also glabrous and diploid, than with the glandular plants from India and Pakistan that also go under the name *G. jessoense* ssp. *jessoense* in Sarvela's (1978) treatment.

In his survey of the genus *Gymnocarpium*, Sarvela (1978) described for the first time the taxon *G. jessoense* ssp. *parvulum* Sarvela which had previously been included in *G. robertianum sensu lato*. Pryer (1981) recognizes both of these as "good" taxa based on morphological data and their distinctiveness is supported by the cluster analysis of the phenolic data. Together they make up the larger part of the so-called glandular group; both *G. robertianum* and *G. jessoense* ssp. *parvulum* are, however, clearly demarcated within this group to form separate and distinct subgroups (Fig. 2).

North American material of the glandular interspecific hybrid *G.* × *intermedium* is distinguishable from both *G. robertianum* and *G. jessoense* ssp. *parvulum* and forms a discrete cluster of its own (Fig. 2).

European material of *G. robertianum*, as well as specimens of *G. remote-pinnatum* (Hayata) Ching from Taiwan and *G. jessoense* ssp. *jessoense* from India and Pakistan, clustered variously within the large glandular group. *Gymnocarpium remote-pinnatum*, which is said to be restricted to Taiwan (Sarvela 1978), grouped with the *G. jessoense* ssp. *jessoense* collections from India and Pakistan (Fig. 2). This was not surprising, considering the close morphological similarities that were observed between specimens of these two taxa. Indeed, from the phenolic data, it would seem that the glandular plants referable to *G. jessoense* ssp. *jessoense* have more in common with *G. remote-pinnatum* than with the presumably typical nonglandular *G. jessoense* ssp. *jessoense* plants from Japan. Although together they form a discrete cluster of their own, the *G. remote-pinnatum* and glandular *G. jessoense* ssp. *jessoense* subgroup subsequently links up with the North American representatives of *G. jessoense* ssp. *parvulum*.

The two samples "RE02" and "RE03" correspond to

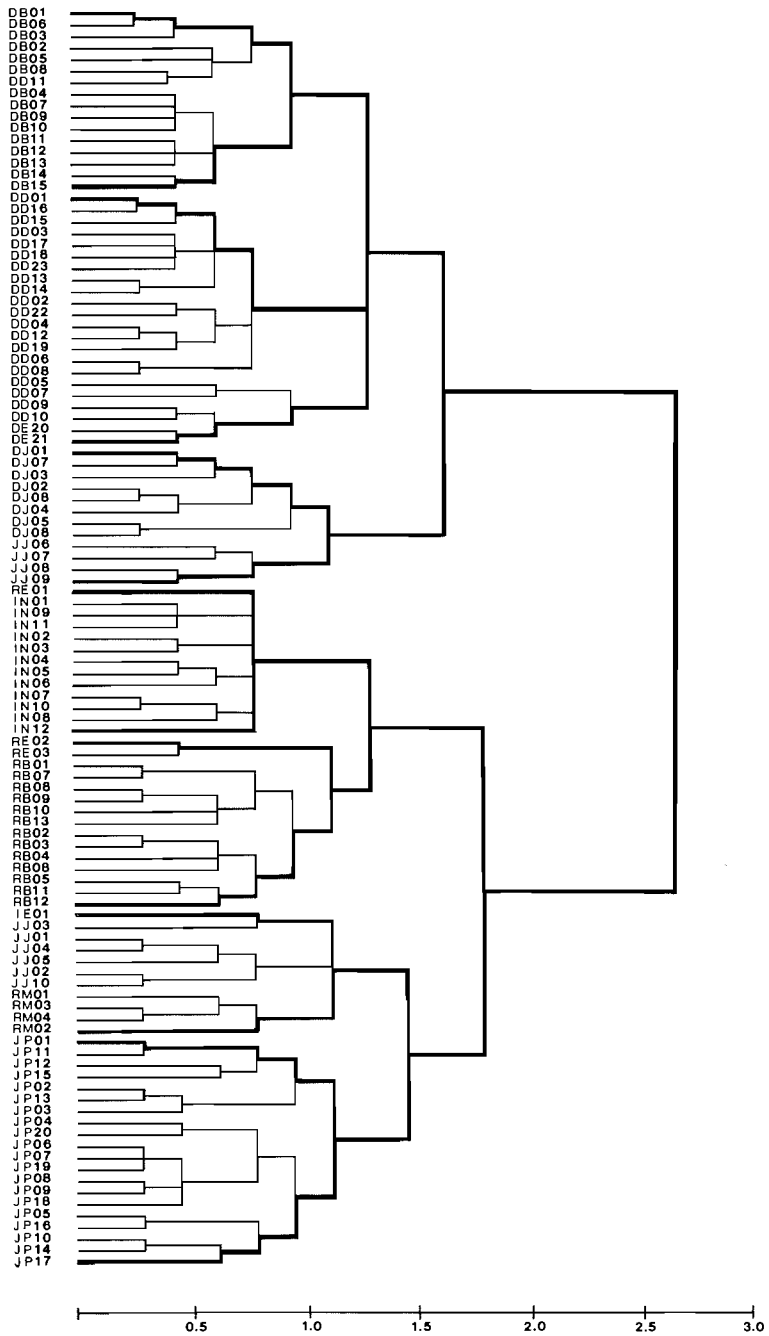


FIG. 2. Dendrogram derived by the flexible sorting method (with $\alpha = 0.625$ and $\beta = -0.25$), from the profile data of the 109 chromatograms of *Gymnocarpium* taxa (for further explanation see text). Each chromatogram is coded and listed in Table 1. The letter symbols represent the following *Gymnocarpium* taxa: DB, *G. dryopteris* ssp. × *brittonianum*; DD, *G. dryopteris* ssp. *dryopteris* (North American); DE, *G. dryopteris* ssp. *dryopteris* (European); DJ, *G. dryopteris* ssp. *disjunctum*; IE, *G.* × *intermedium* (European); IN, *G.* × *intermedium* (North American); JJ, *G. jessoense* ssp. *jessoense*; JP, *G. jessoense* ssp. *parvulum*; RB, *G. robertianum* (North American); RE, *G. robertianum* (European); and RM, *G. remote-pinnatum*.

*G. robertianum* material from France which clustered with North American representatives of *G. robertianum* (Fig. 2).

"RE01" and "IE01" represent collections from Finland identified, respectively, as *G. robertianum* and *G.* × *intermedium*. Although they do not cluster with the North American representatives of these taxa, too few European specimens were available in this analysis for firm conclusions to be drawn on their relationships.

Results very similar to those discussed here were obtained with other clustering techniques (e.g., UPGMA (Sneath and Sokal 1973)) and also with clustering of data on phenolic spot presence without reference to intensity. The major difference in the latter analysis was that two additional *G. dryopteris* ssp. *dryopteris* samples ("DD03" and "DD23") clustered with the *G. dryopteris* ssp. × *brittonianum* subgroup.

Although large clusters of related subgroups are shown in Fig. 2, these could possibly be an artifact of the clustering method. Moreover, the linear sequence of OTUs and clusters is to some extent arbitrary, and so no information is obtainable from Fig. 2 as to whether, for example, *G.* × *intermedium* (IN) might be intermediate between the glandular and nonglandular taxa. Clustering methods transform the original metric character-state matrix to a dendrogram by preserving the close relationships at the expense of possibly distorting major groupings.

Ordination methods such as principal-coordinate analysis (PCO) tend to do the opposite in that the projection onto the first few principal axes reflects the major patterns of variation at the expense of the close interpoint distances. The relationships between the *Gymnocarpium* taxa were, therefore, further explored using ordination methods. A principal-coordinate analysis was conducted using the pairwise distances as input (Gower 1966). In this case, there is a good correspondence between the clusters already discerned in the dendrogram (Fig. 2) and the pattern revealed by the principal-coordinate analysis (Fig. 3). Projection onto the first two axes allows the recognition of four major groups. There is a close association among the subspecies of the *G. dryopteris* complex, which together form a group at the far right side of the first axis. The only taxon which forms a clear-cut subgroup within this complex is *G. dryopteris* ssp. *disjunctum*. Linked with the *G. dryopteris* complex are the nonglandular samples of *G. jessoense* ssp. *jessoense* from Japan.

In Fig. 3, the principal axis (the horizontal one) is that which provides the main separation of the glandular and nonglandular taxa. On the left side, farthest from the *G. dryopteris* complex are two distinct groups, one representing *G. robertianum* and the other *G. jessoense* ssp. *parvulum*. The second axis evidently represents the variation which markedly distinguishes these two taxa from one another.

As in the cluster analysis, the subgroup that comprises *G. remote-pinnatum* from Taiwan and the representatives of the glandular *G. jessoense* ssp. *jessoense* from India and Pakistan demonstrates a close affinity to the *G. jessoense* ssp. *parvulum* group (Fig. 3).

The interspecific hybrid taxon *G.* × *intermedium* has an intermediate position on the PCO plot in Fig. 3. This suggests that its chromatographic profile is intermediate between the glandular and nonglandular elements of this genus, as might be expected from its putative parentage (*G. dryopteris* ssp. *disjunctum* × *G. jessoense* ssp. *parvulum*).

Subsequent axes did not reveal any variation associated with the groups recognized in the clustering procedures.

The distribution and occurrence of the phenolic constituents in taxa of *Gymnocarpium* are given in Table 2. Composite diagrams of the chromatographic profiles of each North American taxon studied are shown in Fig. 4.

The diploid taxon *G. dryopteris* ssp. *disjunctum* as well as all three tetraploid taxa, *G. dryopteris* ssp. *dryopteris*, *G. jessoense* ssp. *parvulum*, and *G. robertianum*, show distinct chromatographic profiles (Fig. 4). The constancy with which these profiles was obtained was striking, considering that the material was selected so as to include different fronds from one clone, fronds from separate clones in one geographical area, individuals of the same taxon from different geographical areas, herbarium and fresh material, and fronds at different stages of maturity.

The two subspecies of *G. dryopteris* showed very similar patterns (Figs. 4B, 4C), the diploid *G. dryopteris* ssp. *disjunctum* lacking, however, spots 8, 9, 12, and 13 common to the tetraploid *G. dryopteris* ssp. *dryopteris* and notably lacking spot 14 which is common to all other North American species and hybrids of *Gymnocarpium* (Table 2). The intersubspecific hybrid *G. dryopteris* ssp. × *brittonianum* has a profile most similar to that of *G. dryopteris* ssp. *dryopteris* and indeed is almost identical with it, but for spots 4 and 12. By using morphological criteria, these two taxa can be very difficult to separate (Pryer 1981). As demonstrated by the cluster analysis, ordination, and phenolic profiles, the taxa that make up the *G. dryopteris* complex have a very close affinity one to another.

The chromatographic profiles of the glandular taxa, *G. jessoense* ssp. *parvulum* and *G. robertianum*, may, at first, appear somewhat similar, but *G. robertianum* can always be readily distinguished from *G. jessoense* ssp. *parvulum* by the presence of spots 5, 6, 7, and 15 and the absence of spot 17 (Figs. 4A, 4D). This is

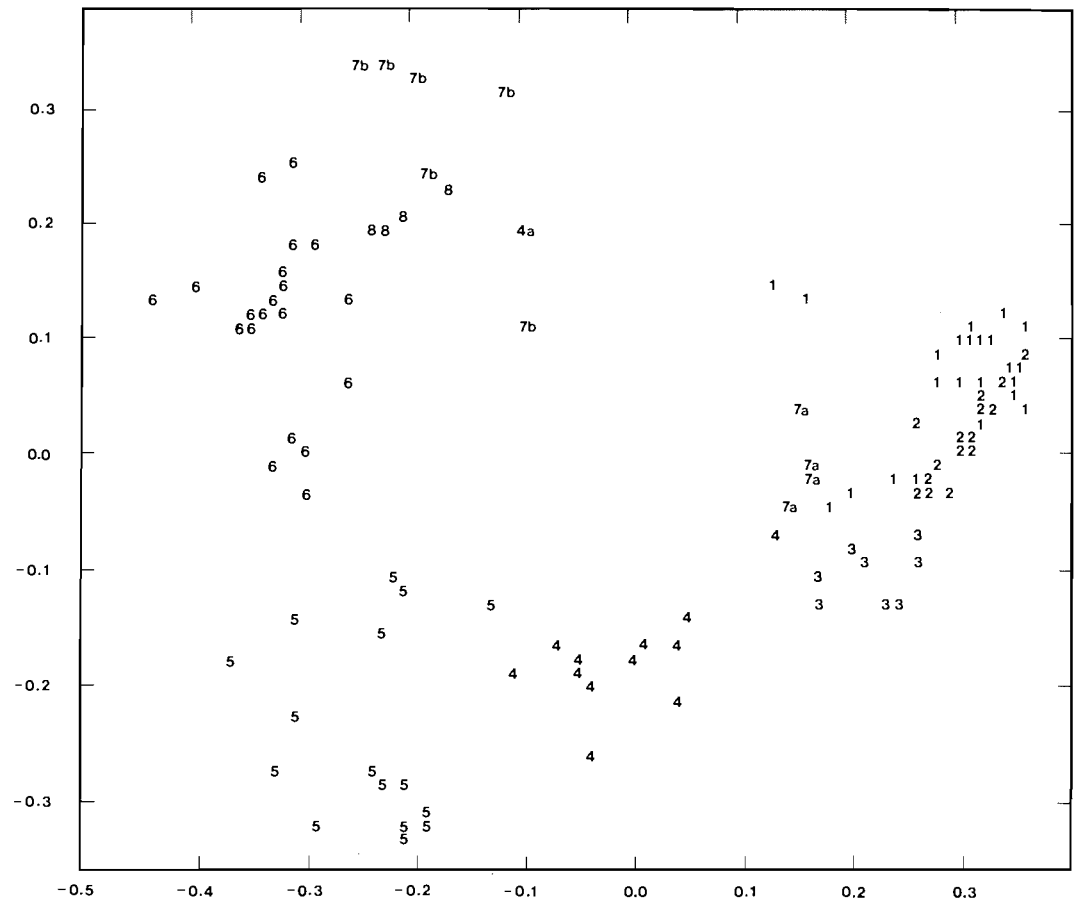


FIG. 3. Projection of the phenolic data of the 109 *Gymnocarpium* chromatograms onto principal coordinate axes; the horizontal axis represents the first principal axis and the vertical the second. The number symbols represent the following *Gymnocarpium* taxa: *1*, *G. dryopteris* ssp. *dryopteris*; *2*, *G. dryopteris* ssp. × *brittonianum*; *3*, *G. dryopteris* ssp. *disjunctum*; *4*, *G.* × *intermedium*; *4a*, *G.* × *intermedium* (Finland); *5*, *G. robertianum*; *6*, *G. jessoense* ssp. *parvulum*; *7a*, *G. jessoense* ssp. *jessoense* (Japan); *7b*, *G. jessoense* ssp. *jessoense* (India and Pakistan); and *8*, *G. remote-pinnatum*.

reflected in their separation in the cluster analysis and PCO ordination and agrees well with their morphological distinctiveness (Pryer 1981; Sarvela 1978).

The interspecific hybrid *G.* × *intermedium* clearly shows an additive profile of the phenolic constituents of its two putative parental taxa, *G. jessoense* ssp. *parvulum* (4*x*) and *G. dryopteris* ssp. *disjunctum* (2*x*) (Fig. 4E).

Although no chemical identifications were carried out, it is still possible to assign tentatively some of the compounds to broad phenolic groups, based upon $R_f$ values and color reactions (Ribéreau-Gayon 1972). The UV-invisible spots that "fume" blue with $NH_3$ vapor (spots 1–7) as well as the UV-visible blue–green spots (spots 20–24) are most likely phenolic acids (Harborne 1973). The UV purple spots that "fume" green (spots 14 and 15) as well as those that "fume" to yellow–green (spots 8–13) are undoubtedly flavonoids (Mabry et al. 1970).

From this study of the chromatographic profile data, and in particular from the numerical analyses, the following conclusions can be drawn. (*i*) Morphologically distinguishable North American taxa of *Gymnocarpium* can be identified by their chromatographic profiles. (*ii*) The chromatographic profiles of *G. jessoense* ssp. *parvulum* and *G. robertianum* are clearly different and distinguishable from one another. This provides supportive evidence for their recognition as two separate taxa. (*iii*) The chromatographic profile of the hybrid taxon *G.* × *intermedium* is a virtual summation of its putative parental profiles (*G. dryopteris* ssp. *disjunctum* × *G. jessoense* ssp. *parvulum*). (*iv*) The three subspecies that comprise *G. dryopteris* are very similar morphologically (Pryer 1981), and their chromato-

TABLE 2. Phenolic constituents of *Gymnocarpium* taxa [a]

| Phenolic constituents | $R_f$ (×100) | | Color | | Taxa [b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BAW | $HCO_2H$ | UV | $NH_3$ | DD | DB | DJ | IN | JP | RB |
| 1 | 33 | 80 | I | B | | | | ● | ● | ● |
| 2 | 30 | 59 | I | B | | | | ● | ● | ● |
| 3 | 52 | 79 | I | B | | | | | ○ | ◐ |
| 4 | 50 | 63 | I | B | | ◐ | | | ○ | ● |
| 5 | 75 | 81 | I | B | ● | ● | ● | ● | | ● |
| 6 | 75 | 67 | I | B | ● | ● | ● | ● | | ● |
| 7 | 82 | 22 | I | B | ○ | ○ | ● | ○ | | ◐ |
| 8 | 57 | 13 | P | YG | ● | ● | | ◐ | ◐ | ○ |
| 9 | 31 | 70 | P | YG | ● | ◐ | | | | |
| 10 | 31 | 50 | P | YG | ● | ● | ● | ● | | |
| 11 | 50 | 40 | P | YG | ● | ● | ◐ | | | |
| 12 | 41 | 57 | I | YG | ● | | | | | |
| 13 | 36 | 35 | I | YG | ○ | ● | | | | |
| 14 | 70 | 18 | P | G | ● | ● | ▲ | ● | ● | ● |
| 15 | 46 | 41 | P | G | | | | | | ● |
| 16 | 24 | 44 | SB | SB | | | | ● | ● | ● |
| 17 | 27 | 06 | O | Y | ● | ● | ● | ● | ◐ | |
| 18 | 41 | 17 | I | Y | ● | ● | ● | ● | | |
| 19 | 49 | 02 | YP | YP | ● | ● | ◐ | ● | ○ | ● |
| 20 | 59 | 56 | SB | BG | ● | ● | ● | ● | ○ | ◐ |
| 21 | 59 | 75 | I | BG | ◐ | ● | ● | ● | | |
| 22 | 74 | 45 | I | BG | ◐ | ● | ◐ | ● | | |
| 23 | 27 | 89 | BG | BG | ◐ | ● | ● | | | |
| 24 | 24 | 72 | SB | BG | | | | ◐ | ● | ● |
| 25 | 31 | 02 | Pk | Pk | ◐ | ◐ | ● | | | |

*a* Key to table: B=blue; BG=blue-green; G=green; I=invisible; O=orange; P=purple; Pk=pink; SB=sky-blue; Y=yellow; YG=yellow-green; YP=yellow-purple.
●=present in 70-100% of chromatograms; ◐=present in 40-69% of chromatograms; ○=present in less than 40% of chromatograms.

*b* For taxa symbols, see Table 1 and Fig. 2.

▲ Occurred on a single chromatogram.

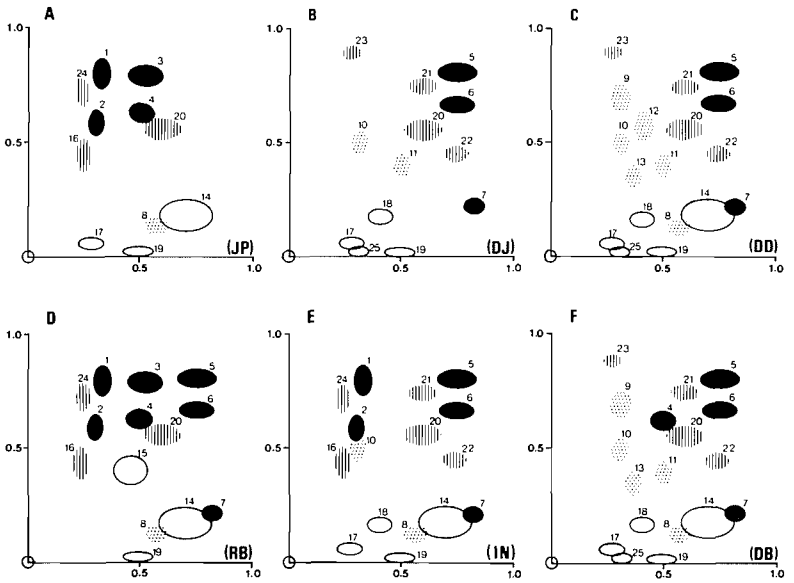


FIG. 4. Composite chromatograms of *Gymnocarpium* taxa. (A) *G. jessoense* ssp. *parvulum*; (B) *G. dryopteris* ssp. *disjunctum*; (C) *G. dryopteris* ssp. *dryopteris*; (D) *G. robertianum*; (E) *G.* × *intermedium*; (F) *G. dryopteris* ssp. × *brittonianum*. Spot numbers same as in Table 2. Shading is based on differences in color responses on the chromatograms; black represents spots that "fume" blue, vertical lines represent those that "fume" blue–green or sky blue, and dot marking represents those that "fume" yellow–green. Other colors are represented by unshaded spots.

graphic profiles support a close affinity of the subspecies. (*v*) Asiatic *G. jessoense* ssp. *jessoense* as circumscribed by Sarvela (1978) possesses heterogeneous chromatographic profiles, and it would appear that plants from India and Pakistan are closer to *G. remotepinnatum* from Taiwan than to *G. jessoense* ssp. *jessoense* from Japan.

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