AN ABSTRACT OF THE THESIS OF

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Kenton L. Chambers

A biosystematic investigation of Cryptogramma section Cryptogramma in western North America has been directed towards resolution of two separate taxonomic questions.

Results of this research support the hypothesis that a previously undescribed diploid (2n = 30 II) taxon, C. cascadensis, is present along with the common and widespread C. acrostichoides. The new species is distinguished by a number of subtle but constant morphological features, including sterile fronds that are soft and deciduous (compared to the coriaceous, evergreen sterile leaves of C. acrostichoides); surficial rather than sunken hydathodes; absence of laminar trichomes, and significantly smaller spores. Data from enzyme electrophoresis confirm the separation of C. cascadensis as a distinct species, as 6 of 13 loci scored were "marker loci", with no alleles held in common between the two species. While different populations of the same species were genetically similar, with genetic identities > 0.85, the mean genetic identity for interspecific population comparisons was 0.36, demonstrating a substantial amount of genetic divergence between the two species. Both species were found to be primarily outcrossing, and the existence of marker loci and a high

degree of allozymic differentiation, even in mixed populations, suggests that the two species are reproductively isolated.

Also investigated was the taxonomy and evolutionary origin of Cryptogramma sitchensis, a taxon previously treated as a variety of C. acrostichoides. Cryptogramma sitchensis was found to be a tetraploid species, with 2n = 60 II, the first report of polyploidy in the genus from North America. Data from enzyme electrophoresis showed fixed heterozygosity, a characteristic of allopolyploid species. Allozyme banding patterns showed that C. sitchensis combines genomes of C. acrostichoides and another distinct species not present in North America. Morphological characters, particularly the dissection of sterile leaves, suggested that this second parent is the eastern Asian C. raddeana. Frequent triploid hybrids, which can be identified by their abortive spores, occur where the ranges of C. sitchensis and C. acrostichoides overlap. Hybrids blur the morphological distinctions between the two species, and are probably responsible for leading earlier workers to conclude that the taxa are only varietally distinct.

These results have implications for systematic treatment of other taxa in section Cryptogramma, suggesting that the best taxonomic approach may be to treat geographic segregates as distinct species, rather than as varieties or subspecies of a single circumboreal species, C. crispa, as has been proposed by some workers.

# Biosystematics of Parsley-ferns, Cryptogramma R. Br., in Western North America

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Edward R. Alverson

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## Chapter I

## General Introduction

*Modern Methods in Fern Systematics.* 1950 was a watershed year in the field of fern systematics. This year brought the publication of Irene Manton's classic book, *Problems of Cytology and Evolution in the Pteridophyta*, which described a new approach to the analysis of confusing species complexes in ferns. Working mainly with the European flora, she showed that many groups of closely related ferns were actually polyploid complexes, in which distinct diploid species had come together through hybridization to form fertile allopolyploid species, intermediate in their morphology between the two diploid species. Without cytological data, such complexes appeared to exhibit a continuous set of variation from one extreme to the other. Chromosome counts revealed patterns of reticulate evolution in which the diploid species were, without a doubt, distinct elements.

In the following years, these techniques were applied to many groups of North American ferns, with equally satisfying results. Patterns of reticulate evolution were uncovered in genera such as *Asplenium* (W.H. Wagner 1954), *Dryopteris* (W.H. Wagner 1971), *Polypodium* (Lang 1971), and *Polystichum* (W.H. Wagner 1973; D.H. Wagner 1979).

1983 marked a second watershed in the field of fern systematics. With the publication of an article in the American Fern Journal, D.

Soltis, C. Haufler, D. Darrow, and G. Gastony brought enzyme electrophoresis, and the study of allozyme variation, to the hands of fern systematists. Earlier attempts to apply electrophoretic techniques to ferns were unsuccessful due to the presence of large amounts of phenolic compounds in fern leaf tissue, which tended to denature enzymes when cells were broken open, resulting in a lack of enzyme activity. The protocol and schedules reported by Soltis et al. (1983) are now widely used in biosystematic research of ferns, as well as many groups of flowering plants.

Allozyme evidence from enzyme electrophoresis is of great value to fern systematists for two reasons. First, it provides a phenotypic character (banding patterns) that is not subject to environmental modification. Ferns have always provided a challenge to systematists because they lack many characters (such as flowers and fruits) commonly used to classify flowering plants, and because many of the remaining characters (such as leaf outline and dissection) are often subject to great environmental modification. Patterns of enzyme bands can be translated into statistics that provide a relatively clearcut means of assessing the closeness of relationship between taxa. Second, banding patterns can be used to identify hybrids and reticulate species. While hybrids are generally morphologically intermediate between parental species, interpretation of hybrids can be ambiguous. With allozymes, hybrids exhibit additive banding patterns, allowing unambiguous determination of parentage.

In recent years, electrophoretic techniques have been applied to a number of genera and complexes that had not been satisfactorily resolved by use of traditional biosystematic techniques. These

include examples of "cryptic speciation", such as the Adiantum pedatum complex (Paris and Windham 1988) and Gymnocarpium dryopteris complex (Pryer and Windham 1988); elucidation of allopolyploidy in Polypodium virginianum (Bryan and Soltis 1987); and studies of autopolyploid evolution in the Pellaea glabella/occidentalis complex (Gastony 1988).

While electrophoretic techniques are very useful to fern systematists, a broad approach that incorporates a variety of types of evidence is best. Morphological evidence is indispensable, because a classification that bears no relevance to morphology has little practical utility. Very often electrophoretic data are correlated with subtle morphological distinctions, and provide an independent line of evidence in support of morphological separation. As a result, researchers are now uncovering many examples of "cryptic speciation" in ferns, in which taxa that are allozymically quite divergent have differentiated morphologically only very slightly (M. Windham, pers. comm.). Thus, morphological differences do exist and must be demonstrated in separating related species, but the degree of evolutionary relationship is not inherently reflected in the degree of morphological difference.

Overview of the Genus Cryptogramma. Cryptogramma is an example of a genus for which the combination of traditional and modern biosystematic techniques could provide a much improved understanding of taxonomic and evolutionary relationships within the genus. It has never been the subject of a thorough monograph, and most of the scant literature is found in local floristic treatments or higher-level taxonomic works.

Ferns of the genus Cryptogramma are characterized by dimorphic sterile and fertile leaves in which the segments of the fertile leaves are pod-like, with underrolled margins protecting the developing sporangia. The sterile vegetative fronds are usually highly dissected, thus explaining the common name, parsley-fern. Other features common to all species in the genus are trilete, prominently verrucate spores, and a base chromosome number of n = 30. The genus is a member of the family Pteridaceae, tribe Cheilantheae of Tryon and Tryon (1982), and is well defined within this group. The plants tend to occur in moist, rocky habitats in mountainous regions (Page 1983, Taylor 1970).

The ten taxa of Cryptogramma that have been described belong to two distinctive sections. The single representative of section Homopteris (Rupr.) C. Chr. is Cryptogramma stelleri, a delicate calciphile with slender creeping rhizomes and ephemeral, membranous leaves. It is widely distributed in the boreal zones of Asia and North America. Because it does not appear to be the subject of any taxonomic controversy, it is not considered further in this thesis.

Section Cryptogramma comprises the remainder of the taxa, including the type species of the genus, C. acrostichoides R. Br., from North America and eastern Asia. Species of this section are characterized by multicipital, decumbent to erect rhizomes producing tufts of firm, often evergreen leaves. In contrast to C. stelleri, species of section Cryptogramma are often calcifuges. Other taxa in this section include C. crispa (L.) R. Brown, widespread in Europe and Asia Minor; C. sitchensis (Rupr.) Moore, from Alaska and adjacent Canada; C. raddeana Fomin from northeast Asia; C. brunoniana Hook. &

Grev. from the Himalayas, C. shensiensis Ching from China, and C. crispa var. japonica Miyabe & Kudo from the mountains of Japan. C. fumariifolia (Phil.) Christ, from the southern Andes, is the lone southern hemisphere representative of the genus. Species of this section have been subject to a variety of taxonomic treatments, and are often treated as subspecies or varieties of a single circumboreal species under the specific epithet of C. crispa.

Thus, two divergent approaches have been applied to the taxonomy of the C. crispa complex. The traditional approach (see Abrams 1940) has been to treat taxa from different geographic regions as distinct species. In recent years this approach has been bolstered by chromosome evidence. Chromosome counts of C. acrostichoides from Washington (Wagner in Fabbri 1963); British Columbia (Taylor and Lang 1963; Taylor and Mulligan 1968); Alberta (Löve 1976); and Colorado (Löve et al. 1971) have all been diploid, with n=30 chromosomes. This contrasts with counts from the European C. crispa sens. str., which is tetraploid, with n=60 chromosomes (Manton 1950). Recent floristic works of North American pteridophytes (Mickel 1979; Lellinger 1985) have followed this traditional taxonomic concept.

On the other hand, many recent regional floras have followed the lead of Hooker (1858) and Fernald (1935) by treating the North American plants as a variety or subspecies of C. crispa. Such works include Hultén (1968); Hitchcock et al. (1969); Cronquist et al. (1972), and Scoggan (1978). This perspective is also espoused by Tryon and Tryon (1982), who consider the complex to consist of a single circumboreal species, with geographic races in Asia, North America, and South America designated as varieties.

Perusal of the literature suggests that authors have chosen their approach to treatment of Cryptogramma according to personal taxonomic biases: those with a more liberal species concept have followed the traditional view, while more conservative workers have followed the more inclusive concept. These treatments have primarily been based upon examination of herbarium specimens. No comprehensive field studies have been conducted (or at least published) to date on any members of the complex, nor have modern biosystematic techniques been applied.

Rationale for this Study. One way to further our knowledge of the C. crispa complex is to narrow the focus of the study to a particular geographic region and a subset of taxa within the C. crispa complex. Information obtained about the characteristics of the complex in a particular region could then be applied to other regions, as working hypotheses to plan the direction of future biosystematic studies.

Western North America is a suitable geographic region in which to conduct this kind of study. First, in addition to the common and widespread C. acrostichoides, I have identified a new species that occurs in subalpine and alpine habitats of the Cascades, Sierra Nevada, and northern Rocky Mountains (Alverson 1989). These plants in fact possess certain features that are more like those of the European C. crispa than the American C. acrostichoides. Second, in Alaska and northwestern Canada there is a taxon that goes by the name of C. acrostichoides var. sitchensis, which appears to be allied to C. acrostichoides, but diverges from that taxon in the direction of C. raddeana (Hultén 1968). Thus, if a regionally based study of this

complex could elucidate the relationships between these differentiated entities, the same systematic approach could be used as a working hypothesis for future researchers to apply to other members of the complex.

This research project has utilized a variety of biosystematic techniques, incorporating morphological, cytological, electrophoretic, ecological, and geographic evidence, in order to test several hypotheses regarding the systematic and evolutionary relationships of Cryptogramma in western North America. One hypothesis is that the C. crispa complex in North America is represented by two divergent evolutionary lineages, one containing C. acrostichoides and C. sitchensis, and the other represented by the new species, C. cascadensis. In addition, the C. acrostichoides lineage has diversified further, producing distinctive geographical forms, of which C. sitchensis is an allopolyploid product of past hybridization between C. acrostichoides and its Asian relative, C. raddeana. This thesis describes the results of this research, and presents a revised taxonomic treatment based upon these results.

Chapter II

## Patterns of Divergence in North American Parsley-Ferns: Evidence for a new species, *Cryptogramma cascadensis*

### ABSTRACT

Biosystematic studies of *Cryptogramma* section *Cryptogramma* in western North America demonstrate that a previously undescribed diploid taxon, *C. cascadensis*, is present along with the common and widespread *C. acrostichoides*. The new species is distinguished by a number of subtle but constant morphological features, including sterile fronds that are soft and deciduous (compared to the coriaceous, evergreen sterile fronds of *C. acrostichoides*); surficial rather than sunken hydathodes; absence of laminar trichomes, and significantly smaller spores. Data from enzyme electrophoresis confirms the separation of *C. cascadensis* as a distinct species, as 6 of 13 loci scored were marker loci, with no alleles held in common between the two species. While different populations of the same species were genetically similar, with genetic identities > 0.85, the mean genetic identity for interspecific population comparisons was 0.36, demonstrating a substantial amount of genetic divergence between the two species. Both species were found to be primarily outcrossing, and the existence of marker loci and a high degree of isozymic differentiation, even in mixed populations, suggests that the two species are reproductively isolated. These results have implications for systematic treatment of other taxa in section *Cryptogramma*, suggesting that the best approach may be to treat geographic

segregates as distinct species, rather than as varieties or subspecies of a single circumboreal species, C. crispa, as has been proposed by some workers.

# INTRODUCTION

Cryptogramma acrostichoides R. Br. was the first species of parsley-fern (Cryptogramma sect. Cryptogramma) to be described from North America. The name was originally published in 1823 based upon specimens collected by Richardson in northwest Canada (Richardson 1823). In 1858, however, W.J. Hooker reduced C. acrostichoides to a form of C. crispa (Hooker 1858). Subsequent taxonomic treatments, reviewed in part by Fernald (1935), show a lack of agreement as to whether the features used to distinguish the two taxa are sufficient to warrant specific status for C. acrostichoides. While some modern pteridologists, such as Lellinger (1985) agree that C. acrostichoides is specifically distinct, others have treated this taxon as a variety of C. crispa (Tryon and Tryon 1982).

The difficulty in reaching a taxonomic consensus regarding the American parsley-fern may largely be due to its propensity for morphological variation, particularly in vegetative characters. Such variation occurs at several levels: within a single clump or clone, within a single population, or between individuals of different populations. Most workers that have passed taxonomic judgement on Cryptogramma have based their taxonomic judgements on herbarium specimens, where intraclonal or intraspecific variation can readily be discerned, but not intrapopulational variation. Thus it is difficult with herbarium material to determine which components of the overall variation are constant within populations, and thus are likely to be genetically fixed, and which characters vary within populations, and thus likely represent or phenotypic variation due to allelic

variation, developmental patterns, or environmental influences. It is only by determining which characters are genetically fixed within a taxon that a satisfactory taxonomic treatment can be constructed.

Without the benefit of field experience, earlier workers often emphasized the similarities of certain sterile fronds of different Cryptogramma taxa, in support of the hypothesis that the taxa are conspecific (Fernald 1935). Such similarities do not inherently imply that the taxa are conspecific. On the other hand, these workers do not appear to have considered the possibility that the difficulty of drawing species' boundaries in Cryptogramma was due to the presence of additional taxa that had not been separated from the known taxa.

Field explorations in the Washington Cascades in August of 1981 first suggested to me that the Cryptogramma story does include more taxa than earlier workers had believed. In several widely scattered subalpine localities I encountered populations of a Cryptogramma that in certain essential features appeared very distinct from typical C. acrostichoides. These observations led me to hypothesize that a second species of parsley-fern was present in western North America; a species harboring a differing set of morphological characteristics and ecological preferences from those of C. acrostichoides. This hypothesis was developed by searching for morphological characters that were constant within a population, or within a set of putatively conspecific populations, and separating these useful characters from taxonomically insignificant variation brought about by environmental or developmental factors.

In this chapter, morphological, electrophoretic, and ecological evidence is presented to test the hypothesis that two divergent

evolutionary lineages are present in what has previously been treated as a single taxon, C. acrostichoides. The morphological evidence is evaluated for constancy from plant to plant and population to population, rather than the overall magnitude of the differences. Electrophoretic data, while also based upon phenotypic characters (enzyme bands), are of particular value, for they provide an estimate of the degree of genetic relatedness between the two taxa that is directly related to differences in the sequences of nucleotides on the DNA molecules. Such data can also provide evidence for reproductive isolation between the taxa. The new species has been designated Cryptogramma cascadensis (Alverson 1989), in reference to the Cascade mountain range, which occupies the center of the new species' geographic distribution.

Not only is the elucidation of this taxon noteworthy in and of itself, but this study also provides an opportunity for reappraisal of the old question of the relationship between C. acrostichoides (now redefined) and its European counterpart, C. crispa.

## MATERIALS AND METHODS

**Morphological Analysis:** Herbarium specimens examined during this study were collected by the author or borrowed from the following herbaria: ALA, ARIZ, ASC, ASU, BRY, CAN, CAS, COLO, CS, DAO, GH, ID, JEPS, MICH, MONT, MONTU, NY, ORE, OSC, POM, RM, RSA, SD, UBC, UC, US, UT, UTC, V, WS, and WTU. During field work in October 1986 and August 1987, samples of living plants were obtained from each population

visited; these plants were maintained in cultivation in a cool greenhouse in the summer, and in a covered cold frame in the winter.

**Chromosomes:** Fertile fronds were collected from wild populations or plants of wild origin cultivated in the greenhouse, fixed in Farmer's formula (a 3:1 solution of 95% ethanol and glacial acetic acid), and stored in a freezer. Chromosome squashes were stained with acetocarmine, and Hoyer's solution was used to make the preparations permanent (Beeks 1955). Slides were examined and photographed under phase-contrast and photographed with Kodak Technical Pan 2415 film.

**Spore Measurements:** Spores were mounted on glass slides in Hoyer's medium. Twenty five spores from each slide were measured along their longest dimension with an ocular micrometer at 640 X. The computer package SAS was used to perform an analysis of variance (ANOVA), and the GT-2 multiple comparison test (Sokal and Rohlf 1981), in order to test for significant differences between mean spore diameters of C. acrostichoides, C. cascadensis, and C. crispa.

**Electrophoresis:** Nine populations of C. acrostichoides and 4 populations of C. cascadensis from widespread localities in western North America (Table 1) were sampled electrophoretically. Where possible, 20 to 25 sporophytes per population were sampled in order to assess intra-populational variability. Starch gel electrophoresis was conducted following the protocol of Soltis et al. (1983). Fronds were placed in plastic bags in the field, and were kept on ice until analyzed. Newly expanding fiddleheads were used when available, but the majority of samples were run with mature fronds. Small pieces of frond tissue were ground in the phosphate grinding buffer of Soltis et al. (1983) and the grindate transferred to small rectangular wicks of

Table 1. Sampling localities for electrophoretic study.

**AK 1:** Alaska, Baranof Island, N of Ptarmigan Peak, 3 km W of Port Walter, elevation 900 m, T63S R68E S22; K. La Bounty s.n., August 1987. (C. acrostichoides)

**AK 2:** Alaska, Baranof Island, Harbor Mtn., 5 km N of Sitka, elevation 500 m, T55S R62E S15; M. Muller s.n., August 1987. (C. acrostichoides)

**CA 1:** California, Eldorado Co., at outlet of Heather Lake, Desolation Wilderness, elevation 2400 m, T12N R17E S19; E.R. Alverson 1289, 26 August 1987. (C. acrostichoides, C. cascadensis)

**CA 2:** California, Siskiyou Co., S side of Mt. Shasta, rocky slopes at timberline above end of road at old ski area, elevation 2450 m, T41N R3W S28; E.R. Alverson 1287, 25 August 1987. (C. acrostichoides)

**CO 1:** Colorado, Gunnison Co., 1.5 km SE of Schofield Pass, 8 km NW of Gothic, elevation 3170 m, T12S R86W; E.R. Alverson 1300, 30 August 1987. (C. acrostichoides)

**NV 1:** Nevada, Elko Co., Ruby Mountains, above Island Lake, elevation 3050 m, T32N R58W S36; E.R. Alverson 1296, 28 August 1987. (C. acrostichoides)

**OR 1:** Oregon, Lane Co., 13 km E of McKenzie Bridge, elevation 650 m, T16S R7E S19; E.R. Alverson 1275, 20 August 1987. (C. acrostichoides)

**OR 2:** Oregon, Deschutes Co., McKenzie Pass Lava Fields ca. 0.5 km SSE of Dee Wright Observatory, elevation 1650 m, T15S R8E S20; E.R. Alverson 1274, 20 August 1987. (C. cascadensis).

**UT 1:** Utah, Salt Lake Co., Secret Lake, 3 km S of Alta, elevation 3020 m, T3S R3E; E.R. Alverson 1297, 29 August 1987. (C. acrostichoides)

**WA 1:** Washington, King Co., Snow Lake Trail, 5 km NW of Snoqualmie Pass, elevation 1100 m, T23N R11E S30; E.R. Alverson 1279, 22 August 1987. (C. acrostichoides, C. cascadensis)

**WA 2:** Washington, Grays Harbor Co., Gibson Slide, Mt. Colonel Bob Wilderness, elevation 820 m, T23N R8W S18; E.R. Alverson 1042, 11 October 1986. (C. acrostichoides)

Whatman filter paper. Wicks were inserted into a vertical slit in a 12% starch gel and subjected to horizontal electrophoresis at 4 C. In order to resolve the slower phosphoglucoisomerase (PGI) locus, a gel buffer pH of 8.8 was required to obtain anodal migration; otherwise all gel buffers were prepared at a pH of 8.0.

PGI, phosphoglucomutase (PGM), and glutamate oxaloacetate transaminase (GOT, also known as aspartate aminotransferase) were run on System 6 of Soltis et al. (1983); triosephosphate isomerase (TPI), leucine aminopeptidase (LAP), and hexokinase (HK) were run on System 8 of Haufler (1985), and shikimate dehydrogenase (SKDH), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGDH), and malate dehydrogenase (MDH) were run on System 11 of Haufler (1985) SKDH, IDH, 6-PGDH, and MDH were also resolved on the Morpholine system of Werth (1985).

IDH, MDH, PGI, PGM, 6-PGDH, SKDH, and TPI were stained in the dark with a 1% agarose solution, and liquid staining solutions were used for the remaining enzymes. All staining schedules followed Soltis et al. (1983). Gels were scored when fresh. Lanes on a given gel were numbered sequentially from left to right. When more than one locus was resolved, loci were numbered sequentially with the fastest (most anodally migrating) isozyme 1. Allozymes of a single locus were labeled alphabetically, with the fastest allele labeled a, the second fastest b, and so on. All gels were photographed using Kodak Technical Pan 2415 film. Statistical analyses of the electrophoretic data was performed by the LYNSPROG program, developed by Andrew Schnabel (Schnabel, unpubl.)

# RESULTS

**Morphological Analysis:** Table 2 summarizes the morphological features by which the new species C. cascadensis can be distinguished from C. acrostichoides and C. crispa. Though it shares many individual characters in common with both of the other species, the overall combination of features present in C. cascadensis is unlike either recognized species.

Seasonal Development - The sterile fronds of C. cascadensis are fully deciduous in the autumn, while those of C. acrostichoides are at least wintergreen, if not fully evergreen. The differences in this trait were retained after 2 years of cultivation in a common garden setting, demonstrating that it has a genetic basis. C. crispa is similar in this regard to C. cascadensis, as its fronds are reported to die down in autumn after the first frosts (Page 1982). Once withered, the fronds of C. cascadensis tend to decay or detach from the rhizome, leaving little accumulation of dead fronds around the base of the plant. The fronds of C. acrostichoides are strongly marcescent (persistent after turning brown and withering), often resulting in a substantial accumulation of old fronds at the base of the plant. Though diagnostic for C. acrostichoides, these old fronds are often removed by collectors in an effort to "tidy up" specimens, so this character may not always be evident in the herbarium. Though deciduous, the fronds of C. crispa do not decay rapidly as in C. cascadensis, but persist in the form of thick mats of dead fronds (Page 1982).

Table 2. Differing features of C. acrostichoides, C. cascadensis and C. crispa. Field characters of C. crispa obtained from Page (1982). An asterisk * denotes traits for which substantial intraspecific variability exists.

| | C. acrostichoides | C. cascadensis | C. crispa |
|---|---|---|---|
| Longevity of sterile fronds | wintergreen to evergreen | deciduous in autumn | deciduous in autumn |
| Detachment of sterile fronds | marcescent, dead fronds accumulating | rapidly decaying or detaching from rhizome | marcescent, accumulating mats of dead fronds |
| Texture of sterile fronds | coriaceous, thick; opaque when dried | soft, thin; translucent when dried | soft, often thin and translucent |
| *Branching of rhizome | multicipital but not strongly so | often strongly multicipital | strongly multicipital, "patches up to 1 m dia." |
| *Degree of dissection of sterile fronds | bipinnate to tripinnate | bipinnate to tripinnate | tripinnate-pinnatifid |
| *Outline of sterile blade | typically ovate-lanceolate | typically deltiod | strongly deltoid |
| *Shape of sterile segments | oblong to ovate-lanceolate | oblong to flabellate | oblong to flabellate |
| Teeth of sterile segments | crenate to dentate on apical 2/3 to 1/2 | regularly dentate on apical 1/2 to 1/3 | crenate to deeply incised w/4-8 oblong to oblanceolate lobes |
| Trichomes on frond surface | present | absent | absent |
| Characteristic color of mature sterile fronds | darker, verdigris green | grass-green | "vivid, bright light-green" |
| Color of abaxial blade surface | lighter than adaxial surface | same as adaxial surface | same as adaxial surface |
| Hydathode position | sunken | surficial | surficial |
| *Hydathode shape | ovate to linear, typically spathulate | clavate to linear, generally elongate | typically clavate and greatly elongate |
| *Positioning of fertile segments | horizontally spreading to ascending | ascending to erect | spreading to erect |
| *Length of fertile segments | 6-14 mm | 4-10 (12) mm | 1-7 (10) mm |
| Petiole width on dried fronds | up to 2 mm (not collapsed) | 1 mm or less (collapsed) | 1.0 to 1.5 mm (collapsed) |
| Scales of rhizome and petiole | bicolorous | bicolorous | concolorous, light brown |
| Mean spore diameter | 54.6 µm | 49.6 µm | 53.6 µm |
| Chromosome number | 2n = 30 pairs | 2n = 30 pairs | 2n = 60 pairs |

Texture and Color - The sterile fronds of C. cascadensis are soft, with a somewhat waxy feel when living, and when pressed and dried become thin and more or less translucent. The sterile fronds of C. acrostichoides (at least at maturity) have a coriaceous texture, and are thick and opaque, even upon drying. The characteristic color of C. cascadensis is a bright grass green, while mature fronds of C. acrostichoides are a darker verdigris green color. Written descriptions suggest that for both of these traits, C. crispa most resembles C. cascadensis (Page 1982). The abaxial surfaces of the sterile fronds of C. acrostichoides are lighter in color than the adaxial surfaces, in contrast to the uniformly colored surfaces of both C. cascadensis and C. crispa. The petioles of C. acrostichoides, particularly those of the fertile fronds, are rigid and straw-like, even when dry, and are up to 2 mm wide. Petioles of C. cascadensis are less firm, and upon drying, collapse inward, so that with dried petioles the diameter is 1 mm or less. Petioles of C. crispa are often collapsed also, but not to the degree of C. cascadensis.

Sterile Frond Morphology - Silhouettes of sterile fronds of C. cascadensis and C. acrostichoides, taken from plants after two years in a common garden setting, are shown in Figure 1. A silhouette of an entire plant of C. crispa is given in Figure 2. Although the typical forms of each species' sterile fronds are distinctive, exceptions occur frequently enough to reduce the utility of this character for systematic purposes.

Sterile fronds of C. cascadensis are typically somewhat deltoid in outline, as are those of C. crispa. Sterile fronds of C.

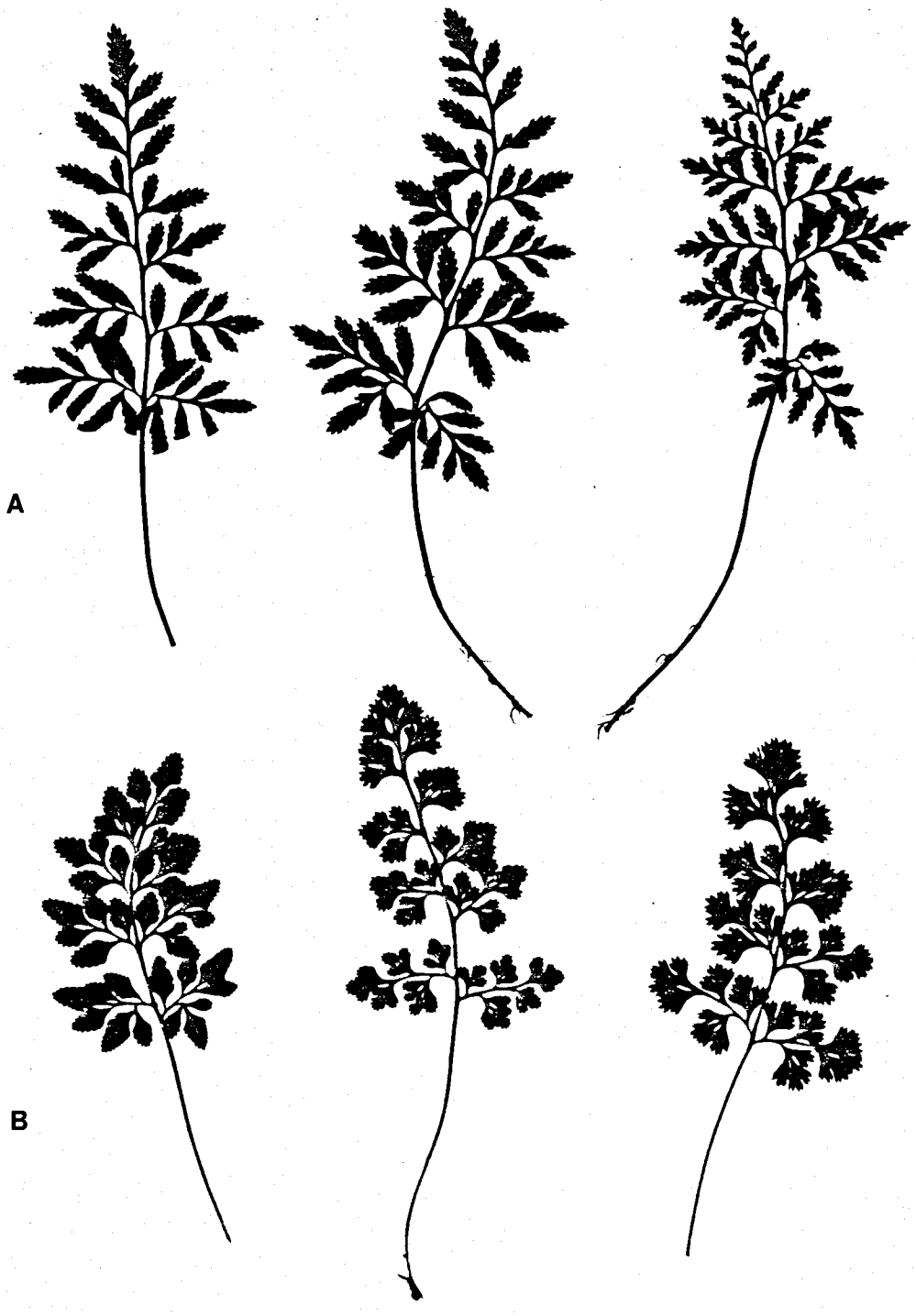


Figure 1. Silhouettes of sterile fronds of Cryptogramma, produced after 2 years of common garden conditions. A: C. acrostichoides. B: C. cascadensis


Figure 2. Silhouette of sterile and fertile fronds of C. crispa (Cumberland, England, P.Taylor 1946, NY).

acrostichoides are often ovate-lanceolate, although fronds with a more deltoid outline do occur. Shape of the ultimate segments varies also; those of C. cascadensis are typically flabellate in outline, while those of C. acrostichoides tend to be elliptical, but with considerable variation in each. Segments of C. crispa tend to be flabellate, and typically are cut with numerous deep incisions or lobes, while both American taxa have regularly and shallowly toothed margins.

Hydathodes - Hydathodes present on the adaxial surface of the pinnules of C. cascadensis are greatly elongate, clavate or even linear, and are flush with the frond surface, the condition present in C. crispa also. The hydathodes of C. acrostichoides are sunken below the frond surface, due to the the thicker, more coriaceous fronds. They are also shorter and wider, typically spathulate to oblanceolate in outline, and only rarely more elongate.

Fertile Fronds - The fertile segments of C. acrostichoides are relatively long, ranging from 6 to 14 mm in length, depending upon the size of the plant. Cryptogramma crispa has rather short fertile segments, typically between 1 and 7 mm long, only occasionally up to 10 mm. Fertile segments of C. cascadensis are typically shorter than C. acrostichoides, but not as short as C. crispa, mostly within 4-10 mm.

Scales - Scales of the rhizome and petiole are a character that has traditionally been used to separate C. acrostichoides from C. crispa, the former with bicolorous scales, due to a darker brown center stripe, and the former with uniformly light brown scales. In this character, the generally bicolorous scales of C. cascadensis

match those of its North American associate, C. acrostichoides. In both of these species, however, there is a tendency for scales on the petiole to partially or completely lose the dark center stripe, rendering these scales essentially concolorous.

Trichomes - Scattered along both surfaces of the fronds of C. acrostichoides are very small, appressed, unbranched cylindrical trichomes. These trichomes are most numerous in the sulca of the rachis and along the costae of the pinnae and pinnules. Such trichomes are essentially lacking in C. cascadensis, and do not appear to occur on C. crispa either. Presence of these trichomes is a useful micromorphological feature distinguishing C. acrostichoides.

**Chromosomes:** Chromosome counts of C. acrostichoides from widely spread localities in North America are uniformly diploid, with n = 30 (Löve et al. 1977). Counts of C. cascadensis show this species is also diploid, with 2n = 30 pairs of chromosomes at meiosis (Figure 3). Counts of C. crispa from Europe are consistently tetraploid, with n = 60 chromosomes (Löve et al., 1977).

**Spore Measurements:** On average, the spores of C. cascadensis are smaller than those of C. acrostichoides (Table 3). Average spore diameter for 16 sporophytes of C. cascadensis ranged from 46.7 μm to 53.3 μm, with a mean of 49.6 μm. Average spore diameter for 22 sporophytes of C. acrostichoides ranged from 49.1 μm to 58.9 μm, with a mean of 54.6 μm, an average of 5.08 μm, or 9.3 %, greater than C. cascadensis. Average spore diameters for 10 sporophytes of C. crispa ranged from 49.8 to 58.1 μm, a range similar to that of C. acrostichoides. An analysis of variance of means of each species was

Figure 3. Meiotic chromosomes of C. cascadensis (Alverson 1306, OSC).

Table 3. Spore size (μm) of C. acrostichoides, C. cascadensis, and C. crispa.

| Taxon | Mean length (s.d.) | N | Range of sporophyte means | Number of sporophytes |
|---|---|---|---|---|
| C. cascadensis | 49.61 (3.03) | 400 | 46.75-53.33 | 16 |
| C. crispa | 53.61 (3.88) | 250 | 49.78-58.10 | 10 |
| C. acrostichoides | 54.64 (4.01) | 550 | 49.07-58.96 | 22 |

Table 4. Tests comparing mean spore length of C. acrostichoides, C. cascadensis, and C. crispa.

A. Analysis of variance

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F |
|---|---|---|---|---|
| Model (among taxa) | 2 | 6087.28 | 3043.64 | 224.08*** |
| Error (within taxa) | 1197 | 16,258.84 | 13.58 | |
| Total | 1199 | 22,346.12 | | |

B. GT-2 test for differences between means (pairwise comparisons)

| Pair | Difference between means (μm) |
|---|---|
| C. cascadensis vs. C. acrostichoides | 5.082*** |
| C. cascadensis vs. C. crispa | 3.997*** |
| C. acrostichoides vs. C. crispa | 1.031*** |

*** = P<0.001

significant at $\alpha = 0.001$ (Table 4). In addition, the multiple comparisons test showed the mean spore diameters of all three species to be significantly different ($P < 0.001$).

**Electrophoretic Analysis:** A total of 170 sporophytes of C. cascadensis and 89 sporophytes of C. acrostichoides were analyzed for this study. Unfortunately, no fresh material of C. crispa was available for analysis. From the 10 enzyme systems examined, 13 putative loci were fully resolved; several additional loci, including all but the least anodally migrating bands of MDH, were incompletely resolved. Allele frequencies at each locus for each population are presented in Table 5.

GOT-1 (Figure 4a) - A monomeric enzyme. Only one allozyme, shared by the two taxa, was found at this locus. A second, slower region of activity possibly representing the chloroplastic isozyme (also invariant) was evident in C. acrostichoides, but not in C. cascadensis.

HK (Figure 4b) - Six allozymes were identified for this monomeric enzyme. Allele c, the common allele in C. cascadensis, was not found in C. acrostichoides. Allele b, found in a few populations of C. cascadensis, was the common allele in C. acrostichoides. Allele a was a rare allele found in one population of C. cascadensis, and alleles d,e,and f were rare alleles found only in C. acrostichoides.

IDH (Figure 4c) - This is a dimeric enzyme with three allozymes identified for the two species. Allele c was the most common allele in both species, and in 6 populations of C. acrostichoides occurred as a fixed homozygote. In C. cascadensis, in contrast, allele c often

Table 5. Allele frequencies and sample sizes (N) for C. acrostichoides and C. cascadensis. Localities are abbreviated as in table 1. Loci are numbered sequentially, and alleles are lettered sequentially, beginning with the most anodally migrating band.

| | | Population | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C. acrostichoides | | | | | | | | C. cascadensis | | | |
| Locus | Allele | CO1 | UT1 | NV1 | CA1 | OR1 | WA1 | WA2 | AK1 | WA1 | OR2 | CA2 | CA1 |
| PGI-1 | a | | | | | | | | | 1.00 | 1.00 | 1.00 | 1.00 |
| | b | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | | |
| | N | 25 | 25 | 8 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |
| PGI-2 | a | | | | | | | | | 0.02 | 0.12 | 0.12 | |
| | b | | | | | | | | | | | | |
| | c | | 0.02 | 0.33 | 0.34 | | | | 0.03 | | | | |
| | d | | | | | | | | | 0.96 | 0.73 | 0.73 | 1.00 |
| | e | | | | 0.02 | 0.08 | 0.15 | 0.24 | 0.28 | | | | |
| | f | | | | | | | | | 0.02 | 0.15 | 0.15 | |
| | g | 1.00 | 0.98 | 0.67 | 0.64 | 0.90 | 0.75 | 0.76 | 0.63 | | | | |
| | h | | | | | 0.02 | 0.10 | | 0.06 | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |
| TPI-1 | a | | 0.02 | | | | | 0.03 | | | 0.02 | 0.02 | 0.12 |
| | b | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 0.89 | 1.00 | 1.00 | 0.98 | 0.98 | 0.88 |
| | c | | | | | | | 0.08 | | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |
| TPI-2 | a | 0.02 | | 0.02 | | | | | | | | | |
| | b | 0.98 | 1.00 | 0.98 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 0.93 | 0.93 | 0.94 | 1.00 |
| | c | | | | 0.02 | | | | | 0.07 | 0.07 | 0.06 | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |
| PGM-1 | a | 0.92 | 0.84 | 0.24 | 0.04 | 0.46 | 0.75 | 0.97 | 0.91 | 0.65 | 0.88 | 1.00 | 0.41 |
| | b | 0.04 | 0.16 | 0.11 | 0.12 | 0.04 | | 0.03 | | | | | |
| | c | | | | | | | | | 0.35 | 0.12 | | 0.59 |
| | d | 0.04 | | 0.65 | 0.84 | 0.50 | 0.25 | | 0.09 | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 23 |
| PGM-2 | a | 0.05 | | 0.09 | 0.08 | | | | | | | | |
| | b | | | | | | | | | 1.00 | 1.00 | 1.00 | 1.00 |
| | c | 0.95 | 0.92 | 0.89 | 0.92 | 0.89 | 0.95 | 0.95 | 1.00 | | | | |
| | d | | 0.08 | 0.02 | | 0.11 | 0.05 | 0.15 | | | | | |
| | N | 21 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 23 |
| GOT-1 | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 9 | 16 | 14 | 20 | 24 | 25 |

Table 5 (con't.)

| | | Population | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *C. acrostichoides* | | | | | | | | *C. cascadensis* | | |
| Locus | Allele | CO1 | UT1 | NV1 | CA1 | OR1 | WA1 | WA2 | AK1 | WA1 | OR2 | CA2 | CA1 |
| LAP | a | | 0.02 | 0.02 | | 0.04 | | | | | | | |
| | b | 0.96 | 0.98 | 0.74 | 0.76 | 0.90 | 0.15 | 0.45 | 0.56 | | | | |
| | c | | | | | | | | 0.11 | | | | |
| | d | 0.04 | | | | 0.06 | 0.85 | 0.45 | | 0.53 | 0.27 | 0.48 | 0.24 |
| | e | | | 0.24 | 0.22 | | | 0.10 | 0.33 | 0.47 | 0.73 | 0.52 | 0.76 |
| | f | | | | | | | | | | | | |
| | g | | | | 0.02 | | | | | | | | |
| | N | 25 | 25 | 23 | 25 | 24 | 10 | 10 | 16 | 20 | 20 | 24 | 25 |
| HK | a | | | | | | | | | 0.55 | | | |
| | b | 0.90 | 1.00 | 0.48 | 0.30 | 1.00 | 1.00 | 1.00 | 1.00 | | 0.15 | | |
| | c | | | | | | | | | | | | |
| | d | | | | 0.08 | | | | | 0.45 | 0.85 | 1.00 | 1.00 |
| | e | 0.10 | | 0.50 | 0.60 | | | | | | | | |
| | f | | | 0.02 | | | | | | | | | |
| | g | | | | 0.02 | | | | | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |
| SKDH | a | | | 0.02 | | | | | | | | | |
| | b | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | | |
| | c | | | | | | | | | 1.00 | 0.95 | 0.96 | 0.38 |
| | d | | | | | | | | | | 0.05 | 0.04 | 0.22 |
| | e | | | | | | | | | | | | 0.40 |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |
| IDH | a | | | 0.02 | | | | | | 0.30 | 0.47 | 0.33 | 0.48 |
| | b | | | | | | | | | | | | |
| | c | 0.98 | 0.96 | 0.96 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 0.70 | 0.53 | 0.37 | 0.52 |
| | d | 0.02 | 0.04 | 0.02 | 0.02 | | | | | | | 0.29 | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |
| 6-PGDH | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | | |
| | b | | | | | | | | | 1.00 | 1.00 | 1.00 | 1.00 |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |
| MDH | a | | | | | | | | | 0.98 | 1.00 | 1.00 | 1.00 |
| | b | | | | | | | | | 0.02 | | | |
| | c | | | | | 0.02 | | | | | | | |
| | d | 1.00 | 1.00 | 0.98 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | | | | |
| | e | | | 0.02 | | | | | | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 20 | 20 | 24 | 25 |

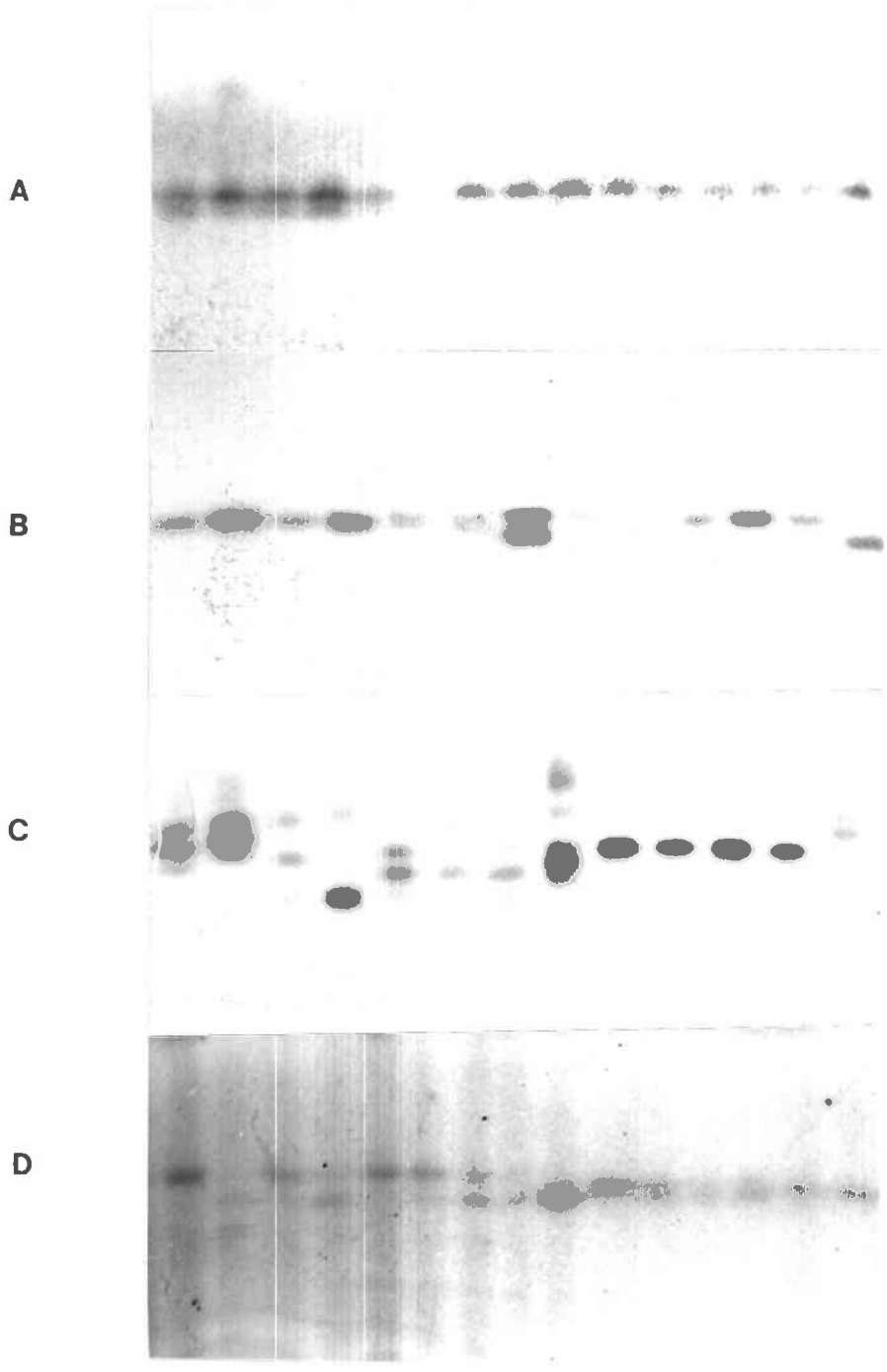


Figure 4. Electrophoretic banding patterns of C. acrostichoides and C. cascadensis. A: GOT. Lanes 1-5, 11-15, C. acrostichoides; lanes 6-10 C. cascadensis. B: HK. Lanes 1-5, C. cascadensis; lanes 6-13, C. acrostichoides. C: IDH. Lanes as in Fig. 4B. D: LAP. Lanes 1-8, C. acrostichoides; lanes 9-15, C. cascadensis.

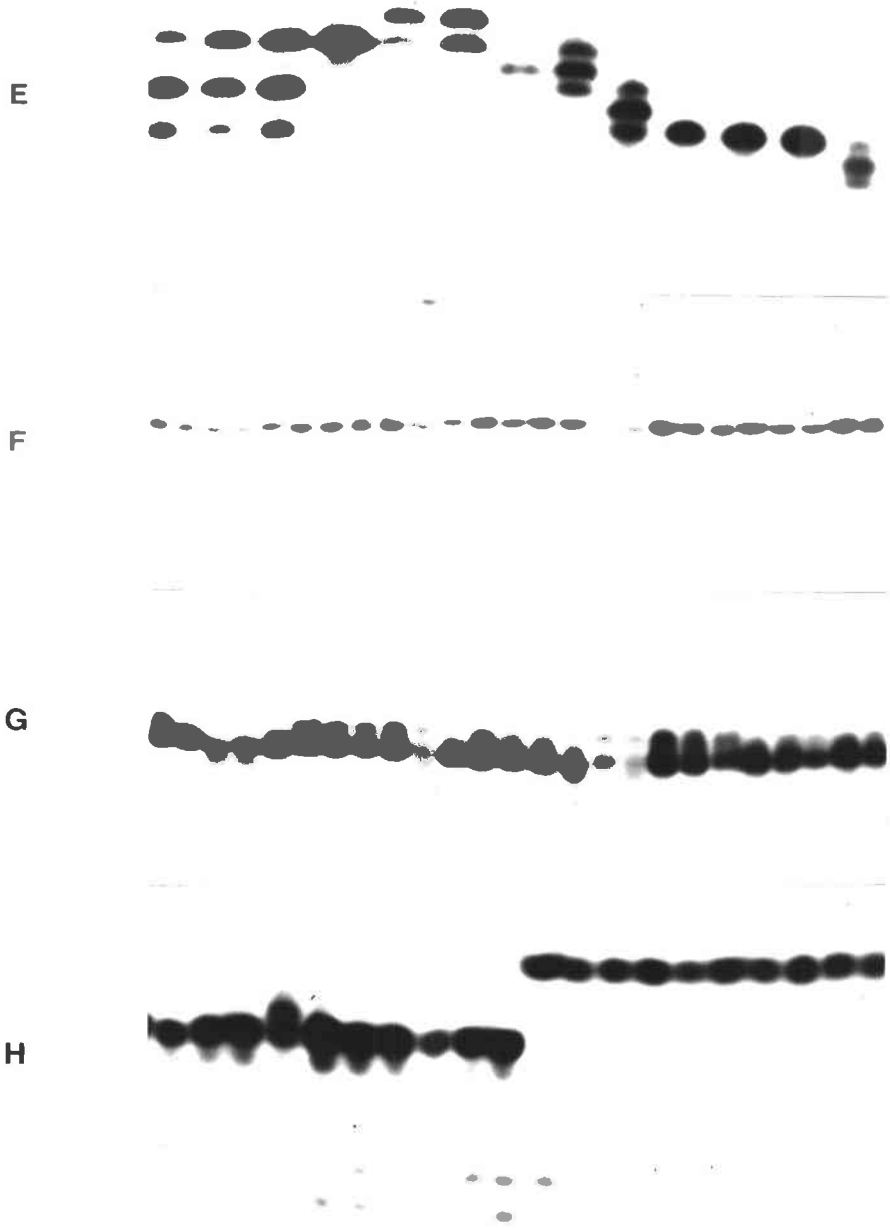


Figure 4 (con't.). E: MHD. Lanes 1-10, C. acrostichoides; lanes 11-20, C. cascadensis. F: 6-PGDH. Lanes 1-5, 11-15, 21-25, C. acrostichoides; lanes 6-10, 16-20, C. cascadensis. G: PGI-1. Lanes as in F. H: PGI-2. Lanes 1-5, C. cascadensis; lanes 6-13, C. acrostichoides.

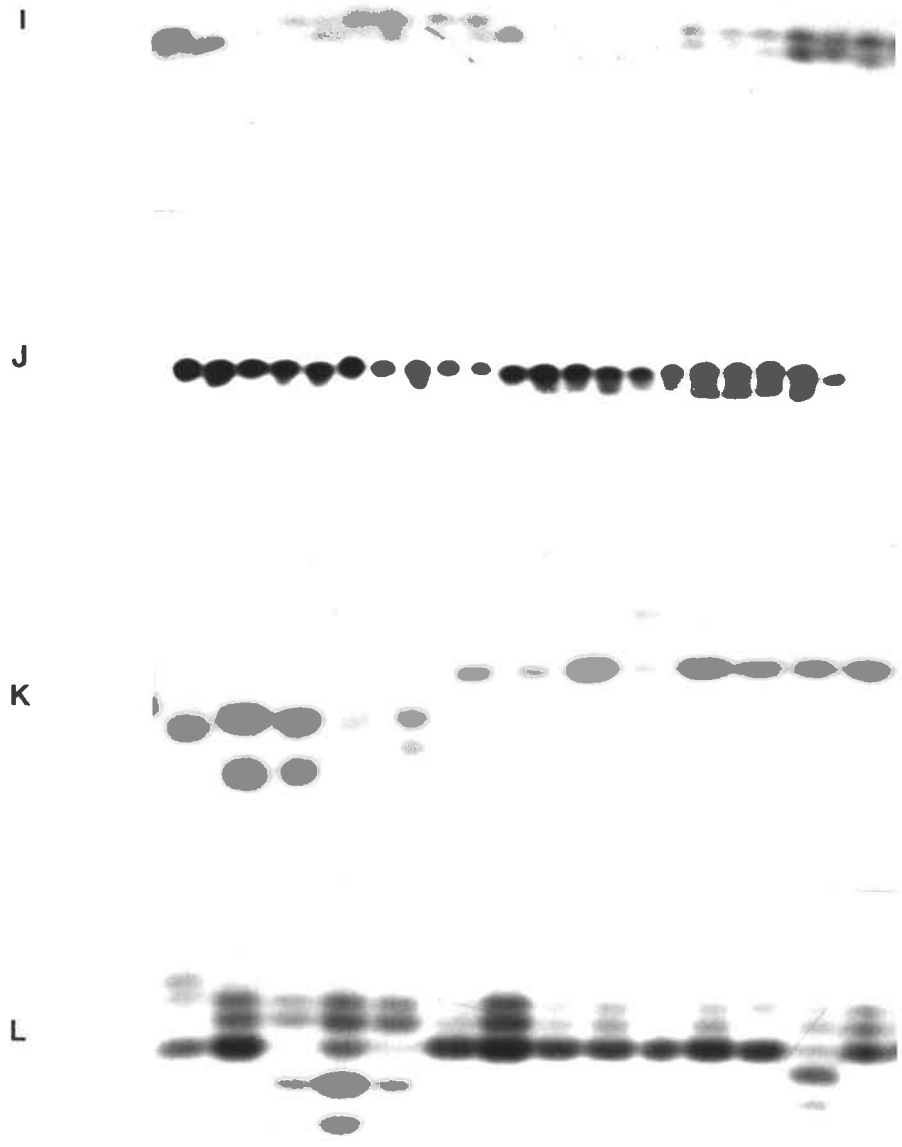


Figure 4 (con't.) I: PGM-1. Lanes 1-10, C. cascadensis; lanes 11-20, C. acrostichoides. J: PGM-2. Lanes 1-5, 11-15, 21, C. acrostichoides; lanes 6-10, 16-20, C. cascadensis. K: SKDH. Lanes 1-5, C. cascadensis; lanes 6-13, C. acrostichoides. L: TPI. Lanes as in K.

occurred as a heterozygote with allele a, which was found only in this species. Allele d was a rare allele found in both species.

LAP (Figure 4d) - Six allozymes were identified for this monomeric enzyme. Only alleles d and e were found in C. cascadensis. Allele b was the most common allele in C. acrostichoides, found in all 9 populations. Alleles d and e occurred in 4 populations of C. acrostichoides each, while a, c, and g were rarer alleles, occurring in 1 or 2 populations only.

MDH (Figure 4e) - Several loci were observed for this enzyme, but only the slowest migrating locus was resolved sufficiently to include in the analysis. This was one of the six "marker loci", in which C. cascadensis and C. acrostichoides shared no alleles in common. Allele a was the common allele in C. cascadensis, but in one population several individuals exhibited a second allele, b. With C. acrostichoides, allele d was predominant, occurring as a fixed homozygote in 7 of 9 populations. Alleles c and e were rare alleles occurring in only one population each of C. acrostichoides.

6-PGDH (Figure 4f) - This was another marker locus. Both species were invariably fixed homozygotes for this enzyme, with the slightly faster allele a found in C. acrostichoides, and allele b found in C. cascadensis. Though the mobilities of the two allozymes differ by only a slight degree, this difference is repeated consistently at every comparison, as shown in Figure 4f, and is thus not an artifact of a non-linear migration of the front.

PGI-1 (Figure 4g) - The pattern with this enzyme was very similar to that of 6-PGDH, again a marker locus with both species fixed homozygotes for slightly different allozymes. This time the faster

allele a was found in C. cascadensis, while allele b was found in C. acrostichoides. Again, numerous comparisons show that the differences in mobility are real.

PGI-2 (Figure 4h) - Eight allozymes of this dimeric isozyme were detected between the two species. Allele d was the common allele in C. cascadensis, with alleles a and f occurring as rare alleles in 3 of the 4 populations. Allele g was the common allele in C. acrostichoides, occurring as a fixed homozygote in 3 populations, but more often accompanied by the rare alleles c, e and h. This was the fourth marker locus, as the two species shared no alleles in common.

PGM-1 (Figure 4i) - Both species shared the common allele a for this monomeric enzyme. Allele c, found only in C. cascadensis, occurred in 3 of the 4 populations in substantial proportions. Alleles b and d occurred only in C. acrostichoides, b in 5 populations at low frequencies, and d in 5 populations, occurring as the most abundant allele in the 3 populations where a did not predominate.

PGM-2 (Figure 4j) - All 4 populations of C. cascadensis were fixed homozygotes exhibiting allele b for this isozyme. Because this allele was not found in C. acrostichoides, this is considered the fifth marker locus. Allele c was the predominant allele in C. acrostichoides, but often accompanied at low frequencies by the rare alleles a and d. Again, the differences in mobility between allozymes a and c are consistent at every comparison shown, thus justifying the claim that this is a marker locus.

TPI-1 (Figure 4k) - Interpretation of this locus is complex, in part because it was overlapping with the second TPI isozyme, TPI-2, and because of the asymmetrically stained, 3-banded pattern exhibited

by the TPI-1 locus. Although it has been suggested (Haufler 1985) that this 3-banded pattern is the result of a gene duplication, Gastony (1988) presents a convincing argument that the three-banded pattern is the result of post-translational modification of the isozyme. Thus when the locus is homozygous, the cathodal, most strongly staining band of the three represents the normal coded dimeric protein, a less abundant charge-modified homodimer occupies the most anodal position, and the central band represents the heterodimeric protein. When the locus is heterozygous, a much more complex pattern results; the four dimeric combinations are joined by six heterodimeric combinations, giving a total of 10 bands. In reality, fewer bands are actually evident, due to overlapping of bands and faint staining. Seven populations of C. acrostichoides and one population of C. cascadensis were fixed homozygotes for allele b, exhibiting only the typical three banded pattern. A faster allele a was found in several populations of both species, whereas the slower allele c was found only in one population of C. acrostichoides.

TPI-2 (Figure 4k) This is a normal, dimeric isozyme, but its interpretation in this study was complicated by its migrating in overlap with TPI-1. Again allele b was the common allozyme, occurring as a fixed homozygote in six populations of C. acrostichoides and one population of C. cascadensis. A faster allele a occurred only in C. acrostichoides, while the slower allele c was a rare allele in both species.

SKDH (Figure 4l) This monomeric enzyme was the sixth marker isozyme that distinguished the two species. Allele b, the common allele in C. acrostichoides, was a fixed homozygote in 7 of the 8

populations, with allele a found only in a single individual in one population. Cryptogramma cascadensis, in contrast, was somewhat more variable; Allele c was the common allozyme but allele d occurred as a rare allele in three populations, and allele e occurred only in the southernmost California population, where it was the most common allele.

Table 6 gives Nei's genetic identity (I) and Nei's genetic distance (D) statistics (Nei 1972) for comparisons of the 13 Cryptogramma populations sampled. Mean genetic identity in comparisons between populations of C. cascadensis was 0.944, and in comparisons between populations of C. acrostichoides was 0.938. Both values are fairly close to 1, which results when both alleles and allele frequencies are identical. In contrast, interspecific comparisons between C. acrostichoides and C. cascadensis were much lower, with a mean value of 0.0358, denoting a much greater degree of genetic differentiation between taxa than within taxa. A similar pattern is expressed in the Nei's genetic distance statistics.

In most populations sampled, outcrossed individuals predominated. Because intragametophytic fertilization leads to complete homozygosity across all loci, any individual with at least one heterozygous locus can be assumed to be a product of outcrossing, and percentage of individuals with at least one heterozygous locus provides a minimum estimate of the percentage of outcrossed individuals in the sample. From a sample of 89 sporophytes of C. cascadensis, at least 93% of the plants were a result of outcrossing; population means ranged from 80%

Table 6. Nei's genetic identity (I) and genetic distance (D) statistics. Localities are abbreviated as in Table 1. The population AK2 has not been included due to the small number of sporophytes sampled (2).

A. Pairwise comparisons for 12 populations of C. acrostichoides and C. cascadensis. Values for I are located above the diagonal, and values for D are located below the diagonal.

| | C. acrostichoides | | | | | | | | C. cascadensis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CO1 | UT1 | NV1 | CA2 | OR1 | WA1 | WA2 | AK1 | WA1 | OR2 | CA2 | CA1 |
| CO1 | | 0.998 | 0.923 | 0.883 | 0.976 | 0.934 | 0.974 | 0.981 | 0.371 | 0.378 | 0.360 | 0.332 |
| UT1 | 0.002 | | 0.916 | 0.874 | 0.974 | 0.929 | 0.972 | 0.980 | 0.368 | 0.376 | 0.357 | 0.330 |
| NV1 | 0.080 | 0.088 | | 0.992 | 0.957 | 0.872 | 0.892 | 0.918 | 0.341 | 0.346 | 0.326 | 0.325 |
| CA2 | 0.125 | 0.135 | 0.008 | | 0.926 | 0.833 | 0.850 | 0.876 | 0.325 | 0.326 | 0.309 | 0.318 |
| OR1 | 0.024 | 0.026 | 0.044 | 0.077 | | 0.931 | 0.953 | 0.964 | 0.351 | 0.354 | 0.336 | 0.318 |
| WA1 | 0.069 | 0.074 | 0.137 | 0.183 | 0.072 | | 0.980 | 0.931 | 0.421 | 0.400 | 0.405 | 0.351 |
| WA2 | 0.026 | 0.029 | 0.114 | 0.163 | 0.048 | 0.020 | | 0.973 | 0.417 | 0.407 | 0.402 | 0.358 |
| AK1 | 0.019 | 0.020 | 0.086 | 0.132 | 0.036 | 0.072 | 0.027 | | 0.369 | 0.377 | 0.357 | 0.331 |
| WA1 | 0.911 | 1.001 | 1.077 | 1.125 | 1.047 | 0.864 | 0.876 | 0.929 | | 0.952 | 0.947 | 0.920 |
| OR2 | 0.973 | 0.979 | 1.062 | 1.121 | 1.038 | 0.916 | 0.899 | 0.883 | 0.050 | | 0.982 | 0.945 |
| CA1 | 1.022 | 1.029 | 1.122 | 1.172 | 1.091 | 0.905 | 0.911 | 0.952 | 0.055 | 0.018 | | 0.920 |
| CA2 | 1.104 | 1.108 | 1.123 | 1.145 | 1.145 | 1.046 | 1.026 | 0.999 | 0.083 | 0.057 | 0.083 | |

B. Means of genetic identity and genetic distance statistics for pairwise comparisons given above. Standard deviations are in parentheses.

| | Within C. cascadensis | Within C. acrostichoides | Between C. acrostichoides and C. cascadensis |
|---|---|---|---|
| Genetic identity (I) | 0.944 (0.023) | 0.938 (0.046) | 0.358 (0.031) |
| Genetic distance (D) | 0.058 (0.024) | 0.069 (0.050) | 1.019 (0.093) |

to 100% (Table 7). For C. acrostichoides, with a sample of 170 sporophytes, a minimum of 62% of the plants were the products of outcrossing, with population means ranging from 0% to 100%, though six of nine populations had outcrossing rates of >50%.

Gene diversity statistics (Nei 1973, Hamrick et al. 1979)) for C. cascadensis and C. acrostichoides are summarized in Table 8, where the values given are the means for all loci. $H_T$ represents total allelic diversity for each species, $H_S$ is the mean allelic diversity within populations, and $G_{ST}$ is the percentage of allelic diversity attributable to the among population component. Low values of $G_{ST}$ for both C. acrostichoides and C. cascadensis show that the majority of genetic variation present in each species lies within populations. Also given are mean values for $A_P$, the number of alleles maintained at polymorphic loci; $P_A$, the proportion of the total number of alleles found within each population; and PI, a polymorphic index, which is equivalent to the expected heterozygote frequency under Hardy-Weinberg equilibrium.

## DISCUSSION

Evidence for Divergent Speciation. The process of speciation, whether in ferns or in seed plants, requires both phenotypic divergence between two taxa, and sufficient reproductive isolation to maintain the distinguishing features (Crawford 1985). While it had not previously been distinguished by botanists, this study showed that C. cascadensis can be separated from its congener, C. acrostichoides, by a suite of morphological features, including seasonality, color and

Table 7. Amount and distribution of allozyme variation in C. acrostichoides and C. cascadensis. Values given are means over all variable loci and all populations[1]

| Species | $H_T$ | $H_S$ | $G_{ST}$(%) | $A_P$ | $P_A$ | PI |
|---|---|---|---|---|---|---|
| C. acrostichoides | 0.187 | 0.128 | 16.0% | 3.50 | 0.549 | 0.097 |
| C. cascadensis | 0.275 | 0.228 | 14.4% | 2.44 | 0.789 | 0.159 |

[1] $H_T$ = total allelic diversity; $H_S$ = mean allelic diversity within populations, $G_{ST}$ = the percentage of total allelic diversity apportioned among populations; $A_P$ = mean number of alleles per polymorphoc locus; $P_A$ = the proportion of the total number of alleles in the species that were found in each population; PI = a polymorphic index (equivalent to the expected heterozygote frequency under Hardy-Weinberg equilibrium).

Table 8. Levels of heterozygosity and inferred minimum outcrossing percentages for populations of C. acrostichoides and C. cascadensis. Populations are abbreviated as in Table 1.

| Population | # of heterozygous sporophytes | # of sporophytes sampled | minimum % of outcrossed plants |
|---|---|---|---|
| C. acrostichoides: | | | |
| AK2 | 0 | 2 | 0% |
| CO1 | 5 | 25 | 20% |
| UT1 | 12 | 25 | 48% |
| WA2 | 10 | 19 | 53% |
| OR1 | 15 | 25 | 60% |
| WA1 | 8 | 10 | 80% |
| NV1 | 19 | 23 | 83% |
| CA1 | 21 | 25 | 84% |
| AK1 | 16 | 16 | 100% |
| Total: | 106 | 170 | 62% |
| C. cascadensis: | | | |
| OR2 | 16 | 20 | 80% |
| WA1 | 18 | 20 | 90% |
| CA1 | 25 | 25 | 100% |
| CA2 | 24 | 24 | 100% |
| Total: | 83 | 89 | 93% |

texture, and micromorphology. This phenotypic divergence has a genetic basis, as shown by common garden trials in which the distinguishing features of the two taxa were maintained. Electrophoretic banding patterns of metabolic enzymes provided a more direct link between phenotypic and genotypic differentiation between the two taxa. Electrophoretic evidence from sympatric populations of C. cascadensis and C. acrostichoides also implies these two diploid species are reproductively isolated. Furthermore, the evidence indicates that the two species do not exhibit a progenitor-derivative relationship.

Allozyme analysis provides a powerful tool for assessing the degree of genetic differentiation between taxa. In this study, C. cascadensis and C. acrostichoides were found to be genetically strongly differentiated. The mean Nei's Genetic Identity statistic of 0.358 for interspecific comparisons lies within the range of genetic identities reported by previous workers for a variety of congeneric fern species (Haufler 1987). The pattern of low similarity between populations of two species and high genetic similarity between populations of the same species was consistent for every population of both species (Table 6). Similarity of conspecific populations held true even over large geographic distances.

Both species exhibited moderate levels of genetic diversity. Values of $A_P$ and $P_A$ for both species, and PI for C. cascadensis are comparable to mean values for outcrossing seed plants (Hamrick et al. 1979, Hamrick 1983). While the number of alleles per polymorphic locus was slightly lower for C. cascadensis than for C.

acrostichoides, this difference could be attributed to the smaller number of populations of C. cascadensis that were sampled.

For both species, inter-populational genetic identities were high because the genetic variability present in each species was largely apportioned within populations. This is indicated by the relatively low values of $G_{ST}$ obtained for each species, showing that only 14% to 16% of the total allelic diversity of each species is apportioned between populations (Table 8). Low values of $G_{ST}$ also are characteristic of species with outcrossing breeding systems. High levels of outcrossing maintain heterozygosity, reduce loss of alleles due to genetic drift, and increase effective neighborhood size. In species where selfing predominates, genetic variability tends to be apportioned in the opposite way, with a greater proportion of alleles unique to a single population (Hamrick 1983).

With ferns, a minimum estimate of outcrossing percentage for each population can be obtained from electrophoretic data. Because selfing (intragametophytic fertilization) leads to instant homozygosity across all loci, any sporophyte exhibiting at least one heterozygous locus has arisen via intergametophytic fertilization. In this study, 90% of all C. cascadensis individuals assayed possessed at least one heterozygous locus, showing that this species possesses a highly outcrossing breeding system (Table 7). In C. acrostichoides, outcrossed individuals predominated in the majority of populations sampled, though overall percentage of heterozygous individuals was lower. These results are consistent with recent studies that indicate that many ferns are strongly outcrossing even though the bisexual

gametophytes of ferns are theoretically capable of selfing (Soltis & Soltis 1987).

With species that are strongly outcrossing, electrophoretic data provide a means of inferring reproductive isolation between sympatric populations of two taxa. Both species were found growing together at two of the localities from which samples for the electrophoretic study were obtained: along the Snow Lake trail in the Washington Cascades (WA1), and at Heather Lake in the Sierra Nevada of California (CA2). Presumably these circumstances occur fairly often, considering that C. acrostichoides occurs throughout the range of C. cascadensis. Despite the fact that both species were highly outcrossing at both localities, with minimum outcrossing rates of 80% or greater, no evidence of hybridization or gene flow between the two species was found at either locality. The maintenance of genetic markers distinguishing the two species at 6 of the 13 loci (46%), despite sympatry, indicates that no gene flow occurs between the two species. This constitutes strong evidence that some mechanism maintains reproductive isolation between the two species.

Because the alleles of C. cascadensis are not primarily a subset of those of C. acrostichoides, the gene frequency data (Table 5) show that the two taxa are divergent species, rather than a progenitor-derivative species pair. Genetic identity comparisons suggest that the two taxa are not even closely related, despite the fact that for over a century the two taxa have been confused with one another. To determine the true affinities of each species within the genus will require an analysis of morphological and biochemical data for the entire world-wide complex.

Implications for Systematics of Cryptogramma. Evidence for speciation in North American Cryptogramma contradicts the work of the many botanists over the past century and a half who have concluded that all of the parsley-ferns are varieties of a single species, C. crispa. This history has been reviewed in Fernald (1935), who cites statements by prominent European botanists such as W.J. Hooker, Milde, C.B. Clarke, and C. Christensen in support of this interpretation. For example, Christensen (in Hulten 1927) echoes many of the previously listed authors in stating "the differences between (C. acrostichoides) and the European C. crispa are so small that I am inclined...to consider it a variety of C. crispa". He then goes on to cite several characters of micromorphology that consistently distinguish the two taxa. Christensen's decision seems to have been a subjective one; because the degree of morphological difference between the two taxa appeared to him to be slight, he concluded that the two taxa could not be separate species.

On the other hand, many American workers, Fernald (1935) notes, have taken the opposite approach. He cites D.C. Eaton (1880) as a case in point: "While it is indisputable that there may be specimens from one continent much resembling the type usually seen in the other, the normal type of C. acrostichoides is so different from that of C. crispa that...it is better to keep them apart".

A century of intense study of systematics and evolution has led to a greater knowledge of what kinds of processes lead to speciation, how species maintain themselves, and what kinds of features species exhibit. Knowledge of chromosome numbers, breeding systems, crossing relationships, and genetic relatedness provides additional information

with which to weigh the significance of morphological evidence. In addition, field experience with a group of taxa provides an advantage over herbarium work, as it is possible to both search for characters not evident on herbarium specimens, and study the degree and nature of morphological variation in natural populations.

With the benefit of such insights, it is possible to make a stronger argument for treating segregate taxa in Cryptogramma as distinct species. Isozyme data corroborate the separation of C. cascadensis from C. acrostichoides on the basis of a series of morphological characters that are subtle, but constant. The Eurasian species, C. crispa, differs from C. acrostichoides even more markedly than C. cascadensis, for not only is C. crispa a deciduous species, but it also has more finely dissected sterile fronds and concolorous rhizome scales (Table 2). In addition to the morphological differences, C. crispa is tetraploid, while both of the American species are diploid. At this point it is not known whether C. crispa is an allotetraploid or autotetraploid; however, the difference in chromosome number suggests a distinct evolutionary history for the Eurasian taxon. Given the evidence currently available, it appears highly unlikely that C. crispa is conspecific with either of the American species.

Other taxa of Cryptogramma, such as C. fumariifolia (Baker) Christ from South America, C. raddeana Fomin from eastern Asia, and C. brunoniana Hook. & Grev. from the Himalayas, possess evergreen and marcescent sterile fronds, and thus are probably allied to C. acrostichoides rather than to C. crispa. Assuming that patterns of evolution and differentiation are consistent throughout the genus, it

seems most appropriate in turn to treat these additional taxa as distinct species, rather than as subspecies or varieties of C. crispa.

Patterns of Intraspecific Differentiation. Given that so much controversy has centered around the issue of how significant the differences are between the named taxa, little attention has been given to patterns of intraspecific differentiation in any taxa of Cryptogramma. Yet, a species with as wide a range as C. acrostichoides is likely to have a long evolutionary history with repeated events of migration and geographical isolation.

Not surprisingly, patterns of morphological differentiation emerge from the examination of several thousand herbarium specimens. Most notably, many of the specimens from California and Nevada possessed less finely dissected (bipinnate) sterile fronds with relatively large, broad segments. Some slight, but perhaps significant electrophoretic differences were correlated with these morphological observations. The closeness of the California and Nevada populations of C. acrostichoides is reflected in their genetic identity of 0.992. In contrast, the mean of all other comparisons involving the California population was 0.874, while the mean of all other comparisons involving the Nevada population was 0.913. These values are relatively low, in comparison to the mean of genetic identities for all possible comparisons except those involving the Nevada and California populations, which was 0.963.

These results suggest a case of incipient speciation in the Pacific Southwest, involving populations that have likely been geographically isolated from the species' main range farther north and east. This isolation is particularly evident in the Great Basin

region, as shown by the relatively low genetic identity (0.916) between the Nevada and Utah populations, even though separated by a distance of only 320 km (200 mi). Long distance dispersal and the resulting interpopulational gene flow acts to slow divergence and the speciation process, while geographic barriers and selective pressures (such as increased aridity, or a mediterranean climate) act to impede gene flow between geographic regions, and thus promote divergence, and ultimately, speciation.

No patterns of morphological or genetic divergence were observed in C. cascadensis. This taxon appears to be morphologically relatively uniform throughout its range. Interpopulational comparisons of genetic identities showed that the three Cascade Range populations were quite similar, with identities greater than 0.947. The Sierra Nevada population was slightly more divergent, but no comparisons were less than 0.920. This may be due in part to the smaller sample size involved (4 populations), but the highly outcrossed breeding system of C. cascadensis may also be important in maintaining these similarities. Not only are populations of outcrossed species less likely to lose alleles due to genetic drift, but long distance dispersal, and the resulting founder effects, is much less likely to occur with obligate outcrossers.

Ecology and Geography of C. cascadensis and C. acrostichoides. Cryptogramma cascadensis is a species of open rocky habitats at high elevations along the entire length of the Cascade Mountains, from southern British Columbia, through Washington and Oregon, to the volcanic peaks of Mt. Shasta and Mt. Lassen (Figure 5), typically growing on the granitic and volcanic rocks that are characteristic of

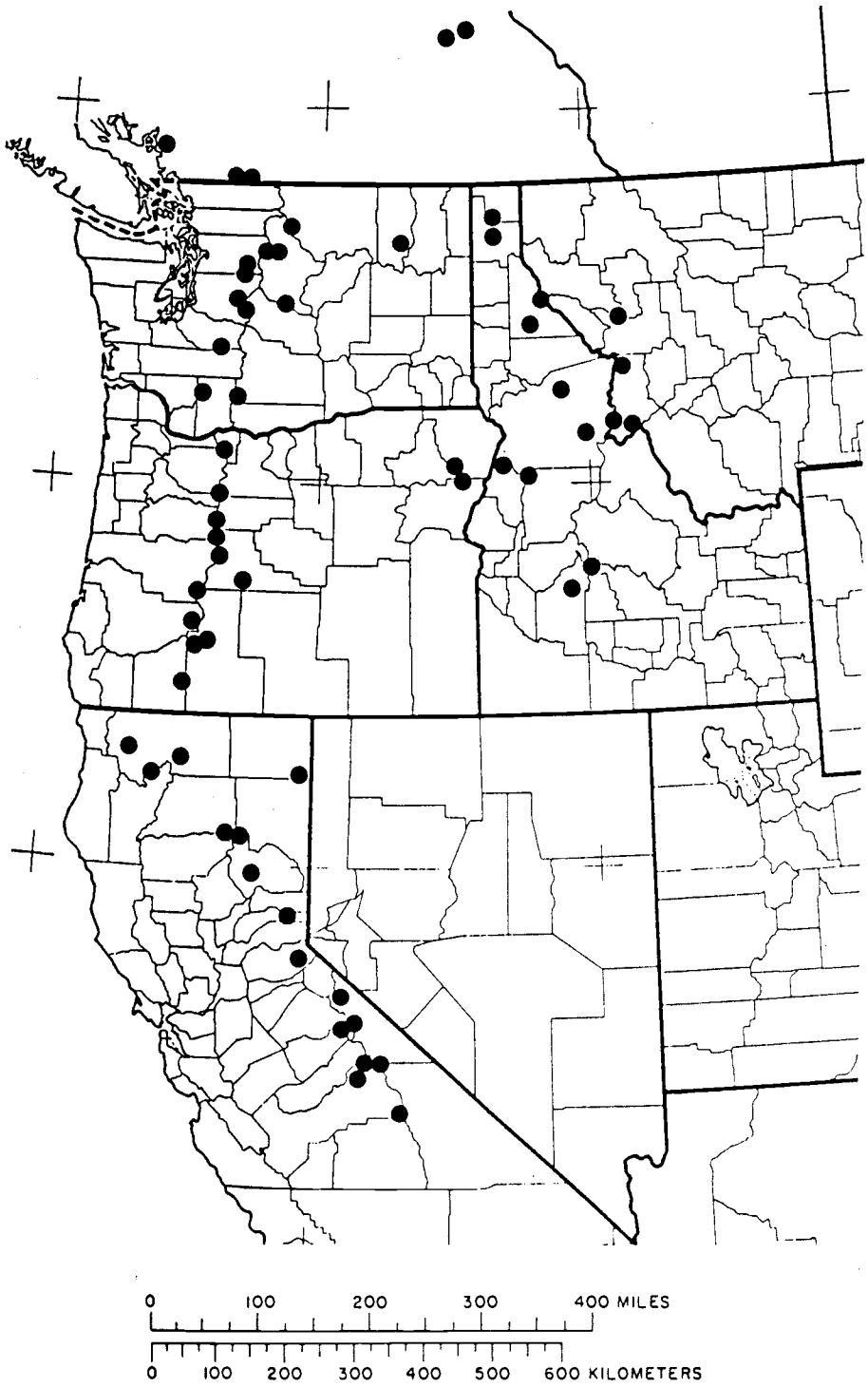


Figure 5. Geographic distribution of C. cascadensis. Each dot represents one or more herbarium specimens.

this Range. The distribution of C. cascadensis extends southward in California along the high Sierra as far as Fresno County. A second center of distribution for this species is in the northern Rocky Mountains of Idaho, western Montana, southeast British Columbia, northeast Washington, and northeast Oregon. Here C. cascadensis is a representative of the coastal floristic element that is disjunct in the high rainfall regions of the northern Rockies (Daubenmire 1975). A complete listing of herbarium specimens annotated as C. cascadensis is presented in Appendix I.

Cryptogramma cascadensis is not known with certainty from the Olympic Mountains of Washington, though suitable habitats probably occur. Two herbarium sheets with C. cascadensis labelled as having been collected in the Olympic Mountains were examined (without specific locality, Piper 1905, WTU; Elwha Basin, Leach & Leach s.n., ORE), but both are mixed sheets also containing plants of C. acrostichoides. In the absence of further corroborating evidence, these records are presumed to be in error.

Cryptogramma acrostichoides has an extremely wide range in North America, occurring as far north in Alaska as 65 N latitude, southward through the western mountains to southern California (San Bernardino Mts, 34 N latitude) and southern Arizona (Pinaleno Mts, 32 N latitude). I have not been able to verify reports of this species from Baja California. C. acrostichoides extends westward through the Aleutian Islands to the Kamtchatka Peninsula in eastern Asia (Hultén 1927). From the northern stations in Alaska and the Yukon, the range extends eastward into northern Manitoba, with outlying populations in western Ontario and near Lake Superior in Michigan and Minnesota.

C. acrostichoides occupies an equally wide range of ecological conditions, occurring in the Pacific Northwest over an elevational range of at least 2400m (8000 ft.). The species occurs near sea level in the Columbia River Gorge, on open coastal headlands in Oregon, and in the San Juan Islands. It is perhaps most common at middle elevations in the Cascade and Olympic Mountains, where the species can be found on just about every rock outcrop and talus slope. Populations can also be found in the subalpine and alpine zones, where it may co-exist with C. cascadensis. In these situations, however, the two species tend to occupy different habitats.

Morphology of C. cascadensis, particularly the deciduous habit and thin frond texture, suggests that this is a species most suited to mesophytic habitats in regions with deep winter snow accumulations. In contrast, C. acrostichoides is a relatively xerophytic species, with thick evergreen fronds that withstand significant moisture stress, and can photosynthesize in autumn, winter, and early spring, if not covered by snow.

This assessment is supported by field observations. At the type locality in Washington near Snoqualmie Pass, where both species are present, colonies of C. cascadensis are generally found in habitats that are released from the snowpack later in the season, either because of concave microtopography, or because of heavy snow accumulation due to winter avalanches. In the subalpine of the Sierra Nevada, at the outlet of Heather Lake, Eldorado Co., California, C. cascadensis grows in its typical habitat on a cool, north-facing talus slope. At the same locality, C. acrostichoides is abundant on the

opposing south-facing slope, where in late August of 1987 the plants were completely withered by drought.

Like many cheilanthoid ferns, C. acrostichoides regularly survives periods of drought with dehydrated, curled, and brittle fronds that readily rehydrate and resume normal functioning when moisture becomes available. C. cascadensis apparently does not possess this drought tolerance mechanism, and must avoid moisture stress by occupying mesic microsites, such as at Heather Lake. When cultivated plants of C. cascadensis were left unwatered, their fronds of first wilted, then withered and died, and did not revive when watered again. In the color and texture of its fronds, C. cascadensis is remarkably similar to Athyrium distentifolium, a common associate in moist subalpine habitats, a convergence attributable to selection for similar ecological conditions.

Correlated with these ecological differences is a difference in mean spore diameter of the two species. Spore size often differs with species of different ploidy (Barrington et al. 1986), but in this case both species are diploid.

Three ecological explanations for variation in spore size among diploid taxa have been proposed: adaptation for dispersal, increased allocation of nutritional resources, and environmental parameters (Barrington et al. 1986). In relating environmental parameters to spore size in Isoetes, Cox & Hickey (1984) found that plants growing in colder, higher, and shadier habitats had smaller spores. A similar pattern was found in Cryptogramma; the mean spore diameter (49.6 μm) of the more mesophytic species, C. cascadensis, is significantly less

than the mean spore diameter (54.6 μm) of the relatively xerophytic species, C. acrostichoides.

A closer look at the spore data shows that there is a great deal of intraspecific variation in spore size, as well. With C. cascadensis, the range in mean spore diameter between different populations was 6.6 μm, greater than the 5.0 μm size difference between the means for the two species. For C. acrostichoides, the difference between the largest and smallest samples was even greater, 9.9 μm. These observations suggest that there is a phenotypic component to spore size that varies with environmental conditions. Just as with frond outline and dissection, variation due to environment appears to overwhelm any inherent genetic differences between species; as a result, spore size is not a diagnostic character that can be used to separate the two species.

Though no data were collected to relate spore size specifically to environment, evidence from herbarium material suggested that such a correlation does exist. For example, one sheet of C. cascadensis from the Sierra Nevada of California [Alexander and Kellogg 4165 (UC)] has two different plants mounted on it. One plant has shorter, predominantly less dissected sterile fronds, suggesting that it is a sun form, while the other is larger, with more dissected sterile fronds, and is apparently a shade form. Spores were measured from both plants. The mean spore diameter of the plant of the sun form was 53.3 μm, 4.7 μm greater than the 48.6 um mean for the shade form, nearly as much as the difference between the means for C. acrostichoides and C. cascadensis.

Mean spore diameter of C. crispa was similar to that of C. acrostichoides. The former species is tetraploid, so the similarity in spore size probably does not suggest a relationship between the two species, since (as noted above) polyploidy often leads to an increase in spore size. In fact, the diploid progenitor(s) of C. crispa may actually have had relatively small spores, similar in size to those of C. cascadensis.

The reasons for the observed differences in spore sizes of diploid species in this study were not fully evident. All three of the hypotheses described above may be applicable. Given the importance and utility of spore size to fern systematics, these hypotheses are worthy of further study and testing.

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Chapter III

# Reticulate Evolution in North American Parsley Ferns: A Reevaluation of *Cryptogramma sitchensis* (Rupr.) Moore

## ABSTRACT

A biosystematic approach was used to investigate the taxonomy and evolutionary origin of *Cryptogramma sitchensis*, a taxon previously treated as a variety of *C. acrostichoides*. *Cryptogramma sitchensis* was found to be a tetraploid species, n = 60, the first report of polyploidy in the genus from North America. Data from enzyme electrophoresis showed fixed heterozygosity, a characteristic of allopolyploid species. Allozyme banding patterns showed that *C. sitchensis* combines genomes of *C. acrostichoides* and another distinct species not present in North America. Morphological characters, particularly the dissection of sterile leaves, suggested that this second parent is the eastern Asian *C. raddeana*. Frequent triploid hybrids, which can be identified by their abortive spores, occur where the ranges of *C. sitchensis* and *C. acrostichoides* overlap. Hybrids blur the morphological distinctions between the two species, and are probably responsible for leading earlier taxonomists to conclude that the taxa are only varietally distinct. Similar biosystematic approaches may be useful in determining the origin of *C. crispa*, the only other known polyploid species in the genus.

# INTRODUCTION

A distinctive Cryptogramma from Alaska and adjacent Canada was first described as Allosorus sitchensis by F.J. Ruprecht in 1845. The species was transferred to Cryptogramma by Thomas Moore in 1857, but most floristic works (e.g., Scoggan 1978, Lellinger 1985) have concurred with Carl Christensen (in Hultén 1937), treating it as a variety of the widespread North American species Cryptogramma acrostichoides R. Br. Seldom collected and largely inaccessible in its native habitat, this taxon has received little study beyond morphological appraisals based on herbarium specimens.

The primary morphological difference between C. sitchensis and C. acrostichoides is the dissection of the sterile leaves and the shape of their segments. Cryptogramma acrostichoides is essentially 2 or 3 times pinnate, but the sterile leaves of C. sitchensis are more finely dissected, and typically 3 or 4 times pinnate, with each segment divided into 4 to 8 small, obovate lobes. Only a few other minor morphological features separate the two taxa; they are are essentially alike in habit, frond texture, indument, and color.

Christensen (in Hulten 1937), in combining sitchensis as a variety of C. acrostichoides, noted that the finely dissected sterile leaves of C. sitchensis resemble those of C. crispa (L.) R. Br. from Europe, but that C. sitchensis belongs with C. acrostichoides because of similarities in color, texture, and scales. Hultén (1941) suggested that C. sitchensis is "closely akin to" Cryptogramma raddeana Fomin, an eastern Asian species, which also is very finely dissected with small, obovate ultimate segments.

Although C. sitchensis exhibits similarities with both C. acrostichoides and C. raddeana, it is identical to neither species. I hypothesize that C. sitchensis arose by allopolyploidy, as a result of hybridization between C. acrostichoides and C. raddeana, followed by chromosome doubling to restore fertility.

Cryptogramma sitchensis and C. acrostichoides are connected by backcross hybrids, which may be common where the two species are sympatric. Readily identified by their abortive spores, such hybrids often provide valuable insights into the relationships between the species involved (Wagner 1969), and have often been used in pteridophyte systematics to interpret patterns of reticulate evolution (Manton 1950).

This chapter discusses morphological features of the three species involved in this complex, along with cytological data, biochemical data from enzyme electrophoresis, and patterns of geographical distribution, and studies of hybridization, to investigate the validity of the hypothesis of an allopolyploid origin for C. sitchensis.

# MATERIALS AND METHODS

**Morphological analysis:** Herbarium specimens were borrowed from ALA, BRY, CAN, CAS, COLO, DAO, GH, MICH, MONTU, NY, ORE, OSC, RM, UBC, UC, US, V, WS, and WTU. Living plants were obtained from representative populations throughout the species' ranges; these plants were maintained in cultivation in a cool greenhouse in the summer, and in a covered cold frame in the winter.

**Chromosomes:** Fertile fronds were collected from wild populations or plants cultivated in the greenhouse and fixed in Farmer's formula (a 3:1 solution of 95% ethanol and glacial acetic acid), and stored in a freezer. Chromosome squashes were stained with acetocarmine, and Hoyer's medium was used to make the preparations permanent (Beeks 1955). Slides were examined under phase-contrast and photographed with Kodak Technical Pan 2415 film.

**Spore measurements:** Spores were mounted on glass slides in Hoyer's medium. Twenty-five spores from each slide were measured along their longest dimension with an ocular micrometer at 640 X. The computer package SAS was used to perform an analysis of variance (ANOVA), and the GT-2 multiple comparison test of Sokal and Rohlf (1981), in order to test for significant differences between mean spore diameters of C. sitchensis, C. acrostichoides, and C. raddeana.

**Electrophoresis:** Thirteen populations were sampled electrophoretically from widespread localities in western North America (Table 9). Where possible, 20 to 25 sporophytes per population were sampled in order to assess intra-populational variability. Starch gel electrophoresis was conducted following the

protocol of Soltis et al. (1983). Fronds were placed in plastic bags in the field and kept on ice until analyzed. Newly expanding fiddleheads were used when available, but the majority of samples were run with mature fronds. Small pieces of frond tissue were ground in the phosphate grinding buffer of Soltis et al. (1983) and the grindate transferred to small rectangular wicks of filter paper. Wicks were inserted into a vertical slit in the 12% starch gel and subjected to horizontal electrophoresis at 4 C. To resolve the slower

Table 9. Sampling localities for electrophoretic study.

**AK 1**: Alaska, Baranof Island, N of Ptarmigan Peak, 3 km W of Port Walter, elevation 900 m, T63S R68E S22; K. La Bounty s.n., August 1987. (C. acrostichoides, C. sitchensis, C. acrostichoides x sitchensis)

**AK 2**: Alaska, Baranof Island, Harbor Mtn., 5 km N of Sitka, elevation 500 m, T55S R62E S15; M. Muller s.n., August 1987. (C. acrostichoides, C. sitchensis, C. acrostichoides x sitchensis)

**AK 3**: Alaska, West Chichagof Island, Whitestripe Ridge, 1.5 km N of Whitestripe Lake, elevation 500 m, T47S R57E S36; M. Muller s.n., 20 September 1986. (C. sitchensis)

**CA 1**: California, Eldorado Co., at outlet of Heather Lake, Desolation Wilderness, elevation 2400 m, T12N R17E S19; E.R. Alverson 1289, 26 August 1987. (C. acrostichoides)

**CO 1**: Colorado, Gunnison Co., 1.5 km SE of Schofield Pass, 8 km NW of Gothic, elevation 3170 m, T12S R86W; E.R. Alverson 1300, 30 August 1987. (C. acrostichoides)

**NV 1**: Nevada, Elko Co., Ruby Mountains, above Island Lake, elevation 3050 m, T32N R58W S36; E.R. Alverson 1296, 28 August 1987. (C. acrostichoides)

**OR 1**: Oregon, Lane Co., 13 km E of McKenzie Bridge, elevation 650 m, T16S R7E S19; E.R. Alverson 1275, 20 August 1987. (C. acrostichoides)

**UT 1**: Utah, Salt Lake Co., Secret Lake, 3 km S of Alta, elevation 3020 m, T3S R3E; E.R. Alverson 1297, 29 August 1987. (C. acrostichoides)

**WA 1**: Washington, King Co., Snow Lake Trail, 5 km NW of Snoqualmie Pass, elevation 1100 m, T23N R11E S30; E.R. Alverson 1279, 22 August 1987. (C. acrostichoides)

**WA 2**: Washington, Grays Harbor Co., Gibson Slide, Mt. Colonel Bob Wilderness, elevation 820 m, T23N R8W S18; E.R. Alverson 1042, 11 October 1986. (C. acrostichoides)

phosphoglucoisomerase (PGI) locus, a gel buffer pH of 8.8 was required to obtain anodal migration; otherwise, all gel buffers were prepared at a pH of 8.0.

PGI, phosphoglucomutase (PGM), and glutamate oxaloacetate transaminase (GOT, also known as aspartate aminotransferase) were run on System 6 of Soltis et al. (1983); triosephosphate isomerase (TPI), leucine aminopeptidase (LAP), and hexokinase (HK) were run on System 8 of Haufler (1985), and shikimate dehydrogenase (SKDH), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGDH), and malate dehydrogenase (MDH) were run on System 11 of Haufler (1985). SKDH, IDH, 6-PGDH, and MDH were also resolved on the Morpholine system of Werth (1985).

IDH, MDH, PGI, PGM, 6-PGDH, SKDH, and TPI were stained in the dark with a 1% agarose solution, and liquid staining solutions were used for the remaining enzymes. All staining schedules followed Soltis et al. (1983). Gels were scored when fresh. When more than one locus was resolved, loci were numbered sequentially with the most anodally migrating isozyme 1. Allozymes of a single locus were labeled alphabetically, with the fastest allele labeled a, the second fastest b, and so on. Lanes on a given gel were numbered sequentially from left to right. Gels were photographed using Kodak Technical Pan 2415 film. Statistical analysis of the electrophoretic data was performed by the LYNSPROG program, developed by Andrew Schnabel (Schnabel, unpubl.)

# RESULTS

**Morphological analysis:** Table 10 summarizes the diagnostic features of C. acrostichoides, C. sitchensis, and C. raddeana. Cryptogramma sitchensis combines the morphological profiles of its two putative parents, having the color, frond texture, and habit of C. acrostichoides but with the dissection of sterile leaves approaching that of C. raddeana.

Sterile fronds - Figure 6 shows silhouettes of typical sterile fronds of C. acrostichoides, C. sitchensis, and their putative hybrid; the sets of fronds representing the latter two taxa were obtained from the same plants as the chromosomes and spores illustrated in Figures 8A and 9A, and 8B and 9B, respectively. Figure 7 shows the silhouette of an entire plant of C. raddeana.

The outline of sterile blades of C. acrostichoides is typically ovate-lanceolate; those of C. raddeana are typically more triangular. Blade outlines of C. sitchensis are variable, ranging from ovate-lanceolate to triangular but are typically more triangular than in C. acrostichoides. C. raddeana is characterized by finely dissected sterile leaves, in which the pinnules are pinnatifid into 4-8 narrow lobes with acute apices. On a single plant of C. sitchensis, the sterile leaves may range from having pinnules deeply incised to the pinnules nearly pinnatifid. Leaves of the latter type bear a strong resemblance to those of C. raddeana, except that the individual lobes tend to be shorter, not quite so narrow, often obovate with a more broadly acute apex. The ultimate segments of C. sitchensis are generally 1 to 1.5 times wider than the rachis of the pinnule or pinna

Table 10. Differing features of C. acrostichoides, C. sitchensis, and C. raddeana.

| | C. acrostichoides | C. sitchensis | C. raddeana |
|---|---|---|---|
| Outline of sterile blade | typically lanceolate | triangular to ovate-lanceolate | triangular |
| Dissection of sterile segments | 2-3 pinnate | 2-4 pinnate | 3-4 pinnate |
| Ratio of width of sterile segments to width of costules | 1.5:1 to 2:1 | 1:1 to 1.5:1 | 1:1 |
| Shape of sterile segments | with 6-12 or more teeth or shallow lobes | often deeply incised or with 2-8 obovate lobes | dissected into 4-8 small obovate lobes |
| Size of fertile segments | 1.5-2 X 6-14 mm | 1.5-2 X 6-8 mm | 1.5 X 5 mm |
| Shape of fertile segments | typically linear | linear to narrowly oblong | narrowly oblong |
| Hydathode position | sunken | surficial to slightly sunken | surficial |
| Hydathode shape | obovate to linear | obovate to narrowly spathulate | obovate to spathulate |
| Rhizome & petiole scales | mostly bicolorous, with dark brown center stripe | bicolorous or concolorous | concolorous, light brown |
| Chromosome number | 2n=30 II | 2n=60 II | (not known) |
| Mean spore diameter | 54.6 µm | 59.9 µm | 48.1 µm |
| Geographic distribution | western N. America to northeastern Asia (Kamtchatka Peninsula) | Alaska & adjacent NW Canada | widely scattered in eastern Asia |

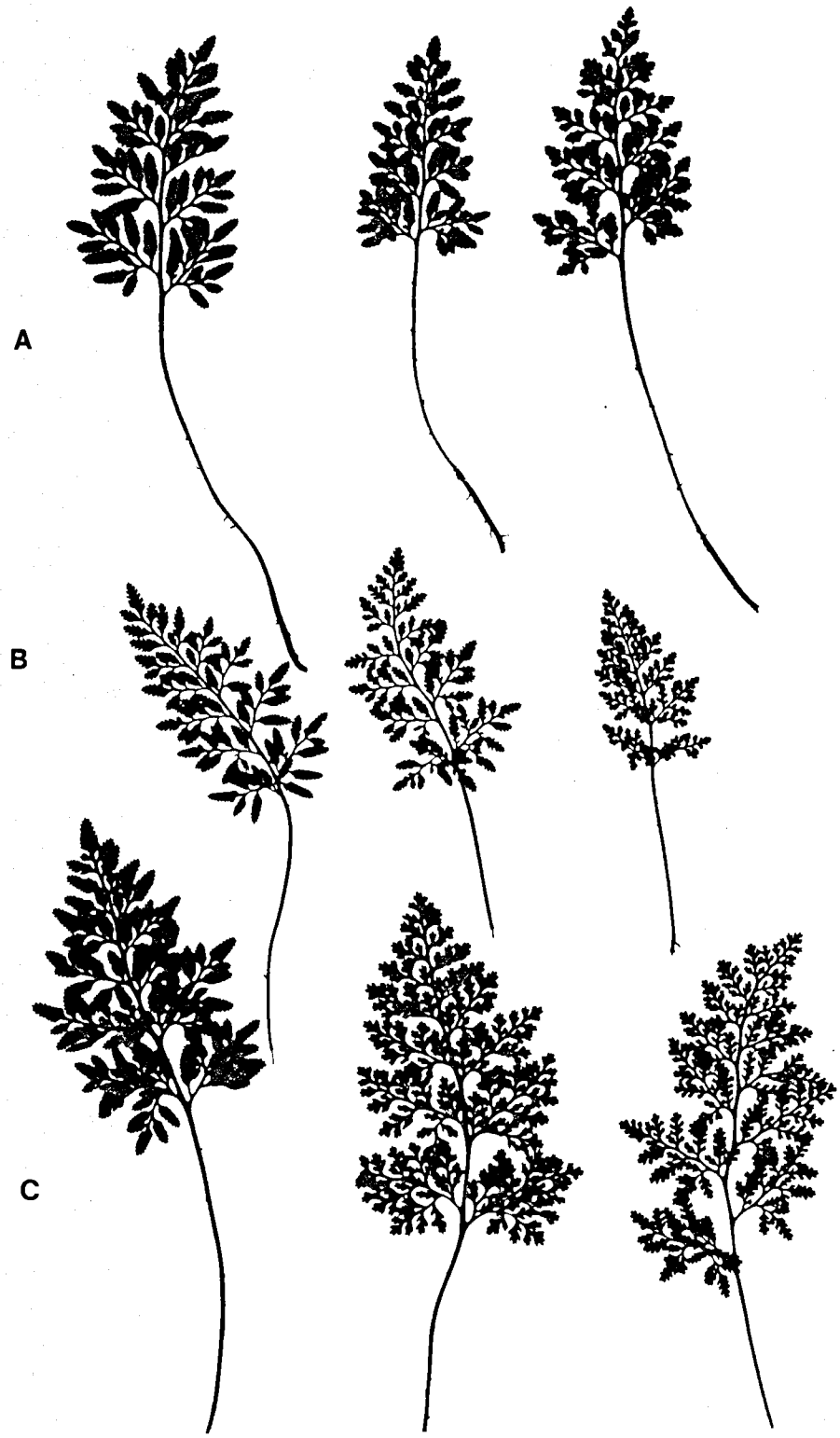


Figure 6. Silhouettes of sterile fronds of Cryptogramma from Alaska. A: C. acrostichoides (Mendenhall Glacier, Juneau, Alverson 1380). B: C. acrostichoides x sitchensis Harbor Mtn., Sitka, Alverson 1385). C: C. sitchensis (Harbor Mtn., Sitka, Alverson 1387).

Figure 7. Silhouette of Cryptogramma raddeana (Hupeh Province, China, A. Henry 6948, NY)

to which they are attached. This is intermediate between the approximately equally wide segments and rachises of C. raddeana and 1.5 to 2 times wider segments of C. acrostichoides.

Hydathodes - In C. acrostichoides, the hydathodes at vein endings on adaxial blade surfaces are distinctly sunken or depressed below the surface of the blade. They are sometimes fairly short and ovate in outline, but more often they are narrowly spathulate to oblanceolate or even linear. Hydathodes of C. raddeana are more or less flush with the lamina surface, generally short and stout, and obovate to broadly spathulate in outline. Cryptogramma sitchensis is intermediate for both characters; the hydathodes are at most slightly sunken below the lamina surface and range from obovate to narrowly spathulate in outline.

Fertile fronds - The fertile pinnules of C. raddeana are relatively small compared to those of the other two species. They are generally no more than 5 mm long and, when the margins are underrolled, are about 1.5 mm wide, giving the pinnules a narrowly oblong outline. Fertile pinnules of C. acrostichoides are typically linear and much longer, up to 14 mm long. Those of C. sitchensis are generally shorter than C. acrostichoides, 6-8 mm long, and may be linear to narrowly oblong in outline.

Scales - Scales of both the rhizome and petiole of C. raddeana are concolorous, a uniform light brown. Cryptogramma acrostichoides tends to have bicolorous scales, with a dark brown center stripe and lighter brown margins, though on scales above the base of the petiole, the dark brown portion may be reduced or lacking. C. sitchensis generally expresses the bicolorous habit of C. acrostichoides, though

the relative dominance of the dark brown center stripe may be reduced over scales of equivalent positions on C. acrostichoides.

**Chromosomes:** Reported counts of C. acrostichoides have shown this species to be uniformly diploid, with 2n = 30 pairs of chromosomes (Löve et al. 1971). The chromosome number of C. sitchensis from Alaska, not previously reported, is tetraploid, with 2n = 60 pairs of chromosomes (Figure 8A). Certain plants from Alaska populations harboring both C. acrostichoides and C. sitchensis are triploid, with meiotic chromosome figures showing 30 bivalents and 30 unpaired chromosomes (Figure 8B). The chromosome number of C. raddeana is unknown, but it must be diploid in order to have participated in the formation of C. sitchensis.

**Spore measurements:** Cryptogramma sitchensis has the largest spores of the three taxa (Table 11). Average spore diameter for C. sitchensis was 59.9 μm, with means for the 12 specimens measured ranging from 57.7 to 62.3 μm. The spores of C. acrostichoides were about 10% smaller, with the mean of 22 specimens being 54.6 μm, from a range of 49.1 to 58.9 μm. Spores of C. raddeana were smaller than either of the other species, with a mean diameter of 48.1 μm from the two specimens measured. The number of collections sampled was small because of the paucity of herbarium specimens. An analysis of variance of means of each species was significant at $\alpha = 0.001$ (Table 12). In addition, the multiple comparisons test showed the mean spore diameters of all three species to be significantly different ($P < 0.001$).

Spores of Cryptogramma species are tetrahedral (trilete), and, when viewed in quantity, the majority of spores are fully filled and

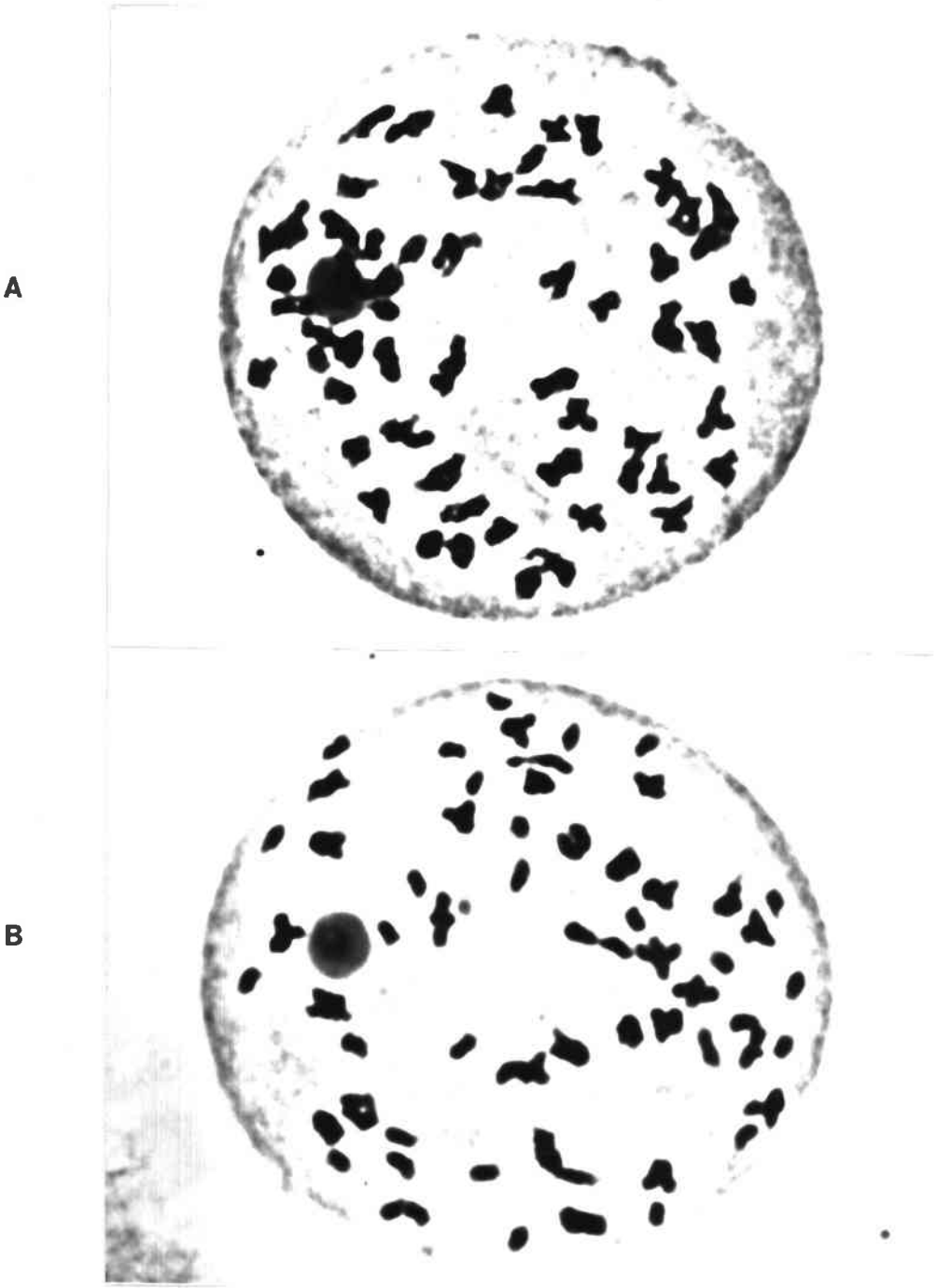


Figure 8. Chromosomes of Cryptogramma from Alaska. A: C. sitchensis (Alverson 1387). B: C. acrostichoides x sitchensis (Alverson 1385).

of relatively uniform size (Figure 9A). Certain plants from Alaska localities harboring both C. acrostichoides and C. sitchensis, however, exhibit abortive spores (Figure 9B). Indicative of sterile hybrids, plants with abortive spores are recognized by an abundance of collapsed or shriveled spores, great variability in spore size, and variable shape, particularly the occasional large, round spores (Wagner et al. 1986). This abortive-spore syndrome is diagnostic of the triploid hybrid C. sitchensis x acrostichoides; the spores illustrated in Figure 9B were obtained from the same plant that provided the triploid chromosome figure illustrated in Figure 8B.

**Electrophoresis:** Only populations of C. acrostichoides and C. sitchensis were sampled electrophoretically, because living material of C. raddeana was not available for analysis. Although populations of C. acrostichoides exhibited the variable electrophoretic profiles characteristic for an outcrossing, diploid species, all individuals of C. sitchensis from the three populations sampled were genetically identical, exhibiting identical banding patterns. Thus, at the five polymorphic loci, C. sitchensis exhibited fixed heterozygosity. Allele frequencies for C. sitchensis and the two Alaska populations of C. acrostichoides that were sampled are given in Table 13. For the majority of the 13 loci, the alleles found in C. sitchensis were a subset of those present in C. acrostichoides. For 8 of the 13 loci, C. sitchensis exhibited a fixed homozygote pattern involving the most common allozyme found in C. acrostichoides. Only the loci that were heterozygous in C. sitchensis are discussed below. For a full description of the electrophoretic profiles of C. acrostichoides, refer to Chapter 2.

Table 11. Spore size (μm) of C. acrostichoides, C. raddeana, and C. sitchensis.

| Taxon | Mean length (s.d.) | N | Range of sporophyte means | Number of sporophytes |
|---|---|---|---|---|
| C. raddeana | 48.11 (3.63) | 50 | 45.84-50.38 | 2 |
| C. acrostichoides | 54.64 (4.01) | 550 | 49.07-58.96 | 22 |
| C. sitchensis | 59.86 (3.76) | 300 | 57.71-62.31 | 12 |

Table 12. Tests comparing mean spore length of C. acrostichoides, C. raddeana, and C. sitchensis.

A. Analysis of variance

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F |
|---|---|---|---|---|
| Model (among taxa) | 2 | 8596.08 | 4298.04 | 280.70*** |
| Error (within taxa) | 897 | 13,734.63 | 15.31 | |
| Total | 899 | 22,330.71 | | |

B. GT-2 test for differences between means (pairwise comparisons)

| Pair | Difference between means (μm) |
|---|---|
| C. acrostichoides vs. C. sitchensis | 5.281*** |
| C. acrostichoides vs. C. raddeana | 6.530*** |
| C. sitchensis vs. C. raddeana | 11.748*** |

*** = P<0.001

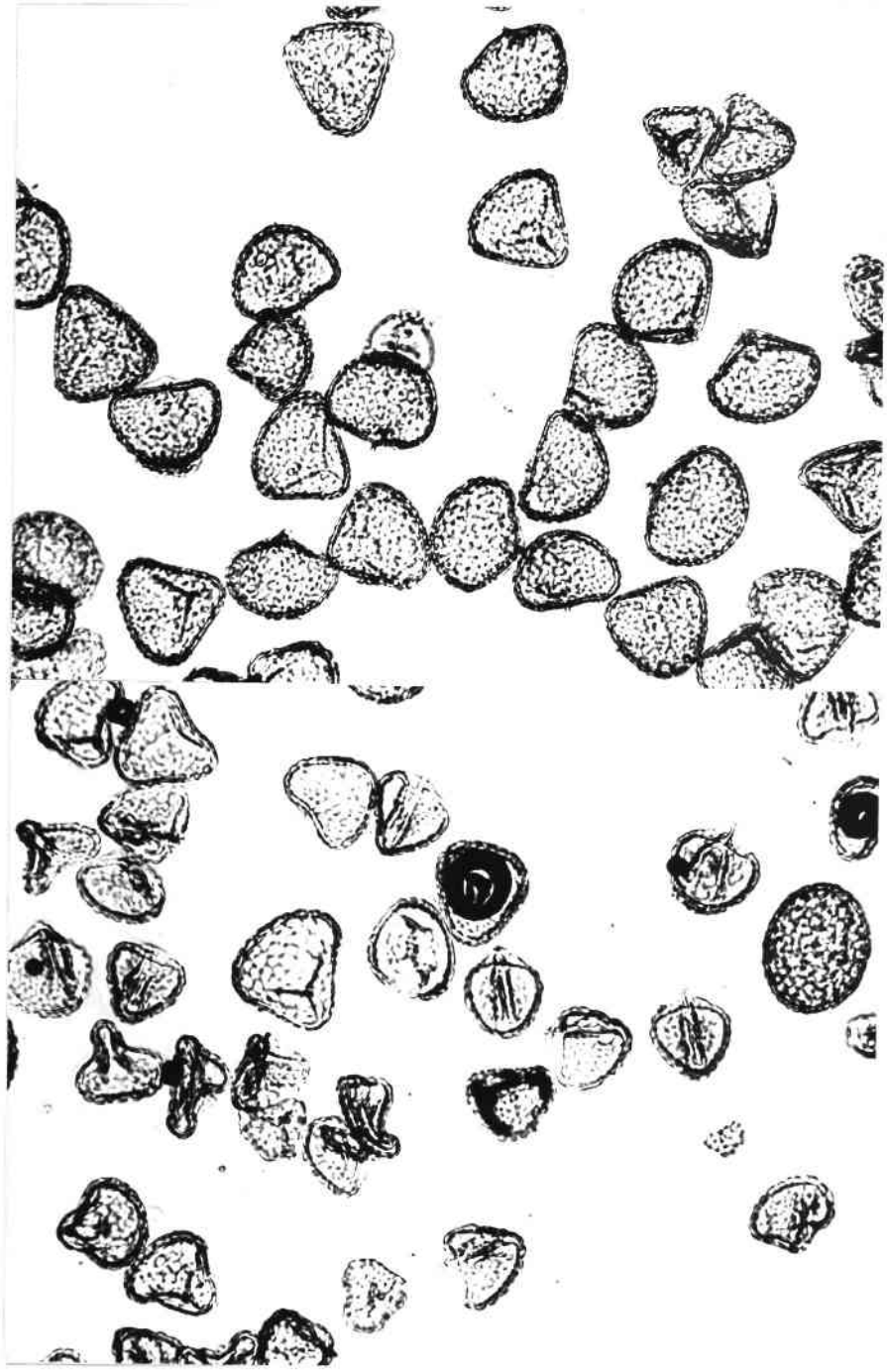


Figure 9. Light microscope photographs of spores of Cryptogramma from Alaska. A: C. sitchensis (Alverson 1387). B: C. acrostichoides x sitchensis (Alverson 1385).

Table 13. Allele frequencies and sample sizes (N) for C. acrostichoides and C. sitchensis. Localities are abbreviated as in Table 9. Loci are numbered sequentially, and alleles are lettered sequentially, beginning with the most anodally migrating band.

| | | Population | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C. acrostichoides | | | | | | | | | C. sitchensis | | |
| Locus | Allele | CO1 | UT1 | NV1 | CA1 | OR1 | WA1 | WA2 | AK1 | AK2 | AK1 | AK2 | AK3 |
| PGI-1 | b | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | N | 25 | 25 | 8 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| PGI-2 | b | | | | | | | | | | 0.50 | 0.50 | 0.50 |
| | c | | 0.02 | 0.33 | 0.34 | | | | 0.03 | | | | |
| | e | | | | 0.02 | 0.08 | 0.15 | 0.24 | 0.28 | | | | |
| | g | 1.00 | 0.98 | 0.67 | 0.64 | 0.90 | 0.75 | 0.76 | 0.63 | 1.00 | 0.50 | 0.50 | 0.50 |
| | h | | | | | 0.02 | 0.10 | | 0.06 | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| TPI-1 | a | | 0.02 | | | | | 0.03 | | | | | |
| | b | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 0.89 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | c | | | | | | | 0.08 | | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| TPI-2 | a | 0.02 | | 0.02 | | | | | | | | | |
| | b | 0.98 | 1.00 | 0.98 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | c | | | | 0.02 | | | | | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| PGM-1 | a | 0.92 | 0.84 | 0.24 | 0.04 | 0.46 | 0.75 | 0.97 | 0.91 | 1.00 | 1.00 | 1.00 | 1.00 |
| | b | 0.04 | 0.16 | 0.11 | 0.12 | 0.04 | | 0.03 | | | | | |
| | d | 0.04 | | 0.65 | 0.84 | 0.50 | 0.25 | | 0.09 | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| PGM-2 | a | 0.05 | | 0.09 | 0.08 | | | | | | 0.50 | 0.50 | 0.50 |
| | c | 0.95 | 0.92 | 0.89 | 0.92 | 0.89 | 0.95 | 0.95 | 1.00 | 1.00 | 0.50 | 0.50 | 0.50 |
| | d | | 0.08 | 0.02 | | 0.11 | 0.05 | 0.15 | | | | | |
| | N | 21 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| GOT | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 9 | 16 | 2 | 9 | 4 | 1 |
| LAP | a | | 0.02 | 0.02 | | 0.04 | | | | | | | |
| | b | 0.96 | 0.98 | 0.74 | 0.76 | 0.90 | 0.15 | 0.45 | 0.56 | 1.00 | 0.50 | 0.50 | 0.50 |
| | c | | | | | | | | 0.11 | | | | |
| | d | 0.04 | | | | 0.06 | 0.85 | 0.45 | | | | | |
| | e | | | 0.24 | 0.22 | | | 0.10 | 0.33 | | | | |
| | f | | | | | | | | | | 0.50 | 0.50 | 0.50 |
| | g | | | | 0.02 | | | | | | | | |
| | N | 25 | 25 | 23 | 25 | 24 | 10 | 10 | 16 | 2 | 9 | 4 | 1 |

Table 13 (con't)

| | | Population | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *C. acrostichoides* | | | | | | | | | *C. sitchensis* | |
| Locus | Allele | CO1 | UT1 | NV1 | CA1 | OR1 | WA1 | WA2 | AK1 | AK2 | AK1 | AK2 | AK3 |
| HK | b | 0.90 | 1.00 | 0.48 | 0.30 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.50 | 0.50 | 0.50 |
| | c | | | | | | | | | | 0.50 | 0.50 | 0.50 |
| | d | | | | 0.08 | | | | | | | | |
| | e | 0.10 | | 0.50 | 0.60 | | | | | | | | |
| | f | | | 0.02 | | | | | | | | | |
| | g | | | | 0.02 | | | | | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| SKDH | a | | | 0.02 | | | | | | | | | |
| | b | 1.00 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| IDH | a | | | 0.02 | | | | | | | | | |
| | b | | | | | | | | | | 0.50 | 0.50 | 0.50 |
| | c | 0.98 | 0.96 | 0.96 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.50 | 0.50 | 0.50 |
| | d | 0.02 | 0.04 | 0.02 | 0.02 | | | | | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| 6-PGDH | a | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |
| MDH | c | | | | | 0.02 | | | | | | | |
| | d | 1.00 | 1.00 | 0.98 | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | e | | | 0.02 | | | | | | | | | |
| | N | 25 | 25 | 23 | 25 | 25 | 10 | 19 | 16 | 2 | 9 | 4 | 1 |

HK (Figure 10A) - C. sitchensis exhibited a fixed heterozygote pattern with allele b, the common allele in seven of the nine C. acrostichoides populations, and allele c, which was not detected in any other North American species of Cryptogramma.

IDH (Figure 10B) - All individuals of C. sitchensis were heterozygotes possessing allele c, the common allele in every C. acrostichoides population, and allele b, which was not found in any other North American species of Cryptogramma.

LAP (Figure 10C) - At this locus, C. sitchensis was a fixed heterozygote with allele b, the common allele in all but one of the C. acrostichoides populations, and allele f, which was not encountered in any other species.

PGI-2 (Figure 10D) - All individuals of C. sitchensis were heterozygotes possessing allele g, the common allele in C. acrostichoides, and allele b, not present in any other North American species of Cryptogramma.

PGM-2 (Figure 10E) - The fixed heterozygote pattern exhibited here by C. sitchensis combined allele c, the common allozyme in C. acrostichoides, with allele a, which in C. acrostichoides occurred only at low frequencies in populations in California, Nevada, and Colorado, and was not detected in populations in Alaska or the Pacific Northwest.

Nei's genetic identity and genetic distance statistics comparing populations of C. sitchensis and C. acrostichoides are presented in Table 14. Interspecific comparisons of genetic identity range from 0.78 to 0.89, with a mean value of 0.85, reflecting a higher degree of similarity than that reported in Ch. 2 between C. acrostichoides and

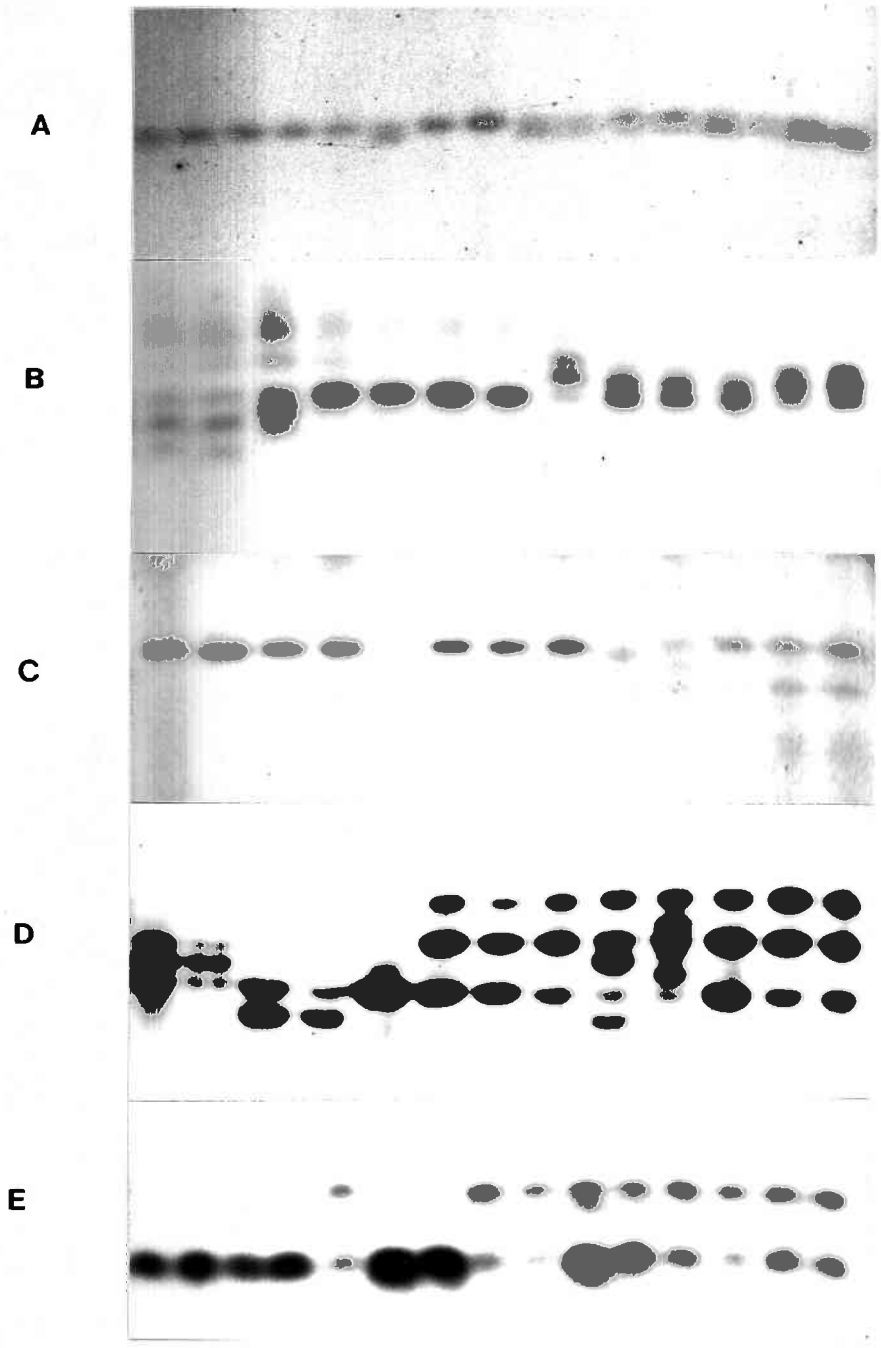


**Figure 10.** Electrophoretic banding patterns of *Cryptogramma*. A: HK. Lanes 1, 11-12, *C. acrostichoides* x *sitchensis*; lanes 2-5, 7-8, *C. acrostichoides*; lanes 6, 9-10, 13-16, *C. sitchensis*. B: IDH. Lanes 1-8, *C. acrostichoides*; lanes 9-11, *C. acrostichoides* x *sitchensis*; lanes 12-13, *C. sitchensis*. C: LAP. Lanes as in B. D: PGI-2. Lanes as in B. E: PGM-2. Lanes 1-4, 6-7, *C. acrostichoides*; lanes 10-11, *C. acrostichoides* x *sitchensis*; lanes 5, 8-9, 12-15, *C. sitchensis*.

Table 14. Nei's genetic identity (I) and genetic distance (D) statistics. Localities are abbreviated as in Table 1.

A. Pairwise comparisons for 12 populations of C. acrostichoides and C. sitchensis. Values for I are located above the diagonal, and values for D are located below the diagonal.

| | C. acrostichoides | | | | | | | | | C. sitchensis | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CO1 | UT1 | NV1 | CA2 | OR1 | WA1 | WA2 | AK1 | AK2 | AK1 | AK2 | AK3 |
| CO1 | | 0.998 | 0.923 | 0.883 | 0.976 | 0.934 | 0.974 | 0.981 | 0.922 | 0.889 | 0.889 | 0.889 |
| UT1 | 0.002 | | 0.916 | 0.874 | 0.974 | 0.929 | 0.972 | 0.980 | 0.368 | 0.886 | 0.886 | 0.886 |
| NV1 | 0.080 | 0.088 | | 0.992 | 0.957 | 0.872 | 0.892 | 0.918 | 0.341 | 0.817 | 0.817 | 0.817 |
| CA2 | 0.125 | 0.135 | 0.008 | | 0.926 | 0.833 | 0.850 | 0.876 | 0.325 | 0.780 | 0.780 | 0.780 |
| OR1 | 0.024 | 0.026 | 0.044 | 0.077 | | 0.931 | 0.953 | 0.964 | 0.351 | 0.862 | 0.862 | 0.862 |
| WA1 | 0.069 | 0.074 | 0.137 | 0.183 | 0.072 | | 0.980 | 0.931 | 0.421 | 0.837 | 0.837 | 0.837 |
| WA2 | 0.026 | 0.029 | 0.114 | 0.163 | 0.048 | 0.020 | | 0.973 | 0.417 | 0.868 | 0.868 | 0.868 |
| AK1 | 0.019 | 0.020 | 0.086 | 0.132 | 0.036 | 0.072 | 0.027 | | 0.369 | 0.874 | 0.874 | 0.874 |
| AK2 | 0.081 | 0.082 | 0.174 | 0.226 | 0.108 | 0.087 | 0.084 | 0.088 | | 0.833 | 0.833 | 0.833 |
| AK1 | 0.117 | 0.121 | 0.203 | 0.249 | 0.149 | 0.178 | 0.142 | 0.136 | 0.183 | | 1.000 | 1.000 |
| AK2 | 0.117 | 0.121 | 0.203 | 0.249 | 0.149 | 0.178 | 0.142 | 0.136 | 0.183 | 0.000 | | 1.000 |
| AK3 | 0.117 | 0.121 | 0.203 | 0.249 | 0.149 | 0.178 | 0.142 | 0.136 | 0.183 | 0.000 | 0.000 | |

B. Means of genetic identity and genetic distance statistics for pairwise comparisons given above. Standard deviations are in parentheses.

| | Within C. acrostichoides | Within C. sitchensis | Between C. acrostichoides and C. sitchensis |
|---|---|---|---|
| Genetic identity (I) | 0.938 (0.046) | 1.000 (0.000) | 0.855 (0.035) |
| Genetic distance (D) | 0.069 (0.050) | 0.000 (0.000) | 0.164 (0.041) |

C. cascadensis. These values, however, are lower than the intraspecific comparisons between populations of C. acrostichoides, most of which are >0.90, because of alleles present in C. sitchensis and not in C. acrostichoides.

## DISCUSSION

Polyploidy and the Origin of C. sitchensis. Although C. sitchensis has been recognized as a morphologically distinct entity by previous workers (Christensen, in Hultén 1937), the discovery of a tetraploid chromosome number for this taxon casts a new light on its distinctness and poses new questions on its evolutionary origin.

The two types of polyploidy distinguished by botanists are characterized by their different modes of origin. Allopolyploid taxa originate when two related but distinct evolutionary lineages (typically congeneric species) hybridize, producing a hybrid plant. This hybrid then must undergo spontaneous doubling of its chromosomes to produce the fertile allopolyploid. Polyploidy may alternatively occur via autopolyploidy, either by the doubling of chromosomes in a cell in an apical meristem of a normal diploid individual, or through the fusion of two unreduced (diploid) gametes from the same species.

Having demonstrated that C. sitchensis is a tetraploid taxon, I questioned whether it originated as a result of allopolyploidy or autopolyploidy. Both types of polyploidy are known to occur in ferns; allopolyploidy has been shown to be important in forming new fern species. This question is not just of academic interest, because the

outcome has a bearing on the appropriate taxonomic placement of C. sitchensis.

Electrophoretic data provided the strongest evidence that C. sitchensis is an allopolyploid. All individuals sampled were genetically identical, possessing the same electrophoretic phenotypes, suggesting that they all originated from a single event of hybridization and polyploidy. Eight of the thirteen loci analyzed were both monomorphic and homozygous. The other five loci that were heterozygous all exhibited the same fixed heterozygous pattern, the pattern characteristic of allopolyploids. Absence of evidence for segregation, such as homozygous loci or unbalanced heterozygotes, suggests that crossing over and segregation of genes occurs only between homologous chromosomes, and not between homeologous chromosomes. This inference can be tested directly by electrophoresis of fern gametophytes, because uniform heterozygosity at loci where sporophytes are fixed heterozygotes indicates an absence of segregation. Such data, however, were not obtained in this study.

The alleles at heterozygous loci provide further evidence for allopolyploidy in C. sitchensis. For four of the heterozygous loci (HK, IDH, LAP, and PGI-2), C. sitchensis combines the allele most common in C. acrostichoides with an allele not found in C. acrostichoides or any other North American Cryptogramma. This pattern suggests that the second genome present in C. sitchensis was contributed by a species not present in North America. The second diploid parent of C. sitchensis should have these "orphan alleles". With the fifth heterozygous locus, PGM-2, both alleles found in C. sitchensis were present in C. acrostichoides, a pattern that is

consistent with an allopolyploid hypothesis if one of the two alleles (most likely allele a, which is uncommon in C. acrostichoides) occurs also in the second parent, which thus contributed that allele to C. sitchensis.

The eight homozygous loci in C. sitchensis represent loci for which both the diploid parents contributed the same allele, indicating that the second parent is a species fairly closely related to C. acrostichoides. The two diploid genomes that combined to form C. sitchensis contributed different alleles at 5 of the 13 loci investigated, and at 4 of these divergent loci one of the pair of alleles was one that did not occur in C. acrostichoides. These 4 loci (HK, IDH, LAP, PGI-2) may be marker loci that distinguish C. acrostichoides from the second parent. This second parent is thus more closely related to C. acrostichoides than C. cascadensis, the new species discussed in Ch. 2, for which six marker loci exist that distinguish it from C. acrostichoides.

Allozyme analysis would be an ideal technique to confirm which other diploid species hybridized with C. acrostichoides to give rise to C. sitchensis, by searching for a species that has these "orphan alleles" at HK, IDH, LAP, and PGI-2, which were found in C. sitchensis but not in C. acrostichoides. Unfortunately, living material of other taxa could not be obtained to perform this analysis. Still, morphological data from herbarium specimens is sufficient to develop a hypothesis on the origin of C. sitchensis. These data suggest that C. raddeana best fits the expected morphology of the second diploid parent.

Cryptogramma raddeana, a species known from scattered stations in the eastern Soviet Union and China, appears to be allied to C. acrostichoides by virtue of its coriaceous lamina and marcescent sterile leaves. It differs from that species in frond outline, degree of dissection, shape of ultimate segments, shape and position of hydathodes, size of fertile pinnules, and color of rhizome scales. In all of these characters, C. sitchensis occupies a position between C. raddeana and C. acrostichoides (Table 10).

Spore size is one character in which C. sitchensis does not occupy a position intermediate between the two putative parents, but the spore data do fit a polyploid hypothesis. Often spores of polyploid species are larger than their diploid relatives because of the increase in cell size with an increase in ploidy (Barrington et al. 1986). Mean spore diameter of C. sitchensis is significantly greater than either of the putative parents, 5.3 μm greater than those of C. acrostichoides and 11.8 μm greater than those of C. raddeana. Apparently, ploidy is not the only factor that affects spore size in Cryptogramma, for the difference in size between the two putative parents is greater than the difference between C. acrostichoides and C. sitchensis. Increase in spore size from polyploidy in C. sitchensis, however, is in a sense an increase over the average (that is, intermediate condition) of spore diameters of the two parents. Taking 51.35 μm as the average of the spore diameters of C. acrostichoides and C. raddeana, we find that the 59.9 μm diameter of C. sitchensis is 1.16 times greater than 51.35 μm. This difference is somewhat less than expected if, as Barrington et al. (1986) assume, a doubling in chromosome number brings a doubling of cell volume,

because a doubling in the volume of a sphere brings about an increase in diameter of 1.26.

Although the chromosome number of C. raddeana is unknown, the small spore size of C. raddeana suggests that it is diploid, the condition necessary for the species to have participated as the second parent of C. sitchensis.

In addition to the morphological evidence, geographical circumstances also point to C. raddeana as having joined with C. acrostichoides to form C. sitchensis. First, no other species of Cryptogramma section Cryptogramma occur in or near the trans-Beringian region. Cryptogramma acrostichoides is a very widespread species in western North America, but it also extends along the Aleutian Islands and on into eastern Asia locally on the Kamtchatka peninsula (Hultén 1927). Although at present the range of C. raddeana (Siberia to China) does not overlap with that of C. acrostichoides, the possibly exists that, at some time in the past, the two species were sympatric and hybridization was able to occur, with subsequent chromosome doubling resulting in the formation of C. sitchensis.

Current practice among fern systematists is to confer species status to fertile allopolyploids; many examples of reticulate complexes have been described in other genera (Lellinger 1985), and this taxonomic approach is widely applied. The data described here are sufficient to suggest that treatment of C. sitchensis as a distinct species is the most appropriate approach.

Hybridization Between C. sitchensis and C. acrostichoides. One significant result of this study has been the identification of hybridization between C. sitchensis and C. acrostichoides where the

ranges of the two species overlap. Because the hybrids involve an allopolyploid species and one of its diploid progenitors, they are considered to be backcross hybrids.

These hybrids are easily distinguished from the two species present in Alaska in three ways: abortive spores, chromosome number, and electrophoretic phenotypes. Unfortunately, distinguishing hybrids from either of the parental species on the basis of morphology alone is difficult. Chromosome squashes require special equipment and expertise, as does electrophoretic analysis; in addition, both techniques require living material. Spores can be obtained from dried herbarium specimens, but because the spores of Cryptogramma mature relatively late in the season, most herbarium specimens lack mature spores. Thus, no certain means by which to identify these hybrids is available, even though they are very important in understanding the systematics and evolution of this complex.

Once identified, however, hybrids provide chromosome-pairing data, which may indicate whether or not a diploid species has contributed to the formation of a particular polyploid. The meiotic chromosomes of the C. sitchensis x acrostichoides hybrid showed the formation of n bivalents and n univalents, where n = 30 (fig. 8B), a pattern characteristic of backcross hybrids between allopolyploid species and one diploid progenitor (Manton 1950). This configuration is interpreted to result from the occurrence of two sets of homologous chromosomes in the hybrid, one set contributed by the polyploid parent and one set contributed by the diploid parent. The set of univalents represents the genome of the second diploid progenitor contributed to the hybrid by the polyploid parent; it represents chromosomes

homeologous (structurally different, and thus unable to form pairs at meiosis) rather than homologous to those contributed by the first diploid parent.

While this widely held hypothesis regarding the mechanism of chromosome pairing has recently been called into question (Jackson 1982), the chromosome pairing behavior observed in the hybrid C. acrostichoides x sitchensis is in accordance with traditional criteria for distinguishing allopolyploids from autopolyploids.

While hybrids are of importance because of the insights they may provide into evolutionary patterns, they are also are of importance to taxonomists and field botanists. Though sterile fern hybrids are usually rare and sporadic in occurrence, evidence from enzyme electrophoresis shows that the C. acrostichoides x sitchensis hybrid may occur in surprisingly high proportions in populations containing both parent species. Hybrids are easily recognized on electrophoretic gels because of their distinctive banding patterns, and in the process of electrophoretically examining several population samples, it was possible to assess the proportion of hybrids occurring in each population.

The electrophoretic banding patterns allowed identification of triploid hybrids in two ways. For loci in which the pool of potential parental plants of C. acrostichoides possessed alleles not found in C. sitchensis, a single triploid hybrid could express three different alleles. This pattern was found with PGI-2 (Figure 10D, lanes 9 and 10), in which five bands, representing three allozymes and two heterodimers, were expressed by hybrid plants.

The second means of identifying hybrids was by dosage effects. With loci in which C. sitchensis was heterozygous, a hybrid involving a C. acrostichoides gametophyte that carried the same allele as one of the alleles in C. sitchensis would still exhibit the same banding pattern as C. sitchensis. Only the two alleles would be expressed, but, as shown in Figure 10D, lane 11, two doses of one allele (here, PGI-2, allele g) would be present, compared to only one dose of the other allele (allele b). Thus, the band representing allele b is relatively faint compared to the band representing allele g.

Population samples were obtained from two Alaska localities harboring both C. sitchensis and C. acrostichoides. Five hybrids were identified from a sample of 30 plants from Baranof Island (AK1), amounting to 16.7% of the population. On Harbor Mountain, near Sitka (AK2), 3 of the 9 plants sampled were hybrids, forming 33.3% of the population.

The frequent occurrence of hybrids creates an impression of a morphological intergradation between C. acrostichoides and C. sitchensis, which would not exist if hybrids were never formed. This is a matter of practical importance to field botanists and taxonomists who wish to identify plants in the field or herbarium. For these workers, examining the spores of their specimens is essential, for once hybrids are identified, separating C. sitchensis from C. acrostichoides is much easier.

Interpretation of Morphological Variation. Lacking the insights gained from the techniques described above, earlier workers (e.g., Christensen, in Hultén 1937) supported the interpretation of C. sitchensis as a variety of C. acrostichoides because they could find

no sharp break between the morphology of the two taxa. Having determined that backcross hybrids are the key, the interpretations of such workers can be accorded their proper place in history.

Still, the distinctions between taxa are not as absolute as in many other fern genera. I believe this is due in large part to the pattern of fertile-sterile dimorphism found in the genus. For example, in combining C. sitchensis as a variety of C. acrostichoides, Christensen (in Hultén 1937) noted that in one plant can be found, "...besides the finely cut leaves of sitchensis some older ones...which agree with those of the type [e.g. C. acrostichoides]. Such leaves...are, so to speak, intermediate between the sterile and fertile ones and are not infrequently met with in other forms of the genus...." This is because the type of dimorphism that occurs in Cryptogramma is not an absolute type, but gradual; in addition to the extremes of sterile and fertile fronds, a range of morphological intermediates also occurs (Figure 6). Because fertile fronds of Cryptogramma are conservative--that is, relatively uniform from species to species--the sterile leaves that are intermediate in the continuum from extreme fertile to extreme sterile leaves will tend to lose the distinctive morphology that characterizes the extreme fertile leaves of each species. But to argue that the existence of these "intermediate" sterile leaves indicate an evolutionary closeness between different taxa in not an accurate use of the evidence; all that is indicated is that fertile leaves are more conservative than sterile ones. Accordingly, systematists should look to the most extreme sterile leaves, i.e. those that are most differentiated, for making systematic comparisons.

The situation is somewhat more complicated in allopolyploid taxa because they combine expression of two divergent genomes. Normally, the phenotype of a hybrid or allopolyploid is assumed to be a combination of the two parental genomes, in which the extremes of each species are diluted by the influence of the other species. But this may not be true in Cryptogramma, where a single plant produces many leaves, and, because of the non-absolute fertile-sterile dimorphism, each apparently is the result of a different transcription of the genetic information coded in the species' chromosomes. Thus, the part of the C. acrostichoides genome that codes for sterile frond dissection, which by definition is also present in C. sitchensis, may be expressed discretely and separately from the expression of the C. raddeana genome, in individual fronds of plants of C. sitchensis.

A third consideration may account for the presence of multiple forms of sterile fronds in plants of C. sitchensis. When spores were examined from several samples that produced electrophoretic banding patterns identifying the plants as triploid hybrids, the spores turned out to be normal and well-formed, rather than shriveled and abortive, as would be expected for hybrids. One explanation could be human error, that the fertile and sterile fronds came from different plants and were accidentally mixed up. Another, more likely hypothesis is that two different plants, one hybrid and one fertile species, were growing together in the same clump. The mechanism by which this could happen becomes clear when the process by which the triploid hybrids come about is considered. Triploid hybrids are formed only when two gametophytes, one from the diploid species and one from the tetraploid species, develop and mature in close proximity. But at the same time

the C. sitchensis gametophyte is providing sperm to fertilize an egg on the C. acrostichoides gametophyte, the C. sitchensis gametophyte may also be self-fertilizing, so that each gametophyte produces a sporophyte, one a triploid hybrid and one the tetraploid C. sitchensis. By the time the plants grew to mature size, distinguishing the individual plants might be impossible; the entire clump could be interpreted as a single plant and, thus, misleadingly confer hybrid morphology to the tetraploid species. Taken in the extreme, by these means a single Cryptogramma clump could combine a diploid individual of C. acrostichoides and a tetraploid individual of C. sitchensis! Such clumps could mistakenly be considered as evidence for a lack of clearcut morphological distinction between the two taxa.

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# Chapter IV

## Systematic Treatment

Cryptogramma R. Brown in Richardson, Bot. App. in Franklin, Narr. Journey Polar Sea. 767. 1823. Parsley fern, Rock-brake, Cliff-brake. [From Greek cryptos, hidden + gramme, line]

Plants epipetric or terrestrial in rocky habitats. Rhizomes stout, decumbent to erect, or slender and creeping; with lanceolate scales. Fronds dimorphic, the fertile fronds exceeding the sterile, scattered or densely tufted. Petioles dark brown below, light brown to green above; lanceolate scales present at base, becoming sparse above. Blades triangular, lanceolate, or elliptic; 2-4 times pinnate; deciduous or evergreen. Segments of sterile fronds ovate, elliptic, obovate, or flabellate; dentate or shallowly to deeply lobed; fertile segments strongly differentiated from sterile ones, lanceolate to linear, revolute or with reflexed margins forming false indusia, protecting young sporangia, then often becoming plane at maturity. Veins free, often ending in hydathodes on the adaxial surface. Sori borne on or near vein endings, round to oblong, often becoming confluent at maturity; spores tetrahedral, yellow, perispore verrucose; stalked, capitate, unicelluar receptacular paraphyses, and yellowish, filamentous farina often present in sorus.

Between 8 and 11 species worldwide; 4 in North America, the others in temperate regions of Europe, Asia, and South America.

1. Rhizomes slender, creeping; fronds scattered, delicate and ephemeral; petioles mostly dark brown, greenish only on upper 1/2 to 1/3; plants often in calcareous habitats [section Homopteris (Rupr.) C. Chr.].....1. C. stelleri

1. Rhizomes stout, decumbent to erect, multicipital; fronds strongly tufted, soft to coriaceous, but present throughout growing season; petioles dark brown only near base; plants mostly of non-calcareous habitats [section Cryptogramma].

2. Fronds soft, thin and translucent when dried; deciduous in autumn, not marcescent; hydathodes surficial; trichomes absent from adaxial frond surface.....2. C. cascadensis

2. Mature fronds coriaceous, opaque; evergreen and marcescent; hydathodes on dried fronds sunken below surface; small, appressed, cylindrical trichomes present in sulca, costae, and costules of adaxial frond surface.

3. Sterile fronds 2-3 times pinnate; segments oblong to ovate-lanceolate, with 6-12 or more teeth or shallow lobes; widespread in N. America.....3. C. acrostichoides

3. At least some sterile fronds 3-4 times pinnate, segments obovate, with 2-6 deep lobes; Alaska and adjacent NW Canada.....4. C. sitchensis

1. Cryptogramma stelleri (S.G. Gmelin) Prantl, Bot. Jahrb. Syst. 3:413. 1882. - slender rock-brake or cliff-brake, Steller's rock brake, fragile rock-brake.

[Pteris stelleri S.G. Gmelin, Novi. Comment Acad. Petrop. 12. 519, pl. 12, fig. 1. 1768. (presumably collected or observed by

Steller, the locality unspecified); Cryptogramma gracilis (Michaux) Torrey, Rep. Bot. Dept. Surv. N.Y. Assembly, 50:196. 1839; Pteris gracilis Michaux., Fl. Bor. Am. 2:262. 1803.]

Rhizomes creeping, slender, up to 1.5 mm wide, greenish-yellow, succulent-brittle, shriveling in the second year following emergence of fronds; sparsely clothed with hyaline-reticulate scales. Fronds scattered, the fertile fronds 5-20 X 1.5-5 cm, sterile fronds 3-15 X 1.5-3 cm; yellow-green; glabrous. Petioles dark brown from the base to more than 1/2 way to the blade, becoming greenish above. Blade broadly lanceolate to ovate-lanceolate, pinnate-pinnatifid to bipinnate, thin and membranous, hydathodes clavate, surficial, often poorly developed or absent. Segments of sterile fronds ovate-lanceolate to flabellate; segments of fertile fronds lanceolate to linear, often only partially differentiated from sterile ones. Sori round to oblong, often discreet, segment margins reflexed, forming continuous false indusia. $2n$ = 30 II (Wagner 1963).

New growth arising in spring, dying back by late summer. Sheltered calcareous cliff crevices and rock ledges, typically associated with coniferous forest or other boreal habitats, from near sea level to 3000 m. Alberta, British Columbia, New Brunswick, Newfoundland, Nova Scotia, District of Mackenzie, Ontario, Prince Edward Island, Quebec, Yukon, Alaska, Colorado, Connecticut, Illinois, Iowa, Massachusetts, Maine, Michigan, Minnesota, Nevada, New Hampshire, New Jersey, New York, Oregon, Pennsylvania, Utah, Vermont, Washington, West Virginia, Wisconson; Asia.

2. Cryptogramma cascadensis E. Alverson, Amer. Fern J., in press. (Alverson 876, 5 km NW of Snoqualmie Pass, Cascade Mts., King Co., Washington) - Cascade parsley fern.

Rhizomes decumbent to erect, strongly multicipital, stout, 4-8 mm wide including old attached frond bases; bearing broadly lanceolate to linear scales up to 6 mm long, the scales often bicolorous, dark brown medially, light brown along the margins. Fronds strongly tufted, the fertile ones erect, 5-25 X 1-4 cm, the sterile ones spreading, 3-20 X 1-6 cm; grass-green, deciduous, soft, glabrous. Petioles ca 1 mm wide when dry, collapsing and strongly furrowed; mostly green to stramineous, dark brown only at very base; base of petiole with scales similar to those of the rhizome or more or less concolorous, with the dark brown central median stripe reduced or absent; scales becoming sparse above. Blades deltoid to ovate-lanceolate, 1/2 to equaling petiole, 2-3 times pinnate, thin and translucent when dried. Segments of sterile fronds cuneate-based, oblong to flabellate, 2-10 mm long, ca. 1/2 as wide as long, the apical 1/2 to 1/3 regularly dentate, and often more deeply incised every 2nd to 4th tooth; segments of fertile fronds ascending to erect, strongly differentiated from sterile fronds, linear, 3-12 X 1-2 mm; hydathodes typically elongate, clavate, not sunken below frond surface. Sori coalescing at maturity, fertile segments revolute, protecting sporangia, at maturity often becoming plane with drying and exposing sporangia. $2n$ = 30 II (Alverson in press.)

New growth arising in spring, spores maturing in late summer and autumn, fronds dying back in autumn. Talus slopes and cliff crevices, often on igneous rocks, typically in relatively mesic habitats, mostly

subalpine, 900-3500 m. British Columbia, California, Idaho, Montana, Oregon, Washington.

3. Cryptogramma acrostichoides R. Brown in Richardson, Bot. App. in Franklin, Narr. journey Polar Sea 754, 767. 1823. (Richardson, "in shady rocky woods, between latitude 56 and 60 N, the Nelson or Mackenzie drainage Systems, Canada) - American parsley fern.

[Cryptogramma crispa (Linnaeus) R. Brown ex Hooker var. acrostichoides (R. Brown) C.B. Clarke; Cryptogramma crispa (Linnaeus) R. Brown ex Hooker ssp. acrostichoides (R. Brown) Hultén.]

Rhizomes decumbent to erect, multicipital, stout, 10-20 mm wide, including attached frond bases; bearing broadly lanceolate scales up to 6 mm long and 2 mm wide at the base; scales bicolorous, dark brown medially, light brown along the margins. Fronds strongly tufted, the fertile ones erect, 5-25 X 1-5 cm, the sterile fronds spreading, 3-17 X 1-5 cm, sterile fronds green to verdigris-green, evergreen and marcescent; small, appressed, cylindrical trichomes scattered along sulca of petiole and along costae and costules of the adaxial blade surface. Petioles firm and straw-like, not collapsed, 1-2 mm wide; green to stramineous, dark brown only at very base; base of petiole with scales similar to those of rhizome or more or less concolorous, with the dark brown median stripe less prominent; scales becoming sparse above. Blades triangular to ovate-lanceolate, 2/3 to equaling the length of the petiole; 2-3 times pinnate; abaxial surface with a spongy texture, lighter green than adaxial surface; coriaceous, opaque. Segments of sterile fronds cuneate-based, oblong to ovate-lanceolate, 4-6 X 2-5 mm, from 1/4 to equally as wide as long; apical

2/3 to 1/2 crenate to dentate, often somewhat more deeply incised every 2nd tooth; segments of fertile fronds horizontal to ascending, strongly differentiated, linear, 3-12 X 1-2 mm; hydathodes mostly relatively short, obovate to spathulate or clavate, sunken below frond surface. Sori coalescing at maturity, fertile segments revolute, protecting sporangia, at maturity often becoming plane with drying and exposing sporangia. 2n = 30 II (Löve et al. 1971).

New growth arising in spring, spores maturing in summer, sterile fronds evergreen, dying back the following summer. Non-calcareous cliff crevices, rock outcrops, and talus, often in relatively dry habitats; typically montane but ranging from lowland to alpine, 0-3700 m, depending upon location. Alberta, British Columbia, District of Mackenzie, Manitoba, Ontario, Saskatchewan, Yukon, Alaska, Arizona, California, Colorado, Idaho, Michigan, Minnesota, Montana, New Mexico, Nevada, Oregon, Utah, Washington, Wyoming; reported from Baja California, Mexico; also in eastern Asia.

4. Cryptogramma sitchensis (Ruprecht) T. Moore, Index fil. lxvii. 1857. - Alaska parsley fern.

[Allosorus sitchensis Ruprecht, Distr. crypt. vasc. Ross. (Beitr. Pflanzenk. Russ. Reiches) 3:47. 1845. (Mertens, Sitka, Alaska, possibly collected above Sitka on Mt. Verstovia). Cryptogramma acrostichoides R. Brown var. sitchensis (Ruprecht) C. Christensen]

Rhizomes decumbent to erect, multicipital, stout, 10-20 mm wide, including attached frond bases; bearing broadly lanceolate scales up to 7 mm long and 2 mm wide at the base; scales bicolorous, dark brown medially, light brown along margins. Fronds strongly tufted, the

fertile ones erect, 5-25 X 1-4 cm, the sterile fronds spreading, 3-17 X 1-5 cm, sterile fronds bright green, evergreen and marcescent; small, appressed, cylindrical trichomes scattered along sulca of petiole and along costae and costules of adaxial blade surface. Petioles firm and straw-like, not collapsed, 1-2 mm wide; green to stramineous, dark brown only at very base; base of petiole with scales similar to those of rhizome or more or less concolorous, with the dark brown median stripe less prominent; scales becoming sparse above. Blades triangular to ovate-lanceolate, 2/3 to equaling the length of the petiole; two types of sterile blades, one type 2-3 times pinnate, the other 3-4 times pinnate; abaxial surface with a spongy texture, lighter green than adaxial surface; coriaceous, opaque. Segments of less dissected sterile fronds ovate-lanceolate, regularly dentate to incised with 8-16 teeth or lobes; segments of more finely dissected sterile fronds pinnatifid with 4-8 small, obovate lobes with acute apices; segments of fertile fronds ascending, strongly differentiated, linear, 3-10 X 1-3 mm; hydathodes obovate to spathulate, only slightly sunken below the frond surface. Sori coalescing at maturity; fertile segments revolute, protecting sporangia, at maturity often becoming plane with drying and exposing sporangia. 2n = 60 II (Alverson, unpublished data).

New growth arising in spring, spores maturing in late summer; sterile fronds evergreen, senescing the following spring and summer. Cliff crevices and talus slopes, lowland to alpine, from near sea level to 1800 m. British Columbia, District of Mackenzie, Yukon, Alaska.

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# Chapter V

## Conclusions

A multifaceted approach has provided new insights into the systematic and evolutionary relationships of the parsley-ferns, Cryptogramma section Cryptogramma, in western North America. These results are one example of a series of dramatic new perspectives into the systematics of various fern groups that have resulted from application of electrophoretic techniques in conjunction with critical field studies.

Separation of C. cascadensis from C. acrostichoides is supported by careful examination of subtle, but constant morphological differences, particularly the deciduous vs. evergreen fronds, surficial vs. sunken hydathodes, absence vs. presence of laminar trichomes, and small vs. large spores. The two species show a high degree of genetic differentiation at loci coding for electrophoretically assayed enzymes. The two species exhibit low genetic identities, which are comparable to other congeneric fern species. Though both species possess outcrossing breeding systems, genetic differentiation is maintained even when the the two species grow together, suggesting that they form reproductively isolated populations.

The Alaskan parsley-fern that has been treated as C. acrostichoides var. sitchensis is a tetraploid, the first reported case of polyploidy in North American Cryptogramma. Evidence from morphology (particularly the degree of frond dissection), and from enzyme electrophoresis (in particular fixed heterozygosity) suggest

that C. sitchensis is an allotetraploid, combining the genomes of C. acrostichoides and a second diploid species. Further cytological and electrophoretic evidence are needed to confirm the role of C. raddeana as the second diploid parent in this case of reticulate evolution.

Future research into the biology and evolutionary history of Cryptogramma can build upon the data base of morphological and electrophoretic evidence presented here. Comparison of breeding systems of C. cascadensis and C. acrostichoides may cast some light onto patterns of their geographic distribution. While both species were primarily outcrossing, intragametophytic fertilization may have predominated in some of the populations of C. acrostichoides that were studied. This may render C. acrostichoides more suited to long distance dispersal, which requires initiation of a population from a single gametophyte. In comparison, if C. cascadensis is indeed exclusively outcrossing, the presence of two gametophytes would be required to initiate a new population. These characteristics of breeding systems may have enabled C. acrostichoides to colonize the entire portion of western Canada following Pleistocene glaciation, while C. cascadensis remained primarily restricted to areas south of the limit of continental glaciation.

Also of interest would be a more intensive study of electrophoretic variation in C. acrostichoides, incorporating both greater sample sizes and greater number of population samples distributed throughout the species' range. Such a study would have a bearing upon the possible incidence of "incipient speciation" occurring in the southwestern U.S., as well as provide opportunities

for comparing relatedness of populations with possible routes of migration.

As stated above, further evidence is needed to confirm the role of C. raddeana in the formation of C. sitchensis. Evidence obtained from further electrophoretic work could show whether there have been multiple origins for C. sitchensis (all populations sampled here were genetically identical and thus originated from a single hybridization event). Such evidence could also give an idea of the relative age of this allopolyploid, by comparing the alleles present in C. sitchensis with their current frequencies of occurrence at the same loci in the two parental species.

Ultimately an approach similar to the one described here should be applied to the investigation of evolutionary patterns in parsley-ferns worldwide. Only then will the question of the appropriate taxonomic treatment (separate species, or varieties of a single worldwide species) be settled to the satisfaction of the most taxonomically conservative workers. Such an investigation would also provide insights into the relationships of taxa on different continents. For example, this study suggests that the parsley-ferns can be separated into two groups: species with evergreen fronds, and species with deciduous fronds. Whether these similarities (such as between C. cascadensis and the Eurasian C. crispa ) are a reflection of common ancestry, or of convergent evolution in similar environments, is an interesting question for future research.

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# APPENDIX

# APPENDIX I

## Collection Data from Herbarium Specimens of C. cascadensis Examined in the Course of this Study

CANADA. **British Columbia:** Selkirk Range, Macoun s.n., 20 Aug 1885 (CAN, NY); Mt. Cheops, 7500 ft, Heacock 459, 13 Aug 1904 (COLO, GH, NY, RM, US); Rogers Pass, Mt. Cheops, Heacock s.n., 20 Aug 1904 (WTU); Revelstoke, Eva Lk., 7000 ft, Mrs. McT. Cowan s.n., 13 Jul 1937 (V); Mt. Revelstoke, 6000 ft, Eastham 16,041, 6 Aug 1947 (DAO); The Lions, 4600 ft, Peterson s.n., 27 Jul 1961 (UBC); Mt. Revelstoke N.P., between Millar Lk. and Jade Lk., 7000 ft, Soper, Shchepanek & Szczawinski 12,491, 15 Aug 1969 (CAN, CAS, V); Glacier N.P., Avalanche Mtn., 7300 ft, Haber & Shchepanek 1662, 28 Jul 1972 (CAN); Slesse Mtn., SW of Chilliwack, Ogilvie & Ceska 18,346, 18,398, 13 Sep 1984 (V-mixed with C. acrostichoides); Mt. Lindeman, W of Chilliwack Lk., Ceska & Ogilvie 18,487, 14 Sep 1984 (V). U.S.A. **California:** Eldorado Co., Susie Lk., 7650 ft, McGregor 108, 13 Aug 1909 (CAS, NY, US); Heather Lk., above Fallen Leaf, 7900 ft, Jepson 8167, 28 Aug 1918 (ASU, JEPS); Camp Sacramento, 6000-8000 ft, Vortride s.n., Jul-Aug 1931 (CAS); E of Medley Lks., Desolation Wilderness, 8500 ft, Robbins 1293, 25 Jul 1943 (UC-mixed with C. acrostichoides); Blue Mtn., 8722 ft, Smith & Neilson 2568, 26 Jul 1970 (JEPS); Echo Peak, 8895 ft, Smith & Neilson 2509, 27 Jul 1970 (JEPS); Pyramid Pk., 9983 ft, Smith & Neilson 2621, 20 Aug 1970 (JEPS); Fresno Co., on Mono Trail, 9000-10,000 ft, Bolander 6241, Sep 1866 (CAS, GH, NY, UC, US, WS); Vidette Meadows, Campbell s.n., 25 Jul 1916 (CAS); Kaiser Pk., 10,000 ft,

Grant 1439, 19 Jul 1918 (JEPS-mixed with C. acrostichoides); Black Peak, 9600 ft, Quibell 2947, 25 Jul 1953 (DAO, WTU); lakelet 1 mi SE of Silver Pk., 10,700 ft, Quibell 4148, 5 Aug 1954 (JEPS-mixed with C. acrostichoides); between Gold Lk. & Mono Mesa, Mono Pass, 11,000-12,000 ft, Thomas 6890, 3 Sep 1959 (CAS, RSA, US); Modoc Co., below Eagle Lk., Warner Mts, 7600 ft, Jepson 7961, 13 Aug 1918 (JEPS, MICH); Mono Co., Slate Cr. basin, E of Mt. Conness, 11,000 ft, Clausen 993, 9 Sep 1934 (CAS, US); Saddlebag Lk, 10,000 ft, Lewis s.n., 31 Aug 1940 (DAO); below McMillan Lk., 8800 ft, Alexander & Kellogg 4165, 27 Aug 1944 (DAO, UC); 1 mi E of Tioga Pass, Warnock 125, 28 Aug 1951 (RSA); Nevada Co., 0.5 mi W of Basin Pk., 8200 ft, True 6997, 6 Sep 1971; Plumas Co., Spanish Peak, Austin 318, Jul 1876 (UC); Mt. Harkness, Lassen Volcanic N.P., 7400 ft, Gillett 887, 23 Jul 1957 (CAS, JEPS, MICH); Shasta Co., Lassen's Peak, Bruce s.n., Aug 1896 (US); Mt. Lassen, timberline, Copeland 1424, 9 Aug 1931 (CAS, POM, UC); Bumpass Peak, 8700 ft, Jensen 385, 6 Oct 1934 (UC); Terrace Lk, Lassen Volcanic N.P., 7725 ft, Leschke s.n., 22 Sep 1956 (CAS); NE shoulder of Lassen Peak, 8300 ft, Gillett 990, 8 Aug 1957 (CAS, JEPS); Siskiyou Co., Mt Shasta, 8000 ft, Brewer 1394, 13 Sep 1862 (UC); Mt. Shasta, 8500 ft, Howe s.n., 4 Aug 1894 (NY, UC); Horse Camp, Mt. Shasta, 8000 ft, Grant 5098, Sep 1902 (CAS); Shasta, 8500 ft, Copeland 7924, 16 Jul 1903 (MICH); Mt. Shasta, Bohmanubon s.n., Jul 1926 (CAS); Virginia Lakes, Leach 3905, 9 Jul 1930 (ORE); above Horse Camp, Mt. Shasta, 8250 ft, Cooke 11,502, 18 Aug 1938 (CAS, GH, UC); valley of Horse Camp Cr., Mt. Shasta, Cooke 13,832, 8 Aug 1939 (NY); Deep Lk., Marble Mts., Wilkes s.n., Aug 1940 (CAS); above Horse Camp, Mt. Shasta, Cooke & Cooke 17,639, 2 Sep 1946 (WS, WTU), Cooke 17,824, 20 Aug 1947 (WS,

WTU); Mt. Shasta, 8000 ft, Howe 3401, 14 Aug 1948 (SD); Paradise Lk., Marble Mts., Alexander & Kellogg 5835, 22 Jul 1949 (UC, US); E of Ski Bowl Lodge, Mt. Shasta, 7700 ft, Bacigalupi & Bacigalupi 7188, 1 Jul 1959 (JEPS); Panther Meadows, Mt. Shasta, 8300 ft, Munz 24,192, 22 Aug 1960 (RSA, UC); above Ski Lodge, Mt. Shasta, 7800-8000 ft, Thorne & Oettinger 38,980, 10 Aug 1969 (RSA); at ski lift, Mt. Shasta, 2450 m, Fosberg 51,438, 17 Aug 1969 (US); Mt. Shasta, 7000 ft, van Royen 10,289, 17 Aug 1969 (US); Upper Boulder Cr. Lake, Ferlatte & Howard 1291, 9 Aug 1970 (JEPS, RSA); Tehama Co., Brokeoff Mtn., 9000 ft, Grinnell s.n., 25 Jul 1925 (JEPS); Brokeoff Mtn. Trail, Lassen Volcanic N.P., 7000 ft, Gillett 1056, 13 Aug 1957 (CAS, JEPS, MICH); Tuolumne Co., W. of Fairview Dome, Yosemite N.P., Hall 170, 22 Aug 1922 (UC); county unknown, Sierra Nevada Mts., Brewer s.n., 1863 (NY); Summit CPRR, Curran s.n., Sep 1883 (NY); "Big Trees", collector unknown (UC-mixed with C. acrostichoides); Sierra Nevada Mts., Grant 237 (ARIZ). **Idaho:** Adams Co., Black Lk., Seven Devils Mts., Johnston s.n., 20 Jul 1931 (CAS); Blaine Co., Mt. Parks, 9400-10,400 ft, Everman 382, 5 Aug 1895 (US-mixed with C. acrostichoides); divide between Alpine Creek and Twin Lakes, Sawtooth Primitive Area, 10,000 ft, Hitchcock & Muhlick 10,500, 30 Jul 1944 (NY, UC, UTC, WS, WTU); Bonner Co., Priest River Experimental Forest, Daubenmire 43,261 22 Jul 1943 (NY, WS, WTU); Elmore Co., 1 mi S of Lower Spangle Lk., Sawtooth Primitive Area, Hitchcock & Muhlick 10,146 19 Jul 1944 (CAN, CAS, GH, NY, RM, UC, US, UTC, WS, WTU); Big Roaring River Lake, Boise Mts., Ertter & Grimes 4545 16 Jul 1981 (CAS, NY, UTC, WTU); Idaho Co., Cool Water Mtn., Gail s.n., 11 Jul 1936 (ID); 3 mi NW of Salamander Mtn., Baker 12681, 17 Aug 1954 (WTU); Kootenai Co., without locality, J.B.L.

11, 1891 (UC); Shoshone Co., Near Stevens Peak, Coeur D'Alene Mts., Leiberg 1451, 3 Aug 1895 (NY, US); Freezeout Summit, Baker 15,415, 27 Jul 1958 (ID, NY, WTU); 12 mi E of Clarkia, Daubenmire 61,104, 1 Jul 1961 (WS); Valley Co., Brundage Mtn., 7600-7800 ft, Pennell & Constance 20,749, 5 Jul 1937 (US); Bruin Mtn., 8000 ft, Wellner 1618, 4 Aug 1978 (RM); county unknown, Oxyer Mines, Dunkel 1330, 5 Aug 1919 (ID). **Montana:** Missoula Co., Squaw Peak, 2375 m, Harvey & Pemble 7075, 7 Jul 1964 (MONTU); Ravalli Co., Piquett Mtn., 2635 m, Pemble 227, 25 Aug 1964 (WTU); Bitterroot Mts., T8N R22W S26, 7000 ft, White 866-22, 3 Aug 1966 (MONTU), Jermyn 6667, 6669, 11 Aug 1966 (MONTU); Mt. Jerusalem, 9000 ft, Lackschewitz & Fageraas 693, 11 Aug 1968 (MONTU); county unknown, Holzinger Basin, Lake McDonald, Umbach 331/01, 21 Aug 1901 (NY, US). **Oregon:** Baker Co., source of Kettle Cr., 7500 ft, Cusick 3374, 11 Aug 1909 (WS, WTU); East Pine Creek, 7500 ft, Peck 18,236, 30 Aug 1915 (WILLU); near Cornucopia, Wallowa Mts., Thompson 13,318, 18 Jul 1936 (CAS, NY, WS, WILLU); Krag Mtn., Wallowa Mts., Head 1273, 24 Jul 1957 (OSC), Head 1661, 15 Sep 1957 (OSC); W base of Red Mtn., Wallowa Mts, 7500 ft, Head 1637, 5 Sep 1957 (NY, OSC); Clackamas Co., Breitenbush Lake area, Rodin 6926, 24 Aug 1962 (ARIZ); Deschutes Co., Hidden Lake, Paulina Mts., Detling 28, 8 Jul 1928 (ORE); Devil's Garden, near S. Sister, Andrews s.n., 27 Aug 1929 (ORE); near Green Lakes, E side Three Sisters, Henderson 14,178, 15 Aug 1931 (ORE); Sisters Mirror Lake area, 6000 ft, Merkle 59-94, 15 Aug 1959 (OSC); Douglas Co., Old Bailey Mtn., Applegate 4125, 7 Jul 1924 (CAS, WILLU); Hood River Co., near Eden Park, Mt. Hood, 6000 ft, English 174, 5 Jul 1926 (WS); Jackson Co., Mt. Pitt, 7000 ft, Colville & Applegate 233, 27 Jul 1887 (US); Mt. Pitt (McLoughlin), Evans 459, 2

Aug 1931 (ORE); Mt. McLoughlin, Pengelly 888, 2 Sep 1957 (SOC); Jefferson Co., Three-Fingered Jack, 6500-7000 ft, Crosby 1073, 5 Sep 1976 (OSC); Klamath Co., bluffs near Crater Lake, Bruce 1160, Aug 1891 (NY) Mt Scott, Crater Lake, Applegate 834, 19 Aug 1896 (CAS, US); Cathedral Cliffs, Crater Lake, 8150 ft, Gorman 113, 19 Aug 1896 (ORE, US, WTU); Crater Lake, Austin 1636, 10 Aug 1897 (POM, US); Wizard Island, Crater Lake, Alexander s.n., 13 Aug 1917 (UC); Rim, Crater Lake, Sweetser s.n., 7 Aug 1919 (ORE); Union Peak, 8000 ft, Applegate 4767, 9 Jul 1926 (CAS); rim, Crater Lake, Maguire, Maguire, and Maguire 15,052, 11 June 1934 (UTC); Wizard Island, Crater Lake N.P., Applegate 8966, 28 Jun 1934; W of Government Camp, Crater Lake N.P., Applegate 9030, 4 Jul 1934 (CAS, WILLU); Slick Rock, Crater Lake N.P., Applegate 9157, 1 Aug 1934 (CAS, UC); Union Peak, Crater Lake N.P., Applegate 10,084, 8 Aug 1935 (CAS); Garfield Peak, Crater Lake N.P., Applegate 10,190, 30 Sep 1935 (CAS); Garfield Peak trail, Crater Lake N.P., Baker 6174, 22 Jul 1949 (ID); Wizard Island, Crater Lake N.P., Baker 6363, 21 Aug 1949 (ID, NY, OSC, RSA, UC, WS, WTU); Wizard Island, Crater Lake N.P., Rodgers 123, 16 Aug 1951 (OSC); Lane Co., Camp Agoseris, Three Sisters, Sheldon 12,554, 20 Jul 1903 (ORE); McKenzie Pass, 5000 ft, Gorman 3864, 15 Aug 1916 (WS); McKenzie Pass, Hall 1229, 3 Aug 1924 (RSA, UC); McKenzie Pass, Gilkey s.n., Jul 1927 (OSC); Mesa Creek, Three Sisters, Easton s.n., 6 Aug 1931; Cascade Summit, McKenzie Hwy., Cole 5, 21 Aug 1932 (GH); McKenzie Pass, Henderson 16,434, 3 Jun & 7 Aug 1934 (ORE); Wickiup Plains, S of South Sister, 5000 ft, Henderson 16,693, 17 Jul 1934 (ORE); McKenzie Pass, Jones 5735, 17 Aug 1934 (WTU); West Lava Camp, McKenzie Pass, 5200 ft, Ireland 1025, 22 Aug 1937 (ORE); W of North Sister, 7300 ft, Van

Vechten 161, Sep 1957; Diamond Peak, 8000 ft, Wagner 892, 11 Sep 1976 (ORE); Diamond Peak, Crosby 956, 22 Aug 1977 (OSC); Linn Co., Mt. Washington, 6000-7000 ft, Crosby 991, 31 Aug 1976 (OSC); Wallowa Co., Chimney Lake, Wallowa Mts., Kruckeberg 2367, 21 Jul 1950 (CAN, COLO, ID, NY, RSA, WS, WTU); W of Blue Lake, Wallowa Mts, Hitchcock & Muhlick 21,399, 28 Jul 1957 (WTU); Horton Pass, Wallowa Mts., 7900 ft., Mason 1636, 2 Aug 1961 (US); pass between Mirror and Minam Lakes, Mason 3040, 23 Aug 1961 (ASU); county unknown, Gayhart Buttes, 2250 m, Colville & Leiberg 274, 8 Aug 1896 (US, ORE); Eastern Oregon, Cusick 1802, 1897 (CAS, UC, US); Wallowa Mts, Cusick 3374a, 11 Aug 1909 (ORE); without locality, Detling s.n., Jul 1928. **Washington:** Chelan Co., Slopes of Mt. Stuart, 1520 m, Sandberg & Leiberg 1821, 28 Aug 1893 (NY); Mt. Stuart, Elmer s.n., Aug 1898 (WTU); Mt. Stuart region, 5000 ft, Thompson 7707, 27-31 Jul 1931 (MICH); Ice Creek, Morrill 383, 23 Aug 1933 (WTU); Ferry Co., Twin Lakes, 3500 ft, St. John 8874, 5 Sep 1927 (WS); King Co., Chair Peak, 5800 ft, Jones s.n., 12 Aug 1934 (UC); Kittitas Co., Stafford Creek drainage, 1.5 mi SE of Earl Peak, 5800 ft, Alverson 534, 14 Aug 1981 (ORE); Lewis Co., Reflection Lake, Mt. Rainier, 5000 ft, Flett 1923, 23 Aug 1901 (NY, WTU); near Snow Lake, Mt. Rainier N.P., Leschke 1846, 28 Aug 1961 (CAS); Pierce Co., Mt. Rainier, 5400 ft, Flett s.n., 8 Oct 1918 (MICH); Glacier Basin, Mt. Rainier, 6700 ft, Grant s.n., Aug 1925 (CAS, WTU); Paradise Valley, Mt. Rainier N.P., 5900 ft, Parks & Parks 21,040, Aug 1828 (UC); above Nisqually Glacier, 5000 ft, Thompson 7626, 26 Jul 1931 (WTU); Wapowety Cleaver, Mt. Rainier N.P., 9000 ft, Lindsay 4526, 18 Aug 1933 (RM, UTC); E Fork Edith Creek, Mt. Rainier, 5900 ft, Smith 2214, 14 Aug 1938 (WTU); Skamania Co., Mt. St. Helens, 4500 ft, St.

John et al. 7453, 4 Aug 1925 (WS); Snohomish Co., Twin Lakes, above Monte Cristo, Broadbent s.n., 2 Sep 1935 (CAS, WTU); Lake Serene, 2600 ft, Alverson 584, 10 Jul 1983 (ORE); Yakima Co., Mt. Adams, Suksdorf 3208, Aug 1880 (WS), Suksdorf 5438, 29 Aug 1886 (WS); Wodan's Vale, Mt. Adams, Suksdorf 2793, 4 Oct 1902 (WS); Hellroaring Meadows, Mt Adams, Mastrogiuseppe et al. 721, 15 Sep 1976 (WS); county unknown, Stevens Pass, 2133 m, Sandberg & Leiberg 781, 20 Aug 1893 (CAN, CAS, UC, WTU); Stampede Tunnel, Henderson s.n., 27 Jul 1892 (WS).