

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* ssp. *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 μ L 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

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

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 $D = (1 - S^2)^{1/2}$.

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

