AN ABSTRACT OF THE THESIS OF

Wes Messinger for the degree of Master of Science in Botany and Plant Pathology presented on November 18, 1994. Title: Molecular Systematic Studies in the Genus *Ribes* (Grossulariaceae).

Redacted for Privacy

Abstract approved: _____

Aaron Liston

Infrageneric classification in *Ribes* has previously relied on a few, often conflicting, morphological markers, such as spines, glands, and inflorescence morphology. Suggestions that hybridization drives the evolution of the genus have not been tested using phylogenetic methods. To assess the validity of infrageneric classifications and the importance of hybridization to the evolution of the group, and to develop an explicit phylogenetic hypothesis, I surveyed exemplars from all subgenera for restriction site variation in two cpDNA regions. Parsimony analysis shows that red currants, European alpine currants, golden currants, true gooseberries, and western gooseberries appear on separate clades. A less well supported clade includes the western North American ornamental currant group and a portion of the dwarf currants. The presence of distinct lineages in *Ribes* is strongly supported by characters with very high consistency, suggesting that hybridization among infrageneric groups is not common in the genus. Unexpectedly, spiny currants and true gooseberries are united, suggesting either a sister group relationship or the possibility that one of these groups arose by reticulate evolution. The four black

currant species examined exhibit surprisingly high divergence, and are not monophyletic in the analysis. Maximum likelihood analysis supports these results. Basal relationships of these lineages are not well resolved.

A similar analysis of a portion of the nuclear ribosomal repeat produced very few characters. Although these data are highly homoplasious, their analysis bears some resemblance to that of the chloroplast DNA. Most prominently, the ornamental currant clade has identical membership.

Brief reviews of the scattered palynological and paleontological literature concerning *Ribes* are presented.

Copyright by Wes Messinger
November 18, 1994
All Rights Reserved

Molecular Systematic Studies in the Genus *Ribes* (Grossulariaceae)

by

Wes Messinger

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed November 18, 1994
Commencement June 1995

Master of Science thesis of Wes Messinger presented on November 18, 1994

APPROVED:

Redacted for Privacy

Aaron Liston, representing Botany and Plant Pathology

Redacted for Privacy

Chair of Department of Botany and Plant Pathology

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Wes Messinger, Author

DEDICATION

This work is dedicated to my Mom and Dad, who made it easy for me to figure out that evolution is the neatest thing that ever happened.

ACKNOWLEDGMENT

I gratefully acknowledge the assistance provided by Aaron Liston and Kim Hummer, whose insight, support, and patience made this project possible.

TABLE OF CONTENTS

CHAPTER I: RESTRICTION SITE ANALYSIS OF CHLOROPLAST DNA PCR PRODUCTS REVEALS DISCRETE LINEAGES IN *RIBES* L. (GROSSULARIACEAE) 1

INTRODUCTION 2

Distribution 2
Conservation Status 2
Taxonomy 4
Interspecific Hybridization 7
A Brief Introduction to the Methods of Molecular Systematics . . . 9

MATERIALS AND METHODS 13

Taxonomic Sampling 13
Choice of Outgroup 13
DNA Isolation 17
Polymerase Chain Reaction 18
Restriction Digests 20
Deriving the Data 20
Mapping Length Variation 21
Data Analysis 21

RESULTS 23

DISCUSSION 33

CONCLUSION 42

REFERENCES 44

CHAPTER II: *RIBES* PHYLOGENETICS BASED ON RESTRICTION SITE MAPPING OF NUCLEAR RIBOSOMAL DNA 52

INTRODUCTION 53

MATERIALS AND METHODS 54

Study Group 54
Laboratory Methods 55
Data Analysis 56

TABLE OF CONTENTS (CONTINUED)

RESULTS 56

Restriction Map 56
Parsimony Analysis 57
Maximum Likelihood Analysis 60

DISCUSSION 60

REFERENCES 65

CHAPTER III: BRIEF REVIEWS 67

POLLEN MORPHOLOGY OF *RIBES* 68

A PROVISIONAL KEY TO *RIBES* POLLEN 73

REFERENCES 74

RIBES IN THE FOSSIL RECORD 75

REFERENCES 78

BIBLIOGRAPHY 80

APPENDICES 90

APPENDIX I: DATA MATRIX OF CHLOROPLAST DNA RESTRICTION SITES 91

APPENDIX II: DATA MATRIX OF NUCLEAR RIBOSOMAL DNA RESTRICTION SITES 94

APPENDIX III: DETAILED PROTOCOL FOR ISOLATION OF GENOMIC DNA FROM *RIBES* 97

APPENDIX IV: CHECKLIST OF *RIBES* SPECIFIC EPITHETS 101

LIST OF FIGURES

Figure 1.1. Strict consensus of twelve most-parsimonious trees produced by weighted parsimony from chloroplast gene fragment restriction site data. 26

Figure 1.2. Majority rule consensus tree identical to one of the most-parsimonious trees 28

Figure 1.3. Major lineages of *Ribes* as indicated by parsimony analysis. 30

Figure 1.4. Maximum likelihood tree produced from chloroplast gene fragment restriction site data 34

Figure 2.1 Most-parsimonious tree identical to majority rule consensus tree retaining compatible groups. Produced from nuclear ribosomal data by weighted parsimony 58

Figure 2.2. Maximum likelihood tree produced from nrDNA restriction site data 61

LIST OF TABLES

Table 1. Taxonomic sampling of the genus *Ribes* 14

Table 2. Chloroplast DNA primers used for PCR 19

Table 3. Concordance of sectional names (Berger 1924) with major lineages of *Ribes* 68

PREFACE

This work is divided into three parts. The first is the main body of research, a manuscript reporting the phylogenetic analysis of restriction site patterns in two chloroplast genes. The second is a similar study of the nuclear ribosomal repeat. A third section includes brief reviews of the scattered palynological and paleontological knowledge concerning the genus.

MOLECULAR SYSTEMATIC STUDIES IN THE GENUS *RIBES* (GROSSULARIACEAE)

CHAPTER I

RESTRICTION SITE ANALYSIS OF CHLOROPLAST DNA PCR PRODUCTS REVEALS DISCRETE LINEAGES IN *RIBES* L. (GROSSULARIACEAE)

INTRODUCTION

Distribution

The genus *Ribes* (Grossulariaceae), which includes the cultivated currants and gooseberries, contains 120 to 150 species distributed in the temperate regions of the Northern Hemisphere and South America (Mesler and Sawyer 1993, Mabberly 1987, Sinnott 1985). Approximately thirty species are recognized in Andean South America (Janczewski 1907). One or two species occur in Central America at high elevations (D'Arcy 1987a&b, Standley 1937) giving the genus a continuous range from Alaska to Tierra del Fuego. A few of the European species are found in western North Africa (Janczewski 1907, Sinnott 1985, Spongberg 1972).

Conservation Status

A number of species have relatively narrow distributions, or are restricted to specialized habitats. For example, *Ribes viburnifolium* is limited to the Channel Islands of California and Northwest Baja California (Wallace 1985). The poorly known *R. erythrocarpum* is found only in Oregon's high Cascades (Applegate 1939, Wynd 1939, Hickman 1969) and ranges immediately to the east (Applegate 1939, Messinger pers. obs.). One North American species, *R. echinellum* (Coville) Rehd. of the Florida panhandle and southeast Georgia, has federal status (it is proposed threatened (Smith and Sinnott 1984)). *R. canthariforme* Wiggins is known from a

single site in southern California, and is a candidate for listing (Sinnott 1985). Other species, including *R. tularense* (Coville) Fedde of California (Norris 1987), *R. watsonianum* Koehne, found in Washington and Oregon, and *R. cognatum* Greene (=*R. oxyacanthoides* ssp. *cognatum* (Greene) Sinnott), which occurs in riparian areas of the Palouse regions of Washington, Idaho, and Oregon, appear on the watch lists of conservation organizations such as The Nature Conservancy and state and federal agencies.

Many western North American species occur in riparian habitats. The continuing degradation of these habitats by farming, grazing, and urban development is an acute threat to the genetic integrity and survival of these species. *Ribes cognatum* has undergone drastic range reduction in the twentieth century, and may be nearing extinction in the United States (Sinnott 1985, Messinger unpublished data). In Sinnott's (1985) treatment, this plant is a subspecies of *R. oxyacanthoides* L., the source of several traits important to cultivated gooseberry breeding programs (Brennan 1991). *R. hudsonianum* var. *petiolare*, a putative relative of the cultivated black currant, is restricted to this shrinking riparian habitat as well. While not under immediate threat of extinction, rigorous evaluation of the conservation status of this species is acutely relevant to future black currant breeding efforts. The conservation status of the poorly collected and little studied South American and Asian taxa cannot be evaluated.

Taxonomy

Ribes, traditionally placed in the polyphyletic family Saxifragaceae in the Englerian classification system (Cronquist 1981), is now well established in Grossulariaceae. The delineation of this family is controversial. Cronquist (1981) circumscribes the Grossulariaceae broadly, with 24 genera, including *Itea*, *Brexia*, *Tetracarpaea*, *Penthorum*, *Escallonia*, and *Montinia*. Takhtajan (1980) assigns these genera to eight families. These systems, as well as that of Thorne (1992), are incongruent with the results of recent molecular analyses. Studies of the chloroplast gene *rbc*L demonstrate the polyphyletic nature of Saxifragaceae *sensu lato*, placing its woody members sister to various divergent rosid and asterid groups. *Ribes* is variously placed in these studies. In an analysis of nearly 500 seed plant sequences (Chase et al. 1993) the genus is sister to Crassulaceae/*Penthorum-Myriophyllum-Tetracarpaea* and Saxifragaceae *sensu stricto* clades with *Itea* sister to all of these [i.e., (*Itea* (*Ribes* (Crassulaceae),(*Penthorum-Myriophyllum-Tetracarpaea*)))]. It is sister to an *Itea-Pterostemon* clade which is in turn sister to a core of herbaceous Saxifragaceae in an analysis of Saxifragaceae *sensu lato* (Morgan and Soltis 1993). In another reconstruction in which *Ribes* is part of a large and phylogenetically diverse outgroup to Saxifragaceae *sensu stricto* (Soltis et al. 1993), *Itea* and *Pterostemon* are similarly placed, but *Ribes* is sister to Crassulaceae. However, the two nodes removing *Ribes* from the status of immediate sister to *Itea* are each supported by bootstrap values of less than 19 per cent (Soltis et al. 1993). Long branch attraction (see Swofford and Olsen 1990) may account for the difference in these two trees:

Ribes is apparently related to Saxifragaceae *sensu stricto*, *Pterostemon*, *Itea*, and the Crassulaceae but the distance (at least 63 steps to the nearest neighbor in the above reconstructions) obscures the nature of the relationships.

An excellent generic diagnosis of *Ribes* is available in Spongberg (1972). The members of this genus are prostrate to erect shrubs, often with nodal spines and internodal bristles. The stems are decurrently ridged from the nodes, the leaves are alternate or clustered on spurs and almost always deciduous and palmately lobed. The calyx forms a rotate to tubular hypanthium which is adnate to the ovary. Petals and stamens are inserted alternately on this floral tube (or disc). The ovary consists of two fused carpels, and the two styles are often fused for at least part of their length. The fruit is a spiny, bristly, glandular-pubescent or glabrous red, orange, yellow, green, or purplish to black berry, sometimes with a waxy bloom. Most species are hermaphroditic, but all South American species and many high-elevation Eurasian species are dioeceous.

The genus has been the subject of little monographic work. Janczewski (1907) monographed the genus worldwide. Berger (1924) treated the hermaphroditic species. Sinnott (1985) monographed the North American members of section *Grossularia*.

Numerous infrageneric classifications have been proposed for *Ribes* (e.g., Spach 1835, 1838, Berlandier 1826, Janczewski 1906a&b, 1907, Coville and Britton 1908, Berger 1924, Poyarkova 1939). See Table 1 for a synopsis of Berger's (1924) system, with genera and subgenera demoted to subgenera and sections. The gooseberries (spiny species with few-flowered racemes) and the currants (many-

flowered, mostly unarmed species) are traditionally separated, and have been recognized at times as separate genera (e.g., Coville and Britton 1908, Berger 1924, Poyarkova 1939). Sinnott (1985), in the most recent monographic work in the genus, recognizes two subgenera, corresponding to the currants and gooseberries, and formally treats those North American gooseberries characterized by pubescent styles (section *Grossularia*).

Essential morphological traits of traditional taxonomy include presence and absence of spines; petiole disarticulation; raceme length; vestiture type and distribution; depth of calyx tube; leaf, anther, and style morphology; and berry color. These traits are highly inconsistent: if traditional classifications are drawn as cladistic hypotheses, the traits exhibit nearly complete homoplasy. For example, the black currants (section *Coreosma* (Spach) Jancz.) are defined by the presence of sessile yellow glands, which also occur in some members of section *Parilla*. Subgenus *Grossularia* (the gooseberries) is defined by the presence of spines, which also occur in two unrelated groups of currants.

Traditional taxonomic methods have not produced consensus concerning evolutionary relationships or infrageneric classification. Anatomy (Bates 1933, Stern, Sweitzer, and Phipps 1970), cytology (Meurman 1928, Darlington 1929, Zielinski 1953, Goldschmidt 1964), secondary chemistry (Bate-Smith 1976, Bohm 1993), and pollen morphology have been equally uninformative, although the scanty pollen data available (Erdtman 1966, 1969, Heusser 1971, Hideux and Ferguson 1976, Pastre and Pons 1973, Verbeek-Reuvers 1980; see also Agababian 1963) appears to support the

distinction between gooseberries and currants (see Chapter III). These studies suffer from some combination of: narrow taxonomic sampling, insufficient variation of characters, lack of taxonomic or evolutionary intent, and lack of formal phylogenetic analysis. No explicit hypothesis concerning the phylogenetic history of the genus has been previously proposed.

Interspecific Hybridization

Sinnott (1985), Henry (1919), and Anderson (1943) reported natural hybrids of North American *Ribes*. The putative hybrids were sterile, or reproductive status was unreported. Three instances of hybridization among four species of west North American gooseberries have been demonstrated by morphometric analysis. These are *R. lobbii* A. Gray X *R. roezlii* Regel var. *cruentum* (E. Greene) Rehder, *R. binominatum* Heller X *R. marshallii* E. Greene, and *R. binominatum* X *R. lobbii*. These hybrids appear to be partially fertile, but no back-crosses or further recombinants were apparent (Mesler, Cole, and Wilson 1991). These species are members of an informal group consisting of smooth-styled species excluded from section *Grossularia* by Sinnott (1985). This group roughly corresponds to *Robsonia*, *Hesperia*, and *Lobbia* of Berger (1924), and is largely confined to western North America. Spontaneous garden hybrids are not uncommon in the genus (Janczewski 1907, Berger 1924).

Hybridization is thought to characterize the evolutionary history of the genus (Grant 1971, Raven and Axelrod 1978). The uniformity of chromosome number and

the absence of obvious breeding barriers as well as a degree of morphological intergradation among related taxa have given rise to the scientific folklore that hybridization is rampant in the genus. Perhaps in part on these grounds, evolution in *Ribes* has been regarded as following the *Ceanothus* pattern, in which species sharing a single chromosome number are interfertile, forming a homoploid complex (Raven and Axelrod 1978, Grant 1971, Sinnott 1985). Nonetheless, extensive crossing experiments, [performed or reviewed by Keep (1962, 1975, Brennan 1992)] failed to produce fertile progeny among most recognized subgenera and sections. Sterile hybrids exhibit reduced chromosome pairing in pollen mother cell meiosis (Meurman 1928, Goldschmidt 1964), indicating that *Ribes* chromosomes are divergent in structure, if not in number. Thus, while reticulation may or may not characterize the evolution of the genus among closely related species, it is probably not a factor at higher taxonomic levels, making them appropriate for cladistic analysis.

This paper reports the results of a molecular study and explicit phylogenetic analysis for the genus as a whole. The study has the following major objectives: to explore the validity of the various infrageneric taxa; to search for monophyletic groups (both as a guide to further phylogenetic study and to assess the extent to which reticulation influences the evolution of the genus); and to produce a phylogeny estimate for those infrageneric groups supported by the molecular data.

A Brief Introduction to the Methods of Molecular Systematics

Nucleotide sequences and restriction sites of the entire chloroplast genome are currently the most common data of plant molecular systematics (Olmstead and Palmer 1994), the former most effective among families and higher levels, the latter among genera (although both have been useful at the species level). Restriction site analysis of PCR-generated chloroplast gene fragments has also proven useful in phylogeny estimation, both among and within genera (Rieseberg et al. 1992, Fritsch and Rieseberg 1993, Liston 1992, Liston and Wheeler 1994, Schwarzbach and Kadereit in review, Wolfe et al. 1993). This method has the advantages of being rapid, inexpensive, and nonradioactive. One potential disadvantage is the relatively low number of base pairs surveyed, which can yield low levels of resolution when study taxa are insufficiently diverged.

Several explicit methods for generating phylogenetic hypotheses are available (reviewed in Swofford and Olsen 1990). Phenetic methods require production of distance or similarity matrices and calculate relationships based on these distances. These cannot explicitly be interpreted as a series of evolutionary events. Cladistic methods directly analyze raw character data, grouping taxa based on shared derived character states (synapomorphies). One advantage of such analyses, particularly of DNA data, is that a direct genetic interpretation of evolutionary branching patterns becomes possible: sequence mutations can be directly mapped onto phylogenetic trees. A number of optimization criteria may be employed in cladistic analysis. Parsimony minimizes the number of character state changes on a reconstructed tree.

Various weighting schemes are intended to approach realistic models of nucleotide evolution (Albert, Chase and Mishler 1993). Maximum likelihood methods produce a tree congruent with a specific model of evolutionary change (Felsenstein 1992). Likelihood is maximized by considering all possible pathways that could produce a given topology.

Of all parsimony-based tree estimation methods, Wagner parsimony is often considered the least burdened by assumptions. It is actually an unrealistic weighting system (Olmstead and Palmer 1994) in which all changes are equally likely. Systems of character weighting often produce subsets of Wagner parsimony results, unless homoplasy is high (Olmstead and Palmer 1994). DeBry and Slade (1985) argued that Dollo parsimony, in which sites can be gained only once (equivalent to a weighting scheme of 1:0), is appropriate for the analysis of restriction site data, because restriction site loss is more likely than gain. The assumptions of Dollo parsimony are, however, so strict as to be unrealistic (Albert, Chase, and Mishler 1992).

Differential weighting of transitions and transversions in sequence data, or restriction site gains over losses (Albert, Chase, and Mishler 1993) has been recently applied in several studies (e.g., Potter and Doyle 1994, Downie and Palmer 1994). Weighting restriction site gains over losses is based on an explicit model of evolution with some empirical support. For restriction site gain, a short sequence differing from a site by one base pair must undergo a single, specific mutation, while change of any residue will lead to site loss. Trees with many site gains become less parsimonious under this weighting system, which thus produces fewer trees than

Wagner parsimony. Recent work with mathematical and biological model systems suggests that, of all available tree-building methods, weighted parsimony is the most apt to recover the true phylogeny over a wide range of evolutionary rates (Huelsenbeck and Hillis 1993; Hillis, Huelsenbeck and Cunningham 1994; Hillis, Huelsenbeck, and Swofford 1994).

Maximum likelihood methods build or test trees by matching them to an explicit probabalistic model of character evolution (Swofford and Olsen 1990). They have the appeal of offering confidence intervals and significance values for branch lengths, as well as a total likelihood score (based on the summation of likelihoods of all possible mutational paths to a particular topology) for the tree (Felsenstein 1992). These methods are only as realistic as their models. This is, however, true of all methods of generating phylogenetic hypotheses, many of which depend on less than obvious assumptions and rely on optimality criteria not based in explicit statistical considerations. Studies with model systems (see above) rate maximum likelihood almost as highly as weighted parsimony.

Of the methods for polarizing cladograms, outgroup rooting has become by far the most popular. It is unsuitable in two particular situations: either when the study set lacks an obvious sister group, or when the available sister groups are so distant that extreme branch length causes root placement to approach that of a taxon with a random set of character states (Felsenstein 1978). Lundberg rooting (Lundberg 1972) chooses the most parsimonious root (and may thus designate several options for placement of the root). It has been useful when long branches to an outgroup cause

difficulty (Hibbett and Vilgalys 1993). Midpoint rooting places the root at the midpoint of the longest possible path between two taxa of the study set (Avise 1994).

When levels of homoplasy are low and data sets simple, the most straightforward method of assessing support for a given tree is by counting synapomorphies on each clade. However, when multiple most-parsimonious trees are produced, this approach can lead to ambiguities: mapping mutations onto every most-parsimonious tree becomes impractical, and mapping mutations onto consensus trees is essentially meaningless, since these trees represent several evolutionary pathways simultaneously.

Many more sophisticated methods of assessing support for phylogenetic trees and clades within them have been recommended, for example, the bootstrap (Felsenstein 1985) and the decay index (Donoghue et al. 1992; Mishler, Donoghue and Albert 1991). Most, however, measure the same thing, (Olmstead and Palmer 1994): the consistency of the tree(s) with the data. They do not, therefore, measure the statistical support for, nor the accuracy of, the tree to which they are applied.

Parsimony analysis employs 'hill-climbing' algorithms, and hence can get stuck in local optima, or 'islands' of parsimonious trees, without finding the global optimum. Searching for such multiple islands of most-parsimonious trees has become *di rigueur* in phylogenetic analysis. Islands are, however, unlikely to occur when retention indices are greater than 0.67 (Maddison 1991).

MATERIALS AND METHODS

Taxonomic Sampling

To include as much morphological diversity as possible, several species from each subgenus recognized by Berger (1924) were chosen for the study (see Table 1). These taxa are treated here as sections of the single genus *Ribes* with two subgenera. Berger's (1924) system recognizes more subdivisions than that of Janczewski (1907), particularly among the west North American gooseberries. This increases its utility as a guide to sampling. Several species in each subgenus were sampled, and species from much of the geographic range of each subgenus were included. Broad taxonomic distribution within the infrageneric classification of Coville and Britton (1908) was also achieved. In addition, two varieties of *R. velutinum* were included in order to assess divergence between closely related taxa. Preliminary results indicating extremely low levels of variation among species demonstrated that sampling several individuals within species was unnecessary.

Choice of Outgroup

Itea virginica was chosen as outgroup based on published *rbc*L sequence and cpDNA restriction site analyses. The most closely related available woody member of Saxifragaceae *sensu lato*, *Itea* differs from *Ribes* by 63 nucleotide changes in the chloroplast gene encoding the large subunit of ribulose bisphosphate decarboxylase

Table 1. Taxonomic sampling of the genus *Ribes*

Infrageneric Taxon[1]	Included Species	Range[2]	NPGR Accession and Voucher[3]
Currants (subgenus *Ribes*)		**Circumboreal, South America**	
Ribes	--	--(Circumboreal)	
	R. triste Pallas	N. America, E. Asia	(Messinger 313)
	R. sativum (Reichb.) Syme cv. Diploma	Northern Europe	RIB747 (M. Thompson 46)
Calobotrya	--	--(W. N. America)	
	R. cereum Douglas	W. N. America	RIB237.001
	R. mogollonicum Greene	W. N. America	RIB294.001
	R. sanguineum Pursh	West Coast, N.America	RIB46
	R. viscossisimum Pursh	W. N. America	RIB281.001 (N. Fredricks 394)
	R. ciliatum Humb. & Bonpl.	Central Mexico	RIB670.001 (Messinger 311)
Heritiera	--	--(W. N. America, E. Asia)	
	R. erythrocarpum Coville & Leiberg	Cascade Range, N. America	RIB860.001 (Messinger 249)
	R. howellii Greene	W. N. America	RIB449.001 (Messinger 333)
	R. laxiflorum Pursh	North America	
	R. glandulosum Grauer	North America	RIB231
Grossularioides	--	(W. N. America, E. Asia)	
	R. lacustre (Pers.) Poiret	W. N. America, East Asia	RIB45
	R. montigenum McClatchie	W. N. America	RIB864.001 (Messinger (254)

Table 1. (Continued).

Infrageneric Taxon[1]	Included Species	Range[2]	NPGR Accession and Voucher[3]
Coreosma	--	--(Circumboreal)	
	R. americanum Mill.	North America	RIB93
	R. nigrum L.	North Eurasia	RIB215.001
	R. hudsonianum A. Richards var. *petiolare* (Douglas) Jancz.	W. N. America	RIB278 (N. Fredricks 390)
	R. viburnifolium A. Gray	Channel Islands and Baja California	RIB762.001
Symphocalyx	--	--(W. N. America)	
	R. aureum Pursh	W. N. America (Rockies west)	RIB769
	R. odoratum Wendl.	North America (Rockies east)	RIB691
Berisia	--	--(Eurasia)	
	R. alpinum L.	Europe	RIB6640
	R. maximowiczii Batalin	East Asia	RIB267
	R. diacantha Pall.	East Asia	RIB34 (Messinger 315)
Parilla	--	--(South America: Andes)	
	R. andicola Jancz.	South America: Andes	(Luteyn 14094)
	R. valdivianum Phil.	South America: Andes	(Messinger 314)
Gooseberries (subgenus *Grossularia*)		**Circumboreal**	
Grossularia	--	--(Circumboreal)	
	R. oxyacanthoides L. ssp. *irriguum* (Douglas) Sinnot	W. N. America	RIB773.001 (Messinger 221)

Table 1. (Continued).

Infrageneric Taxon[1]	Included Species	Range[2]	NPGR Accession and Voucher[3]
	R. niveum Lindl.	W. N. America	RIB777.001 (Messinger 226)
	R. burejense Fr. Schmidt	East Asia	RIB259.001 (Messinger 334)
Hesperia	--	--(W. N. America)	
	R. speciosum Pursh	California	RIB901.001 (U.C. Berkeley Botanic Garden 84.0004 location 24)
Robsonia	--	--(W. N. America)	
	R. menziesii Pursh	California, Oregon	RIB769.001 (Messinger 233)
	R. roezlii Regel var. *cruentum* (E. Greene) Regel	California, Oregon	RIB772.001 (Messinger 217)
Lobbia	--	--(W. N. America)	
	R. binominatum A. A. Heller	Oregon Cascade Range	RIB867.001 (Messinger 260)
	R. velutinum E. Greene var. *velutinum*	Great Basin (N. America)	RIB865 (Messinger 255.1)
	R. velutinum var. *goodingii* (Peck) Hitchc.	Snake River Watershed (N. America)	RIB781 (Messinger 233)
OUTGROUP:	*Itea virginica*	E. North America	(Messinger 337)

[1]Sections (these are subgenera in Berger's 1924 two-genus system) preceded by dash. [2]Sectional ranges in parentheses, preceded by dash. [3]U.S.D.A/A.R.S National Plant Germplasm Repository accession numbers e.g., RIB777.001. Vouchers housed at OSC.

(*rbc*L) (Morgan and Soltis 1993), and by 76 cpDNA restriction sites (Soltis et al. 1993). *Heuchera*, an herbaceous genus of Saxifragaceae *sensu stricto*, differs from *Ribes* by only 50 *rbc*L sites (Morgan and Soltis 1993), but the two genera are separated by several hypothesized cladogenesis events. These high levels of divergence in relatively conservative coding sequence indicate that *Ribes* is a phylogenetically isolated group.

DNA Isolation

Laboratory procedures were largely derived from those of Liston (1992). Total DNAs were isolated using variations of the CTAB method of Doyle and Doyle (1987). For some taxa, a modification of this method to a maximum volume of 1.5 ml during the chloroform extraction step was adequate to isolate high-quality DNA. However, *Ribes* leaves contain phenolic compounds and tannins (Stern, Sweitzer, and Phipps 1970; Bate-Smith 1976) and many species appear high in complex polysaccharides (mucilage). All of these may inhibit PCR, and marked differences in ease of amplification were noted among species. For material recalcitrant to PCR amplification, a number of extensions and modifications of the Doyle and Doyle method were necessary. Additional organic extractions and high percentages of PVP and sodium bisulfite were helpful, and very high ratios of CTAB to tissue were essential. The additional CTAB/PEG precipitation steps of Rowland and Nguyen (1993) were also very effective.

Polymerase Chain Reaction

Two plastid sequences were amplified. Primers homologous to bases 1-30 of *rbcL* and to 22 bases of ORF 106 (*zfp*A) (see Table 2 for primer sequences) are designed to amplify the entire coding region of *rbc*L and the intervening sequence(Arnold et al. 1991). These primers are expected to produce a sequence of about 3,000 base pairs in dicots, of which nearly 2,000 are noncoding intervening sequence (Rieseberg et al. 1992). This region of the target sequence corresponds to part of a mutational 'hot spot' in the plastid genome of *Triticum* and *Aegilops* (Ogihara, Terachi and Sasakuma 1991), and may be expected to exhibit high levels of variation, although the amplified region includes the conservative *rbc*L coding sequence.

The primers *rpo*C1-195 and *rpo*C2-1364 (Table 2) amplify about 90% of *rpo*C1 and 30% of *rpo*C2, as well as the intervening sequence between the two genes and the intron in *rpo*C1 (Liston 1992). In Astragalus (Liston 1992), the Galegeae (Liston and Wheeler 1994) and other dicots (Schwarzbach and Kadereit in review; C. Asmussen, A. Liston, J. Wheeler unpublished data) these primers produce a fragment of about 4100 base pairs.

PCR amplification of target sequences followed the procedure of Arnold, Buckner, and Robinson (1991) with minor modifications, including the "hot start" procedure, which can inhibit priming to heterologous sites (Erlich, Gelfand, and Sninsky 1991). DMSO may inhibit the formation of unwanted secondary structure in template DNA and improve specificity of primer binding. Addition of 5% DMSO to

Table 2. Chloroplast DNA primers used for PCR

Primer sequence 5' to 3'	position and strand[a]	name	predicted size	features
ATG TCA CCA CAA ACA GAA ACT AAA GCA AGT	57586-57615 B	*rbc*L-Z1	3200 bp	
ACT ACA GAT CTC ATA CTA CCC C	60860-60839 A	ORF106 (=*zfp*A)		
TAG ACA TCG GTA CTC CAG TGC	19967-19986	*rpo*C2-1364	4100 bp	740 bp intron &
AAG CGG AAT TTG TGC TTG TG	24071-24052	*rpo*C1-195		160 bp IGS

[a]Relative to the tobacco chloroplast genome (Shinozaki et al. 1986).
[b]From Arnold et al. (1991).
[c]Derived from Shimada et al. (1990); see Liston (1992).

the reaction mixture was necessary to amplify the nrDNA of several species (Messinger, Liston and Hummer 1993), but was not helpful for amplification of chloroplast sequences.

Restriction Digests

Amplified regions were digested with 15 restriction enzymes. An initial survey of enzymes determined that following enzymes revealed variation in a portion of the species sampled: *Alu*I, *Bsa*JI, *Bst*UI, *Hae*III, *Hha*I, *Msp*I, *Rsa*I, *Sau*96I, *Scrf*I (4-cutters), *Bsr*I (5-cutter), *Bam*HI, *Bst*BI, *Cla*I (6-cutters) in the *rpo*C fragment, with the addition of *Hin*fI (a 4-cutter), and *Ase*I (a 6-cutter) in the *rbc*L fragment. *Cla*I and *Bst*BI revealed no variation in *rbc*L. Reactions were performed directly in the PCR buffer, with the addition of a matching buffer supplied by the manufacturer. BSA was often added and incubation was occasionally extended for up to 48 hours. Restriction fragments were separated on 1.4-2.0% agarose gels cast with ethidium bromide, and photographed over UV light.

Deriving the Data

Restriction sites were added to the data matrix (Appendix I) only when unambiguous interpretations could be made. Bands absent in some taxa were not scored as mutations unless corresponding bands were present whose length summed to that of

the missing band. This method is adequate to ensure the homology of restriction sites, making restriction mapping of many small fragments unnecessary. Sequences of both *rpo* and *rbc*L are available with the sequence of the entire chloroplast genome in a few species, such as *Nicotiana tabacum* (Shinozaki et al. 1986). In particular, *rbc*L of *Itea virginica*, *Ribes aureum*, and *R. sanguineum* have been recently sequenced (Chase et al. 1993, Morgan and Soltis 1993). By comparison of restriction maps of these sequences generated by GCG (Genetics Computer Group 1991) to gel photos, questions of homology or redundancy could often be conclusively resolved.

Mapping Length Variation

Length mutations, discovered when similar variation was revealed by multiple restriction enzymes, were mapped by double digests to assess their homology. They were not included in the data matrix and phylogenetic analyses. Length change and point mutation do not conform to similar models of evolutionary process, and combining them unnecessarily confounds analysis. In addition, the frequent association of length change with mutational 'hot spots' (Ogihara, Terachi and Sasakuma 1991) makes homology assessment, as well as character state assignment and polarization even more problematic (Golenberg et al. 1993).

Data Analysis

Except the maximum likelihood procedure, all phylogeny estimates were performed using PAUP version 3.0s+4(ß) for UNIX (Swofford 1992). To estimate most-parsimonious trees, heuristic searches were performed with site gains and losses weighted equally (Wagner parsimony), and with gains weighted 1.3 to 1 over losses (Albert, Chase, and Mishler 1993). Outgroup rooting, Lundberg rooting with ancestral states estimated by comparison to the outgroup, and midpoint rooting were compared. [Lundberg rooting (Lundberg 1972) chooses the most parsimonious root (and may thus designate several options for placement of the root). It has been useful when long branches to an outgroup cause difficulty (Hibbett and Vilgalys 1993)]. Both strict and majority rule consensus trees were produced. The latter was identical to one most-parsimonious tree, on which mutations were mapped.

A bootstrap test (Felsenstein 1985) with 100 replicates using a heuristic search with PAUP's SWAP=SPR (subtree pruning-regrafting: intermediate in speed and reliability to nearest neighbor interchange and tree bisection-reconnection algorithms) and STEEPEST descent options in effect was performed with 1.3 to 1 weighting of restriction site gains to losses. Decay indices (Donoghue et al. 1992) were computed to assess support for parsimony estimates. Decay indices were calculated with the weighting system in effect.

If most-parsimonious trees conflicted with traditional ideas of sectional circumscription, monophyly was forced by designating constraint trees, and the effect on length of most-parsimonious trees was assessed.

A maximum likelihood estimate based on Kimura's (1980) two-parameter model of restriction site change (Smouse and Li 1987) was produced with the PHYLIP program 'restml' (Felsenstein 1991), with input order randomized, global rearrangements in effect, and an extrapolation factor of 100. Runs were performed with site length set to four and six, and the results compared, since restriction enzymes that recognize four, five and six base-pair sites were included in the study.

RESULTS

Products of approximately 3200 bp were obtained with the *rbc*L primers. The *rpo*C primers yielded fragments of approximately 4100 b.p. The *rpo*C fragment could not be amplified from herbarium material of *Ribes andicola*, and this species was not included in the cpDNA analysis.

Approximately 720 b.p., 253 in *rbc*L and 467 in *rpo*C, were surveyed with 16 restriction enzymes. Within the ingroup, 38 (28 in *rbc*L and 20 in *rpo*C) sites were interpretable and variable, and in 30 sites (*rbc*L: 13; *rpo*C: 17) this variation was shared among taxa, representing potential phylogenetic information. An additional 16 mutations (9 in *rpo*C) separated the ingroup from *Itea virginica*. The data matrix derived from these sites is presented as Appendix I. Inspection of this matrix reveals several patterns: 1) most shared mutations are distributed among taxa in precisely the same configuration as other mutations (i.e., the data have very high consistency); 2) the groups supported by these mutations match certain traditional taxa; 3) few

mutations are shared within or among these infrageneric groups. In addition, *Itea* shares the majority pattern or has a unique character state in all but one case.

Two length mutations were detected in the *rbc*L fragment. An insertion in *R. viburnifolium* relative to the rest of the study group, and an insertion in *Itea* (really a deletion in *Ribes* as a whole if the polarization is correct) occur in the intervening sequence. These changes are of different lengths, occur on different *Ase*I fragments, and are thus autapomorphic. Extensive length variation in the *rpo*C fragment maps to the intron in *rpo*C1. So many lengths are present that their homology could not be determined without sequence data, and they are here considered autapomorphic and uninformative.

Wagner parsimony analysis of the restriction site data produced 220 trees of 62 steps. Weighting site gains over site losses 1.3 to 1 reduced the number of trees to 12 (each of 685 steps, equivalent to 62 unweighted steps; C.I. =0.887, excluding autapomorphies 0.816, R.I. =0.926). The strict consensus of these 12 trees is given as Figure 1.1. The majority rule consensus tree with compatible clades retained is identical to one most-parsimonious tree (Figure 1.2).

Parsimony analysis confirms the impression produced by inspection of the matrix. Homoplasy is very low, and there are a number of discrete clades which correspond well to previously suggested infrageneric taxa: the golden currant clade, the true gooseberry clade, the European alpine currant clade, and the western gooseberry clade (Figure 1.3). The placement of several species is unclear, however, and little confidence can be placed in branching order at the base of the tree. Within the larger

clades most mutations are autapomorphies, allowing little resolution of the branching order among species. Levels of support indicated by bootstrap percentages for the various clades are given in Figure 1.2, and essentially parallel the majority rule consensus percentages. Decay indices greater than one could not be computed due to excessive computer time required. Outgroup and midpoint rooting with and without *Itea* gave identical results. Lundberg rooting gave several possible roots per most-parsimonious tree including those of the other two methods.

In order of descending support (as measured by number of synapomorphies mapped to each branch), the following clades appear in the strict consensus: the golden currants (*Symphocalyx*), represented by the close relatives *R. aureum* and *R. odoratum*, appear on the longest branch within *Ribes*. Surprisingly, the taxonomically distant true gooseberries and spiny currants are united on a single branch: the true gooseberries (*Grossularia*) appear on the second longest branch united with *R. montigenum* (*Grossularioides*). *R. lacustre* (also *Grossularioides*) is sister to this group. The dioeceous species *R. alpinum*, *R. diacantha*, and *R. maximowiczii*, the European alpine currants (*Berisia*) are united. A single mutation causes R. *alpinum* to be placed sister to the other two species. The two red currants, *R. triste* and *R. sativum*, are united. All sections of gooseberry other than *Grossularia* are united, with the unique Californian *R. speciosum* sister to the rest. The clade on which the members of *Calobotrya* (the ornamental currants) appear with two dwarf currants (*Heritiera*) is supported by only a single character.

Figure 1.1. Strict consensus of twelve most-parsimonious trees produced by weighted parsimony from chloroplast gene fragment restriction site data.

Figure 1.1

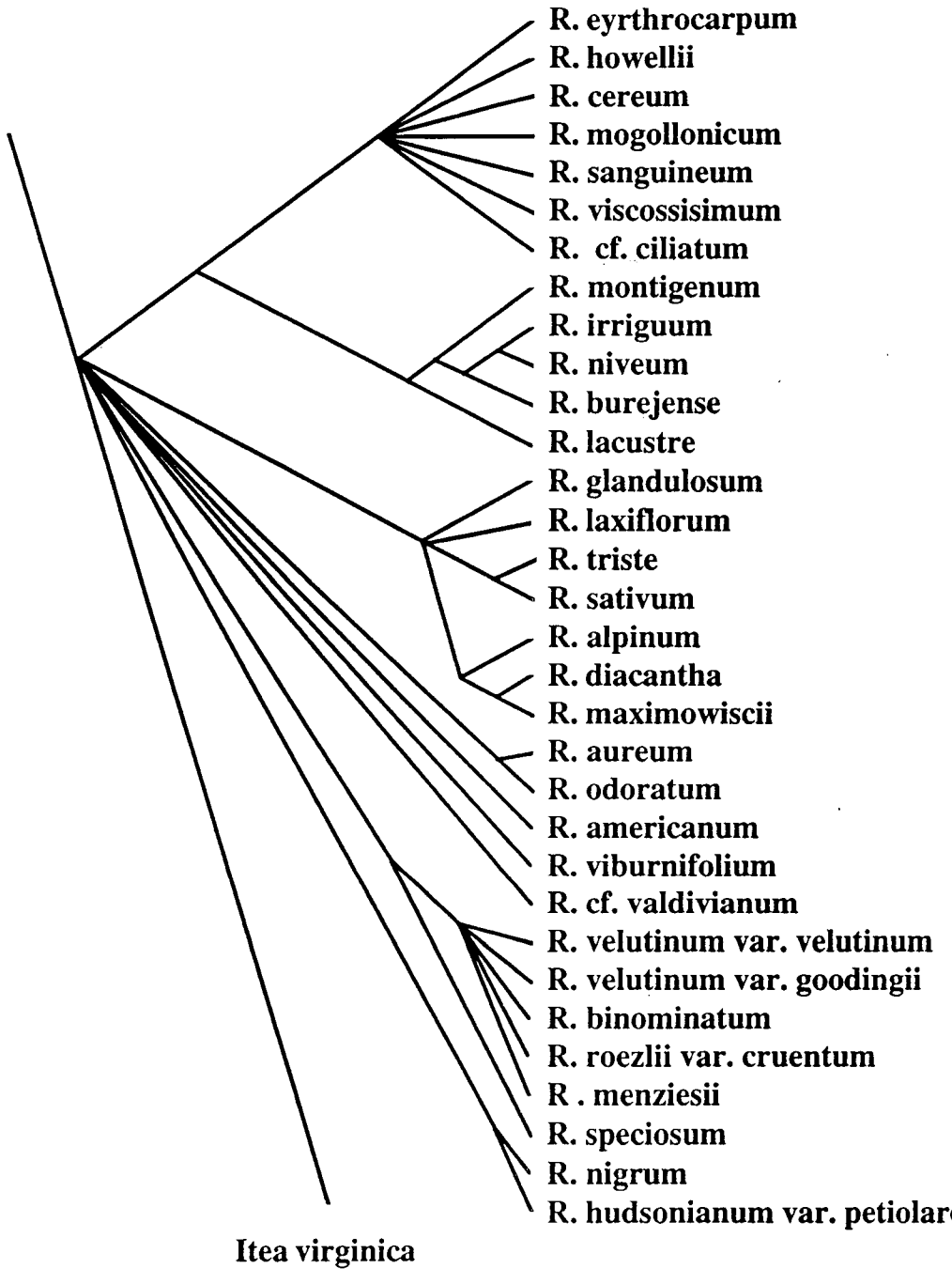

R. eyrthrocarpum
R. howellii
R. cereum
R. mogollonicum
R. sanguineum
R. viscossisimum
R. cf. ciliatum
R. montigenum
R. irriguum
R. niveum
R. burejense
R. lacustre
R. glandulosum
R. laxiflorum
R. triste
R. sativum
R. alpinum
R. diacantha
R. maximowiscii
R. aureum
R. odoratum
R. americanum
R. viburnifolium
R. cf. valdivianum
R. velutinum var. velutinum
R. velutinum var. goodingii
R. binominatum
R. roezlii var. cruentum
R . menziesii
R. speciosum
R. nigrum
R. hudsonianum var. petiolare
Itea virginica

Figure 1.2. Majority rule consensus tree identical to one of the most-parsimonious trees. Upward triangles are site gains; downward triangles are site losses. Hollow triangles are homoplasious character states. Bootstrap percentages are given above the branches found in a majority of bootstrap trees. Bootstrap percentages based on Wagner parsimony differ by only one or two percentage points except that *R. speciosum* is united with the rest of the western gooseberries in 64% of the trees. The tree is of 62 steps, with a consistency index excluding autapomorphies of 0.816 and a retention index of 0.926.

Figure 1.2

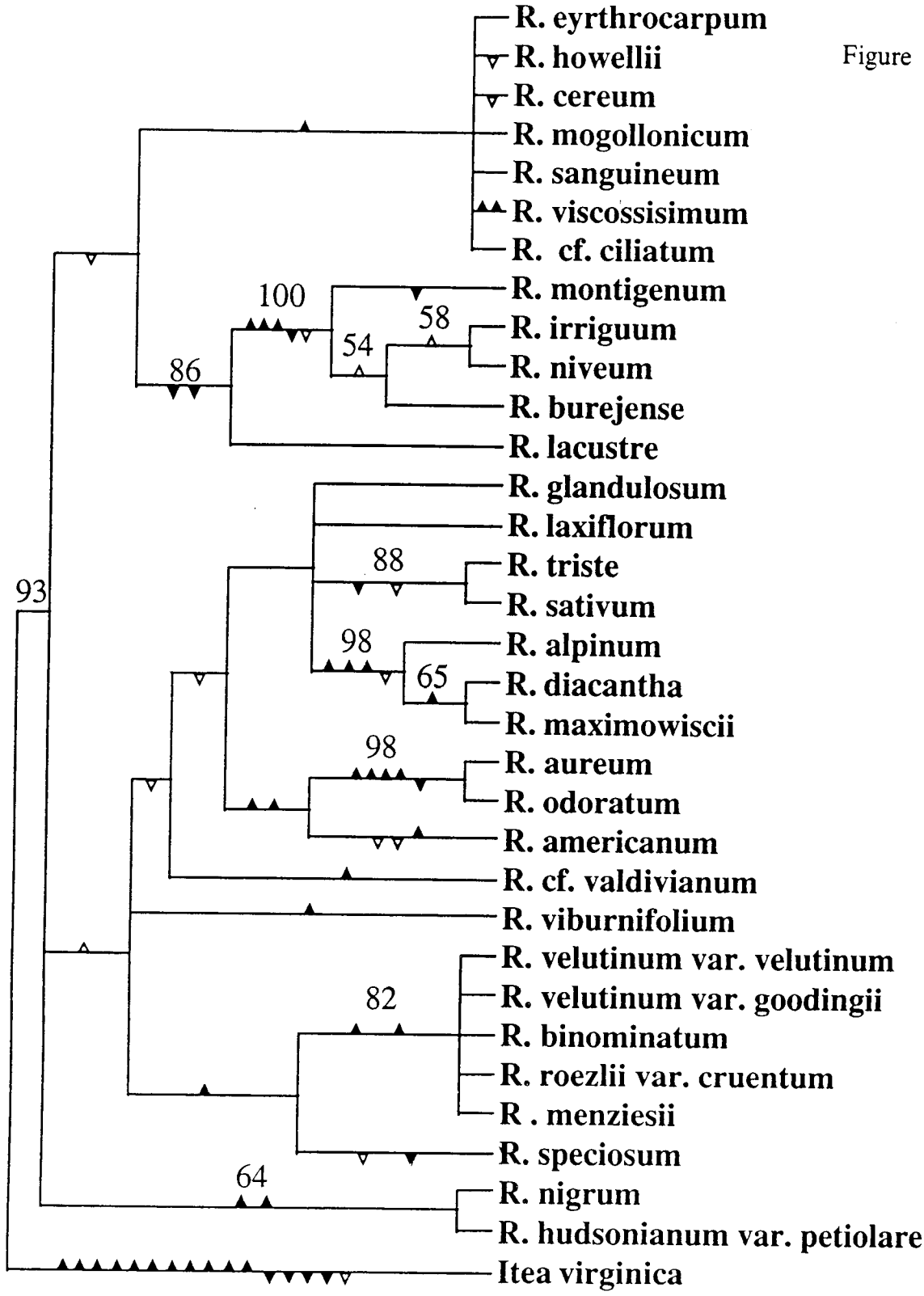
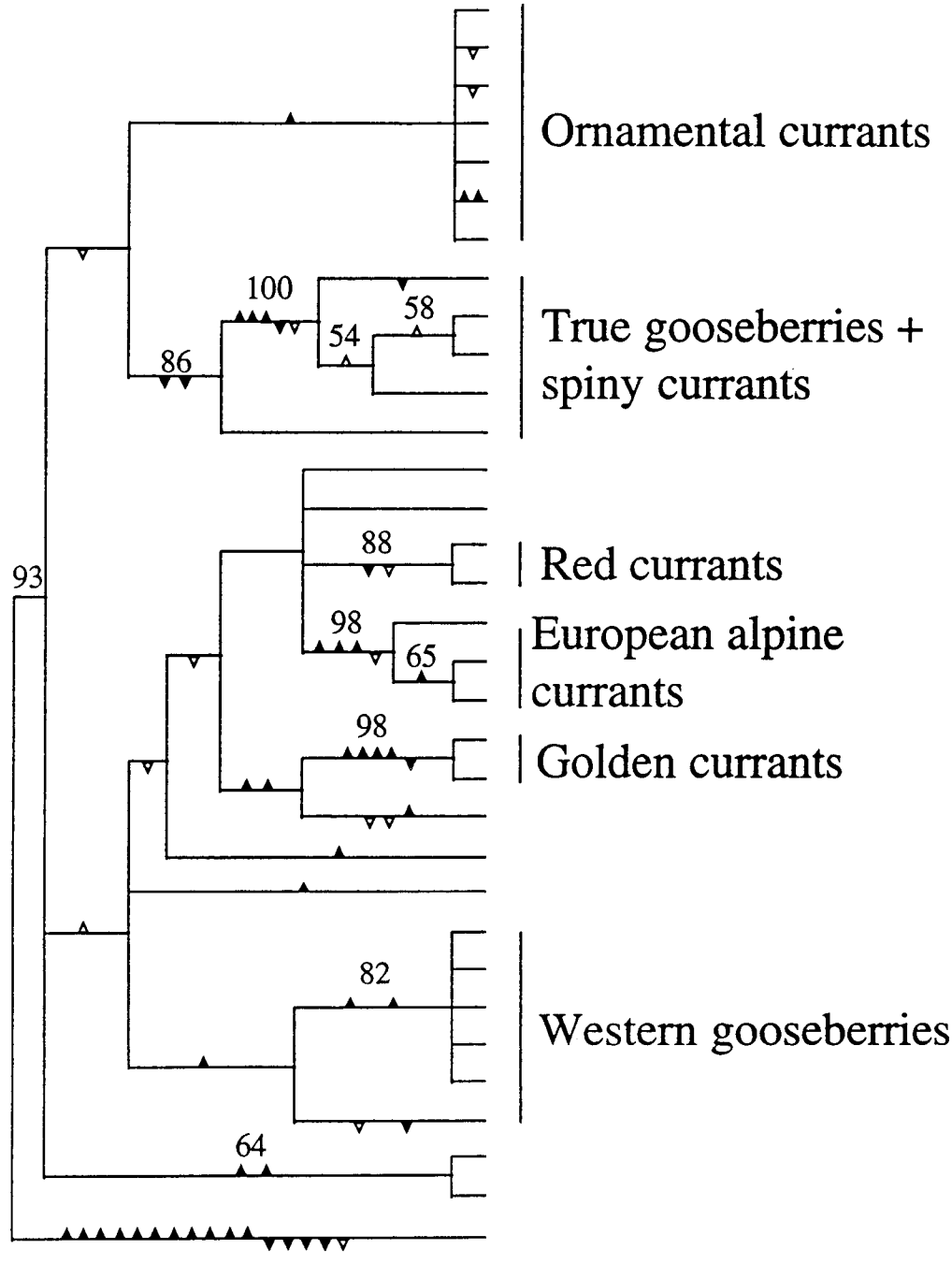

R. eyrthrocarpum
R. howellii
R. cereum
R. mogollonicum
R. sanguineum
R. viscossisimum
R. cf. ciliatum
R. montigenum
R. irriguum
R. niveum
R. burejense
R. lacustre
R. glandulosum
R. laxiflorum
R. triste
R. sativum
R. alpinum
R. diacantha
R. maximowiscii
R. aureum
R. odoratum
R. americanum
R. cf. valdivianum
R. viburnifolium
R. velutinum var. velutinum
R. velutinum var. goodingii
R. binominatum
R. roezlii var. cruentum
R . menziesii
R. speciosum
R. nigrum
R. hudsonianum var. petiolare
Itea virginica
100
58
54
86
88
93
98
65
98
82
64

Figure 1.3. Major lineages of *Ribes* as indicated by parsimony analysis. Character states and bootstrap percentages as in Figure 1.2.

Figure 1.3


Ornamental currants
100
58
54
86
True gooseberries +
spiny currants
88
93
Red currants
98
65
European alpine
currants
98
Golden currants
82
Western gooseberries
64

Other features of the tree are less robust. Two other dwarf currants appear in a polychotomy with the red currants and the European alpine currants. The black currants (*Coreosma*) are scattered across the tree: no character unites them all, although a pair of sites unites *R. nigrum* and *R. hudsonianum* var. *petiolare*. Placement of the single South American currant also varies wildly among most-parsimonious trees. It also shares the morphological trait (presence of sessile yellow glands) that unites section *Coreosma*. Whether or not *R. valdivianum* is included, constraining section *Coreosma* to monophyly yields six trees of 64 steps (C.I.=03859, 0.775 excluding autapomorphies, R.I.=0.904). This is an increase in length of only two steps (about 3.2%) over the most-parsimonious tree.

Results of Maximum Likelihood analysis (Figure 1.4) are nearly identical to the strict consensus of parsimony based trees, whether recognition site length was set to four or six b.p. (These two analyses differ only in whether *Itea* appears on its own branch or on a clade including *Ribes nigrum* and *R. hudsonianum* var. *petiolare*.) All of the well-supported groups from the parsimony analysis are present, and their distal branching patterns are entirely congruent. These groups include the Alpine currants, the golden currants, and true gooseberries, and the ornamental currants. Groups with less support in the parsimony analysis also appear, specifically the ornamental currant clade, which is supported in the parsimony analysis by a single site.

DISCUSSION

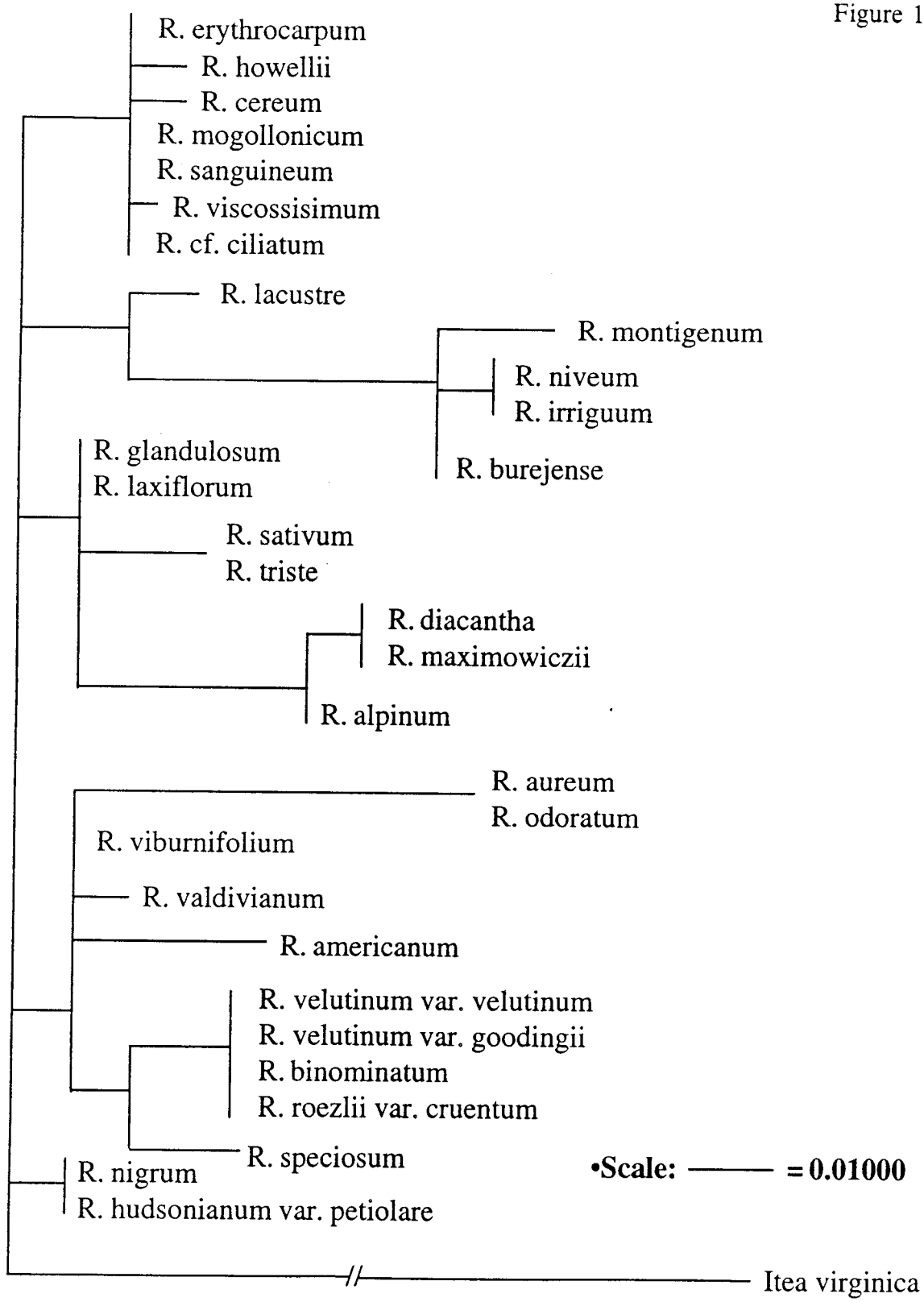
These data support the existence of several discrete lineages in the genus *Ribes*. Most species share a restriction profile 'groundplan' from which various lineages and species diverge at several sites. While branching order of these groups is not robustly indicated by parsimony analysis, species least divergent from the restriction profile groundplan, such as *Calobotrya* or some *Coreosma*, may be ancestral, especially considering that the outgroup species shares this groundplan (or exhibits a unique character state). The remarkable consistency of the data set lends confidence to these estimates, even though they are based on small numbers of variable sites. On the other hand, since few sites are shared among the larger groups, basal phylogenetic relationships in *Ribes* are not resolved.

Maximum likelihood analysis produced a tree entirely consistent with both the original data set inspection and parsimony analysis. The differences are all in poorly resolved basal branches. Congruence of trees produced by different methods may be considered further support for the patterns resolved (Avise 1994, Patterson, Williams and Humphries 1993). Maximum likelihood analysis thus bolsters the evidence for discrete lineages in *Ribes* while confirming the lack of resolution among these lineages.

Two possible, and not necessarily mutually exclusive, evolutionary scenarios could explain the pattern seen in these analyses, in which robust clades are combined with poor resolution at both branch bases and tips. The first is that *Ribes* evolution is characterized by long periods of stasis interrupted by sudden radiations. In this

Figure 1.4. Maximum likelihood tree produced from chloroplast gene fragment restriction site data. Branches approximately proportional to length. Branch angles are arbitrary, and branches whose significance is $P>0.05$ are collapsed. Log likelihood = -350.57242. Restriction enzyme recognition site set to 4 bp. An analysis with recognition site set to 6 bp differs only in that the branch with *R. hudsonianum* var. *petiolare* and *R. nigrum* is collapsed, while a branch separating these two taxa and *Itea virginica* from the rest of the tree has significant length.

Figure 1.4


R. erythrocarpum
R. howellii
R. cereum
R. mogollonicum
R. sanguineum
R. viscossisimum
R. cf. ciliatum
R. lacustre
R. montigenum
R. niveum
R. irriguum
R. burejense
R. glandulosum
R. laxiflorum
R. sativum
R. triste
R. diacantha
R. maximowiczii
R. alpinum
R. aureum
R. odoratum
R. viburnifolium
R. valdivianum
R. americanum
R. velutinum var. velutinum
R. velutinum var. goodingii
R. binominatum
R. roezlii var. cruentum
R. speciosum
R. nigrum
R. hudsonianum var. petiolare
•Scale: —— = 0.01000
Itea virginica

scenario, the molecular dissimilarity, lack of resolution, and lack of apparent monophyly in the black currants are the result of a sudden radiation early in the history of the genus. The circumboreal (worldwide if the similar South American species are included) distribution of the black currants is also consistent with its early, sudden, radiation. Similar, more recent radiations account for the high similarity of clades that appear monophyletic in the analysis. For example, *Calobotrya* and the western gooseberries, both western North American groups, might have diversified in this manner during the Pleistocene.

The second scenario requires us to consider hybridization as an evolutionary force. Rather than (or as well as) a force for diversification, as postulated by Anderson and Stebbins (1954), gene flow due to hybridization contributes to molecular (if not morphological) homogeneity within compatible lineages. In this case, long branches, which in the iconography of the phylogenetic tree represent single common ancestors, actually represent all terminal taxa linked in a tangle of flowing genes. Such homogenizing hybridization need not be frequent or regular, and must be distinguished from rampant contemporary hybridization which would obscure the identity and relationships of lineages, especially if it occurred among less closely related species. Evidence for such modern gene flow in *Ribes* is scant. In addition, nuclear markers, although few and ambiguous, suggest patterns similar to this analysis (Messinger, Liston and Hummer1993; see Chapter II).

This second scenario could be excluded for a given lineage only if hybridization is shown to be difficult or impossible. Such data are incomplete, since most species are

not presently of commercial importance and have thus not been included in intensive controlled crossing programs.

It is most likely that these two scenarios both contributed to the history of the genus, to differing degrees in different lineages. To tease apart their relative importance, further taxonomic and genetic sampling may be necessary. In addition, biogeographic evidence will be suggestive.

While the parsimony analysis neither clearly resolves the sequence of divergence of clades nor the relationships of species within clades, it suggests several natural groups. In the strict consensus tree, which is the most conservative evolutionary hypothesis available from parsimony analysis, six clades correlate with traditional classification: the golden currant, the true gooseberry, the alpine currant, the western gooseberry, the red currant, and the ornamental currant.

As expected, the golden currants are united in this analysis: the two included species are virtually identical morphologically in addition to their lack of molecular differentiation. Other taxa in the group are poorly known Mexican species, but share similar morphology. The great molecular divergence of these two species from the rest of the genus correlates with morphology as well: the extremely long calyx tube, yellow flowers, and lack of obvious odor, an informal gauge of secondary chemistry, set this group well apart from all other *Ribes*. That the longest branch within the genus bears a western North American group implies relatively early divergence of the genus in this region, although the extreme diversity of the circumboreal section *Coreosma* suggests that its origin may have been still earlier.

The true gooseberries (all members of section *Grossularia* sampled) are robustly united in the analysis. Suggestions that smooth-styled European members of this circumboreal group are distinct (Sinnott 1985) are not supported. Further sampling of these species will be necessary to confirm the pattern.

The presence of European alpine currants on a long branch suggests that this may be a third early diverging lineage, and implies early development of dioecy in the genus. (Although a constraint tree uniting *Itea* and section *Berisia* is 8 steps (12.5%) longer than the most-parsimonious tree, the only character which unites *Itea* with any subset of the ingroup is a single *Bsr*I site gain shared with these three species, further hinting at the possibility of their early origin.)

The smooth-styled gooseberries of West North America form a distinct and very well supported lineage. This group reaches its greatest development in California: its diversity here parallels that of several other taxa, for example members of the Onagraceae and Hydrophyllaceae (Raven and Axelrod 1978). Adaptive radiations during the physiographic and climatic upheavals of the Pleistocene are implicated in California's floristic diversity (Raven and Axelrod 1978), and may have contributed to that of *Ribes*. Unsampled in this study were various Great Basin species subject to many of the same Pleistocene events as the California species. The Florida/Georgia endemic *R. echinellum*, which may be relictual (Radford 1959) should be sampled in any further work to determine its status relative to the western gooseberry clade. Sinnott (1985) suggests the latter species is more closely related to this western group

than to the true gooseberries. The position of these species relative to the western gooseberry lineage may further illuminate the timing of this radiation.

The red currants are a circumboreal clade of few species. Many segregate taxa have been recognized among the European taxa. Few morphological traits unite this group (fruit color, sparse vestiture, lack of odor). The molecular characters reported here provide additional evidence of the relationship. Association of this group with the European alpine clade and with *Ribes glandulosum* and *R. laxiflorum* is not well supported. The ornamental currant clade (including section *Calobotrya* and *Heritiera* in part) is perhaps the most weakly supported clade in the strict consensus tree. It is supported only by a single site, does not appear in the bootstrap consensus tree, and has a decay index of only one. Although the floral morphology is quite diverse, the group is geographically homogeneous. Pleistocene radiation in intermountain North America may account for the paucity of synapomorphies observed.

Several features of this tree conflict with previous ideas of *Ribes* evolution and classification. These are the placement of spiny currants with the true gooseberries; the relative paucity of evidence uniting the black currants, the placement of *Ribes glandulosum* and *R. laxiflorum* sister to red currants and European alpine currants, and the separation of gooseberries into two distinct groups.

The two species in section *Grossularioides* (spiny currants) exhibit currant inflorescence morphology: the pedicels are jointed, with disarticulating fruits, and the racemes are many-flowered. The spininess of the group separates it from most other currants (although a small subsection of *Berisia* including *R. diacantha* is also armed).

Because inflorescence morphology is considered more reliable than vegetative traits such as spines, these species are traditionally classified with the currants. Given this taxonomic framework, it is quite surprising that these species and the true gooseberries (section *Grossularia*) have similar cpDNA haplotypes. This association has two possible causes: either the cpDNA tree reflects historical branching patterns, i.e., the gene tree is congruent with the species tree; or the two spiny currants arose by hybridization. If the latter is the case, some introgression beyond chloroplast capture undoubtedly occurred, considering that morphological traits such as spines are, in a sense, nuclear markers. One possible explanation is introgression from members of the circumboreal gooseberries into currants, even though such crosses are difficult in cultivation (Keep 1962, 1975) and probably very rare in the wild. The analysis further suggests the possibility of two separate introgression events, the first producing the widespread, mesic, mid-elevation west North American and east Asian *R. lacustre* and the second the high elevation xeric-tolerant *R. montigenum* of west North America. The present data do not distinguish between the possibilities. Conflict between chloroplast ad nuclear trees may be taken as evidence of isolated hybridization events (Rieseberg and Brunsfeld 1992). Restriction site characters of a region of nuclear rDNA (Messinger, Liston and Hummer 1993) are highly ambiguous. They are few in number and highly homoplasious: Wagner parsimony weakly suggests the pattern seen here (Messinger, Liston and Hummer 1993), but weighted parsimony and maximum likelihood do not (see Chapter II). Explanation of this intriguing pattern is a high priority in further evolutionary studies of *Ribes*, but

requires the development of multiple reliable nuclear markers. Also, intensive taxonomic sampling in these two groups will be required to complete the picture.

The placement of two sections of gooseberries in separate lineages does not exclude the possibility that they are monophyletic: the basal relationships are simply unresolved. The extent of divergence measured in this study, however, suggests at least that they have been separate for a large portion of the history of the genus. Pollen characters may illuminate both this relationship and that between sections *Grossularia* and *Grossularioides*

Few characters unite the black currants in traditional taxonomic schemes. This is true of most of the infrageneric groups, however, and section *Coreosma* is as robust as any. Most workers have placed all northern hemisphere species with sessile yellow glands together in subgenus or section *Coreosma*. While *R. nigrum* and *R. hudsonianum* var. *petiolare* are weakly united by the data, *R. americanum*, *R. viburnifolium*, and *R. valdivianum* are not well placed. Coville and Britton (1908) removed *R. americanum* from the black currants based on its deeper floral tube, and placed it with a subgroup of *Calobotrya*. (Many taxonomic schemes have been proposed for the black currants. It is possible, considering the long and active history of taxonomy, that a morphological hypothesis could be found to match any molecular tree (Patterson, Williams and Humphries 1993)). If true, this would make comparisons between morphological and molecular trees moot.) *R. viburnifolium* is an island endemic, which shares the distribution of many isolated relicts (Stebbins and Major 1965, Wallace 1985). The evolutionary events that led to the establishment of

South American *Ribes* lineages must be further explored before their relationships may be discussed. Isolation may account for the unresolved placement of *R. viburnifolium* and *R. valdivianum*. The remainder of the group may also have diverged anciently, or perhaps did not participate in homogenizing hybridization: breeders have encountered difficulty in crossing within this group (see Keep 1962, 1975). In any case, neither the traditional idea of *Coreosma* nor the reliance on the single vestiture trait are acceptable according to these data, and further examination of the group is in order.

Section *Heritiera*, the dwarf currants, is also based on a single trait, even more doubtful than vestiture and odor: growth form. Such groups are unlikely to cohere in analyses employing multiple characters, and the group's polyphyletic placement in part on the ornamental currant clade and in part on the red currant/alpine currant clade is therefore unsurprising.

CONCLUSION

This study supports recognition of the following infrageneric taxa, here named informally: golden currants, European alpine currants, western gooseberries, and red currants. The true gooseberries are monophyletic, but intriguingly associated with the spiny currants. A group of west North American currants roughly corresponding to section *Calobotrya* is less well supported. Insufficient sampling does not allow discussion of the validity of placing the dioeceous South American species in a single

taxon. The dwarf currants are a polyphyletic group. Serious problems may exist in the concept of the black currants as a single group.

In addition, these results are strongly suggestive of roles for both reticulation and sudden radiation in the history of the genus. Both the association of spiny currants and true gooseberries and the extreme homogeneity of the various clades indicate that hybridization cannot be ignored in future studies of evolution in *Ribes*. Phylogenetic research within any of these groups may be hindered by the difficulty in disentangling branches from anastomoses. The existence of several diverse lineages indicates a number of radiations, both early (the event producing the golden currants) and late (among the ornamental currants and western gooseberries) in the North American West, a pattern observed in other genera.

Further work should concentrate on the relationships of the black currants, the origins of the diverse South American species, and particularly the nature of the relationship between the spiny currants and the true gooseberries. A comprehensive effort to find markers appropriate for resolving both the deeper branches in the genus and the relationships within species groups will also be valuable.

REFERENCES

Agababian, V.S. 1963. Pollen morphology of the genus *Ribes* L. Izv. Akad. Nauk Arm. S.S.R., Biol. Nauki 16: 93-98.

Albert, V.A., M.W. Chase, and B.D. Mishler. 1993. Character-state weighting for cladistic analysis of protein-coding DNA sequences. Annals of the Missouri Botanical Garden 80: 752-766.

Anderson, E., and G.L. Stebbins. 1954. Hybridization as an evolutionary stimulus. Evolution 8: 378-388.

Anderson, J.P. 1943. Two notable plant hybrids from Alaska. Proceedings of the Iowa Academy of Sciences 50: 155-157.

Applegate, E.I. 1939. Plants of Crater Lake National Park. The American Midland Naturalist 22: 225-314.

Arnold, M.L., C.M. Buckner, and J.J. Robinson. 1991. Pollen mediated introgression and hybrid speciation in Louisiana irises. Proceedings of the National Academy of Sciences, USA 88: 1398-1402.

Avise, J.C. 1994. Molecular Markers, Natural History, and Evolution. Chapman & Hall, New York.

Bates, J.C. 1933. Comparative anatomical research within the genus *Ribes*. The University of Kansas Science Bulletin 21: 369-398.

Bate-Smith, E.C. 1976. Chemistry and taxonomy of *Ribes*. Biochemical Systematics and Ecology 4: 13-23.

Berger, A. 1924. A taxonomic review of currants and gooseberries. Technical Bulletin of the New York State Agricultural Experiment Station 109: 1-118.

Berlandier, J.L. 1826. Mémoire sur les Grossulariées. Mémoires de la Société de physique et d'Histoire Naturelle de Genève 3: 43-60.

Bohm, B.A. 1993. External and vacuolar flavonoids of *Ribes viscossisimum*. Biochemical Systematics and Ecology 21: 745.

Brennan, R.M. 1991. Currants and Gooseberries (*Ribes*). In J.N. Moore, and J.R. Ballington [eds.], Genetic Resources of Temperate Fruit and Nut Crops, 457-488. International Society of Horticultural Sciences, Wageningen, The Netherlands.

Chase, M.V., D.E. Soltis, R.G. Olmstead, D. Morgan, D.H. Les, B.D. Mishler, M.R. Duvall, R. Price, H.G. Hillis, Y.-L. Qui, K.A. Kron, J.H. Rettig, E. Conti, J.D. Palmer, J.R. Manhart, J. Sytsma, H.J. Michaels, W.J. Kress, K.G. Karol, W.D. Clark, M. Hedren, B.S. Gaut, R.K. Jansen, K.-J. Kim, C.F. Wimpee, J.F. Smith, G.R. Furnier, S.J. Strauss, Q.-Y. Xiang, G.M. Plunkett, P.S. Soltis, S.E. Williams, P.A. Gadek, C.J. Quinn, L.E. Equiarte, E. Golenberg, G.H. Learn, S. Graham, S.C.H. Barrett, S. Dayanadan, and V.A. Albert. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Annals of the Missouri Botanical Garden 80: 528-580.

Coville, F.V., and N.L. Britton. 1908. Grossulariaceae. North American Flora 22: 193-225.

Cronquist, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York.

D'Arcy, W.G. 1987a. Flora of Panama: checklist and index part I: The introduction and checklist. Missouri Botanical Garden, St. Louis.

_____ 1987b. Flora of Panama: checklist and index part II: index. Missouri Botanical Garden, St. Louis.

Darlington, C.D. 1929. A comparative study of the chromosome complement in *Ribes*. Genetica 11: 267-272.

DeBry, R.W., and N.A. Slade. 1985. Cladistic analysis of restriction endonuclease cleavage maps within a maximum-likelihood framework. Systematic Zoology 34: 21-34.

Donoghue, M.J., R.G. Olmstead, J.F. Smith, and J.D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Annals of the Missouri Botanical Garden 79: 333-345.

Downie, S.R., and J.D. Palmer. 1994. A chloroplast DNA phylogeny of the Caryophyllales based on structural and inverted repeat restriction site variation. Systematic Botany 19: 236-252.

Doyle, J.J., and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

Erdtman, G. 1966. Pollen morphology and plant taxonomy: angiosperms. Hafner, NY.

_____ 1969. Handbook of Palynology: Morphology-Taxonomy-Ecology. Munksgaard, Copenhagen.

Erlich, H.A., D. Gelfand, and J.J. Sninsky. 1991. Recent advances in the polymerase chain reaction. Science 252: 1643-1651.

Felsenstein, J. 1978. Cases in which parsimony and compatibility methods will be positively misleading. Systematic Zoology 27: 401-410.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

_____ 1991. PHYLIP: Phylogeny Inference Package (version 3.4). Computer program distributed by the author, University of Washington, Seattle.

_____ 1992. Phylogenies from restriction sites: a maximum-likelihood approach. Evolution 46: 159-173.

Fritsch, P., and L.H. Rieseberg. 1993. Chloroplast DNA restriction site analysis of *Styrax* series *Imbricatae* (Styracaeae): Implications for the Madrean-Tethyan hyothesis. (abstract). American Journal of Botany 80: 149.

Genetics Computer Group (1991): Program manual for the GCG package version 7, April 1991. 575 Science Drive, Madison, Wisconson.

Goldschmidt, E. 1964. Cytological species and interspecific hybrids of the genus *Ribes*. Hereditas 52: 139-150.

Golenberg, E.M., M.T. Clegg, M. Durbin, J. Doebley, and D.P. Ma. 1993. Evolution of a non-coding region of the chloroplast genome. Molecular Phylogenetics and Evolution 2: 52-64.

Grant, V. 1971. Plant Speciation. Columbia University Press, New York.

Henry, J.K. 1919. *Ribes divaricatum* X *Ribes lobbii*. Canadian Field Naturalist 19: 94.

Heusser, C.J. 1971. Pollen and Spores of Chile: Modern types of the Pteridophyta, Gymnospermae and Angiospermae. University of Arizona Press, Tucson.

Hibbett, D.S., and R. Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. Systematic Botany 18: 409-433.

Hickman, J.C. 1969. Disjunction and endemism in the flora of the central western Cascades of Oregon: an historical and ecological approach to plant distributions. Ph.D. Thesis, University of Oregon, Eugene.

Hideux, M.J., and I.K. Ferguson. 1976. The stereostructure of the exine and its evolutionary significance in Saxifragaceae *sensu lato*. In I.K. Ferguson, and J. Muller [eds.], The Evolutionary Significance of the Exine. Academic Press, New York.

Hillis, D.M., J.P. Huelsenbeck, and C.W. Cunningham. 1994. Application and accuracy of molecular phylogenies. Science 264: 671-677.

Hillis, D.M., J.P. Huelsenbeck, and D.L. Swofford. 1994. Hobgoblin of phylogenetics? Nature 369: 363-364.

Huelsenbeck, J.P., and D.M. Hillis. 1993. Success of phylogenetic methods in the four-taxon case. Systematic Biology 42: 247-264.

Janczewski, E. de. 1903. Essai d'une disposition naturelle des espèces dans le genre *Ribes* L. Bulletin international de l'Academie des sciences de Cracovie, Classe des sciences mathematiques et naturelles : 232-241.

_____ 1906a. Species generis *Ribes* L. II Subgenera *Ribesia* et *Coreosma*. Bulletin International de l'Académie des Sciences de Cracovie. Classe des Sciences Mathématique et Naturelles : 1-13.

_____ 1906b. Species generis *Ribes* L. III. Subgenera *Grossularioides*, *Grossularia*, et *Berisia*. Bulletin International de l'Académie des Sciences de Cracovie. Classe des Sciences Mathématique et Naturelles : 280-293.

_____ 1907. Monographie des groseilliers *Ribes* L. Mémoires de la Société de physique et d'Histoire Naturelle de Genève 35: 199-517.

Keep, E. 1962. Interspecific hybridization in *Ribes*. Genetica 33: 1-23.

Keep, E. 1975. Currants and gooseberries. In J. Janick, and J.N. Moore [eds.], Advances in Fruit Breeding, 197-268. Purdue University Press, West Lafeyette, Indiana.

Kimura, M. 1980. A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111-120.

Liston, A. 1992. Variation in the chloroplast gene *rpo*C1 and *rpo*C2 of the genus *Astragalus* (Fabaceae): evidence from restriction mapping of a PCR-amplified product. American Journal of Botany 79: 953-971.

Liston, A., and J.A. Wheeler. 1994. The phylogenetic position of the genus *Astragalus* (Fabaceae): evidence from the chloroplast genes *rpo*C1 and *rpo*C2. Biochemical Systematics and Ecology 22: 377-388.

Lundberg, J. 1972. Wagner networks and ancestors. Systematic Zoology 21: 398-413.

Mabberly, D.J. 1987. The plant-book. Cambridge University Press, Cambridge.

Maddison, D. 1991. The discovery and importance of multiple islands of most parsimonious trees. Systematic Zoology 40: 315-328.

Mesler, M.R., J.C. Cole, and P. Wilson. 1991. Natural hybridization in western gooseberries (*Ribes* subgenus *Grossularia*: Grossulariaceae). Madroño 38: 115-129.

Mesler, M.R., and J.O. Sawyer. 1993. Grossulariaceae: gooseberry family. In J.C. Hickman [ed.], The Jepson Manual: Higher Plants of California. University of California Press, Berkeley.

Messinger, W., A. Liston, and K. Hummer. 1993. Restriction site mapping of *Ribes* nuclear ribosomal DNA. Acta Horticulturae 352: 175-184.

Meurman, O. 1928. Cytological studies in the genus *Ribes*. Hereditas 11: 289-356.

Mishler, B., M. Donoghue, and V. Albert (1991): The decay index as a measure of relative robustness within a cladogram. Tenth Meeting of the Willi Hennig Society, Toronto. (abstract)

Morgan, D.R., and D.E. Soltis. 1993. Phylogenetic relationships among members of the Saxifragaceae *sensu lato* based on *rbc*L sequence data. Annals of the Missouri Botanical Garden 80: 631-660.

Norris, LL. 1987. Status of five rare plant species in Sequoia and Kings Canyon National Parks. In Conservation and Management of Rare and Endangered Plants. California Native Plant Society.

Ogihara, Y., T. Terachi, and T. Sasakuma. 1991. Molecular analysis of the hot spot region related to length mutations in wheat chloroplast DNAs. I. Nucleotide divergence of genes and intergenic spacer regions located in the hot spot region. Genetics 129: 873-884.

Olmstead, R.G., and J.D. Palmer. 1994. Chloroplast DNA systematics: a review of methods and data analysis. American Journal of Botany 81: 1205-1224.

Pastre, A., and A. Pons. 1973. Quelques aspects de la systématique des Saxifragacées à lumières des données de la palynologie. Pollen et Spores 15: 117-133.

Patterson, C., D.M. Williams, and C.J. Humphries. 1993. Congruence between molecular and morphological phylogenies. Annual Review of Ecology and Systematics 24: 153-188.

Potter, D., and J.J. Doyle. 1994. Phylogeny and systematics of *Sphenostylis* and *Nesphostylis* (Leguminosae: Phaseolae) based on morphological and chloroplast DNA data. Systematic Botany 19: 389-406.

Poyarkova, A.I. 1939. *Ribes*ioideae. In V.L. Komarov, and S.V. Yuzepchuk [eds.], Flora of the U.S.S.R, 175-208. Israel Program for Scientific Translations, Jeruselam, for the Smithsonian Institute and National Science Foundation, Washington D.C.

Radford, A.E. 1959. A relict plant community in South Carolina. Journal of the Elisha Mitchell Scientific Society 75: 35-43.

Raven, P.H., and D.I. Axelrod. 1978. Origin and relationships of the California flora. University of California Publications in Botany 72: 1-134.

Rieseberg, L.H., M. Hanson, and C.T. Philbrick. 1992. Androdioecy is derived from dioecy in Datiscaceae; evidence from restriction site mapping of PCR-amplified chloroplast DNA fragments. Systematic Botany 17: 324-336.

Rowland, L.J., and B. Nguyen. 1993. Use of polyethylene glycol for purification of DNA from leaf tissue of woody plants. Biotechniques 14: 735-736.

Schwarzbach, A. and J.W. Kadereit. In Press. Rapid radiation of North American desert genera of the *Papaveraceae*: evidence from restriction site mapping of PCR-amplified chloroplast DNA fragments. Plant Systematics and Evolution

Shimada, K., M. Fukuta, M. Ishikawa, and M. Sugiura. 1990. Rice chloroplast RNA polymerase genes: the absence of an intron in *rpo*C1 and the presence of an extra sequence in *rpo*C2. Molecular and General Genetics 221: 395-402.

Shinozaki, K., M. Ohme, M. Tanaka, T. Wakasugi, N. Hayashida, T. Matsubayashi, N. Zaita, J. Chunwongse, J. Obokota, K. Yamaguchi-Shinozaki, C. Ohto, K. Torazawa, B.-Y. Meng, M. Sugita, H. Deno, T. Kamagoshira, K. Yamada, J. Kusuda, F. Takaiwa, A. Kato, N. Tohdoh, H. Shimada, and M. Suguira. 1986. The

complete nucleotide sequence of the tobacco chloroplast genome: its organization and expression. EMBO Journal 5: 2043-2049.

Sinnott, Q.P. 1985. A revision of *Ribes* L. Subg. *Grossularia* (Mill.) Pers. sect. *Grossularia* (Mill.) Nutt. (Grossulariaceae) in North America. Rhodora 87: 189-286.

Smith, E.L., and Q.P. Sinnott. 1984. Department of the Interior, Fish and Wildlife Service, 50 CFR Part 17: Endangered and threatened wildlife and plants; *Ribes echinellum* (Miccosukee Gooseberry) proposed to be a threatened species. Federal Register 49: 34535-34537.

Smouse, P.E., and W.-H. Li. 1987. Likelihood analysis of mitochondrial restriction-cleavage patterns for the human-chimpanzee-gorilla trichotomy. Evolution 41: 1162-1176.

Soltis, D.E., P.S. Soltis, M.T. Clegg, and M. Durbin. 1990. *rbc*L sequence divergence and phylogenetic relationships in Saxifragaceae sensu lato [*sic*]. Proceedings of the National Academy of Sciences, USA 87: 4640-4644.

Soltis, D.E., E.R. Morgan, A. Grable, P.S. Soltis, and R. Kuzoff. 1993. Molecular systematics of Saxifragaceae *sensu lato*. American Journal of Botany 80: 1056-1081.

Spach, E. 1835. Revisio Grossularierum. Ann. Sci. Nat. Bot. II 4: 16-31.

_____ 1838. Histoire Naturelle des Végétaux. Phanerogames. Revet, Paris.

Spongberg, S.A. 1972. The genera of Saxifragaceae in the southeastern United States. Journal of the Arnold Arboretum 53: 109-498.

Standley, P.C. 1937. Flora of Costa Rica. Field Museum of Natural History, Chicago.

Stebbins, G.L., and J. Major. 1965. Endemism and speciation in the California flora. Ecological Monographs 35: 1-35.

Stern, W.L., E.M. Sweitzer, and R.E. Phipps. 1970. Comparative anatomy and systematics of woody Saxifragaceae. *Ribes*. In N.K.B. Robson, D.F. Cutler, and M. Gregory [eds.], New Research in Plant Anatomy. Academic Press, London.

Swofford, D.L. (1992): PAUP: Phylogenetic Analysis Using Parsimony, version 3.0s+4(beta). Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Swofford, D.L., and G.J. Olsen. 1990. Phylogeny reconstruction. In D.M. Hillis, and C. Moritz [eds.], Molecular Systematics, 411-501. Sinauer Associates, Sunderland, Massachusetts.

Takhtajan, A.L. 1980. Outline of the classification of flowering plants (Magnoliophyta). Botanical Review 46: 225-359.

Thorne, R.F. 1992. Classification and geography of the flowering plants. Botanical Review 38: 225-348.

Verbeek-Reuvers, A.A.M.L. 1980. Grossulariaceae. In W. Punt, and G.C.S. Clarke [eds.], The Northwest European Pollen Flora, 107-116. Elsevier Scientific Publishing Company, New York.

Wallace, G.D. 1985. Vascular plants of the Channel Islands of Southern California and Guadalupe Island, Baja California, Mexico. Natural History Museum of Los Angeles County, Los Angeles.

Wolfe, A.D., W.J. Elisens, L.E. Watson, and C.W. dePamphilis. 1993. Using RFLPs of chloroplast gene PCR-products for phylogenetic analysis: when is enough, enough? (abstract). American Journal of Botany 80: 183.

Wolfe, A.D., W.J. Elisens, L.E. Watson, C.W. dePamphilis, and C.E. Freeman. 1993. A phylogenetic and biogeographic analysis of the North American Cheloneae (Scrophulariaceae): evidence from chloroplast PCR-product restriction site variation. (abstract). American Journal of Botany 80: 183.

Wynd, F.L. 1936. The flora of Crater Lake National Park. The American Midland Naturalist 17: 881-949.

Zielinski, Q.B. 1953. Chromosome numbers and meiotic studies in *Ribes*. Botanical Gazette 114: 265-274.1

CHAPTER II

RIBES PHYLOGENETICS BASED ON RESTRICTION SITE MAPPING OF NUCLEAR RIBOSOMAL DNA

INTRODUCTION

Chloroplast DNA markers (mainly restriction site analysis of the entire chloroplast genome and sequence of the chloroplast gene *rbc*L) have been the workhorses of plant systematics in recent years (Olmstead and Palmer 1994). While their utility is indisputable, a number of problems with relying on a single gene or tight linkage group (such as the chloroplast genome) to reconstruct evolutionary history are known. In general, the potential lack of correspondence between gene phylogeny and organismal phylogeny requires that single-gene trees be interpreted with extreme caution (Doyle 1992). Chloroplast markers are most often maternally inherited, and thus may give a distorted picture of organismal phylogeny. Hybridization events may be obscured by lack of nuclear markers in a study, and chloroplast capture resulting from such events can obscure phylogeny (Rieseberg 1991, Rieseberg and Brunsfeld 1992). For all of these reasons, use of nuclear markers in conjunction with chloroplast markers is preferred.

The nuclear ribosomal genes in angiosperms occur as a tandem repeat of 6 distinct regions. The region coding for the ribosomal small subunit (18s nrDNA) is separated by a transcribed spacer (ITS-1) from the 5.8s nrDNA, which is in turn separated from the large subunit (26s nrDNA) by a transcribed spacer (ITS-2). Between the 18s and 26s nrDNA is a long, highly variable region that is not transcribed, the intergenic spacer (IGS). The bulk of the IGS consists of short tandem repeats, and much of the variation in this region is in number of these repeats.

In plants, restriction mapping of the entire nrDNA repeat has been informative within genera (Kim and Mabry 1991) and even within species (Schaal and Learn 1988). Much of the useful variation at these levels is found in the IGS. Sequence of the two ITS regions is currently the systematic marker of choice within genera of plants (Baldwin 1992, 1993; see Baum et al. 1994).

Restriction site variation of a region of nrDNA, amplified by PCR, was examined as a nuclear marker in *Ribes*. High copy number and availability of PCR primers make the nrDNA repeat relatively straightforward to amplify by PCR. The region examined includes the 18S and 5.8S coding regions, and the two internal transcribed spacers (ITS-1 and ITS-2). ITS-1 exhibits about twice the variation of ITS-2 in some vascular plant groups (Baldwin 1992, 1993). The IGS could not be amplified, possibly due to excessive length or secondary structure associated with extensive tandem repeats.

MATERIALS AND METHODS

Study Group

The study group is similar to that of the chloroplast DNA study (Chapter I). A number of species were not included in this earlier work because they were not yet available. These include *Ribes triste* Pallas, *R. cf. valdivianum* Phil, *R. velutinum* Greene var. *velutinum*, and *R. cf. ciliatum* Humb. & Bonpl. A second sample in section *Ribes*, the cultivar 'Cherry' (NPGR Accession RIB19) was included. The

outgroup of choice, *Itea virginica* L., was not yet available. However, this taxon is distant from *Ribes*. In the parsimony analysis of cpDNA restriction sites (Chapter I), midpoint rooting gave the same result as rooting with *Itea* as outgroup, in other words, the root was placed at the longest branch in the ingroup. This suggests that long branch attraction may be operating and the root so designated may not differ from that of a random outgroup. Midpoint rooting is used in this analysis to aid comparison with the cpDNA results.

Laboratory Methods

Laboratory methods are described in Chapter I. Addition of 5% DMSO to PCR reactions was necessary in many taxa. Primer sequences were from Nickrent and Franchina (1990) and Nickrent (pers. comm.): CTG GTT GAT CCT GCC AG corresponding to positions 4-24 in the *Glycine* 18S sequence (Eckenrode et al. 1985), and TAT GCT TAA ACT CAG CGG GT corresponding to positions 45-26 in the *Oryza* 26S sequence (Takaiwa et al. 1985).

Frequent cutters revealing variation in this study were: *Bgl*I, *Bsm*aI, *HaeIII*, *Rsa*I, *Sau*96I, and *Scr*fI. Restriction maps were constructed by double digests with an array of infrequent cutters: *Ase*I, *Bam*HI, *Bsr*I, *Cla*I, *Eco*RI, *Eco*RV, *Kpn*I, *Sac*I, *Xba*I, and *Xmn*I.

Data Analysis

Variable restriction sites were scored as binary characters. Phylogenetic analysis was carried out with the UNIX version of PAUP 3.0s+4 (beta) (Swofford 1992) using the heuristic search algorithm and both Wagner and weighted parsimony. When parallel events are extremely common (as in this restriction site data set based on a small, highly variable sequence), parsimony methods can fail to give the most likely tree (Felsenstein 1978). Maximum likelihood methods based on explicit models of restriction site evolution are designed to overcome this problem.

The data was subject to the maximum-likelihood method of Felsenstein (1992) as implemented in the PHYLIP (version 3.4) program RESTML (Felsenstein 1991).

RESULTS

Restriction Map

Sites of restriction enzymes which revealed mutations were mapped. Most of these sites map to ITS-1. Thirty-nine sites including a total of 167 nucleotides were mapped. Map locations of each restriction site are given with the data matrix derived from them (Appendix II). Of these, 15 were variable, and 13 exhibited shared variation: these potentially represent phylogenetic information. An important feature of this data set is that, except for three pairs and a trio of close relatives, each species

exhibits a unique haplotype, or restriction site profile, based on only seven endonucleases.

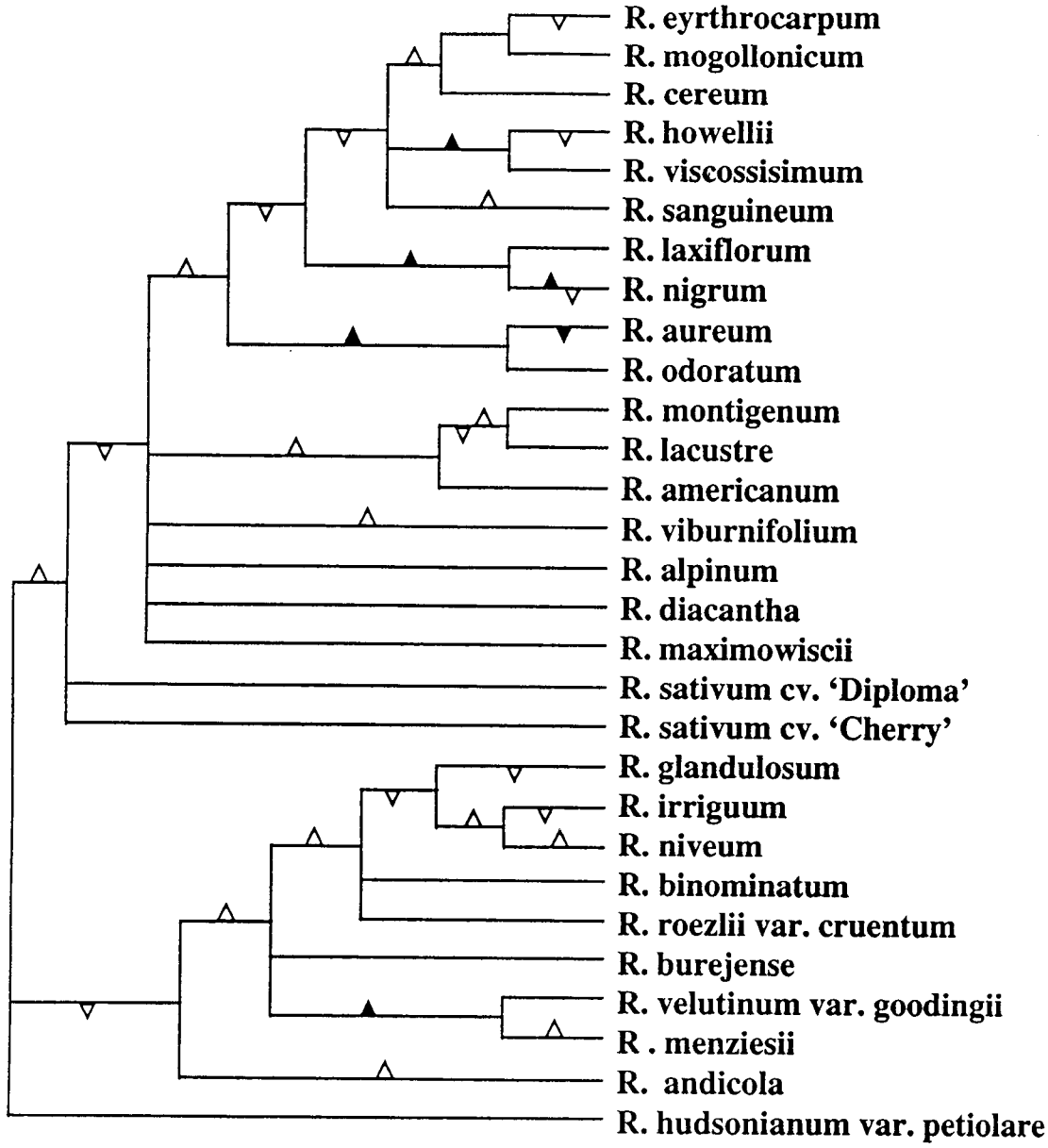
Parsimony Analysis

Weighted parsimony produced 40 most-parsimonious trees (CI 0.533, CI excluding autapomorphies 0.500, RI 0.754). The strict consensus of these trees is unresolved except for six pairs of taxa: *Ribes howellii* plus *R. viscossisimum*, *R. laxiflorum* plus *R. nigrum*, *R. montigenum* plus *R. lacustre*, *R. aureum* plus *R. odoratum*, and *R. velutinum* var. *goodingii* plus *R. menziesii*. A majority-rule consensus tree of the weighted parsimony results, identical to one of the most-parsimonious trees, is presented as Figure 2.1. Neither number of characters nor appearance in the strict consensus tree lend significant support to any branch. Bootstrap analysis was not performed. Problems with mapping mutations onto consensus trees have already been discussed.

Wagner parsimony produced 2,592 most-parsimonious trees of 30 steps. The strict consensus of these trees is even less resolved than that produced by weighted parsimony, retaining only the species pair *Ribes menziesii-R. velutinum* var. *goodingii*. The majority rule consensus differs primarily in that the North American members of *Grossularia* are sister to *Grossularioides* in 52% of the trees.

Figure 2.1 Most-parsimonious tree identical to majority rule consensus tree retaining compatible groups. Produced from nuclear ribosomal data by weighted parsimony. One of 40 trees 30 steps in length, CI 0.533, CI excluding autapomorphies 0.500. RI 0.754. Upward triangles are site gains; downward triangles are site losses. Open triangles are homoplasious sites. To facilitate comparison with cpDNA results, the tree is rooted at its midpoint. This is identical to functional outgroup rooting with *R. hudsonianum* var. *petiolare*.

Figure 2.1


R. eyrthrocarpum
R. mogollonicum
R. cereum
R. howellii
R. viscossisimum
R. sanguineum
R. laxiflorum
R. nigrum
R. aureum
R. odoratum
R. montigenum
R. lacustre
R. americanum
R. viburnifolium
R. alpinum
R. diacantha
R. maximowiscii
R. sativum cv. 'Diploma'
R. sativum cv. 'Cherry'
R. glandulosum
R. irriguum
R. niveum
R. binominatum
R. roezlii var. cruentum
R. burejense
R. velutinum var. goodingii
R . menziesii
R. andicola
R. hudsonianum var. petiolare

Maximum Likelihood Analysis

Results of maximum likelihood analysis are presented as an unrooted tree (Figure 2.2). Branches of zero length or no statistical significance are collapsed. Some similarities to parsimony analysis are apparent. A prominent feature of this analysis is the separation of a group including all the members of section *Calobotrya*. The three members of section *Berisia* appear together on branches of zero length, as do two cultivated red currants and the *Ribes menziesii-R. velutinum* var. *goodingii* species pair.

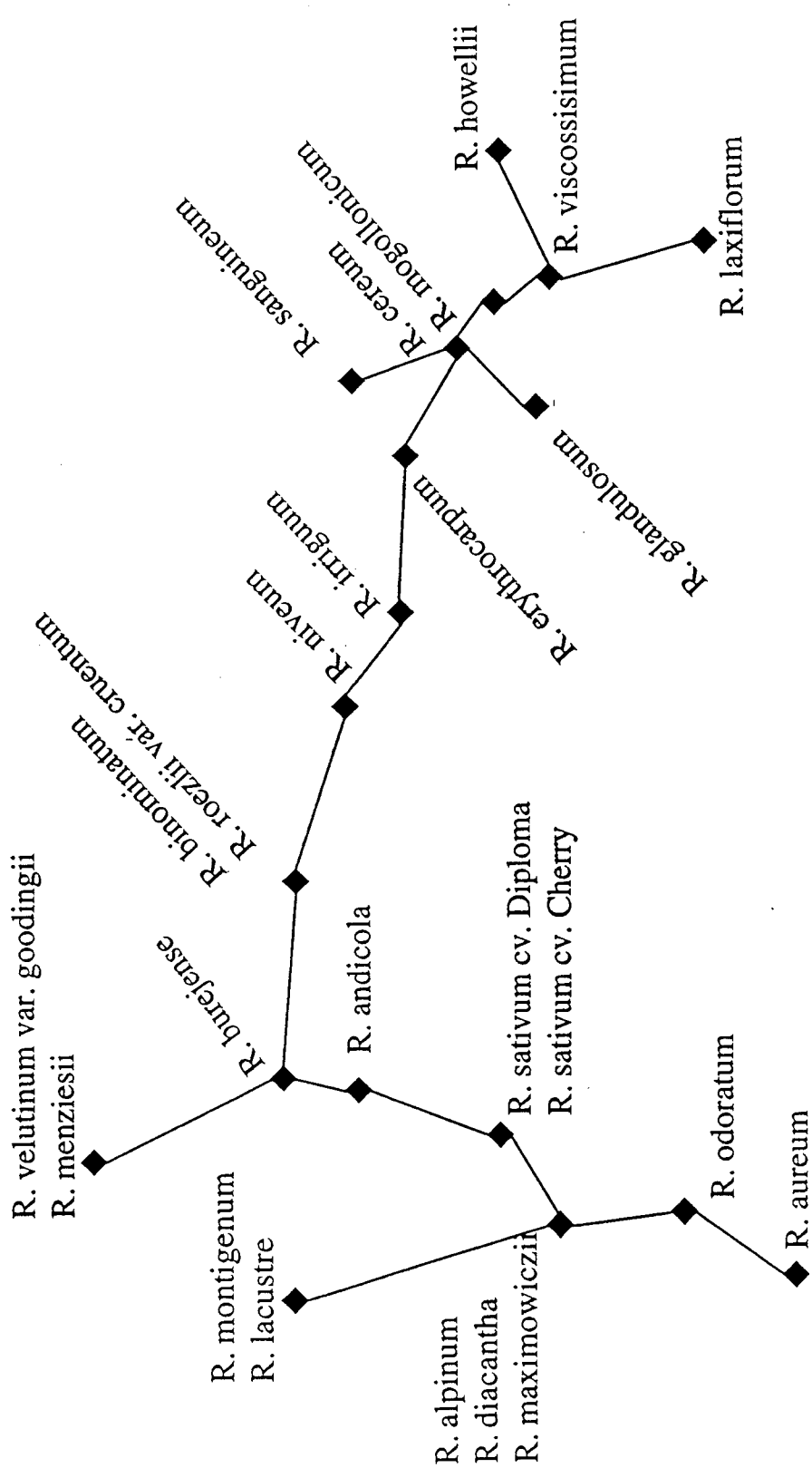
DISCUSSION

This data set does not give well-resolved phylogenies due to its low ratio of informative characters to terminal taxa and the high levels of homoplasy in those characters. This level of homoplasy suggests that ITS-1 may be changing too rapidly to be taxonomically useful at the sectional level in *Ribes*, at least when sampled with restriction enzymes. Sequencing this gene would yield more characters, but problems of homoplasy might persist even with such increased sampling.

Several features of this analysis are relevant to problems in *Ribes* taxonomy, in spite of the need for more characters before definitive conclusions can be drawn. The following discussion refers to the parsimony analyses. Section *Heritiera* was previously treated an independent group (Berger 1924) and as a section of subgenus *Coreosma* (the black currants) (Janczewski 1907). This analysis suggests that some of its members are allied with section *Calobotrya*, while others are not. Section

Figure 2.2. Maximum likelihood tree produced from nrDNA restriction site data. Branches are approximately proportional to distance, those with non-significant length are collapsed. Branch angles are arbitrary and tree is unrooted.

Figure 2.2


R. velutinum var. goodingii
R. menziesii
R. montigenum
R. lacustre
R. alpinum
R. diacantha
R. maximowiczii
R. burejense
R. andicola
R. sativum cv. Diploma
R. sativum cv. Cherry
R. odoratum
R. aureum
R. binominatum
R. roezlii var. cruentum
R. niveum
R. inermis
R. erythrocarpum
R. glandulosum
R. sanguineum
R. cereum
R. mogollonicum
R. howellii
R. viscossisimum
R. laxiflorum

Grossularia includes both European and North American species. The North American and some Eurasian species (including the section's type) have pubescent styles. *R. burejense*, a smooth-styled Eurasian member of the section, is separated from the hairy-styled North American species in this analysis (Figures 2.1 and 2.2), and appears to be more closely related to the smooth-styled western North American species. Monophyly of section *Calobotrya* is supported. Monophyly of section *Berisia* (the Old World alpine currants), including the spiny *R. diacantha* is also supported: these species exhibit identical haplotypes.

The value of comparing these results to the cpDNA trees (Chapter I) is limited by low character number and high homoplasy as well. The main conflict between the two analyses is the separation of the spiny currants and the true gooseberries in the weighted nrDNA tree. This separation does not appear in the Wagner parsimony trees. The conflict in these analyses is a result of excessive homoplasy in the data set, and the nature of the association between the two groups must remain ambiguous until more markers are developed. *Ribes burejense* is not united with other members of *Grossularia* (the true gooseberries). Several groups observed in the cpDNA tree are preserved, however. These include a clade uniting the members of *Grossularioides* (oddly including *R. americanum)*, a clade with *Symphocalyx*, and a *Calobotrya* clade including *Heritiera* in part which is identical to that in the cpDNA tree.

Even though few characters are produced by restriction site mapping of this nrDNA region, the extent to which their analysis supports the cpDNA results is

remarkable. However, due to the small number of characters, and their extreme homoplasy, these trees are not proposed as phylogenetic hypotheses. Neither do they constitute strong evidence for or against the cpDNA hypotheses.

REFERENCES

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. Molecular Phylogenetics and Evolution 1: 3-16.

_____ 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on its sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. American Journal of Botany 80: 222-238.

Baum, D.A., K.J. Sytsma and P.C. Hoch. 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. Systematic Botany 19: 363-388.

Berger, A. 1924. A taxonomic review of currants and gooseberries. Technical Bulletin of the New York State Agricultural Experiment Station. 109: 1-118.

Doyle, J.J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Systematic Botany 17: 144-163.

Eckenrode, V.K, J. Arnold and R.B. Meagher. 1985. Comparison of the nucleotide sequence of soybean 18S rRNA with the sequences of other small-subunit rRNAs. Journal of Molecular Evolution. 21: 259-269.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Systematic Zoology 27:401-410.

_____ 1991. PHYLIP: Phylogeny Inference Package (version 3.4). Computer program distributed by the author, University of Washington, Seattle.

_____ 1992. Phylogenies from restriction sites: a maximum-likelihood approach. Evolution 46: 159-173.

Janczewski, E. de. 1907. Monographie des groseilliers *Ribes* L. Mémoires de la Société de physique et d'Histoire Naturelle de Genève 35: 199-517.

Nickrent, D., and C.R. Franchina. 1990. Phylogenetic relationships of the Santalales and relatives. Journal of Molecular Evolution 31: 294-301.

Rieseberg, L.H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. American Journal of Botany 78:1218-1237.

Rieseberg, L.H., and S.J. Brunsfeld. 1992. Molecular evidence and plant introgression. IN P.S. Soltis, D.E. Soltis and J.J. Doyle [eds.] Molecular Systematics of Plants. Chapman and Hall, New York.

Swofford, D.L. 1992. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.0s+4(beta). Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Takaiwa, F., K. Oono, and M. Sugiura. 1985. Nucleotide sequence of the 17S-25S spacer region from rice rDNA. Plant Molecular Biology 4: 355-364.

CHAPTER III

BRIEF REVIEWS

POLLEN MORPHOLOGY OF *RIBES*

No comprehensive survey of *Ribes* pollen exists. The available information is largely in the context of regional floras (Verbeek-Ruevers 1980, Huesser 1971, Adams and Morton 1974). Work with a taxonomic focus is limited to consideration of the disposition of Saxifragaceae *sensu lato* (e.g. Hideux and Ferguson 1976). From these works, and a number of basic palynological references, a sketch of the essential features of *Ribes* pollen and its variation can be made.

Table 3. Concordance of sectional names (Berger 1924) with major lineages of *Ribes*

Subgenus, Section	Common Name	Major Lineage
Ribes	**currants**	
Ribes	red currants	red currant
Coreosma	black currants	none
Symphocalyx	golden currants	golden currant
Calobotrya	ornamental currants	ornamental currant
Berisia	European alpine currants	European alpine currants
Parilla	Andine currants	none
Heritiera	Dwarf currants	polyphyletic: ornamental currant lineage or none
Grossularioides	spiny currants	true gooseberry + spiny currant
Grossularia	**gooseberries**	
Grossularia	true gooseberries	true gooseberry + spiny currant
Hesperia, *Lobbia*, *Robsonia*	western gooseberries	western gooseberries

Of the infrageneric taxa suggested by Berger (1924), eight are represented in the literature by a figure or description. These represent the European gooseberries of commerce (*Grossularia*, represented by *R. uva-crispa* (Verbeek-Ruevers 1980)), a species of western North American gooseberry (section *Hesperia*: *R. roezlii* (Erdtman 1969)), two groups of west North American currants (*Calobotrya*, represented by *R. pringlei* of Mexico (Erdtman 1966) and *R. sanguineum* (Hideux and Ferguson 1976, Verbeek-Ruevers 1980)), and *Symphocalyx*, represented by *R. aureum* (discussed in Verbeek-Ruevers 1980). The South American currants (*Parilla*), are represented by *R. valdivianum* (Huesser 1971), and the European dioecious currants (*Berisia*) are represented by *R. alpinum* (Verbeek-Ruevers 1980). *Ribes rubrum* (Hideux and Ferguson 1976, Pastre and Pons 1973, Verbeek-Ruevers 1980) represents section *Ribesia*, the red currants, although Verbeek-Ruevers (1980) also discusses *R. petraeum* and *R. spicatum*. Section *Coreosma*, the black currants, is represented by *R. nigrum* (Verbeek-Ruevers 1980). Some of these taxa are represented by a single drawing, without explanation, others by excellent descriptions and micrographs (Verbeek-Ruevers 1980 being the best). Sections *Grossularioides*, *Heritiera*, *Lobbia*, and *Robsonia* are not represented.

All *Ribes* pollen is little ornamented (psilate), with pores (endoapertures) surrounded by thinner, rugose regions of the exine (ectoapertures). Of the morphological differences, the most striking is between the currants and gooseberries. Pollen of all currants is isodiametric, with evenly spaced pores (panto- or periporate). *R. uva-crispa* pollen is elliptic, equatorially zonocolporate (the pores situated in

furrows confined to a single area) (Verbeek-Ruevers 1980). A single drawing of the pollen of *R. roezlii* (Erdtman 1969) appears virtually identical to that of *R. uva-crispa*.

Currant pollen may be distinguished by size, shape, number of endoapertures, and number and shape of ectoapertures. *Ribes nigrum* has more or less cubical, 6-porate pollen. Each pore is situated in a more or less circular endoaperture (Verbeek-Ruevers 1980). *R. valdivianum* has virtually identical pollen (Huesser 1971), while the pollen of *R. alpinum* is morphologically similar, but smaller (Verbeek-Ruevers 1980). Red currant pollen is similar in size to that of *R. nigrum*, but with 8-14 pori present. These are situated singly in round ectoapertures and in pairs in the ends of dumbbell-shaped ectoapertures. It appears from inadequate data that *R. aureum* (Verbeek-Ruevers 1980), *R. pringlei* (Erdtman 1966), and *R. sanguineum* (Hideux and Ferguson 1976, Verbeek-Ruevers 1980) most closely resemble this type, but the pores of the latter are most often single in round ectoapertures.

It is apparent from these descriptions that pollen morphology may have taxonomic value in *Ribes*, particularly at the infrageneric level. Several relationships are tentatively suggested by the data. The most important of these is the distinctiveness of the gooseberries. Pollen morphology may be added to few-flowered racemes, unjointed pedicels, and spines as a character that distinguishes all groups of gooseberries from the rest of *Ribes*. Such consistency supports the validity of these characters. Of course, it is possible that the factors controlling inflorescence morphology also affect pollen morphology. If this were the case, these traits would reduce to a single

character, losing much of their power. The two gooseberries represented have been placed in separate groups based largely on stigma vestiture: these pollen traits do not support that distinction.

While differences among currant pollen types are less obvious, the contrasts in aperture number and shape may be biologically meaningful. *Ribes nigrum* shares endoaperture number and ectoaperture shape (as well as cubical spore shape) with *R. valdivianum* of Chile. These species are generally classified in separate subgenera, but they share the sessile yellow glands diagnostic of black currants (indeed, Janczewski (1907) terms this group of South American currants 'dioecious *Eucoreosma*'). *R. alpinum* has significantly smaller pollen spores than these two species, but its aperture traits are the same. The small spore size may, however, limit pore number, making morphological similarity inevitable.

It is not surprising that the closely related European red currants have identical pollen morphology. Both pore number and ectoaperture shape may unite them with the two groups of west North American currants. Unfortunately, the pollen of the latter groups is poorly studied, and the nature of their pollen morphology and the relationships it might suggest remain ambiguous. Further study might uncover more variation in these traits, as well as new traits, perhaps of internal surface of endoapertures, or exine stratification (as Hideux and Ferguson 1976), aperture margins, colpus membrane sculpturing, margo presence or absence, aperture cover, or columella size and shape.

This body of work is both exciting and frustrating: it hints at a suite of valuable characters, relatively straightforward to extract, whose implications will remain obscure until a thorough survey of the genus is made. Of particular interest is the pollen of section *Grossularioides*, since its morphology may be relevant to the relationship of this group with section *Grossularia*.

A PROVISIONAL KEY TO *RIBES* POLLEN

Prepared from the literature by W. Messinger, April 1994. Many of these taxa were represented only by single figures.

I. grains isodiametric, pantoporate

A. Endoapertures 6, single in ± circular ectoapertures, arranged as on the faces of a cube (currants with various sessile glands)

a. Grains 27-41 microns . . . . . *R. nigrum, R. valdivianum*

b. Grains 18-24 microns . . . . . *R. alpinum*

B. Endoapertures usu. >6

a. Endoapertures single in ± circular ectoapertures (North American golden and ornamental currants) . . . . . *R. sanguineum, R. pringlei, R. aureum*

b. Some endoapertures paired in dumbbell-shaped ectoapertures (european red currants) . . . . . *R. rubrum* and allies

II. grains elliptic to obtusely rectangular, zonocolporate (i.e., having vertical equatorial colpi each with two pori) (gooseberries) . . . . . *R. uva-crispa, R. roezlii*

REFERENCES

Adams, R.J., and J.K. Morton. 1974. An atlas of pollen of the trees and shrubs of eastern Canada and the adjacent United States. University of Waterloo, Waterloo, Ontario.

Agababian, V.S. 1963. Pollen morphology of the genus Ribes L. Izv. Akad. Nauk Arm. S.S.R., Biol. Nauki 16: 93-98.

Berger, A. 1924. A taxonomic review of currants and gooseberries. Technical Bulletin of the New York State Agricultural Experiment Station 109: 1-118.

Erdtman, G. 1966. Pollen morphology and plant taxonomy: angiosperms. Hafner, NY.

_____ 1969. Handbook of Palynology: Morphology-Taxonomy-Ecology. Munksgaard, Copenhagen.

Heusser, C.J. 1971. Pollen and Spores of Chile: Modern types of the Pteridophyta, Gymnospermae and Angiospermae. University of Arizona Press, Tucson.

Hideux, M.J., and I.K. Ferguson. 1976. The stereostructure of the exine and its evolutionary significance in Saxifragaceae *sensu lato*. In I.K. Ferguson, and J. Muller [eds.], The Evolutionary Significance of the Exine. Academic Press, New York.

Pastre, A., and A. Pons. 1973. Quelques aspects de la systématique des Saxifragacées à lumières des données de la palynologie. Pollen et Spores 15: 117-133.

Verbeek-Reuvers, A.A.M.L. 1980. Grossulariaceae. In W. Punt, and G.C.S. Clarke [eds.], The northwest European pollen flora, 107-116. Elsevier Scientific Publishing Company, New York.

RIBES IN THE FOSSIL RECORD

Saxifragaceae *sensu lato* is a heterogeneous group that may occupy a central, relatively early place in angiosperm evolution. Palynological (Hideux and Ferguson 1976), chemical (Bate-Smith 1976), and anatomical (Stern et al. 1970) characters of members of this group can be considered plesiomorphic. Phylogenetic hypotheses based on chloroplast restriction fragment and *rbc*L sequence also indicate fairly deep branching for the members of this group (Chase et al. 1993). If this is the case, occurrence of Grossulariaceae and its relatives would be expected early in the fossil record (although perhaps rarely, in view of its relatively minor role in contemporary plant communities).

The distinctive pollen (Cronquist 1981), as well as leaves (Wolfe and Wehr 1987) of *Itea* are found in Eocene deposits, 48-49 million years old.

Ribes-like fossils first appear in the late Cretaceous, in both North and South America. *Riboidoxylon*, fossil wood similar to that of modern *Ribes*, is known from late Upper Cretaceous strata in California, 70 to 80 million years ago (Cronquist 1981). A Maastrichtian (late Cretaceous) flora from Chubut and Santa Cruz, Argentina, and Cerro Guido, Chile includes leaves identified as *Ribes* (Menendez 1969, Vakhrameev 1991). Most of the other South American fossils do not match contemporary taxa, suggesting either great age for *Ribes* or problems with the determinations.

Ribes fossils are known from the Tertiary of west North America, China, Holland, and Germany (Darrah 1939, who gives their age as Miocene). The Creede

flora of Colorado is a well-studied example, now considered Oligocene (Axelrod 1987; Wolfe and Schorn 1989, 1990). Various leaf specimens have been identified as *Ribes* by Axelrod (1987). Eight of Axelrod's *Ribes* species, a *Rubus*, two species of *Physocarpus*, a *Holodiscus* (in part), and an *Acer* were replaced with three species of *Ribes* (Wolfe and Schorn 1989, 1990). These are compared to the contemporary western North American species *R. lacustre* (section *Grossularioides*), *R. amarum*, and *R. speciosum*, members of the western gooseberry lineage (see Chapter I). This work represents the only rigorous morphological examination of any fossil ascribed to *Ribes*.

In California and Nevada strata suggested to be lower to middle Miocene, two leaves out of about 5200 were considered similar to *Ribes lacustre* (LaMotte 1936).

Axelrod and Raven (1985) and Wolfe and Wehr (1987) assign the Florissant beds of Colorado to the early Oligocene or late Eocene (Graham 1993). MacGinitie (1953) describes *Ribes errans* from this material, and compares it to the contemporary *R. inerme* and *R. leptanthum*, as well as to a *Ribes* sp. from the Oligocene Molalla flora.

Later occurrences of fossils considered as *Ribes* include *Ribes stanfordianum*, from the Pliocene of California. This plant's morphological traits and associated taxa suggest affinities with *R. viscossisimum* and *R. nevadense* (Dorf 1930).

None of the identifications are accompanied by explicit reasoning except that of *Itea* leaves (Wolfe and Wehr 1987) and the reidentifications in the Creede Flora (Wolfe and Schorne 1989, 1990). This taxonomic mayhem in the Creede flora also

indicates a lack of reliability in older attributions. However, taking the body of work as a whole, *Ribes* may have been present at least in North and South America prior to the K-T boundary, and had achieved its present distribution by the Miocene. A more conservative approach to these data is merely to acknowledge that the genus was well established in North America by the Oligocene.

Perhaps he most interesting feature of *Ribes* paleontology is that the first fossils described are similar to spiny species, either gooseberries or spiny currants, suggesting that this may be the plesiomorphic condition in the genus, at least in North America. However, unlike the methods for identifying fossils, the extent and rigour of comparisons to contemporary *Ribes* in these references (Wolfe and Schorn 1990) is not clear. Several Eurasian and South American currants share the unlobed leaf morphology of *R. speciosum*, for example, and *R. laxiflorum* is similar to *R. lacustre* in leaf dissection. Whether this pattern holds must await the explicit analysis of fossil collections previously identified on intuitive grounds.

REFERENCES

Axelrod, D.I. 1950. Studies in Late Tertiary Paleobotany. Carnegie Institution of Washington, Washington, D.C.

_____ 1987. The late Oligocene Creede Flora, Colorado. Univ. Calif. Publ. Geol. Sci. 130: 1-235.

_____ and P.H. Raven. 1985. Origins of the cordilleran flora. Journal of Biogeography 12: 21-47.

Bate-Smith, E.C. 1976. Chemistry and taxonomy of *Ribes*. Biochemical Systematics and Ecology 4: 13-23.

Chase, M.V., D.E. Soltis, R.G. Olmstead, D. Morgan, D.H. Les, B.D. Mishler, M.R. Duvall, R. Price, H.G. Hillis, Y.-L. Qui, K.A. Kron, J.H. Rettig, E. Conti, J.D. Palmer, J.R. Manhart, J. Sytsma, H.J. Michaels, W.J. Kress, K.G. Karol, W.D. Clark, M. Hedren, B.S. Gaut, R.K. Jansen, K.-J. Kim, C.F. Wimpee, J.F. Smith, G.R. Furnier, S.J. Strauss, Q.-Y. Xiang, G.M. Plunkett, P.S. Soltis, S.E. Williams, P.A. Gadek, C.J. Quinn, L.E. Equiarte, E. Golenberg, G.H. Learn, S. Graham, S.C.H. Barrett, S. Dayanadan, and V.A. Albert. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Annals of the Missouri Botanical Garden 80: 528-580.

Cronquist, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York.

Darrah, W.C. 1939. Textbook of Paleobotany. D. Appleton-Century, New York.

Dorf, E. 1930. Pliocene floras of California. Carnegie Institution of Washington Publication 412: 1-108.

Graham, A. History of the Vegetation: Cretaceous (Maastrichtian)-Tertiary. In Flora of North America Editorial Committee [eds.], Flora of North America vol. 1. pp. 57-70. Oxford.

Hideux, M.J., and I.K. Ferguson. 1976. The stereostructure of the exine and its evolutionary significance in Saxifragaceae *sensu lato*. In I.K. Ferguson, and J. Muller [eds.], The Evolutionary Significance of the Exine. Academic Press, New York.

LaMotte, R.S. 1936. The upper Cedarville flora of northwestern Nevada and adjacent California. Carnegie Institution Contributions to Palaeontology 455: 57-142.

MacGinitie, H.D. 1953. Fossil plants of the Florissant beds, Colorado. Carnegie Institution of Washington, Washington, D.C.

Menendez, C. A. 1969. Die Fossilen floren Südamericas. In E. J. Fittkau, J. Illies, H. Klinge, G. H. Schwab, H. Sioli, [eds.]. Monographiae biologicae 19: Biogeography and Ecology in South America.

Raven, P.H., and D.I. Axelrod. 1978. Origin and relationships of the California flora. University of California Publications in Botany 72: 1-134.

Stern, W.L., E.M. Sweitzer, and R.E. Phipps. 1970. Comparative anatomy and systematics of woody Saxifragaceae. Ribes. In N.K.B. Robson, D.F. Cutler, and M. Gregory [eds.], New Research in Plant Anatomy. Academic Press, London.

Vakhrameev, V.A. 1991. Jurassic and Cretaceous Floras and Climates of the Earth. Cambridge University Press, Cambridge.

Wolfe, J.A.and H.E.Schorn. 1989. Paleoecologic, paleoclimatic, and evolutionary significance of the Oligocene Creede flora, Colorado. Paleobiology 15: 180-198.

_____ . 1990. Taxonomic revision of the Spermatopsida of the Oligocene Creede flora, southern Colorado. U.S. Geological Survey Bulletin 1923.

Wolfe, J.A., and W. Wehr. 1987. Middle Eocene dicotyledonous plants from Republic, northeastern Washington. U.S. Geological Survey Bulletin 1597.

BIBLIOGRAPHY

Adams, R.J., and J.K. Morton. 1974. An atlas of pollen of the trees and shrubs of eastern Canada and the adjacent United States. University of Waterloo, Waterloo, Ontario.

Agababian, V.S. 1963. Pollen morphology of the genus *Ribes* L. Izv. Akad. Nauk Arm. S.S.R., Biol. Nauki 16: 93-98.

Albert, V.A., M.W. Chase, and B.D. Mishler. 1993. Character-state weighting for cladistic analysis of protein-coding DNA sequences. Annals of the Missouri Botanical Garden 80: 752-766.

Anderson, E., and G.L. Stebbins. 1954. Hybridization as an evolutionary stimulus. Evolution 8: 378-388.

Anderson, J.P. 1943. Two notable plant hybrids from Alaska. Proceedings of the Iowa Academy of Sciences 50: 155-157.

Applegate, E.I. 1939. Plants of Crater Lake National Park. The American Midland Naturalist 22: 225-314.

Arnold, M.L., C.M. Buckner, and J.J. Robinson. 1991. Pollen mediated introgression and hybrid speciation in Louisiana irises. Proceedings of the National Academy of Sciences, USA 88: 1398-1402.

Avise, J.C. 1994. Molecular Markers, Natural History, and Evolution. Chapman & Hall, New York.

Axelrod, D.I. 1950. Studies in Late Tertiary Paleobotany. Carnegie Institution of Washington, Washington, D.C.

_____ 1987. The late Oligocene Creede Flora, Colorado. Univ. Calif. Publ. Geol. Sci. 130:1-235.

_____ and P.H. Raven. 1985. Origins of the cordilleran flora. Journal of Biogeography 12: 21-47.

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. Molecular Phylogenetics and Evolution 1: 3-16.

_____ 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on its sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. American Journal of Botany 80: 222-238.

Baum, D.A., K.J. Sytsma and P.C. Hoch. 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. Systematic Botany 19: 363-388.

Bates, J.C. 1933. Comparative anatomical research within the genus *Ribes*. The University of Kansas Science Bulletin 21: 369-398.

Bate-Smith, E.C. 1976. Chemistry and taxonomy of *Ribes*. Biochemical Systematics and Ecology 4: 13-23.

Berger, A. 1924. A taxonomic review of currants and gooseberries. Technical Bulletin of the New York State Agricultural Experiment Station 109: 1-118.

Berlandier, J.L. 1826. Mémoire sur les Grossulariées. Mémoires de la Société de physique et d'Histoire Naturelle de Genève 3: 43-60.

Bohm, B.A. 1993. External and vacuolar flavonoids of *Ribes viscossisimum*. Biochemical Systematics and Ecology 21: 745.

Brennan, R.M. 1991. Currants and Gooseberries (*Ribes*). In J.N. Moore, and J.R. Ballington [eds.], Genetic Resources of Temperate Fruit and Nut Crops, 457-488. International Society of Horticultural Sciences, Wageningen, The Netherlands.

Chase, M.V., D.E. Soltis, R.G. Olmstead, D. Morgan, D.H. Les, B.D. Mishler, M.R. Duvall, R. Price, H.G. Hillis, Y.-L. Qui, K.A. Kron, J.H. Rettig, E. Conti, J.D. Palmer, J.R. Manhart, J. Sytsma, H.J. Michaels, W.J. Kress, K.G. Karol, W.D. Clark, M. Hedren, B.S. Gaut, R.K. Jansen, K.-J. Kim, C.F. Wimpee, J.F. Smith, G.R. Furnier, S.J. Strauss, Q.-Y. Xiang, G.M. Plunkett, P.S. Soltis, S.E. Williams, P.A. Gadek, C.J. Quinn, L.E. Equiarte, E. Golenberg, G.H. Learn, S. Graham, S.C.H. Barrett, S. Dayanadan, and V.A. Albert. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbc*L. Annals of the Missouri Botanical Garden 80: 528-580.

Coville, F.V., and N.L. Britton. 1908. Grossulariaceae. North American Flora 22: 193-225.

Cronquist, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York.

D'Arcy, W.G. 1987a. Flora of Panama: checklist and index part I: The introduction and checklist. Missouri Botanical Garden, St. Louis.

_____ 1987b. Flora of Panama: checklist and index part II: index. Missouri Botanical Garden, St. Louis.

Darlington, C.D. 1929. A comparative study of the chromosome complement in *Ribes*. Genetica 11: 267-272.

Darrah, W.C. 1939. Textbook of Paleobotany. D. Appleton-Century, New York.

DeBry, R.W., and N.A. Slade. 1985. Cladistic analysis of restriction endonuclease cleavage maps within a maximum-likelihood framework. Systematic Zoology 34: 21-34.

Donoghue, M.J., R.G. Olmstead, J.F. Smith, and J.D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Annals of the Missouri Botanical Garden 79: 333-345.

Dorf, E. 1930. Pliocene floras of California. Carnegie Institution of Washington Publication 412: 1-108.

Downie, S.R., and J.D. Palmer. 1994. A chloroplast DNA phylogeny of the Caryophyllales based on structural and inverted repeat restriction site variation. Systematic Botany 19: 236-252.

Doyle, J.J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Systematic Botany 17: 144-163.

Doyle, J.J., and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

Eckenrode, V.K, J. Arnold and R.B. Meagher. 1985. Comparison of the nucleotide sequence of soybean 18s rRNA with the sequences of other small-subunit rRNAs. Journal of Molecular Evolution 21: 259-269.

Erdtman, G. 1966. Pollen morphology and plant taxonomy: angiosperms. Hafner, NY.

_____ 1969. Handbook of Palynology: Morphology-Taxonomy-Ecology. Munksgaard, Copenhagen.

Erlich, H.A., D. Gelfand, and J.J. Sninsky. 1991. Recent advances in the polymerase chain reaction. Science 252: 1643-1651.

Felsenstein, J. 1978. Cases in which parsimony and compatibility methods will be positively misleading. Systematic Zoology 27: 401-410.

_____ 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

_____ 1991. PHYLIP: Phylogeny Inference Package (version 3.4). Computer program distributed by the author, University of Washington, Seattle.

_____ 1992. Phylogenies from restriction sites: a maximum-likelihood approach. Evolution 46: 159-173.

Fritsch, P., and L.H. Rieseberg. 1993. Chloroplast DNA restriction site analysis of *Styrax* series *Imbricatae* (Styracaeae): Implications for the Madrean-Tethyan hyothesis. (abstract). American Journal of Botany 80: 149.

Genetics Computer Group (1991): Program manual for the GCG package version 7, April 1991. 575 Science Drive, Madison, Wisconson.

Goldschmidt, E. 1964. Cytological species and interspecific hybrids of the genus *Ribes*. Hereditas 52: 139-150.

Golenberg, E.M., M.T. Clegg, M. Durbin, J. Doebley, and D.P. Ma. 1993. Evolution of a non-coding region of the chloroplast genome. Molecular Phylogenetics and Evolution 2: 52-64.

Graham, A. History of the Vegetation: Cretaceous (Maastrichtian)-Tertiary. In Flora of North America Editorial Committee [eds.], Flora North America vol. 1. Oxford.

Grant, V. 1971. Plant Speciation. Columbia University Press, New York.

Henry, J.K. 1919. *Ribes divaricatum* X *Ribes lobbii*. Canadian Field Naturalist 19: 94.

Heusser, C.J. 1971. Pollen and Spores of Chile: Modern types of the Pteridophyta, Gymnospermae and Angiospermae. University of Arizona Press, Tucson.

Hibbett, D.S., and R. Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. Systematic Botany 18: 409-433.

Hickman (1969): Disjunction and endemism in the flora of the central western Cascades of Oregon: an historical and ecological approach to plant distributions. Ph.D. Thesis, University of Oregon, Eugene.

Hideux, M.J., and I.K. Ferguson. 1976. The stereostructure of the exine and its evolutionary significance in Saxifragaceae *sensu lato*. In I.K. Ferguson, and J. Muller [eds.], The Evolutionary Significance of the Exine. Academic Press, New York.

Hillis, D.M., J.P. Huelsenbeck, and C.W. Cunningham. 1994. Application and accuracy of molecular phylogenies. Science 264: 671-677.

Hillis, D.M., J.P. Huelsenbeck, and D.L. Swofford. 1994. Hobgoblin of phylogenetics? Nature 369: 363-364.

Huelsenbeck, J.P., and D.M. Hillis. 1993. Success of phylogenetic methods in the four-taxon case. Systematic Biology 42: 247-264.

Janczewski, E.de. 1903. Essai d'une disposition naturelle des espèces dans le genre *Ribes* L. Bulletin international de l'Academie des sciences de Cracovie, Classe des sciences mathematiques et naturelles: 232-241.

_____ 1906a. Species generis *Ribes* L. II Subgenera *Ribesia* et *Coreosma*. Bulletin International de l'Académie des Sciences de Cracovie. Classe des Sciences Mathématique et Naturelles: 1-13.

_____ 1906b. Species generis *Ribes* L. III. Subgenera *Grossularioides*, *Grossularia*, et *Berisia*. Bulletin International de l'Académie des Sciences de Cracovie. Classe des Sciences Mathématique et Naturelles: 280-293.

_____ 1907. Monographie des groseilliers *Ribes* L. Mémoires de la Société de physique et d'Histoire Naturelle de Genève 35: 199-517.

Keep, E. 1962. Interspecific hybridization in *Ribes*. Genetica 33: 1-23.

Keep, E. 1975. Currants and gooseberries. In J. Janick, and J.N. Moore [eds.], Advances in Fruit Breeding, 197-268. Purdue University Press, West Lafeyette, Indiana.

Kimura, M. 1980. A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111-120.

LaMotte, R.S. 1936. The upper Cedarville flora of northwestern Nevada and adjacent California. Carnegie Institution Contributions to Palaeontology 455: 57-142.

Liston, A. 1992. Variation in the chloroplast gene *rpo*C1 and *rpo*C2 of the genus *Astragalus* (Fabaceae): evidence from restriction mapping of a PCR-amplified product. American Journal of Botany 79: 953-971.

_____ and J.A. Wheeler. 1994. The phylogenetic position of the genus *Astragalus* (Fabaceae): evidence from the chloroplast genes *rpo*C1 and *rpo*C2. Biochemical Systematics and Ecology 22: 377-388.

Lundberg, J. 1972. Wagner networks and ancestors. Systematic Zoology 21: 398-413.

Mabberly, D.J. 1987. The plant-book. Cambridge University Press, Cambridge.

Maddison, D. 1991. The discovery and importance of multiple islands of most parsimonious trees. Systematic Zoology 40: 315-328.

MacGinitie, H.D. 1953. Fossil plants of the Florissant beds, Colorado. Carnegie Institution of Washington, Washington, D.C.

Menendez, C. A. 1969. Die Fossilen floren Südamericas. In E. J. Fittkau, J. Illies, H. Klinge, G. H. Schwab, H. Sioli, [eds.], Monographiae biologicae 19: Biogeography and Ecology in South America.

Mesler, M.R., J.C. Cole, and P. Wilson. 1991. Natural hybridization in western gooseberries (*Ribes* subgenus *Grossularia*: Grossulariaceae). Madroño 38: 115-129.

_____ and J.O. Sawyer. 1993. Grossulariaceae: gooseberry family. In J.C. Hickman [ed.], The Jepson Manual: Higher Plants of California. University of California Press, Berkeley.

Messinger, W., A. Liston, and K. Hummer. 1993. Restriction site mapping of *Ribes* nuclear ribosomal DNA. Acta Horticulturae 352: 175-184.

Meurman, O. 1928. Cytological studies in the genus *Ribes*. Hereditas 11: 289-356.

Mishler, B., M. Donoghue, and V. Albert (1991): The decay index as a measure of relative robustness within a cladogram. Tenth Meeting of the Willi Hennig Society, Toronto. (abstract)

Morgan, D.R., and D.E. Soltis. 1993. Phylogenetic relationships among members of the Saxifragaceae *sensu lato* based on *rbc*L sequence data. Annals of the Missouri Botanical Garden 80: 631-660.

Norris, LL. 1987. Status of five rare plant species in Sequoia and Kings Canyon National Parks. In Conservation and Management of Rare and Endangered Plants. California Native Plant Society.

Nickrent, D., and C.R. Franchina. 1990. Phylogenetic relationships of the Santalales and relatives. Journal of Molecular Evolution 31: 294-301.

Ogihara, Y., T. Terachi, and T. Sasakuma. 1991. Molecular analysis of the hot spot region related to length mutations in wheat chloroplast DNAs. I. Nucleotide divergence of genes and intergenic spacer regions located in the hot spot region. Genetics 129: 873-884.

Olmstead, R.G., and J.D. Palmer. 1994. Chloroplast DNA systematics: a review of methods and data analysis. American Journal of Botany 81: 1205-1224.

Pastre, A., and A. Pons. 1973. Quelques aspects de la systématique des Saxifragacées à lumières des données de la palynologie. Pollen et Spores 15: 117-133.

Patterson, C., D.M. Williams, and C.J. Humphries. 1993. Congruence between molecular and morphological phylogenies. Annual Review of Ecology and Systematics 24: 153-188.

Potter, D., and J.J. Doyle. 1994. Phylogeny and systematics of *Sphenostylis* and *Nesphostylis* (Leguminosae: Phaseolae) based on morphological and chloroplast DNA data. Systematic Botany 19: 389-406.

Poyarkova, A.I. 1939. Ribesioideae. In V.L. Komarov, and S.V. Yuzepchuk [eds.], Flora of the U.S.S.R, 175-208. Israel Program for Scientific Translations, Jerusalam, for the Smithsonian Institute and National Science Foundation, Washington D.C.

Radford, A.E. 1959. A relict plant community in South Carolina. Journal of the Elisha Mitchell Scientific Society 75: 35-43.

Raven, P.H., and D.I. Axelrod. 1978. Origin and relationships of the California flora. University of California Publications in Botany 72: 1-134.

Rieseberg, L.H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. American Journal of Botany 78: 1218-1237.

Rieseberg, L.H., and S.J. Brunsfeld. 1992. Molecular evidence and plant introgression. In PS Soltis, DE Soltis and JJ Doyle [eds.] Molecular Systematics of Plants. Chapman and Hall, New York.

Rieseberg, L.H., M. Hanson, and C.T. Philbrick. 1992. Androdioecy is derived from dioecy in Datiscaceae; evidence from restriction site mapping of PCR-amplified chloroplast DNA fragments. Systematic Botany 17: 324-336.

Rowland, L.J., and B. Nguyen. 1993. Use of polyethylene glycol for purification of DNA from leaf tissue of woody plants. Biotechniques 14: 735-736.

Schwarzbach, A. and J.W. Kadereit. In Press. Rapid radiation of North American desert genera of the *Papaveraceae*: evidence from restriction site mapping of PCR-amplified chloroplast DNA fragments. Plant Systematics and Evolution.

Shimada, K., M. Fukuta, M. Ishikawa, and M. Sugiura. 1990. Rice chloroplast RNA polymerase genes: the absence of an intron in *rpo*C1 and the presence of an extra sequence in *rpo*C2. Molecular and General Genetics 221: 395-402.

Shinozaki, K., M. Ohme, M. Tanaka, T. Wakasugi, N. Hayashida, T. Matsubayashi, N. Zaita, J. Chunwongse, J. Obokota, K. Yamaguchi-Shinozaki, C. Ohto, K. Torazawa, B.-Y. Meng, M. Sugita, H. Deno, T. Kamagoshira, K. Yamada, J. Kusuda, F. Takaiwa, A. Kato, N. Tohdoh, H. Shimada, and M. Suguira. 1986. The complete nucleotide sequence of the tobacco chloroplast genome: its organization and expression. EMBO Journal 5: 2043-2049.

Sinnott, Q.P. 1985. A revision of *Ribes* L. Subg. *Grossularia* (Mill.) Pers. sect. *Grossularia* (Mill.) Nutt. (Grossulariaceae) in North America. Rhodora 87: 189-286.

Smith, E.L., and Q.P. Sinnott. 1984. Department of the Interior, Fish and Wildlife Service, 50 CFR Part 17: Endangered and threatened wildlife and plants; *Ribes echinellum* (Miccosukee Gooseberry) proposed to be a threatened species. Federal Register 49: 34535-34537.

Smouse, P.E., and W.-H. Li. 1987. Likelihood analysis of mitochondrial restriction-cleavage patterns for the human-chimpanzee-gorilla trichotomy. Evolution 41: 1162-1176.

Soltis, D.E., P.S. Soltis, M.T. Clegg, and M. Durbin. 1990. *rbc*L sequence divergence and phylogenetic relationships in Saxifragaceae sensu lato [*sic*]. Proceedings of the National Academy of Sciences, USA 87: 4640-4644.

Soltis, D.E., E.R. Morgan, A. Grable, P.S. Soltis, and R. Kuzoff. 1993. Molecular systematics of Saxifragaceae *sensu lato*. American Journal of Botany 80: 1056-1081.

Spach, E. 1835. Revisio Grossularierum. Ann. Sci. Nat. Bot. II 4: 16-31.

_____ 1838. Histoire Naturelle des Végétaux. Phanerogames. Revet, Paris.

Spongberg, S.A. 1972. The genera of Saxifragaceae in the southeastern United States. Journal of the Arnold Arboretum 53: 109-498.

Standley, P.C. 1937. Flora of Costa Rica. Field Museum of Natural History, Chicago.

Stebbins, G.L., and J. Major. 1965. Endemism and speciation in the California flora. Ecological Monographs 35: 1-35.

Stern, W.L., E.M. Sweitzer, and R.E. Phipps. 1970. Comparative anatomy and systematics of woody Saxifragaceae. *Ribes*. In N.K.B. Robson, D.F. Cutler, and M. Gregory [eds.], New Research in Plant Anatomy. Academic Press, London.

Swofford, D.L. (1992): PAUP: Phylogenetic Analysis Using Parsimony, version 3.0s+4(beta). Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Swofford, D.L., and G.J. Olsen. 1990. Phylogeny reconstruction. In D.M. Hillis, and C. Moritz [eds.], Molecular Systematics, 411-501. Sinauer Associates, Sunderland, Massachusetts.

Takaiwa, F., K. Oono, and M. Sugiura. 1985. Nucleotide sequence of the 17S-25S spacer region from rice rDNA. Plant Molecular Biology 4: 355-364.

Takhtajan, A.L. 1980. Outline of the classification of flowering plants (Magnoliophyta). Botanical Review 46: 225-359.

Thorne, R.F. 1992. Classification and geography of the flowering plants. Botanical Review 38: 225-348.

Vakhrameev, V.A. 1991. Jurassic and Cretaceous Floras and Climates of the Earth. Cambridge University Press, Cambridge.

Verbeek-Reuvers, A.A.M.L. 1980. Grossulariaceae. In W. Punt, and G.C.S. Clarke [eds.], The Northwest European Pollen Flora, 107-116. Elsevier Scientific Publishing Company, New York.

Wallace, G.D. 1985. Vascular plants of the Channel Islands of Southern California and Guadalupe Island, Baja California, Mexico. Natural History Museum of Los Angeles County, Los Angeles.

Wolfe, A.D., W.J. Elisens, L.E. Watson, and C.W. dePamphilis. 1993. Using RFLPs of chloroplast gene PCR-products for phylogenetic analysis: when is enough, enough? (abstract). American Journal of Botany 80: 183.

Wolfe, A.D., W.J. Elisens, L.E. Watson, C.W. dePamphilis, and C.E. Freeman. 1993. A phylogenetic and biogeographic analysis of the North American Cheloneae (Scrophulariaceae): evidence from chloroplast PCR-product restriction site variation. (abstract). American Journal of Botany 80: 183.

Wolfe, J.A.and H.E.Schorn. 1989. Paleoecologic, paleoclimatic, and evolutionary significance of the Oligocene Creede flora, Colorado. Paleobiology 15: 180-198.

_____ . 1990. Taxonomic revision of the Spermatopsida of the Oligocene Creede flora, southern Colorado. U.S. Geological Survey Bulletin 1923.

Wolfe, J.A., and W. Wehr. 1987. Middle Eocene dicotyledonous plants from Republic, northeastern Washington. U.S. Geological Survey Bulletin 1597.

Wynd, F.L. 1936. The flora of Crater Lake National Park. The American Midland Naturalist 17: 881-949.

Zielinski, Q.B. 1953. Chromosome numbers and meiotic studies in *Ribes*. Botanical Gazette 114: 265-274.

APPENDICES

APPENDIX I
DATA MATRIX OF CHLOROPLAST DNA RESTRICTION SITES

APPENDIX I. Data Matrix of Chloroplast DNA Restriction Sites.

Taxon codes are first three letters of specific epithet (see Table 1). Characters are restriction sites, restriction fragment length(s) found in the majority of taxa are given first, although this notation would not accomadate characters 52 and 53, which represent site losses in *Itea* relative to the majority of *Ribes*. Site presence=1, site absence=0. Characters 1-29 are in *rpo*C, 30-55 in *rbc*L.

rpoC: 1.*Alu*I950=410+540 2.*Bsa*JI750=340+410 3.*Bst*UI590=360+220 4.*Bst*UI690+60 5.*Hae*III460+800 6.*Hae*III1260+100 7.*Hae*III480+220 8.*Hae*III920+120 9.*Hha*I100+4000 10.*Msp*I290+380 11.*Msp*I1400+1200+200 12.*Msp*I730+170 13.*Rsa*I1090=840+210(+insertion) 14.*Rsa*I510+840 15.*Rsa*I420=300+120 16.*Sau*96I940+110 17.*Sau*96I490+220 18.*Sau*96I520+180 19.*Sau*96I520+220 22.*Scr*FI140+170 21.*Bsr*I1200=670+530 22.*Bsr*I650+310 23.*Bam*HI2100=900+1200 24.*Bam*HI900+1150 25.*Bst*BI500+200 26.*Bst*BI800+250 27.*Bst*BI700+350 28.*Cla*I510+1850 29.*Cla*I1500+350

*rbc*L: 30.*Alu*I520+1200 31.*Alu*I380+160 32.*Alu*I1000+750 33.*Alu*I270+270 34.*Bsa*JI150+1250 35.*Bsa*JI480=290+190 36.*Bst*UI400=230+170 37.*Bst*UI250+440 38.*Bst*UI800+1200 39.*Bst*UI925=625+230 40.*Hae*III120+310 41.*Hae*III1020=610+410 42.*Hin*fI900+80 43.*Rsa*I340+270 44.*Rsa*I900+80 45.*Sau*96I720+420 46.*Sau*96I700=210+490 47.*Scr*FI305+650 48.*Scr*FI955+23 49.*Scr*FI190+1450+unobserved fragments totalling 160 deduced from published sequences 50.*Scr*FI850+180 51.*Bsr*I1950=1250+700 52.*Bsr*I 53.*Bsr*I 54.*Ase*I900 55.*Bst*BI400+2900

```
      Site:    1111111111222222222233333333334444444444555555
               1234567890123456789012345678901234567890123456789012345
Taxon
ERY   111000000010101000001010010100000011000010000100001111O
GLA   111000000010101000001010100000000011000010000100001111O
HOW   111000000010101000001010010000000011000010000100001111O
LAX   111000000010101000001010100000000011000010000100001111O
MON   101000000010110001001000000100000100000010000100001111O
LAC   111000000010101000001010000100000000000010000100001111O
CER   111000000010101000001010010100000011000010000100001110O
MOG   111000000010101000001010010100000011000010000100001111O
SAN   111000000010101000001010010100000011000010000100001111O
VIS   111000000010101000001010010100000011100010000100101111O
CIL   111000000010101000001010010100000011000010000100001111O
AUR   111011000011101000111010100000100011000010000000011111O
ODO   111011000011101000111010100000100011000010000000011111O
AME   111000000010101?????100010000100001100001000010000111100
NIG   110000000010101000001010100100000011000010000100001111?
PET   111000000010101000001010100100000011000010000100001111O
VIB   111000000010101000001010100110000011000010000100011111O
TRI   111000000000101000001010100000000011000010000100001110O
SAT   111000000000101000001010100000000011000010000100001110O
AND   ??????????????????????????????000001100001000010000111O
VAL   1?100000001010100000101010O??000001101001000010000111O
ALP   111000000110101000000010100000000011000110001100001111O
DIA   111000000110101000000010100000000011000111001100001111O
MAX   111000000110101000000010100000000011000111001100001111O
IRR   101000000010111001001000100100000100000010100100001111O
BUR   101000000010111001001000100??000010000001000010000111O
NIV   101000000010111001001000100100000100000010100100001111O
VEL   111100000010101000001010100100000011000010010100011111I
GOO   111100000010101000001010100100000011000010010100011111I
BIN   111100000010101000001010100100000011000010010100011111I
CRU   111100000010101000001010100100000011000010010100011111I
MEN   111100000010101000001010100100000011000010010100011111I
SPE   011100000010101000001010100100000011000010000100010111O
ITVI  ????001110100011100001111011000110110010000001110010010
```

APPENDIX II

DATA MATRIX OF NUCLEAR RIBOSOMAL DNA RESTRICTION SITES

APPENDIX II. Data Matrix of Nuclear Ribosomal DNA Restriction Sites. Restriction endonuclease and map position for each site are given. Groups of columns are sites recognized by a single enzyme. Non-variable sites are included in the matrix.

Columns 1-3: BglI1780 BglI2230 BglI2310

Columns 4-8: BsmaI650 BsmaI980 BsmaI1290 BsmaI1860 BsmaI1920

Columns 9-16: HaeIII210 HaeIII790 HaeIII1220 HaeIII1440 HaeIII1790 HaeIII1920 HaeIII2170 HaeIII2270

Columns 17-21: RsaI110 RsaI500 RsaI1600 RsaI1820 RsaI2120

Columns 22-26: Sau96I650 Sau96I1580 Sau96I1790 Sau96I1830 Sau96I1960

Columns 27-36: ScrfI490 ScrfI730 ScrfI1044 ScrfI1450 ScrfI1840 ScrfI1900 ScrfI1910 ScrfI1950 ScrfI2000 ScrfI2200;

ERY	001	11100	11110011	1111	11000	1111011011
GLA	001	11100	11110011	1110	11000	1111011011
HOW	001	11100	11110011	1111	11001	1111001011
LAX	011	11100	11110011	1110	11000	1111001111
MON	001	11100	11110011	1110	11000	1111101011
LAC	001	11100	11110011	1110	11000	1111101011
CER	011	11100	11110011	1111	11000	1111011011
MOG	011	11100	11110011	1111	11000	1111011011
SAN	011	11100	11110011	1111	11000	1111101011
VIS	011	11100	11110011	1111	11001	1111001011
AUR	011	11101	11110011	1110	11100	1111001011
ODO	0?1	11101	11110011	1110	11000	1111001011
AME	001	11101	11110011	1110	11000	1111101011
NIG	001	11110	11110011	1110	11000	1111001111
PET	001	11100	11110011	1110	11010	1111001011
VIB	001	11101	11110111	111?	11000	1111001011
Cherry	001	11101	11110011	1110	11010	1111001011
Diploma	001	11101	11110011	1110	11010	1111001011
ANDc	001	11100	11111011	1111	11010	1111001011
ALP	001	11101	11110011	1110	11000	1111001011
DIA	001	11101	11110011	1110	11000	1111001011
MAX	001	11101	11110011	1110	11000	1111001011
IRR	001	11100	11110111	1110	11000	1111011011
NIV	101	11100	11110111	1110	11010	1111011011
BUR	001	11100	11111011	1110	11010	1111011011
GOO	???	11100	11111011	1110	11010	1111011011
BIN	001	11100	11111011	1110	11010	1111011011
CRU	001	11100	11111011	1110	11010	1111011011
MEN	101	11100	11111011	1110	11010	1111011011

APPENDIX III

DETAILED PROTOCOL FOR ISOLATION OF GENOMIC DNA FROM *RIBES*

APPENDIX III. Detailed Protocol for Isolation of Genomic DNA from *Ribes*.

Protocols for use in systematic and population studies should be quick, due to the often large sample sizes involved, and widely applicable: the ideal protocol would require zero time and be useful for all of life. Unfortunately, wild plants, and woody species in particular, can present special problems for DNA isolation, most often due to idiosyncratic secondary chemistry. Within *Ribes*, species vary widely in ease of DNA isolation. The black currants are particularly recalcitrant. The following procedure was developed during this study, but is applicable to any unfamiliar plant group. By first applying the simplest methods to the entire study group, laborious additional steps are avoided when unnecessary. Additional purification steps are added incrementally until useful DNA is obtained.

For best results, use young, fresh leaves. Fresh leaves may be frozen at -80°C. While useful DNA may be obtained from dried material, it is not preferred due to unreliability.

PROTOCOL I

This is a scaled down version of Doyle and Doyle (1987), using 1.5ul microfuge tubes. It is fast and efficient, and yields DNA of sufficient quality for PCR amplification of known fragments from the majority of plant taxa.

2X CTAB
2% Hexadecyltrimethyl ammonium bromide (CTAB)
1.4M NaCl
20mM EDTA
100mM Tris, ph 8.0

1-4% PVP
1-4% NabiS

1. Grind ca. 0.3g leaf tissue in about 1ml hot (65°C.) CTAB, either in a spot plate or directly in 1.5ml microfuge tubes.

2. Incubate ca. 30 minutes at 65°C.

3. Extract once with ca. 1 volume chloroform:isoamyl alcohol 24:1.

4. Spin at about 6,000 RCF for 10 minutes. Most of the debris in the upper, aqueous phase should be concentrated in the interface. If it is not, try spinning more and faster.

5. Remove the upper, aqueous phase to a clean microfuge tube and add about two-thirds volume isopropanol. Allow to precipitate at room temperature overnight. (As little as 30 minutes *may be* sufficient.)

6. Spin at maximum speed for 30-60 minutes, pour off supernate, and dry thoroughly.

7. Fill tube with 76% EtOH/0.01M ammonium acetate, let stand for 10-30 minutes, spin briefly, pour off supernate, and dry thoroughly.

8. Resuspend in 50-200ul TE.

MODIFICATIONS

Should the above crude preparation fail to yield DNA of adequate quality for PCR, a number of modifications are possible.

1. Add more precipitation steps at the end: after resuspending pellet, add 0.5 volume 7.5M ammonium acetate and 2/3 volume isopropanol or 2 volumes ethanol, precipitate 30 minutes to overnight, spin, remove supernate, and dry as above, and wash with 70% ethanol.

2. Try a high salt precipitation, meant to precipitate DNA away from polysaccharides: bring final solution to 2.5M NaCl, add 2 volumes ethanol, precipitate, spin, decant, dry, wash, and resuspend as above.

3. Add more organic extractions: up to five chloroform/isoamyl alcohol extractions.

Another approach is to increase the ratio of CTAB to tissue. The above procedure is performed in 15ml centrifuge tubes, with similar small amounts of tissue (0.3-1g fresh weight) in about 7ml CTAB.

CTAB AND PEG PRECIPITATIONS

If greater volume, additional extractions, and additional alcohol precipitations are not effective, the procedure of Rowland and Nguyen (1993) may be. This modification was effective at times during the course of this study. The PEG precipitation step may cause unacceptable loss of DNA in some hands, and may be postponed until DNA quality is found to be in inadequate.

1. Following the chloroform extraction(s), add one fifth volume of 5% CTAB, 0.7M NaCl solution to the aqueous phase and precipitate with isopropanol.

2. Following the final resuspension in TE, add 0.4 volumes 5M NaCl and 1.25 volumes 13% PEG (polyethylene glycol MW 8000, Sigma) and allow to precipitate on ice for 1 hour. Wash in 70% ethanol and resuspend in minimal TE.

APPENDIX IV
CHECKLIST OF *RIBES* SPECIFIC EPITHETS

APPENDIX IV. Checklist of *Ribes* specific epithets. After Berger (1924), except sections *Parilla* and *Berisia* after Janczewski (1907). For recent treatments of parts of the North American *Ribes* flora see Hitchcock and Cronquist (1973), Mesler and Sawyer (1993), and Sinnott (1985). A number of taxonomic improvements have been made in the context of such regional floras, and this checklist is not intended as a summary of currently accepted taxonomy. Autonyms are used for infrageneric taxa, rather than the original names (i.e., *Ribesia* should be *Ribes*, and the prefix '*Eu-*' is unnecessary. Species used in the above systematic studies are marked with an asterisk.

RIBES (Red Currants)
- **sativum*
- **triste*
- *multiflorum*
- *manshuricum*
- *warscewisczii*
- *rubrum*
- *petraeum*
- *emodense*
- *latifolium*
- *meyeri*
- *moupinense*
- *setchuense*
- *griffithii*
- *soulieanum*
- *longiracemosum*

COREOSMA (Black Currants)
- **nigrum*
- *ussuriense*
- **americanum*
- *nelsoni*
- *bracteosum*
- *procumbens*
- **petiolare*
- *dikuscha*
- *hudsonianum*
- *fragrans*
- *japonicum*
- **viburnifolium*

SYMPHOCALYX (Golden Currants)
- *chihuahuaense*
- **odoratum*
- **aureum*
- *gracillimum*
- *fontinale*

CALOBOTRYA (Ornamamental Currants)

Series TORTUOSA
- *tortuosum*

Series SANGUINEA
- **wolfii (=mogollonicum)*
- *nevadense*
- *neglectum*
- *rugosum*
- *affine*
- *columbianum*
- *dugesii*
- *ceriferum*
- *brandegeii*
- *orizabae*
- **ciliatum*
- *pringlei*
- **viscosissimum*
- *hallii*
- **sanguineum*
- *glutinosum*
- *malvaceum*
- *indecorum*
- *polystachyum*
- *sanctae-barbarae*

Series CEROPHYLLA
- *reniforme*
- *inebrians*

**cereum*
viscidulum
mescalereum

HERITIERA (Dwarf Currants)
**erythrocarpum*
**howellii*
**laxiflorum*
**glandulosum*
sucheziense
ambiguum

GROSSULARIOIDES (Spiny Currants)
**lacustre*
horridum
**montigenum*

BERISIA (Alpine Currants)
Section DIACANTHA
**diacantha*
pulchellum
giraldii
Section BERISIA
orientale
distans
tricuspe
**alpinum*
vilmorinii
humile
tenue
Section BERISIA (Cont'd)
coeleste
glaciale
luridum
acuminatum
**maximowiczii*
franchettii
kialanum
Section DAVIDIA
davidii
henryi
laurifolium

PARILLA (Andine Currants)
Section HEMIBOTRYA
fasciculatum
sardoum
nubigenum
Section ANDINA
cucullatum
nitidissimum
densiflorum
weddellianum
pentlandii
brachybotrys
bogotanum
peruvianum
dombeyanum
bolivianum
**andicola*
macrostachyum
leptostachyum
ecuadorense
lindenii
albifolium
hirtum
elecans
polyanthes
palenae
cuneifolium
ovalifolium
guyanum
viscosum
glandulosum
incarnatum
catamarcanum
bicolor
weberbaueri
lehmannii
macrobotrys
Section PARILLA
parviflorum
spegazinii
magellanicum
parvifolium
**valdivianum*
integrifolium

punctatum

Genus Grossularia of Berger (note endings)

ROBSONIA
**speciosa*

HESPERIA
roezlii
amara
**cruenta*
greeneiana
victoris
senilis
**menziesii*
hystrix
leptosma
hesperia
californica

LOBBIA
sericea
lobbii
marshallii
pinetorum
watsoniana
tularensis
**binominatum*
madrensis
quercetorum
microphylla
lasiantha
congdoni
leptantha
**velutina*
glandulifera

GROSSULARIA
Series CYNOSBATAE
cynosbati
echinella
Series NIVEAE
missouriensis
rotundifolia
**nivea*
curvata
texensis
echinella
Series DIVARICATAE
divaricata
rotundifolia
parishii
Series SETOSAE
**irrigua*
non-scripta
setosa
cognata
klamathensis
hirtella
inermis
oxyacanthoides
neglecta
Series RECLINATA (Old World)
acicularis
formosana
stenocarpa
grossularioides
reclinata
alpestris
**bureiensis*