FLAVONOIDS AND TAXONOMY OF THE LIMNANTHACEAE

by

WILLIAM HARRISON PARKER

B.A., Reed College, 1968

M.Sc., Univ. British Columbia, 1972

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in the Department
of
Botany

We accept this thesis as conforming to the
required standard.

THE UNIVERSITY OF BRITISH COLUMBIA

August, 1975

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study.

I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of

Botany

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date

August 26, 1975

ABSTRACT

The Limnanthaceae is a small family of North American annual herbs with uncertain relationships. It is generally recognized to consist of two genera, Limnanthes and Floerkea, together containing 10 species with 13 varieties and subspecies. To help clarify relationships within this family, a comparative flavonoid investigation was undertaken. All taxa were compared on the basis of flavonoids occurring in all tissues, and most Limnanthes taxa were additionally compared on the basis of flavonoids occurring in the petals and the ultraviolet reflectance characteristics of their flowers. A total of 48 flavonol glycosides were encountered. Of these, 35 were identified as derivatives of six flavonol aglycone types: syringetin, isorhamnetin, kaempferol, laricytrin (myricetin 3'-methyl ether), quercetin and myricetin, all glycosylated with combinations of glucose and rhamnose. The flavonoid data were analyzed by three numerical taxonomic techniques: clustering by the weighted pair group method, Principal Components Analysis, and Varimax Factor Analysis with rotation. Duplicate comparisons were made according to type of tissue analyzed and concentrations of flavonoids considered. Classifications based on petal flavonoids occurring in relatively higher concentrations were found to reflect most clearly natural relationships in Limnanthes. The ultraviolet reflectance characteristics of flowers were found to distinguish one supra-specific group of Limnanthes taxa from the remainder of the genus. The adaptive significance of ultraviolet patterning is discussed, together with its implications concerning flavonoid compositions. The method and validity of using flavonoids as taxonomic characters is also discussed. Flavonoid and ultraviolet reflectance characteristics are integrated with all other taxonomically significant information known for the Limnanthaceae, and proposals for taxonomic revision are made. A synopsis of the family is presented which recognizes one genus, Floerkea, containing 15 species with 5 varieties..

TABLE OF CONTENTS

Abstract	ii
List of Tables	vi
List of Figures	vii
List of Appendices	ix
Acknowledgement	xiii
Introduction	
Family Description	1
Taxonomic Background	
A. Below the Family Level	3
B. Family Affinities	7
Previous Chemical Investigations	10
Agronomic Evaluation of <u>Limnanthes</u>	11
Thesis Objectives	12
Materials and Methods	
The Plants and Their Sources	14
Plant Growth	18
Plant Tissue Selection	20
Floral Patterning in Ultra Violet Light	
A. Introduction	22
B. Method	24
Flavonoid Isolation and Identification	
A. Introduction	25
B. Plant Extractions	27
C. Identification of the Flayonoids of <u>L. douglasii</u> var. <u>douglasii</u> , OTU 1	
i. Chromatography	28
ii. Spectral Analyses	30

TABLE OF CONTENTS -- Continued

iii. Hydrolyses	31
iv. Acetate Preparation	32
D. Identification of the Remaining <u>Limnanthes</u> Flavonoids	33
Expression of Flavonoids as Taxonomic Characters	33
Treatment of the Flavonoid Data	
A. Introduction	34
B. Method	35
Results	
Floral Patterning in Ultra Violet Light	39
Flavonoid Characterization and Identification	40
A. Data Presentation	44
B. Explanation of the Data	51
Significance of the Flavonoids of the Limnanthaceae	
A. Aglycones	60
B. Glycosides	62
The Contribution to Visible Flower Colour by Syringetin Derivatives	62
Environmental Modification of Flavonoid Composition	63
Comparative Flavonoid Data	65
Flavonoid Differences Between Petal and Whole Plant Material	68
Numerical Taxonomic Results	68
Comparisons of OTUs by Flavonoids	
A. Occurrence Tables	83
B. Cluster Analyses	84
C. Factor Analyses	88
D. Glycosylation Classes	89

TABLE OF CONTENTS -- Continued

Discussion

Method and Validity of Using Flavonoids as Taxonomic Characters

A. Flavonoids as Taximetric Characters

- i. Introduction 92
- ii. Inclusion of Negative Matches 92
- iii. Flavonoid Concentration 94
- iv. Lack of Variation among Characters 95

B. Use of Dry versus Fresh Plant Material 96

C. Petal versus Whole Plant Results 97

D. Factor Analysis versus Conventional Cluster Analysis 98

E. Flavonoid Differences between Duplicate Taxa. 99

Taxonomy of the Limnanthaceae

A. Introduction 101

B. Hypothetical Evolutionary History of the Limnanthaceae 104

C. Assumptions that Led to the Hypothetical History 107

D. Comparison of the Proposed Classification to the Existing Classification

i. Above the Species Level 111

ii. Species Level and Below 116

E. Summary of Proposals for Revision of the Limnanthaceae

i. Introduction 128

ii. Genus Level 129

iii. Section Level 129

iv. Species and Varietal Levels 129

F. Synopsis of the Family Limnanthaceae 130

Conclusions 133

Literature Cited 135

LIST OF TABLES

TABLE I -- Present Day Classification of the Limnanthaceae	8
TABLE II -- Identification and Sources of Operational Taxonomic Units of <u>Limnanthes</u> and <u>Floerkea</u>	16
TABLE III -- Identifications of the Flavonoids of the Limnanthaceae	46
TABLE IV -- Rfs and Colours of Flavonoids of the Limnanthaceae	49
TABLE V -- Products Obtained upon Total Hydrolysis with 1.0 N HCl	52
TABLE VI -- Derivatives Obtained upon Partial Acid Hydrolysis and Hydrolysis with Emulsin	53
TABLE VII -- UV Absorption Maxima of Flavonoids of the Limnanthaceae	54
TABLE VIII -- Proton Chemical Shift Values of TMS Ethers of Six Flavonol β -D-Rutinosides of the Limnanthaceae	55
TABLE IX -- Whole Plant Flavonoids of 30 OTUs of the Limnanthaceae	67
TABLE X -- Petal Flavonoids of 18 OTUs of <u>Limnanthes</u>	69
TABLE XI -- Synopsis of the Results of Four Cluster Analyses Based on Whole Plant Flavonoids of 30 OTUs	86
TABLE XII -- Synopsis of the Results of Four Cluster Analyses Based on Petal Flavonoids of 18 OTUs	87

LIST OF FIGURES

FIGURE I -- Distribution of <u>Limnanthes</u> in Oregon and California	2
FIGURE II -- The Flowers of Six Selected <u>Limnanthes</u> taxa Taken in Visible and Ultraviolet Light	40
FIGURE III -- Composite Two-Dimensional Map of 48 Flavonoid Glycosides of the Limnanthaceae	48
FIGURE IV -- Stepwise Elaboration of the B-Ring of Flavonols of the Limnanthaceae	61
FIGURE V -- TLC Map of Whole Plant Flavonoids of <u>L. montana</u> Taken in Ultraviolet Light	66
FIGURE VI -- TLC Map of Whole Plant Flavonoids of <u>L. montana</u> Taken in Visible Light	66
FIGURE VII -- Weighted Pair Group Clustering of 30 OTUs by Jaccard Coefficient: High Concentrations of Whole Plant Flavonoids	71
FIGURE VIII -- Weighted Pair Group Clustering of 30 OTUs by Simple Match Coefficient: High Concentrations of Whole Plant Flavonoids	72
FIGURE IX -- Weighted Pair Group Clustering of 30 OTUs by Jaccard Coefficient: All Concentrations of Whole Plant Flavonoids	73
FIGURE X -- Weighted Pair Group Clustering of 30 OTUs by Simple Match Coefficient: All Concentrations of Whole Plant Flavonoids	74
FIGURE XI -- Weighted Pair Group Clustering of 18 OTUs by Jaccard Coefficient: High Concentrations of Petal Flavonoids	75
FIGURE XII -- Weighted Pair Group Clustering of 18 OTUs by Simple Match Coefficient: High Concentrations of Petal Flavonoids	76
FIGURE XIII -- Weighted Pair Group Clustering of 18 OTUs by Jaccard Coefficient: All Concentrations of Petal Flavonoids	77
FIGURE XIV -- Weighted Pair Group Clustering of 18 OTUs by Simple Match Coefficient: All Concentrations of Petal Flavonoids	78
FIGURE XV -- Principal Components Analysis of 30 OTUs Based on Whole Plant Flavonoids	79
FIGURE XVI -- Varimax Factor Analysis of 30 OTUs Based on Whole Plant Flavonoids	80
FIGURE XVII -- Principal Components Analysis of 18 OTUs Based on Petal Flavonoids	81

LIST OF FIGURES -- Continued

FIGURE XVIII -- Varimax Factor Analysis of 18 OTUs Based on Petal Flavonoids	82
FIGURE XIX -- Hypothetical Phylogenetic Tree of the Limnanthaceae	108
FIGURE XX -- Principal Components Analysis of 9 OTUs of <u>L. floccosa</u> Based on Whole Plant Flavonoids	121

LIST OF APPENDICES

APPENDIX I -- Mason's Classification of <u>Limnanthes</u> R. Br.	139
APPENDIX II -- Voucher Specimens of <u>Limnanthes</u> Taxa Grown from seed	140
APPENDIX III -- TLC Map of Petal Flavonoids of <u>L. douglasii</u> var. <u>douglasii</u> , OTU 1	141
APPENDIX IV -- TLC Map of Whole Plant Flavonoids of <u>L. douglasii</u> var. <u>douglasii</u> , OTU 1	142
APPENDIX V -- TLC Map of Petal Flavonoids of <u>L. douglasii</u> var. <u>douglasii</u> , OTU 2	143
APPENDIX VI -- TLC Map of Whole Plant Flavonoids of <u>L. douglasii</u> var. <u>douglasii</u> , OTU 2	144
APPENDIX VII -- TLC Map of Petal Flavonoids of <u>L. douglasii</u> var. <u>nivea</u> , OTU 3	145
APPENDIX VIII -- TLC Map of Whole Plant Flavonoids of <u>L. douglasii</u> var. <u>nivea</u> , OTU 3	146
APPENDIX IX -- TLC Map of Petal Flavonoids of <u>L. douglasii</u> var. <u>rosea</u> , OTU 4	147
APPENDIX X -- TLC Map of Whole Plant Flavonoids of <u>L. douglasii</u> var. <u>rosea</u> , OTU 4	148
APPENDIX XI -- TLC Map of Petal Flavonoids of <u>L. douglasii</u> var. <u>sulphurea</u> , OTU 5	149
APPENDIX XII -- TLC Map of Whole Plant Flavonoids of <u>L. douglasii</u> var. <u>sulphurea</u> , OTU 5	150
APPENDIX XIII -- TLC Map of Whole Plant Flavonoids of <u>L. vinculans</u>	151
APPENDIX XIV -- TLC Map of Petal Flavonoids of <u>L. bakeri</u> , OTU 7	152
APPENDIX XV -- TLC Map of Whole Plant Flavonoids of <u>L. bakeri</u> , OTU 7	153
APPENDIX XVI -- TLC Map of Petal Flavonoids of <u>L. striata</u> , OTU 8	154
APPENDIX XVII -- TLC Map of Whole Plant Flavonoids of <u>L. striata</u> , OTU 8	155
APPENDIX XVIII -- TLC Map of Petal Flavonoids of <u>L. macounii</u> , OTU 9	156
APPENDIX XIX -- TLC Map of Whole Plant Flavonoids of <u>L. macounii</u> , OTU 9	157

LIST OF APPENDICES -- Continued

APPENDIX XX -- TLC Map of Petal Flavonoids of <u>L. macounii</u> , OTU 10	158
APPENDIX XXI -- TLC Map of Whole Plant Flavonoids of <u>L. macounii</u> , OTU 10	159
APPENDIX XXII -- TLC Map of Petal Flavonoids of <u>L. alba</u> var. <u>alba</u> , OTU 11	160
APPENDIX XXIII -- TLC Map of Whole Plant Flavonoids of <u>L. alba</u> var. <u>alba</u> , OTU 11	161
APPENDIX XXIV -- TLC Map of Petal Flavonoids of <u>L. alba</u> var. <u>alba</u> , OTU 12	162
APPENDIX XXV -- TLC Map of Whole Plant Flavonoids of <u>L. alba</u> var. <u>alba</u> , OTU 12	163
APPENDIX XXVI -- TLC Map of Petal Flavonoids of <u>L. alba</u> var. <u>versicolor</u> , OTU 13	164
APPENDIX XXVII -- TLC Map of Whole Plant Flavonoids of <u>L. alba</u> var. <u>versicolor</u> , OTU 13	165
APPENDIX XXVIII -- TLC Map of Petal Flavonoids of <u>L. gracilis</u> var. <u>gracilis</u> , OTU 14	166
APPENDIX XXIX -- TLC Map of Whole Plant Flavonoids of <u>L. gracilis</u> var. <u>gracilis</u> , OTU 14	167
APPENDIX XXX -- TLC Map of Petal Flavonoids of <u>L. gracilis</u> var. <u>gracilis</u> , OTU 15	168
APPENDIX XXXI -- TLC Map of Whole Plant Flavonoids of <u>L. gracilis</u> var. <u>gracilis</u> , OTU 15	169
APPENDIX XXXII -- TLC Map of Petal Flavonoids of <u>L. gracilis</u> var. <u>parishii</u> , OTU 16	170
APPENDIX XXXIII -- TLC Map of Whole Plant Flavonoids of <u>L. gracilis</u> var. <u>parishii</u> , OTU 16	171
APPENDIX XXXIV -- TLC Map of Petal Flavonoids of <u>L. montana</u> , OTU 17	172
APPENDIX XXXV -- TLC Map of Whole Plant Flavonoids of <u>L. montana</u> , OTU 17	173
APPENDIX XXXVI -- TLC Map of Petal Flavonoids of <u>L. floccosa</u> ssp. <u>bellingeriana</u> , OTU 18	174
APPENDIX XXXVII -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>bellingeriana</u> , OTU 18	175

LIST OF APPENDICES -- Continued

APPENDIX XXXVIII -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>bellingermana</u> , OTU 19	176
APPENDIX XXXIX -- TLC Map of Petal Flavonoids of <u>L. floccosa</u> ssp. <u>pumila</u> , OTU 20	177
APPENDIX XL -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>pumila</u> , OTU 20	178
APPENDIX XLI -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>pumila</u> , OTU 21	179
APPENDIX XLII -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>grandiflora</u> , OTU 22	180
APPENDIX XLIII -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>floccosa</u> , OTU 23	181
APPENDIX XLIV -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>floccosa</u> , OTU 24	182
APPENDIX XLV -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>floccosa</u> , OTU 25	183
APPENDIX XLVI -- TLC Map of Whole Plant Flavonoids of <u>L. floccosa</u> ssp. <u>californica</u> , OTU 26	184
APPENDIX XLVII -- TLC Map of Whole Plant Flavonoids of <u>F. proserpinacoides</u> , OTU 27	185
APPENDIX XLVIII -- TLC Map of Whole Plant Flavonoids of <u>F. proserpinacoides</u> , OTU 28	186
APPENDIX XLIX -- TLC Map of Whole Plant Flavonoids of <u>F. proserpinacoides</u> , OTU 29	187
APPENDIX L -- TLC Map of Whole Plant Flavonoids of <u>F. proserpinacoides</u> , OTU 30	188
APPENDIX LI -- 100 MHz NMR Spectrum of TMS Ether of Kaempferol 3-O- β -D-Rutinoside	189
APPENDIX LII -- 100 MHz NMR Spectrum of TMS Ether of Quercetin 3-O- β -D-Rutinoside	190
APPENDIX LIII -- 100 MHz NMR Spectrum of TMS Ether of Isorhamnetin 3-O- β -D-Rutinoside	191
APPENDIX LIV -- 100 MHz NMR Spectrum of TMS Ether of Myricetin 3-O- β -D-Rutinoside	192

LIST OF APPENDICES -- Continued

APPENDIX LV -- 100 MHz NMR Spectrum of TMS Ether of Laricytrin 3-O- β -D-Rutinoside	193
APPENDIX LVI -- 100 MHz NMR Spectrum of TMS Ether of Syringetin 3-O- β -D-Rutinoside	194
APPENDIX LVII -- Matrix of Similarity Coefficients Calculated for 30 OTUs by Jaccard Coefficient: High Concentrations	195
APPENDIX LVIII -- Matrix of Similarity Coefficients Calculated for 30 OTUs by Simple Match Coefficient: High Concentrations	196
APPENDIX LIX -- Matrix of Similarity Coefficients Calculated for 30 OTUs by Jaccard Coefficient: All Concentrations	197
APPENDIX LX -- Matrix of Similarity Coefficients Calculated for 30 OTUs by Simple Match Coefficient: All Concentrations	198
APPENDIX LXI -- Matrix of Similarity Coefficients Calculated for 18 OTUs by Jaccard Coefficient: High Concentrations	199
APPENDIX LXII -- Matrix of Similarity Coefficients Calculated for 18 OTUs by Simple Match Coefficient: High Concentrations	200
APPENDIX LXIII -- Matrix of Similarity Coefficients Calculated for 18 OTUs by Jaccard Coefficient: All Concentrations	201
APPENDIX LXIV -- Matrix of Similarity Coefficients Calculated for 18 OTUs by Simple Match Coefficient: All Concentrations	202
APPENDIX LXV -- Coordinates of 30 OTUs Plotted in Figure XV	203
APPENDIX LXVI -- Coordinates of 30 OTUs Plotted in Figure XVI	204
APPENDIX LXVII -- Coordinates of 18 OTUs Plotted in Figure XVII	205
APPENDIX LXVIII -- Coordinates of 18 OTUs Plotted in Figure XVIII	206

ACKNOWLEDGEMENT

I thank the following people who assisted in the completion of this thesis:

Dr. B.A. Bohm for his support and advice throughout the course of this work;

Drs. C.J. Marchant, J.R. Maze, W.B. Schofield, I.E.P. Taylor and R.L. Taylor
for their criticisms of the manuscript and helpful suggestions for
its improvement;

Dr. F.W. Collins for technical advice;

Mr. Steve Borden, Institute of Animal Resource Ecology, University of British
Columbia, for performing the computer analyses;

Dr. Bob Bose, Environment Canada, Department of Fisheries, Vancouver, for
determining the NMR spectra;

Mr. G.A. White, U.S.D.A.; Agricultural Research Service, Beltsville, Maryland,
for providing seed of Limnanthes;

Dr. R. Ornduff, Dept. of Botany, University of California, Berkeley, for
providing dried material of Limnanthes;

and my wife Jane C. Parker for her continued support and assistance.

INTRODUCTION

FAMILY DESCRIPTION

The Limnanthaceae is a small family of annual herbs containing two genera: Floerkea Willd., which is monotypic, and Limnanthes R. Br., which contains 18 recognized taxa. Floerkea proserpinacoides Willd. is widely distributed across the United States and southern Canada. Limnanthes is restricted to southern Oregon, California and the southern tip of Vancouver Island, British Columbia. Figure I is a map showing the distributions of all Limnanthes taxa except L. macounii Trel. which occurs only in the vicinity of Victoria, B.C.

All of the Limnanthaceae are early spring annuals, and most are associated with vernal pools or soil pockets kept moist by seepage. Limnanthes taxa vary in their moisture requirements; some actually grow in standing water, others at the edges of pools or streams, and others on moist hillsides. With the exception of L. montana Jepson and L. striata Jepson, both of which frequently occupy more shaded streamside habitats, Limnanthes grows in open spots. Taxa of Limnanthes occur over a range of elevations from nearly sea level to about 1800 meters (Mason, 1952; Gentry & Miller, 1965; Arroyo, 1973a; Munz & Keck, 1963). Floerkea grows in the shade of shrubs or trees. All of these plants are ephemeral; they appear early in the spring, then flower and set seed, and are soon overtopped by other plants and die.

Although all members of the Limnanthaceae are self-compatible, their breeding systems vary from nearly total outcrossing to complete autogamy. Outcrossing members are adapted to insect pollination. These plants have large numbers of attractive, fragrant flowers, and tend to produce a carpet

FIGURE I

Distribution of Limnanthes
in California and Oregon

of blooms over a meadow. From this character the common name for Limnanthes, "meadow foam", is derived. Additional traits associated with outcrossing species are protandry and the presence of nectar glands at the base of the filaments. Autogamous members of the family tend to have unscented, nectarless, and generally inconspicuous flowers. The extreme is found in L. macounii and F. proserpinacoides, which are totally self-pollinating, have small greenish flowers and reduced numbers of floral parts. These characters, coupled with small stature, make these plants very difficult to find.

TAXONOMIC BACKGROUND

Past taxonomic treatments of the Limnanthaceae recognized that this family comprises a discrete group of closely related plants. However, these treatments have expressed conflicting views regarding affinities with other families and relationships within the family. To some extent, these conflicts remain unsettled today.

A. Below the Family Level

Robert Brown's original circumscription of the family (Brown, 1833) delimits two genera, Limnanthes and Floerkea. Some later authors have questioned the validity of this separation and united all members of the family in the genus Floerkea. This subject is reviewed by Mason in his monograph of Limnanthes (1952). Actually, the question of whether the family consists of one genus or two has not yet been adequately resolved. The primary reason for this is that Mason excluded F. proserpinacoides from his treatment of Limnanthes. Since the modern classification of the Limnanthaceae is based on Mason's treatment, this exclusion has resulted in a de facto separation of the family into two genera. Whether this separation was justified cannot be determined without applying the same taxonomic criteria used by Mason to all

members of the family simultaneously, including Floerkea.

A more recent study by Ornduff and Crovello (1968), based on a taximetric analysis of morphological characters, concluded that the differences between Limnanthes and Floerkea are probably not great enough to distinguish two genera on either phenetic or phylogenetic grounds. However, the two genera have not been formally reunited.

Mason (1952) divided Limnanthes into 8 species and 11 varieties totaling 15 taxa. His classification is presented as Appendix I. Mason's taxonomic scheme is based on the results of artificial crosses and morphological and cytological analyses.

The results of Mason's cytological investigation show uniform patterns of 5 identifiable pairs of chromosomes, indicating a close relationship between all Limnanthes taxa (except L. macounii which was thought to be extinct at that time and for which Mason had no living material). This complete lack of karyotype variation suggests either a comparatively recent divergence from ancestral stock of the taxa investigated, or a strong evolutionary conservancy of karyotype in the family.

Mason's classification of Limnanthes relies heavily on the biological species concept. Thus, in many instances experimentally interfertile taxa, formerly treated as separate species, are reduced to variety rank. Taxa so reduced include two of the four varieties of L. douglasii R. Br., the two varieties of L. alba Benth., and the two varieties of L. gracilis Howell.

According to Mason, Limnanthes can be divided naturally into two sections, the Reflexae and the Inflexae (See Appendix I). Names of these two sections are derived from the petal positions assumed and maintained after pollination. Although Mason did not detect other morphological differences coincident with this division, he concluded that the two sections represent two phylogenetically distinct groups since no successful crosses could be made

across the sectional barrier.

Ornduff and Crovello (1968) agreed with the separation of Limnanthes into two sections, although their presented results only weakly support this separation. Using a taximetric analysis based on 35 morphological characters, all recognized taxa of the Limnanthaceae were clustered using the weighted pair group method and a variation of factor analysis. Two additional factor analyses were also performed, one on 18 floral characters, and the other on the remaining 17, or "vegetative" characters. The results of each clustering analysis are markedly different. Only the results of the vegetative analysis follow Mason's sectional alignment, producing a distinct cluster of taxa corresponding to the section Reflexae and a fairly diffuse grouping corresponding to the section Inflexae. It is notable that in this analysis F. proserpinacoides also grouped with the Reflexae. The results of the analysis utilizing floral characters apparently contradict the presence of a sectional division since no groups were separated. According to the authors, these results are easily explained, if not expected, since in this instance a classification based on floral characters is in effect closely linked to level of autogamy. Therefore, they hypothesize that the two sections are not separated by the floral analysis, since parallel trends toward autogamy have apparently occurred simultaneously in both sections. The analysis based on the combined data of the floral and vegetative analysis separated three clusters of taxa: the first contains L. macounii and F. proserpinacoides, the second contains Mason's three varieties of L. floccosa Howell, and the third contains the remaining Limnanthes taxa. Again the authors explained that the observed phenetic affinities are closely linked to level of autogamy and do not invalidate Mason's sectional division, which they considered an accurate phylogenetic arrangement.

In their 1968 study, Ornduff and Crovello included two undescribed

Limnanthes taxa with affinities to members of the Reflexae. Ornduff has subsequently described one of these as a new species, Limnanthes vinculans Ornduff (1969a). It is a narrow endemic growing sympatrically with L. douglasii R. Br. var. nivea Mason which it closely resembles in all but its leaflet morphology.

Arroyo (1973a) reexamined L. floccosa, elevating Mason's three varieties to the rank of subspecies and creating two new subspecies. Level of autogamy, as demonstrated by a phenetic cluster analysis heavily weighted with floral characters, was the sole criterion used to distinguish the five subspecies, although geographical and ecological evidence was cited to support this separation. Arroyo concluded that the five taxa of L. floccosa represent stages in the trend toward complete autogamy in the Inflexae and form a natural group recently derived from the predominantly outcrossing L. alba Benth. (1973a, 1973b). She did not explain why she chose to divide L. floccosa into subspecies in contrast to the varietal subdivision used uniformly by Mason for the rest of the genus.

In his most recent publication concerning the Limnanthaceae, Ornduff (1971) reported that both L. macounii and F. proserpinacoides have chromosome numbers of $n=5$, the same as the rest of the family. Ornduff (1971) also presented the results of extensive Limnanthes interbreeding trials. In general his results confirmed the work of Mason, demonstrating low interspecific crossability and a high degree of chromosomal homology (including L. macounii). However, Ornduff made three important observations regarding sterility barriers in Limnanthes. 1) In general, the presence of sterility barriers between taxa does not correspond with the morphological differences distinguishing these taxa. 2) Since the cytological differences between taxa are of a relatively minor nature, hybrid sterility, where present, probably has a genetic basis. 3) The presence of a sterility barrier between two taxa is most likely

if the two lack distinct geographical differences. In this last regard, it is of particular interest that L. macounii, which is the most disjunct species of Limnanthes, produced the highest number of interspecific hybrids including the only intersectional hybrid, a sterile offspring resulting from a cross with L. montana.

Table I shows the modern classification of the Limnanthaceae. It is based on Mason's classification of Limnanthes, and it includes the more recent taxonomic additions previously cited. In addition, it lists Floerkea as a distinct genus.

In summary, relationships below the family level in the Limnanthaceae remain unclear for two primary reasons; 1) it is probable that certain similarities have arisen by convergence; the trend toward autogamy has most likely occurred independently in the family at least three times and possibly more often. 2) the biological species concept employed by Mason is probably of limited value in this context, since genetic barriers to interbreeding exist between closely related sympatric taxa, but may not be present between more distantly related allopatric taxa.

B. Family Affinities

In his description of the Limnanthaceae, Robert Brown (1833) was uncertain regarding affinities with other families. He implied that his earlier examinations of Floerkea suggested an association with the perigynous families. However, after the discovery of Limnanthes, he decided that the family was more properly allied with the hypogynous families. Other nineteenth century authors generally agreed with Brown, either linking to, or including members of the Limnanthaceae in, the Geraniaceae. Engler and Prantl (1896) disagreed including the family in the Sapindales.

Most modern authors, including Hutchinson (1926), Cronquist (1968),

TABLE I

Present Day Classification of the Limnanthaceae

Floerkea Willd.

- 1) F. proserpinacoides Willd.

Limnanthes R. Br.

Section Reflexae

- 2) L. bakeri J.T. Howell
- 3) L. douglasii R. Br. var. douglasii
" var. nivea Mason
" var. rosea (Benth.) Mason
" var. sulphurea Mason
- 4) L. macounii Trel.
- 5) L. striata Jepson
- 6) L. vinculans Ornduff

Section Inflexae

- 7) L. alba Benth. var. alba
" var. versicolor (Greene) Mason
- 8) L. floccosa Howell ssp. floccosa
" ssp. bellingeriana (M.E. Peck) Arroyo
" ssp. californica Arroyo
" ssp. grandiflora Arroyo
" ssp. pumila (Howell) Arroyo
- 9) L. gracilis Howell var. gracilis
" var. parishii (Jepson) Mason
- 10) L. montana Jepson

and Takhtajan (1969), agree that the Limnanthaceae constitutes a distinct family belonging to the Geraniales. Maheshwari and Johri (1956) took exception to this viewpoint after comparing the Limnanthaceae to the Sapindales and the Geraniales. Their comparison was based on embryological and morphological features of Limnanthes discovered by Mason (1951) and Mathur (1956), together with their own findings on embryo development, floral morphology, and seed morphology of F. proserpinacoides. The authors showed that the Limnanthaceae has few morphological similarities to either the Geraniales or Sapindales, with regard to embryogenesis, and seed, pollen, and gynoecium morphology. On this basis, Maheshwari and Johri concluded that assignment of the Limnanthaceae to either the Sapindales or Geraniales is unsatisfactory, and that probably the family should be raised to ordinal rank. Unfortunately, the authors do not speculate on the derivation of the Limnanthaceae, nor do they suggest with which orders, if any, the Limnanthaceae shares the greatest affinity.

Warburg (1938) discussed inclusion of the Limnanthaceae in the Geraniales in light of its chromosome cytology. He made chromosome counts, determined karyotype morphology, and observed chromosome behaviour at meiosis for at least one representative of each family in the Geraniales sensu Hutchinson (1926), i.e., Geraniaceae, Oxalidaceae, Tropaeolaceae, Limnanthaceae, Linaceae, Balsaminaceae and Zygophyllaceae. The behaviour of chromosomes of Limnanthes at meiosis, their large size, and small number caused Warburg to propose two alternate hypotheses regarding placement of the Limnanthaceae; either the family is improperly placed in the Geraniales and more likely fits into the Sapindales as proposed by Engler and Prantl; or the family is a primitive relic closely related to ancestral taxa from which members of the Geraniales are derived. Neither of these hypotheses has been supported by more recent discoveries, but the cytological observations on which they were

based remain valuable.

PREVIOUS CHEMICAL INVESTIGATIONS

Considering the small size of the Limnanthaceae, a relatively large amount of research has been directed toward characterizing this family's chemistry. This is not surprising in view of the interesting results that have been obtained.

Members of the family contain glucosinolates, a character known to be shared by only about a dozen other families. m-Methoxybenzyl isothiocyanate was isolated and characterized from seed of L. douglasii by Ettlinger and Lundeen (1956). Actually, this isothiocyanate is not naturally occurring; instead, it is a derivative of the glucoside present in the seed, which is formed by the enzymic release of glucose. Hence, the naturally occurring glucoside of L. douglasii is m-methoxybenzyl glucosinolate, given the trivial name glucolimnanthin. This compound possesses an extremely rare substitution pattern not known outside the Limnanthaceae. Miller et al. (1964) have detected an additional unidentified glucosinolate in L. douglasii var. nivea, and also reported the presence of glucosinolates in all other Limnanthes taxa (Gentry & Miller, 1965).

The United States Department of Agriculture (U.S.D.A.) has conducted extensive research on the seed oil chemistry of Limnanthes (Gentry & Miller, 1965; Miller et al., 1964). Unlike common vegetable oils based on 16 or 18-carbon fatty acids, the seed oil of Limnanthes is based on 20 and 22-carbon fatty acids. Positions of unsaturation of the fatty acids of Limnanthes are also uncommon. Four such fatty acids comprise about 95% of the total seed oil fraction of Limnanthes; of these, three are unknown from other sources, while the fourth is erucic acid, also present in rape and mustard oils.

Before the present investigation was completed, little was known of

the phenolic substances of the Limnanthaceae. Bate-Smith (1962) reported that hydrolyzed extracts of leaves of L. douglasii contained caffeic acid, kaempferol, quercetin, myricetin, leucocyanidin and leucodelphinidin. As part of the present investigation, Parker and Bohm (1975) characterized 18 flavonol glycosides from L. douglasii. The results and implications of that study are presented in later chapters.

AGRONOMIC EVALUATION OF LIMNANTHES

The fatty acids present in the seed oil of Limnanthes are well suited to the production of high quality wax products. Because of this character, and since the oil content of the nutlets is 20-33% (Gentry & Miller, 1965), the U.S.D.A. has conducted extensive trials to determine the suitability of Limnanthes as a crop plant. Trials were made in Alaska, Oregon, California and Maryland, and consisted of planting, harvesting and calculating yields for most of the Limnanthes taxa (Higgins et al., 1971). In addition, various researchers have tried to determine optimal conditions for germination of Limnanthes seed (Toy & Willingham, 1966; 1967; Cole, 1974).

The results of the field trials indicate that most of the wild populations of Limnanthes produce abundant seed in the range of 1000 kg/hectare when cultivated. From an agronomic standpoint L. alba is considered the most desirable species due to its upright form and good seed retention, both qualities being necessary for mechanical harvesting. Although profits are marginal at present, Higgin et al. (1971) concluded that L. alba probably contains sufficient genetic variability such that an artificial selection program could substantially increase seed yields. Since Limnanthes seed contains 15-25% protein, the authors suggested that the extracted meal could be used as stock fodder, if residual levels of toxic glucosinolates are not too high.

THESIS OBJECTIVES

The Limnanthaceae is particularly well suited to the application of chemical taxonomic techniques. Due to the uncertainty of relationships, both within the family and with other families, the discovery and application of new information with taxonomic significance is desirable. Unless new information can be applied, it is probably impossible to fully elucidate family relationships; and therefore, impossible to test the validity of the classification shown in Table I.

The chemical characters of the family are an untapped source of potentially valuable taxonomic information. Secondary chemical constituents have served as useful taxonomic characters for many groups of plants, both large and small, and it is possible that the systematic utilisation of these characters might be useful in the context of the Limnanthaceae. The information available on seed fats suggests that these constituents possess little taxonomic significance (Miller et al., 1964). The possible taxonomic significance of other classes of secondary compounds, including the glucosinolates, cannot be estimated until they are more completely elucidated. The flavonoids of the Limnanthaceae also fall into this latter category.

The primary intentions of the present study are: 1) to determine the flavonoid composition of each member of the Limnanthaceae; 2) to produce an artificial classification(s) of the family based solely on flavonoid compositions; 3) to compare the chemical classification with the existing phenotypic treatments; and 4) to discuss any implications regarding evolution within the family or regarding affinities to other families uncovered in the course of this work. In

There are three secondary objectives of this study: 1) to assess the value of the contribution flavonoid character taxonomy can make to the systematics of the Limnanthaceae; 2) to discuss briefly the method of application

of flavonoids as characters in plant taxonomy; and 3) to assess the taxonomic significance of flower patterning in the family.

MATERIALS AND METHODS

THE PLANTS AND THEIR SOURCES

Whenever possible freshly grown plant material was used for flavonoid comparisons. This practice tends to eliminate several potential sources of chemical variation for the following reasons: the use of fresh material eliminates most ambiguities arising either from compounds being present in low concentration or from individual variation, since a large amount of plant material is usually available and many plants can be analyzed simultaneously; environmentally induced variation in flavonoid composition, if any exists, also is eliminated, since all plants can be grown under nearly uniform conditions; and although flavonoid breakdown is apparently insignificant in pressed specimens of Limnanthes and Floerkea, the use of fresh material for chemical comparisons eliminates this possible source of variation.

Approximately three quarters of the Limnanthes taxa were grown from seed provided by G.A. White, U.S.D.A., Agricultural Research Service, Beltsville, Maryland. This seed was available as a result of the agronomic evaluation being conducted by the U.S.D.A. The taxa for which no seed was available were those described after the U.S.D.A. work was underway, including L. vinculans and the various subspecies of L. floccosa. Unfortunately, seed of Floerkea was unobtainable from any source.

Since it is nearly essential to include all members in a comparative study, herbarium specimens were used for analysis of those taxa of the Limnaceae for which no seed could be obtained. Dried material of L. vinculans and L. floccosa was provided by Dr. R. Ornduff, Department of Botany, University of California, Berkeley, while several collections of Floerkea were

provided by the University of British Columbia Herbarium.

The partial use of dried material causes certain interpretive problems arising from the analysis of non-parallel material. The effects of this practice on the results of this study will be discussed in the last chapter.

In the course of this work, it was realized that Dr. W.B. Schofield, Department of Botany, University of British Columbia, had discovered a population of the extremely rare L. macounii at William Head, Vancouver Island about 25 miles from the only previously known population at Victoria, B.C. (Mr. A. Ceska, Department of Biology, University of Victoria, has informed me that he and his wife have discovered additional populations since that time.) Material was grown from seed taken from the Schofield collection, and additional plants were collected from this new population.

Table II identifies the sources of all plants used in this study. The collectors and their collection numbers are given for the herbarium material. The remaining plants are identified by the original U.S.D.A. plant accession numbers. These numbers are the same as those referred to by Higgins et al. (1971) in their agronomic evaluation study.

Each entry in Table II was treated as a distinct taxon and analyzed separately, and each has been assigned an Operational Taxonomic Unit (OTU) number. The assignment of these numbers roughly follows the accepted classification of the family, but this assignment was made only to identify OTUs.

Certain varieties, subspecies or species are represented by more than one OTU in Table II. These repetitive OTUs represent different collections and almost certainly different populations as well. The decision to analyze separately different collections of a taxon does not reflect any preconceived taxonomic judgements. A primary reason for separate treatment is that some of the taxa were identified according to different taxonomic criteria and conventions. For instance, it was considered unwise to group fresh material

TABLE II

Identification and Sources of Operational Taxonomic Units
Of Limnanthes and Floerkea

OTU	Taxon Name	U.S.D.A. Plant Accession No.	Collector and Collection No.
1	<u>L. douglasii</u> var. <u>douglasii</u>	278170	
2	<u>L. douglasii</u> var. <u>douglasii</u>	283708	
3	<u>L. douglasii</u> var. <u>nivea</u>	283713	
4	<u>L. douglasii</u> var. <u>rosea</u>	283715	
5	<u>L. douglasii</u> var. <u>sulphurea</u>	283718	
6	<u>L. vinculans</u>		Rubtzoff 5699
7	<u>L. bakeri</u>	283706	
8	<u>L. striata</u>	283727	
9	<u>L. macounii</u> (Victoria)	315048	
10	<u>L. macounii</u> (William Head)		Schofield
11	<u>L. alba</u> var. <u>alba</u>	283701	
12	<u>L. alba</u> var. <u>alba</u>	B55689	
13	<u>L. alba</u> var. <u>versicolor</u>	283705	
14	<u>L. gracilis</u> var. <u>gracilis</u>	283722	
15	<u>L. gracilis</u> var. <u>gracilis</u>	283723	
16	<u>L. gracilis</u> var. <u>parishii</u>	283724	
17	<u>L. montana</u>	283725	
18	<u>L. floccosa</u> ssp. <u>bellingeriana</u>	283720	
19	<u>L. floccosa</u> ssp. <u>bellingeriana</u>		Kalin (Arroyo) 7031
20	<u>L. floccosa</u> ssp. <u>pumila</u>	283721	
21	<u>L. floccosa</u> ssp. <u>pumila</u>		Kalin 7033
22	<u>L. floccosa</u> ssp. <u>grandiflora</u>		Kalin 7028
23	<u>L. floccosa</u> ssp. <u>floccosa</u>		Kalin 7026
24	<u>L. floccosa</u> ssp. <u>floccosa</u>		Kalin 7022
25	<u>L. floccosa</u> ssp. <u>floccosa</u>		Kalin 6917
26	<u>L. floccosa</u> ssp. <u>californica</u>		Niehaus 371 & Ornduff 6885
27	<u>F. proserpinacoides</u> (Dutchess Co., N.Y.)		Ahles 76368
28	<u>F. proserpinacoides</u> (Kettle Falls, Wash.)		Beamish 60352
29	<u>F. proserpinacoides</u> (Montreal, Quebec)		Rouleau 4001
30	<u>F. proserpinacoides</u> (Shushan, N.Y.)		Dobbin 1758

of L. floccosa var. bellingeriana, identified on the basis of a three variety subdivision of L. floccosa, with pressed material of L. floccosa ssp. bellingeriana?, identified according to a five subspecies division. Therefore, OTUs 18 and 19 were analyzed separately.

Three taxa obtained from the U.S.D.A. were not identified according to Mason's classification. These taxa are: L. douglasii, OTU 1; L. gracilis, OTU 14; and L. alba, OTU 11. After growth and examination, each of these taxa was identified as the respective typical variety, and they are designated in this fashion in Table II. It is of interest that the seed of OTU 1 was originally from Europe, and probably this taxon is descended from the plants originally collected by David Douglas in the early 1830s from which Brown described the family (Gentry & Miller, 1965). In this case an artificial isolation has probably been maintained for nearly 150 years, which allows an estimation of the effects of genetic drift and inbreeding over this period.

Floerkea proserpinacoides and L. macounii are subdivided into four and two OTUs respectively, as shown in Table II. Both are totally autogamous, and populations from different locations are effectively genetically isolated. For this reason, separate analyses of these populations may reveal differences in flavonoid composition attributable to genetic drift or different selection pressures. In the case of L. macounii, the two populations are only a few miles apart, but in the case of F. proserpinacoides, the most widely distributed element of the family, the populations are from the extremes of its range.

Three different collections of L. floccosa ssp. floccosa were available from various locations. Each of these collections was made by Arroyo and was used in her systematic studies. These collections are treated here as distinct OTUs to assess the variation within this taxon and to test the validity of Arroyo's recent taxonomic treatment of L. floccosa (Arroyo, 1973a).

In the course of growing L. douglasii var. sulphurea, OTU 5, U.S.D.A. Accession #283718, it was observed that these plants had petal patterning differing from Mason's description of this variety. Mason (1952) described it as having completely yellow petals, while the U.S.D.A. plants have a small amount of white at the tips. However, they have a smaller amount than the petals of L. douglasii var. douglasii. Otherwise, these plants correspond to Mason's description of variety sulphurea, including leaflet shape, a character which distinguishes this taxon from other varieties of L. douglasii. Mason described F_1 hybrids of varieties sulphurea and douglasii as having petal tips with an intermediate amount of white. Whether the abnormal colouring of the U.S.D.A. variety results from such crossing is not known, but the identification of OTU 5 as L. douglasii var. sulphurea was tentatively accepted for this study.

Voucher specimens of all plants grown for this investigation are deposited in the U.B.C. Herbarium. Appendix II lists these vouchers and their collection numbers. The four collections of Floerkea are also on file in the U.B.C. Herbarium. Voucher specimens of L. floccosa and L. vinculans, that were provided by Ornduff, are on file at the Herbarium of the University of California, Berkeley.

PLANT GROWTH

Most plants were grown in a greenhouse under nearly uniform conditions. The different taxa were grown in random groups, two to four at a time, from the end of 1972 to the beginning of 1975. One taxon, L. douglasii var. douglasii, OTU 1, was also grown outdoors on a much larger scale in the summer of 1973 to provide a quantity of fresh plant material so that the major flavonoids could be isolated.

Mason (1952) reported that Limnanthes seed will not germinate unless

the soil temperature is kept under 15°. Preliminary germination tests confirmed this observation and also showed that stratification of the seed at about 5-10° for 3-5 days enhanced germination. Therefore, all seed was placed in a refrigerator for a few days before planting in pots. The pots were placed in cold frames on top of the Bio-Sciences Building, University of British Columbia and kept there until germination was complete. This practice was fairly satisfactory except in the middle of winter or the middle of summer, when temperatures in the cold frame exceeded the range in which Limnanthes will germinate.

After germination, pots of young Limnanthes plants were transferred to a greenhouse and allowed a short growth period. After development of the second or third permanent leaf, the young seedlings were transplanted 20 to a flat, using a mixture of about 90% sterilized garden soil and 10% sand. The survival rate of transplanted seedlings was close to 100%, if they were not overwatered. Since the moisture requirements of the different taxa varied, each taxon was watered an appropriate amount arrived at by trial and error; if any signs of damping-off appeared, the amount of water was reduced.

Plants used for flavonoid comparisons were grown on the same bench under the same bank of ordinary fluorescent lights. Like many early spring annuals, taxa of Limnanthes require long daylight periods to flower (Mason, 1952). It was found that all taxa rapidly flowered under 16 hour daylength, so this light duration was used consistently. Also, it was discovered early in the course of this work that should daylength be shortened drastically (i.e., 16 hour to an 8 hour daylength) after the plants have begun flowering, they rapidly cease flowering and die. A complete generation of Limnanthes, from seed germination to seed ripening, occurs in 3-4 months under the conditions outlined above.

The procedure for growing L. douglasii var. douglasii outdoors was

similar to the above, except that seedlings were transplanted directly into the ground instead of flats. Plants were spaced every 15 cm in a single row about 50 m long. Seed for this planting was germinated in late June, and plants were harvested in late September, just prior to flowering. Outdoor-grown plants produced much coarser, more lush vegetation than the corresponding greenhouse grown plants. Whether this difference results from the naturally shortening daylength or some additional factors associated with the outdoor planting is not known.

There are two problems associated with the growth of Limnanthes plants which deserve comment. Their succulent vegetation is particularly attractive to aphids and whiteflies, both common greenhouse pests. These insects selectively infest Limnanthes plants, often occurring in great numbers on them while adjacent plants are unaffected. Therefore, in order to obtain satisfactory yields, it was necessary to spray plants periodically with "Raid" insecticide (S.C. Johnson & Son, Ltd.). Interestingly, the field grown plants of L. douglasii were not attacked by insects.

The second problem associated with Limnanthes culture regards its root collar. In order for a plant to reach maturity, its root collar must remain buried. Since this root collar extends only a short distance below the ground (1-2 cm), care must be taken when watering and weeding not to disturb the soil immediately around the plant. If the root collar is uncovered, the plant will live only a day or so.

PLANT TISSUE SELECTION

The type of tissue used for flavonoid analysis varied with the source of plant material. Since only small amounts of pressed specimens were available, all the material at hand was used. However, for plants grown from seed, choices had to be made regarding 1) whether all tissues should be

included in the flavonoid comparison tests, and 2) whether any tissues should be analyzed separately.

All members of the Limnanthaceae have a basal rosette of succulent, but finely divided, compound leaves and caulescent flowering stems, which are largely indeterminate and may easily produce over 25 blooms in most species. The plants have short root collars with a diffuse network of finely dissected, deeply penetrating roots, a characteristic which makes root collection difficult and tedious. Early in the course of this investigation, the flavonoid contents of the roots of L. douglasii var. nivea, OTU 3, were compared to the contents of the above ground parts to determine whether root recovery was necessary. For this experiment the roots were efficiently recovered using a stream of water to wash away the surrounding soil. This preliminary test showed relatively weak concentrations and a more simplified pattern of flavonoids in the roots than in leaf and stem material. For this reason, no further attempts were made to recover quantitative amounts of root material from other taxa. However, some root material was included with the plants used for comparative study since they were harvested by pulling them from the soil after flowering had begun. Although this procedure yields only small amounts of root tissue, the flavonoids present in this material were considered to comprise the total flavonoid composition of any given taxon, and this composition is hereafter referred to as the "whole plant flavonoid composition".

No root material was collected when L. douglasii var. douglasii was grown in the open field. To facilitate harvesting, these plants were simply cut off at ground level. However, for comparative analysis, this taxon was regrown under the same conditions as the other taxa.

The flowers of many Limnanthes taxa are quite striking, some taxa having flowers with various shades of yellow patterning on otherwise white

to ivory coloured petals. Since there is striking variation between flowers of some taxa, and preliminary tests showed high flavonoid concentrations in the petals, the decision was made to analyze separately the petals of each taxon grown from seed. In this manner the flavonoid compositions of these OTUs could be compared on the basis of petal composition as well as whole plant composition. The petals from 10-20 individuals, collected over a 2-3 week period, yielded quantities of flavonoids adequate for comparative analysis.

FLORAL PATTERNING IN ULTRA VIOLET LIGHT

A. Introduction

Insect-pollinated, outcrossing plants frequently possess special adaptations, such as nectar glands and patterned flowers that are associated with this type of breeding system. Contrasting petal colouring is common in such plants and often results in a "bullseye" effect. This type of pattern tends to draw an observer's eye toward the flower center. Since the goal of the insect pollinator is the nectar glands rather than the reproductive parts of a flower, bullseye patterns have been interpreted as adaptations which attract visiting insects to the center of the flower and have thus been termed "nectar guides".

Only by looking at a flower as a bee sees it, is it possible to fully appreciate colour adaptations that facilitate pollination by this vector. Although this cannot be done directly, it is possible to examine flowers at all wavelengths to which bees respond. Von Frisch's work on honeybee vision (1967) has revealed that bees, unlike humans, possess light receptors sensitive to near ultra violet (UV) light. By photography using a filter transparent only to UV light and film sensitive to this colour range, various investigators have discovered that certain flowers have nectar guides that are visible only in UV light. Flowers with such nectar guides have contrasting

bright and dark regions, corresponding to areas of reflectance and absorption of UV light. To date, two classes of flavonoids, flavonols and chalcones, both with absorption maxima in the near UV range, have been identified as the pigments responsible for the UV absorbing portion of the nectar guides of Rudbeckia and Oenothera visible under UV light (Thompson et al., 1972; Dement & Raven, 1974).

Comparison of the floral patterns of closely related plants as the bee sees them may reveal information with potential systematic importance. Daumer (1958) has found that flowers with patterning visible in UV are more attractive to pollinating bees than unpatterned flowers. This phenomenon has led Ornduff and Mosquin (1970) to conclude that plants which have evolved a system of nectar guides visible in UV light are more highly adapted to an out-crossing habit than related plants without such a system.

Discrimination by pollinating agents on the basis of floral appearance can play an important role in the divergence of populations and the creation of new taxonomic entities. Two closely related populations with no genetic barriers to crossing, differing only in their appearance to bees, may become reproductively isolated on ethological grounds (Macior, 1971). If two populations become isolated by pollinator selection, they may eventually become distinct as a result of genetic drift, and possibly differentiate into new species in response to changing selection pressures.

Differences in flower colouring revealed by UV photography have proven useful for differentiation of closely related taxa. Horovitz and Cohen (1972) have shown that the UV reflectance characteristics of Sinapis alba and S. arvensis help distinguish these two closely related species. Similarly, Ornduff and Mosquin (1970) have shown that closely related, but geographically isolated, elements of the Nymphoides indica complex are distinguished in the same manner.

Several taxa in the Limnanthaceae are almost totally outcrossing; these plants often have visibly patterned flowers, nectar glands, and are bee-pollinated. The varieties of L. douglasii follow this pattern, and it is interesting that the most striking differences between these plants result from variation in floral colours and patterning. Since flower petals of Limnanthes were found to contain high concentrations of flavonoids which might produce UV visible nectar guides, and because information of potential systematic importance might be discovered, the flowers of all Limnanthes taxa grown from seed were examined by UV photography. It was hoped that any floral differences revealed by this technique would complement chemical differences and would be useful in reevaluating the systematics of the family.

B. Method

Flowers of OTUs grown from seed were photographed on colour film, first with no filter, and second with a filter which transmitted UV light only. All photographs were taken with an Exa 35 mm SLR, F 3.5 lens, and extension rings adjusted so that the resulting flower image on the colour transparency was approximately life size. High Speed Ektachrome (Kodak) film, ASA-160 was used throughout. Photography was done in the lab, but only on bright sunny days when a strong natural window light was present. Preliminary trials showed that an exposure time of 1/25 second produced satisfactory pictures in existing daylight under these conditions.

After photography in natural light, each flower was rephotographed through a Wratten No. 18A (Kodak) 2 inch glass filter under the same conditions as above, but with an additional hand-held UV light source (366 m μ). The 18A filter is transparent only to light with wavelengths less than 400 m μ . Since a regular camera lens will transmit light over 350 m μ , with the filter in place the frequency range of light reaching the film is 350-400 m μ .

(Kodak Data Book M-27, 1968). The photographs made by this technique theoretically represent the UV component of a bee's vision.

An exposure time of 30 seconds for UV photographs was selected on the basis of preliminary trials. The films were developed by regular commercial processing. For presentation in this thesis, selected transparencies were copied and printed in black and white.

Before the technique of UV flower photography can produce satisfactory results, some test photographs must be made. This is necessary to determine exposure and focusing details and choice of background. Since the light refraction by an ordinary camera lens is different for UV than for visible light, the lens must be refocused before a UV exposure is made. However, the UV image is invisible through the camera viewfinder. So, unless a fluorescent viewing screen is available, the degree of lens movement must be predetermined by trial exposures. For this work a uniform decrease of extension of about 3 mm produced UV exposures that were reasonably well focused.

Choice of flower background must also be determined by trial and error. This is because the UV reflectance and absorptive characters of the subject flower must be known before a contrasting background can be chosen. For the UV photography of Limnanthes flowers, a white background gave the best results, although a black background was also tried.

FLAVONOID ISOLATION AND IDENTIFICATION

A. Introduction

A comparative survey of the flavonoids of any group of plants consists of two parts: 1) isolating and identifying all the flavonoids present in total, and 2) assessing which compounds occur in each taxon under study. In practice, however, this technique is modified slightly; each taxon is analyzed in sequence, rather than all taxa at once. In closely related groups

of plants, each with similar flavonoid complements, the isolation and identification of the flavonoids of one taxon yields a set of standard compounds. By direct comparison to these standards, it is usually possible to identify most of the compounds present in the remaining taxa. Therefore, only a few additional unknown flavonoids need be isolated and identified from each new taxon. Once these compounds have been identified, they in turn serve as standards for comparison.

The family Limnanthaceae consists of relatively closely related taxa. Preliminary observations indicated that the flavonoid complements of these taxa were very similar. Therefore, L. douglasii var. douglasii, OTU 1, with its large yellow and white patterned flowers was selected for initial flavonoid identifications based on the amount of seed available and the size and colour of its flowers, rather than the uniqueness of its flavonoid pattern.

Plants of OTU 1 were grown outside to provide enough fresh material for flavonoid identification, and four flats of plants were grown in the greenhouse to provide sufficient numbers of flowers for separate analysis of the petals. Since most of the flavonoids present in remaining taxa were identified by comparison to the compounds of OTU 1, less fresh material was required for each taxon grown subsequently, and the material from a single flat of plants was usually adequate. However, in certain cases it was necessary to regrow a taxon to isolate enough of a previously unknown compound(s) for identification.

Unfortunately, a few compounds always occurred in trace amounts or were present in taxa for which no seed was available (e.g., Floerkea). Identification of these compounds was impossible without reference standards for comparison, and these were not available. However, after the flavonoids of the first several taxa were characterized, the flavonoid patterns of the

remaining taxa often could be completely determined on the basis of two chromatograms, one of the petals and one of whole plant material.

In the course of this work, slightly different procedures were used to isolate flavonoids appropriate to the type and amount of material available and the purpose for which the material was used. These different procedures will be described in the following sections.

B. Plant Extractions

Fresh leaf-stem material of field grown L. douglasii var. douglasii, OTU 1, weighing 3.5 kg was repeatedly extracted with hot 90% MeOH. This extract was concentrated in vacuo at 30°, followed by trituration with Celite Analytical Filter Aid to remove chlorophylls. The presence of large quantities of carbohydrates and other components necessitated a step to remove these components before flavonoid separation was possible. This was accomplished by passing the extract dissolved in water through a short column of polyamide (SC-6). Sufficient adsorbent was used to hold all flavonoids while carbohydrates and other non-adsorbing compounds were eluted with water. Subsequently, the flavonoids were removed from the column by repeated elution with 50% aqueous MeOH until no traces could be detected under UV light. The flavonoid containing eluent was then concentrated in vacuo.

The preliminary cleaning of MeOH extracts by means of a short polyamide column as described above proved invaluable for separation of flavonoids of Limnanthes, and this procedure was used as a preliminary step whenever any fresh material was extracted. However, caution had to be exercised while eluting with water, since highly glycosylated flavonoids, as are found in all Limnanthes taxa, have R_fs approaching 1.0 under these circumstances. Therefore, to avoid flavonoid loss or contamination, careful monitoring under UV light was required during elution.

Approximately 10,000 petals were collected from 4 flats of OTU 1 over a period of flowering of 2-3 weeks. The flavonoids were isolated from this material following the technique used for leaf-stem material, except that the trituration step with Celite was eliminated. Although the presence of carbohydrates was not as troublesome in this and other petal extracts, these extracts were eluted from a short polyamide column with 50% MeOH, leaving behind carotenoid pigments which were sometimes present in high concentration.

Following the work on OTU 1, the isolation procedure was changed slightly; rather than extraction with hot MeOH, fresh plant tissues were repeatedly extracted with MeOH at room temperature for a duration of 2-5 days. Hopefully, this prevented any possible breakdown of highly glycosylated flavonoids on exposure to heat. Although less efficient than extraction with hot solvent, acceptable amounts of flavonoids were extracted under the milder conditions.

The procedure used to isolate flavonoids from pressed plant material was simpler than those already outlined. The dried material was extracted with MeOH at room temperature for 2-5 days and then concentrated. No preliminary clean-up steps were required, although such steps might have been necessary had larger amounts of material been available. In every case where a taxon was analyzed from pressed material, only about 0.1 g dry weight of plant was available.

C. Identification of the Flavonoids of *L. douglasii* var. *douglasii*, OTU 1

i. Chromatography

After preliminary cleaning, petal and whole plant extracts were dissolved in small volumes of water and chromatographed on columns of SC-6 polyamide using a linear gradient 0-50% MeOH in H₂O. This practice separated

flavonoids roughly according to glycosylation class -- biosides from triosides, etc. Column fractions, containing compounds with a common glycosylation pattern, were consolidated and concentrated in vacuo. These fractions were taken up in a small volume of 80% MeOH and streaked on TLC plates (20 x 20 cm) using DC 6.6 polyamide (MacNarey-Nagel) as the adsorbent. These plates were run repeatedly in CHCl_3 -MeOH-butanone- H_2O (55:22:20:3). This organic solvent was developed for this purpose and tends to separate flavonoids according to their degree of substitution on the flavonoid nucleus, rather than type of sugar moiety. The resulting bands of pure compounds were marked under UV light and scraped, together with the polyamide, off the glass plates. The compounds were eluted with 80% aqueous MeOH with a vacuum aspirator. The resulting eluents were concentrated, and again taken up in small volumes of 80% MeOH.

Some groups of flavonoids with different glycosylation patterns, could not be separated completely by aqueous column chromatography. Separation was achieved using an aqueous TLC system -- DC 6.6 polyamide with H_2O -n-BuOH-acetone (8:1:1). The chloroform-based TLC system was then used to resolve these classes of compounds into individual components.

The above procedure used to isolate pure flavonoids was successful for most types of flavonoids present in Limnanthes. However, compounds with sugars attached at the 3 and 7 positions of the flavonoid nucleus were not separated by TLC in the above organic solvent system. Unfortunately, efforts to solve the problem were only partially successful; an acidic system using DC 6.6 polyamide and CHCl_3 -isopropanol-butanone-HOAc (10:3:3:4) was developed which will resolve the 3,7-diglycosides, but only if they are applied to plates in small amounts. However, this system made possible the identification of these compounds, even though they were not isolated as individual pure compounds.

The efficiency of compound recovery from the TLC polyamide was variable. Apparently, efficiency of elution is inversely correlated with degree of hydroxylation of the B-ring of the flavonoid nucleus -- i.e., the more hydroxyl groups present on a compound, the lower the efficiency of recovery of that compound. Recovery of myricetin derivatives with their three B-ring hydroxyl groups was probably less than 50%, and the recovery of compounds with two hydroxyl groups on the B-ring, such as quercetin derivatives, was only slightly better. Compounds with only one B-ring hydroxyl group were recovered more efficiently. Attempts were made to find a better method for eluting compounds from TLC polyamide, but no better eluting solvent than 80% MeOH was discovered. It is also worth noting that recovery of flavonoids from polyamide columns was by no means complete.

ii. Spectral Analyses

Ultra violet absorption spectra were determined for all compounds isolated in sufficient quantities (ca. 1 mg). Standard procedures outlined by Mabry et al. (1970) were followed. The technique consists of spectral determination in MeOH, and then in MeOH with various shift reagents added. The data obtained are useful for structural determination of flavonoids. UV spectra of Limnanthes compounds were determined on a Pye-Unicam SP 1800 spectrophotometer.

Nuclear magnetic resonance (NMR) spectroscopy also may be useful for identification of flavonoid compounds. However, satisfactory results using this technique require the isolation of comparatively large amounts of compound (10-20 mg). Six compounds were isolated from field-grown material of OTU 1 in quantities ranging from 7-30 mgs, and their NMR spectra were determined.

To obtain satisfactory NMR spectra, the six Limnanthes flavonoids were crystalized from 80% MeOH after isolation by TLC. Trimethylsilyl (TMS)

ethers of the six compounds were prepared using "Trisil Kits" (Pierce Chemical Company). NMR spectra of the TMS ethers dissolved in CCl_4 were determined on a Varian HA-100 instrument (100 MHz) using tetramethylsilane as internal standard. TMS ethers were hydrolyzed to yield the original flavonoids by addition of aqueous MeOH to the CCl_4 solutions. The resulting solutions of liberated flavonoid compounds were then concentrated in vacuo.

iii. Hydrolyses

The hydrolysis of flavonoid glycosides was accomplished by several different procedures. All hydrolyses were performed to determine either the number and type of sugar moieties attached to a flavonoid nucleus, or, when a compound was glycosylated with more than one sugar, the position and order of attachment of the sugars. Total hydrolyses were accomplished with 1.0 N HCl in water for 30 minutes at 95-100°. Liberated sugars were isolated, neutralized and identified by TLC following a procedure similar to that described by Mabry et al. (1970). Relative amounts of the identified sugars were also estimated using this technique. The liberated aglycones were identified by co-chromatography with standards.

When a glycoside contains more than one sugar, partial hydrolysis may assist in its identification by comparing the resulting derivatives with standard compounds. By this process both the order and position of sugar attachment may sometimes be determined. Limnanthes compounds were partially hydrolyzed by two methods, depending on the mode of glycosylation: hydrolysis for short intervals at 95-100° with either 0.1 N HCl or 20% (v/v) acetic acid.

Enzymic hydrolysis with emulsin proved a useful technique for the characterization of many of the Limnanthes compounds. These hydrolyses were accomplished by mixing a small amount of enzymic mixture (Nutritional Biochemical Corp.) into 1.0 N acetate buffer (pH 5.1) containing dissolved

compound and allowing the suspension to stand overnight at room temperature (Mabry et al., 1970). In this manner glucose was selectively removed by the β -glucosidase contained in the emulsin, when glucose was the only substituent at a given site. (Glucose is also removed if it is the terminal substituent of a glycoside moiety; however, enzymic hydrolyses were not performed on compounds with this mode of glycosylation in the present investigation.)

iv. Acetate Preparation

Two previously unreported compounds, each based on a rare aglycone type, were isolated in comparatively large quantities from L. douglasii var. douglasii. To characterize these compounds more completely, their acetate derivatives were prepared, and their melting points determined. Prior to derivatization of these two compounds, an additional acetate derivative of a third Limnanthes compound possessing the same glycosylation pattern was prepared to test the planned procedure and to compare the melting point of this test derivative with the published value.

The procedure used to prepare acetate derivatives was as follows: 1 ml of acetic anhydride was added to 5 mg of compound in a 25 ml round bottom flask; a few drops of triethylamine were added, and the solution was left overnight at room temperature; this solution was then concentrated, taken up in chloroform and the derivative precipitated by the addition of EtOH. The precipitated derivative was removed from solution by filtration, dried and crystallized from a mixture of benzene and cyclohexane. By following this process, the amount of crystalline derivative recovered was 70-80% in each case. These acetate derivatives were not substituted at all free hydroxyl groups of the flavonoid nucleus since the 5-hydroxyl group remains unacetylated due to hydrogen bonding.

D. Identification of the Remaining Limnanthes Flavonoids

When compounds were encountered which did not occur in L. douglasii, they were isolated and identified by the processes already described, if they were present in sufficient concentration. Compounds consistently occurring in trace amounts remain unidentified. However, the presence of these compounds was still recorded, and they were included with the known compounds for analysis.

The flavonoid complement of each taxon, as it was determined for comparative purposes, was taken to be all the flavonoids that could be resolved by two-dimensional TLC of whole plant and petal extracts. The TLC system used was polyamide DC 6.6, 1st dimension -- H_2O -n-BuOH-acetone-HOAc - (16:2:1:1), 2nd dimension -- $CHCl_3$ -isopropanol-butanone-HOAc - (10:3:3:4). Some plates were run twice in the second dimension. After drying thoroughly, the two-dimensional maps were sprayed with a boronate reagent. This reagent is a modification of that described by Somaroo et al. (1973) and greatly increases the visibility of flavonoids on polyamide under UV light. It was prepared by dissolving a small amount of diphenyl boric acid ethanolamine complex (Aldrich) in MeOH, and then diluting with water to 0.1% (w/v). The compounds resolved by this process were identified whenever possible by comparison to standard compounds using the technique of co-chromatography, if necessary.

EXPRESSION OF FLAVONOIDS AS TAXONOMIC CHARACTERS

Flavonoids are often used as characters in taxonomic studies by simply comparing presence or absence in the taxa under study. In practice, however, the relative concentrations of occurrence are often also considered. In the present investigation, taxa were compared on the basis of 46 flavonoid characters. Two different levels of compound concentration on comparative

two-dimensional chromatograms were recognized and recorded: 1) present in relatively high concentration and visible before spraying with boronate reagent, (several orders of magnitude of concentration are represented in this first classification) and 2) present in trace amount and visible only after spraying. By this process each OTU was described on the basis of flavonoids present in whole plant material and also on the basis of petal material when it was available.

One advantage of using a technique which takes into account concentrations of flavonoid occurrences, is that a better approximation can be made of the results as they actually occur on a chromatogram. Since the relative concentrations of flavonoids seem to be consistent for a given taxon, a procedure which considers concentration data probably conserves more taxonomically significant information than one that does not.

TREATMENT OF THE FLAVONOID DATA

A. Introduction

Small groups of taxa can be compared on the basis of their flavonoids by constructing a matrix of OTUs versus flavonoid characters. The order of OTUs and/or characters can be visually arranged so that taxa with the greatest character similarities cluster together in the matrix. As the number of OTUs and/or characters increases, the technique of manually rearranging OTUs into clusters becomes more difficult and subjective. This is because the eye can only consider so much information at once, and when confronted with excessive data, tends to concentrate on a small portion of the data. This process leads to results prejudiced in favor of the portion of the data considered. For this reason, phenetic comparisons of large numbers of OTUs on the basis of many characters are best made using the techniques of numerical taxonomy. Theoretically, the use of these techniques eliminates the above prejudice.

A most important tool of the numerical taxonomist is cluster analysis, a technique recently made practicable by the development of the computer. There are many variations of computerized cluster analysis, but essentially all of these techniques are designed to consider character similarities between all taxa simultaneously and produce groupings or clusters of taxa based on relative similarities which provide the best "fit" to the data.

It was desirable to use numerical taxonomic techniques for analysis of the flavonoid data of the Limnanthaceae for two reasons: 1) a data matrix containing 30 OTUs described by 46 characters exceeds the size for which an accurate assessment of similarity can be made by eye; and 2) cluster analysis based on flavonoid characters allows a direct comparison to the earlier numerical taxonomic analysis of the Limnanthaceae by Ornduff and Crovello based on morphological data (1968).

B. Method

To cluster taxa by conventional methods, a matrix of similarity coefficients must be calculated between all taxa. Since there are many different ways to calculate similarity coefficients, the results of cluster analysis may vary with the type of coefficient used. Whether or not two taxa are made more similar due to the mutual absence of a given flavonoid character (negative matches are considered), is a question that has not been satisfactorily answered. To help answer this question, cluster analyses of Limnanthes and Floerkea were performed using two types of similarity coefficients: 1) the simple matching coefficient (Sokal & Sneath, 1963, p. 133) which considers negative matches; and 2) the coefficient of Jaccard (Sokal & Sneath, 1963, p. 133) which calculates similarity only on the basis of mutual occurrence.

Flavonoid data were compiled into two Basic Data Matrices listing, in one case, all OTUs (30) as rows versus the flavonoid characters (46) found

found in whole plant material, and in the other case the OTUs grown fresh (18) versus the flavonoid characters (46) found in the fresh petals. From these two matrices the simple matching and Jaccard similarity coefficients were calculated by computer between all taxa. These coefficients were arranged in half matrices of OTUs, either 30 x 30 (whole plant data) or 18 x 18 (petal data). In each instance duplicate matrices were calculated handling the data in two ways: 1) similarities were determined considering only those compounds occurring in relatively high concentrations; 2) similarities were determined on the basis of any occurrence regardless of concentration. By this process a total of eight matrices of similarity coefficients were computed. From this data, eight different cluster analyses were computed using the weighted pair group clustering method, and the results were expressed in eight dendrograms (phenograms).

In their taximetric study of the Limnanthaceae, Ornduff and Crovello (1968) used two techniques to cluster OTUs. The first was a conventional method, the weighted pair group method, that produced a dendrogram expressing overall cophenetic similarities between OTUs. The second method was a form of factor analysis which produced a three-dimensional plot of OTUs in "reduced character space". Distances between OTUs plotted by the second technique are proportional to major sources of variation between taxa. Although the computer program used by Ornduff and Crovello was not available for the present investigation, two other standard types of factor analysis were performed on flavonoid data.

The two techniques, Principal Components Analysis and Varimax Factor Analysis (Both programs are from the Scientific Subroutine Package of IBM.), will be briefly described here since the method differed from standard clustering programs and may not necessarily express overall cophenetic similarities. Instead these techniques are based on key components of the total variation

present between taxa.

The first step in performing any factor analysis is to set up a character-by-character correlation coefficient matrix. Then, overall clustering of characters (not OTUs as by conventional clustering programs) is computed. From the n dimensions of hyperspace, corresponding to the number of characters considered, the relative amount of variability expressed by each dimension is factored. The three (in this case) factors or vectors which together account for the greatest amount of variation between taxa are selected. These factors are not correlated. The "factor loadings" for each character are calculated, i.e., the relative contribution that each character makes to the variation expressed by each of the three selected vectors. Then, the character complement of each OTU is multiplied by the respective factor loadings, producing three coordinates for each OTU. These coordinates are used to plot OTUs in three dimensional space using a standard type of plotting program which pictorializes the three-dimensional plot in two dimensions.

The type of analysis described above is factor analysis by principal components. The other technique used in this study was Varimax Factor Analysis with rotation. The preliminary steps are the same for this technique. After the factors are extracted, they are transformed by rotation to different coordinates which theoretically show interrelationships between OTUs in their simplest form. From these adjusted factors, new factor loadings are calculated, and the OTUs are plotted in three dimensions as above.

Since preliminary results indicated that the cluster analyses were more taxonomically meaningful if only the compounds occurring in relatively heavy concentration were used as characters, the two types of factor analysis were performed only on the basis of flavonoids visible before spraying the chromatograms. Eighteen OTUs were analyzed on the basis of 36 variable

flavonoids occurring in the petals (the remaining 10 flavonoids did not vary under these conditions), while 30 OTUs were analyzed on the basis of 31 variable flavonoids present in whole plant material.

RESULTS

FLORAL PATTERNING IN ULTRA VIOLET LIGHT

Figure II is a composite plate made up of selected photograph pairs of Limnanthes flowers taken in visible and UV light. The taxa represented are the varieties of L. douglasii, OTUs 2,3,4 and 5, L. macounii, OTU 10, and L. gracilis var. gracilis, OTU 14. Of these six taxa, the first five exhibit some form of UV patterning while the last (Figure IIE) is included in the plate to illustrate the pattern of uniform absorption observed for the remaining twelve unpictured taxa.

The photograph pair of L. douglasii var. rosea (Figure IIB) shows the most striking result revealed by the flower photography. Variety rosea has white petals with rose coloured veins and anthers. Under UV light the outer portions of the petals of this variety reflect the light in sharp contrast to the strongly absorbing inner petal zones, thus producing a bullseye effect. This nectar guide visible only under UV light is analogous to the familiar yellow and white pattern of variety douglasii (Figure IIC).

The photographs of L. douglasii var. nivea (Figure IID) show some similarities to those of variety rosea. Variety nivea, which also has white flowers under visible light, also shows the bullseye effect under UV light, but with less contrast than variety rosea. Also contributing to the UV patterning effect of variety nivea are the light coloured regions at the petal bases and the light coloured anthers, both contrasting with the dark flower center. The pale appearance of the petal bases is due in part to the reflectance of the basal hairs. A similar reflectance is also evident for variety rosea, and somewhat evident for variety sulphurea (Figure IIA). Reflecting

FIGURE II -- The Flowers of Six Selected Limnanthes taxa Taken in Visible and Ultra Violet Light.

The upper pictures of the photograph pairs are taken in visible light. The lower pictures are taken through the 18A filter.

A) L. douglasii var. sulphurea, OTU 5; B) L. douglasii var. rosea, OTU 4; C) L. douglasii var. douglasii, OTU 2; D) L. douglasii var. nivea, OTU 3; E) L. gracilis var. gracilis, OTU 14; and F) L. macounii, OTU 10.

hairs at the petal bases were occasionally observed for other unpictured taxa as well, including L. alba var. versicolor.

Both L. douglasii vars. sulphurea (Figure IIA) and douglasii (Figure IIC) have some yellow flower pigmentation in visible light. When photographed under UV light, neither of these varieties has sharply contrasting zones, but each exhibits a certain amount of patterning. Both have petals divided into regions of differing degrees of UV absorption, with the darker zones toward the flower centers. The variety sulphurea also exhibits certain features in addition to the plain pattern of variety douglasii; the petal veins of variety sulphurea appear under UV light as contrasting radial lines, absorbing less UV light than the inner zone but more than the outer, lighter petal parts.

The appearance under UV light of the small plain flowers of L. macounii (Figure IIF) was surprising. Its petal bases reflect to some extent in contrast to the outer petal regions, the petal veins, and the heavily absorbing sepals. Although there are no hairs at the petal bases and no lighter coloured zones at the petal tips of L. macounii, the central zone of UV reflectance and the contrasting veins show similarities to the patterns of L. douglasii vars. nivea and rosea.

Flowers appearing white in the visible spectrum, but which absorb UV light, are common and include those of most taxa of Limnanthes. The net effect to a bee looking at this type of flower is a colour that contrasts with the background vegetation. Presumably, this condition has evolved in many lines of plants resulting from selection pressures to increase the probability of a visit by a pollinator and thus enhance cross fertilization. The elaboration of nectar guides, which function to draw the insect to the center of a flower, is a further adaptation to facilitate cross pollination brought about by the same selection pressures.

The presence of UV-visible nectar guides in the varieties of L. douglasii suggests that these taxa are more highly evolved outcrossers than are other Limnanthes taxa. Of these varieties, rosea, with its sharply contrasting zones, is the most highly evolved of the four. Extrapolating backwards, it is probable that the outcrossing Limnanthes taxa with plain unpatterned flowers most closely resemble the ancestral form of this group, and probably the genus as well.

The observed differences in floral patterning of the four varieties of L. douglasii suggest an important implication regarding distance of relationship in this species. Various sympatric taxa of Limnanthes have evolved genetic barriers to hybridization which were used by Mason (1952) to distinguish species. Since Mason found the four varieties of L. douglasii experimentally interfertile, he grouped them together as varieties of one polymorphic species in spite of the fact that earlier authors had described some of them as distinct species (Bentham, 1848; Loudon, 1855; Greene, 1891; Abrams, 1941). Although Mason stated that these four varieties readily produce fertile natural hybrids, the only substantial evidence he cited in support of this statement ~~was the~~ intermingling of characters between two of the varieties, rosea and nivea, in an area of sympatry. However, the mixing of characters between these two varieties appears to be the extent of intermingling within the species. The other varieties persist as discrete taxonomic entities, even in zones of sympatry. It is possible that the general lack of intermingling among the four varieties is attributable to pollinator specificity resulting from their differently appearing flowers. This specificity may create an ecological barrier to genetic exchange, causing the isolation of these taxa, even though no barriers to hybridization with a genetic basis have evolved. Therefore, the absence of a genetic barrier does not necessarily indicate that all of these four taxa are varieties of one species.

Instead, some of them may be as distantly related as other isolated, recognized species of Limnanthes. The presence of clearcut morphological differences between them tends to support this possibility.

The presence of UV-visible flower patterns in only some taxa of Limnanthes has other implications concerning evolution in this genus. The common occurrence of such patterning in L. macounii and the four varieties of L. douglasii suggests that these five taxa have evolved from a common ancestor with similar flower patterning. If this is true, these taxa constitute a natural supraspecific group distinguished from the rest of the genus by this trait.

Ornduff (1969^b; Ornduff & Crovello, 1968) has indicated that autogamy is a derived condition in Limnanthes. The presence of a UV-visible floral pattern in L. macounii with no obvious selective advantage to this self-pollinating species supports this conclusion. This pattern is probably a remnant of an adaptation valuable only to an outcrossing plant.

There have been several changes of flower morphology accompanying autogamy in L. macounii, such as reduction of size and number of parts. However, the presence of UV-visible nectar guides in this taxon suggests that not all traits associated with the outcrossing habit have been lost. The retention of such traits ~~may~~ be explained by the prior establishment of linkage groups caused by selection pressures to preserve outcrossing.

Like L. macounii, some of the subspecies of L. floccosa are totally autogamous (Kalin, 1973a, 1973b). However, trends to autogamy have occurred independently in the two species, since each belongs to a different section of the genus. The flowers of L. floccosa, a member of the section Inflexae, exhibit UV absorption characteristics identical to outcrossing members of the section; i.e., flowers with uniformly high UV absorption as shown by the photograph of L. gracilis (Figure IIE). Although nectar guides visible in UV

light have not evolved in this section, the observed flower characters still undoubtedly represent an adaptation to the outcrossing habit. Therefore, a conservation of flower pigmentation has operated in L. floccosa parallel to that described above for L. macounii.

Relatively high concentrations of flavonoids with absorption maxima in the near UV were extracted from petals of all Limnanthes taxa. This presence suggests that the absorption recorded by UV photography results directly from these compounds. If correct, this contention agrees with the conclusions of other recent investigations designed to determine the chemical nature of UV-visible nectar guides (Thompson et al., 1972; Dement & Raven, 1974). Assuming that the flower flavonoids do cause the observed absorption exhibited by flowers of Limnanthes taxa, then these flavonoids are adaptations which confer a selective advantage to outcrossing plants. Since the UV absorption characteristics of autogamous members of Limnanthes do not differ significantly from the outcrossing members, an evolutionary conservation has been working on the flower flavonoids of all tested members of this genus.

The argument that the flower flavonoids of Limnanthes are adaptations for cross fertilization, and that these compounds will tend to be conserved by evolution, has profound implications regarding the contribution flavonoid distribution may make to the taxonomy of this group of plants. These implications will be discussed fully in the following chapter.

FLAVONOID CHARACTERIZATION AND IDENTIFICATION

A. Data Presentation

Flavonoid identifications depend on the synthesis of many pieces of information gathered from a variety of techniques. In the first part of this section the data gathered for Limnanthes and Floerkea flavonoids will be tabulated. In the second part of the section, an explanation will be presented

of how the compounds were identified.

A total of 48 flavonoid glycosides were found in taxa of Limnanthes and Floerkea. Table III lists notations assigned to these flavonoids, their identifications, and the abbreviations used in following tables. Where an identification was not possible, the most reasonable guess is provided.

Flavonoid aglycones were present occasionally in some taxa, including those analyzed from pressed material. However, the appearance of aglycones was erratic, and when present they occurred only in low concentration. These observations suggest that the appearance of aglycones was caused by a low level of hydrolytic activity, either in extracts, or in pressed material. For this reason, it was assumed that flavonoid aglycones do not naturally occur in the Limnanthaceae.

Figure III is a composite drawing showing the approximate relative Rfs (20 x 20 cm TLC glass plates of Polyamide DC 6.6; 1st dimension -- H_2O -n-BuOH-acetone-HOAc - 16:2:1:1, 2nd dimension -- $CHCl_3$ -isopropanol-butanone-HOAc - 10:3:3:4) of all flavonoid glycosides occurring in the Limnanthaceae. Although this drawing is a full sized reproduction of a 20 x 20 cm two-dimensional map, it is not meant to represent the results of any actual chromatogram. Each of the flavonoids in the drawing is labeled with the number or letter randomly assigned during the course of this work.

Table IV lists Rfs of the 48 flavonoids as they appear in Figure III. These Rfs are approximations only, since flavonoid Rfs on polyamide are variable, being controlled by many factors; however, the relative positions of these compounds are consistent. Table IV also lists colour characteristics of most flavonoids as they appear on polyamide chromatograms under UV light (366 m μ), with and without NH_3 vapour, and also after spraying with boronate reagent. Colour data are not listed for compounds that occurred only in trace amounts, since no judgement could be made beyond presence or absence.

TABLE III

Identifications of the Flavonoids of the Limnanthaceae

Symbol	Compound	Abbreviation
H	Unknown kaempferol monoside; perhaps acylated	
D	Probably the 7-0-glucoside of kaempferol	
E	Probably the 7-0-glucoside of quercetin	
O	Syringetin-3-0-glucoside	Sg-3-0-G
P	Isorhamnetin-3-0-glucoside	Ir-3-0-G
Q	Kaempferol-3-0-glucoside	Kp-3-0-G
R	Laricytrin-3-0-glucoside	Lc-3-0-G
S	Quercetin-3-0-glucoside	Qu-3-0-G
T	Myricetin-3-0-glucoside	My-3-0-G
C	Unknown 3-0-monoside of quercetin	
A	Probably the 7-0-rutinoside of kaempferol	
B	Probably the 7-0-rutinoside of quercetin	
19	Unknown 3-0-monoside or bioside of syringetin	
20	Unknown 3-0-monoside or bioside of isorhamnetin	
21	Unknown 3-0-monoside or bioside of kaempferol	
22	Unknown 3-0-monoside or bioside of laricytrin	
23	Unknown 3-0-monoside or bioside of quercetin	
U	Syringetin-3-0-rutinoside	Sg-3-0-Rut
V	Isorhamnetin-3-0-rutinoside	Ir-3-0-Rut
W	Kaempferol-3-0-rutinoside	Kp-3-0-Rut
X	Laricytrin-3-0-rutinoside	Lc-3-0-Rut
Y	Quercetin-3-0-rutinoside	Qu-3-0-Rut
Z	Myricetin-3-0-rutinoside	My-3-0-Rut
I	Syringetin-3-0-diglucoside	Sg-3-0-GG

TABLE III -- Continued

Symbol	Compound	Abbreviation
J	Isorhamnetin-3-0-diglucoside	Ir-3-0-GG
K	Kaempferol-3-0-diglucoside	Kp-3-0-GG
L	Laricytrin-3-0-diglucoside	Lc-3-0-GG
M	Quercetin-3-0-diglucoside	Qu-3-0-GG
N	Myricetin-3-0-diglucoside	My-3-0-GG
F	A bioside of kaempferol; probably kaempferol-3-0-glucoside,7-0-glucoside	
G	A bioside of quercetin; probably quercetin 3-0-glucoside,7-0-glucoside	
1	Syringetin-3-0-rhamnosylrutinoside	Sg-3-0-GRR
2	Isorhamnetin-3-0-rhamnosylrutinoside	Ir-3-0-GRR
3	Kaempferol-3-0-rhamnosylrutinoside	Kp-3-0-GRR
4	Laricytrin-3-0-rhamnosylrutinoside	Lc-3-0-GRR
5	Quercetin-3-0-rhamnosylrutinoside	Qu-3-0-GRR
6	Myricetin-3-0-rhamnosylrutinoside	My-3-0-GRR
7	Syringetin-3-0-rutinoside,7-0-glucoside	Sg-3-0-Rut,7-0-G
8	Isorhamnetin-3-0-rutinoside,7-0-glucoside	Ir-3-0-Rut,7-0-G
9	Kaempferol-3-0-rutinoside,7-0-glucoside	Kp-3-0-Rut,7-0-G
10	Laricytrin-3-0-rutinoside,7-0-glucoside	Lc-3-0-Rut,7-0-G
11	Quercetin-3-0-rutinoside,7-0-glucoside	Qu-3-0-Rut,7-0-G
12	Myricetin-3-0-rutinoside,7-0-glucoside	My-3-0-Rut,7-0-G
13	Syringetin-3-0-rutinoside,7-0-rutinoside	Sg-3-0-Rut,7-0-Rut
14	Isorhamnetin-3-0-rutinoside,7-0-rutinoside	Ir-3-0-Rut,7-0-Rut
15	Kaempferol-3-0-rutinoside,7-0-rutinoside	Kp-3-0-Rut,7-0-Rut
16	Laricytrin-3-0-rutinoside,7-0-rutinoside	Lc-3-0-Rut,7-0-Rut
17	Quercetin-3-0-rutinoside,7-0-rutinoside	Qu-3-0-Rut,7-0-Rut

FIGURE III

Composite Two-Dimensional Map¹ of 48
Flavonoid Glycosides of the Limnanthaceae²

1 -- 1st dimension (horizontal) -- H_2O -n-BuOH-acetone-HOAc (16:2:1:1)
2nd dimension (vertical) -- $CHCl_3$ -butanone-isopropanol-HOAc (10:3:3:4)

2 -- Solid circles denote identified compounds; broken circles denote compounds present in quantities insufficient for complete identification.

TABLE IV

Rfs¹ and Colours² of Flavonoids of the Limnanthaceae

Compound	Rf-1	Rf-2	UV	UV/NH ₃	UV/Spray ³
H	0.08	0.10	P	P	G
D	0.14	0.15	○	-	ld
E	0.15	0.07	-	-	ld
O Sg-3-0-G	0.34	0.54	P	PY	YG
P Ir-3-0-G	0.24	0.43	P	G	G
Q Kp-3-0-G	0.20	0.31	P	P	G
R Lc-3-0-G	0.24	0.18	Br	Y	Ro
S Qu-3-0-G	0.18	0.14	Br	Y	Or
T My-3-0-G	0.18	0.06	Br	Y	Ro
C	0.22	0.11	-	-	ld
A	0.31	0.18	Y	Y	G
B	0.30	0.06	Y	Y	Or
19	0.46	0.54	-	-	ld
20	0.41	0.44	-	-	ld
21	0.36	0.34	-	-	ld
22	0.41	0.23	-	-	ld
23	0.38	0.18	-	-	ld
U Sg-3-0-Rut	0.54	0.54	P	PY	YG
V Ir-3-0-Rut	0.50	0.44	P	G	G
W Kp-3-0-Rut	0.43	0.34	P	P	G
X Lc-3-0-Rut	0.50	0.24	Br	Y	Ro
Y Qu-3-0-Rut	0.46	0.18	Br	Y	Or
Z My-3-0-Rut	0.46	0.08	Br	Y	Ro
I Sg-3-0-GG	0.55	0.38	P	PY	YG
J Ir-3-0-GG	0.55	0.31	P	G	G
K Kp-3-0-GG	0.50	0.28	P	P	G
L Lc-3-0-GG	0.55	0.19	-	-	ld
M Qu-3-0-GG	0.52	0.14	Br	Y	Or
N My-3-0-GG	0.51	0.06	-	-	ld
F	0.58	0.23	-	-	ld
G	0.58	0.10	-	-	ld
1 Sg-3-0-GRR	0.69	0.40	P	PY	YG
2 Ir-3-0-GRR	0.70	0.31	P	G	G

TABLE IV -- Continued

Compound	Rf-1	Rf-2	UV	UV/NH ₃	UV/Spray
3 Kp-3-0-GRR	0.62	0.23	P	P	G
4 Lc-3-0-GRR	0.68	0.18	P	Y	Ro
5 Qu-3-0-GRR	0.63	0.13	P	Y	Or
6 My-3-0-GRR	0.65	0.08	P	Y	Ro
7 Sg-3-0-Rut, 7-0-G	0.83	0.38	P	PY	YG
8 Ir-3-0-Rut, 7-0-G	0.80	0.32	P	Y	G
9 Kp-3-0-Rut, 7-0-G	0.77	0.24	P	Y	G
10 Lc-3-0-Rut, 7-0-G	0.82	0.14	Br	Y	Rd
11 Qu-3-0-Rut, 7-0-G	0.77	0.11	Br	Y	Or
12 My-3-0-Rut, 7-0-G	0.78	0.03	Br	Y	Rd
13 Sg-3-0-Rut, 7-0-Rut	0.90	0.38	P	PY	YG
14 Ir-3-0-Rut, 7-0-Rut	0.87	0.31	P	Y	G
15 Kp-3-0-Rut, 7-0-Rut	0.84	0.24	P	Y	G
16 Lc-3-0-Rut, 7-0-Rut	0.88	0.15	Br	Y	Rd
17 Qu-3-0-Rut, 7-0-Rut	0.86	0.10	Br	Y	Or

1 -- Rf-1 = 1 development, Polyamide DC6.6, H₂O-n-BuOH-acetone-HOAc ((16:2:1:1)
Rf-2 = 1 development, Polyamide DC6.6, CHCl₃-butanone-isopropanol-HOAc
(10:3:3:4)

2 -- Colour explanation -- P = purple; Br = brown; Y = yellow; PY = pale
yellow; G = green; Or = orange; Ro = rose; Rd = red; YG = yellow
green; ld = limit of detection

3 -- Spray = 0.1% (w/v) diphenyl boric acid ethanolamine complex in H₂O & MeOH

Table V lists the products obtained upon total hydrolysis with HCl, including flavonoid aglycone, sugar(s), and relative concentration of each sugar where appropriate. Table VI lists the derivatives of partial acid hydrolysis and the derivatives of enzymic hydrolysis with emulsin (β -glucosidase) where these procedures were employed.

Table VII lists UV absorption maxima of the flavonoid glycosides that were isolated in sufficient quantities for determination. Table VII lists maxima in MeOH and also in MeOH following the addition of various shift reagents.

Table VIII is a tabulation of the results of NMR spectroscopy of six flavonoids of Limnanthes. It presents the proton shifts of TMS-ethers of six β -D-rutinosides relative to tetramethylsilane. Reproductions of the NMR spectra of these six derivatives are presented as Appendices LI-LVI.

B. Explanation of the Data

The flavonoids of the Limnanthaceae follow a definite pattern. A series of six flavonol aglycone types repeats many times, each time comprising a class of compounds possessing uniform glycosylation. In total 12 glycosylation patterns were found in the Limnanthaceae. All of these isolated in quantities sufficient for hydrolysis proved to be based on two sugars, glucose and rhamnose.

Using aqueous solvents, flavonoids were resolved into groups, each with uniform glycosylation. The acidic chloroform solvent resolved these groups into six components, each based on a particular aglycone type. Thus, the array of compounds diagrammed in Figure III is divided along the horizontal axis into glycosylation classes (e.g., U-Z, 1-6, ... etc.) that were separated by development in the aqueous solvent. These groups in turn are separated along the vertical axis into individual compounds based on each

TABLE V

Products Obtained upon Total Hydrolysis with 1.0 N HCl

Compound	Aglycone	Sugar(s)
O Sg-3-0-G	Syringetin	Glucose
P Ir-3-0-G	Isorhamnetin	"
Q Kp-3-0-G	Kaempferol	"
R Lc-3-0-G	Laricytrin	"
S Qu-3-0-G	Quercetin	"
T My-3-0-G	Myricetin	"
U Sg-3-0-Rut	Syringetin	Glucose, Rhamnose
V Ir-3-0-Rut	Isorhamnetin	"
W Kp-3-0-Rut	Kaempferol	"
X Lc-3-0-Rut	Laricytrin	"
Y Qu-3-0-Rut	Quercetin	"
Z My-3-0-Rut	Myricetin	"
I Sg-3-0-GG	Syringetin	Glucose
K Kp-3-0-GG	Kaempferol	"
M Qu-3-0-GG	Quercetin	"
1 Sg-3-0-GRR	Syringetin	1 Glucose, 2 Rhamnose
2 Ir-3-0-GRR	Isorhamnetin	"
3 Kp-3-0-GRR	Kaempferol	"
4 Lc-3-0-GRR	Laricytrin	"
5 Qu-3-0-GRR	Quercetin	"
6 My-3-0-GRR	Myricetin	"
7 Sg-3-0-Rut,7-0-G	Syringetin	2 Glucose, 1 Rhamnose
8 Ir-3-0-Rut,7-0-G	Isorhamnetin	"
9 Kp-3-0-Rut,7-0-G	Kaempferol	"
10 Lc-3-0-Rut,7-0-G	Laricytrin	"
11 Qu-3-0-Rut,7-0-G	Quercetin	"
12 My-3-0-Rut,7-0-G	Myricetin	"
13 Sg-3-0-Rut,7-0-Rut	Syringetin	Glucose, Rhamnose
14 Ir-3-0-Rut,7-0-Rut	Isorhamnetin	"
15 Kp-3-0-Rut,7-0-Rut	Kaempferol	"
16 Lc-3-0-Rut,7-0-Rut	Laricytrin	"
17 Qu-3-0-Rut,7-0-Rut	Quercetin	"

TABLE VI

Derivatives Obtained upon
Partial Acid Hydrolysis and Hydrolysis with Emulsin

Compound	Hydrolysis	Derivatives
1 Sg-3-0-GRR	20% HOAc	Sg, Sg-3-0-G, Sg-3-0-Rut
2 Ir-3-0-GRR	"	Ir, Ir-3-0-G, Ir-3-0-Rut
3 Kp-3-0-GRR	"	Kp, Kp-3-0-G, Kp-3-0-Rut
4 Lc-3-0-GRR	"	Lc, Lc-3-0-G, Lc-3-0-Rut
5 Qu-3-0-GRR	"	Qu, Qu-3-0-G, Qu-3-0-Rut
6 My-3-0-GRR	"	My, My-3-0-G, My-3-0-Rut
7 Sg-3-0-Rut, 7-0-G	0.1 N HCl	Sg, Sg-7-0-G, Glucose, Rhamnose
8 Ir-3-0-Rut, 7-0-G	"	Ir, Ir-7-0-G, Glucose, Rhamnose
9 Kp-3-0-Rut, 7-0-G	"	Kp, Kp-7-0-G, Glucose, Rhamnose
10 Lc-3-0-Rut, 7-0-G	"	Lc, Lc-7-0-G, Glucose, Rhamnose
11 Qu-3-0-Rut, 7-0-G	"	Qu, Qu-7-0-G, Glucose, Rhamnose
12 My-3-0-Rut, 7-0-G	"	My, My-7-0-G, Glucose, Rhamnose
7 Sg-3-0-Rut, 7-0-G	Emulsin	Sg-3-0-Rut, Glucose
8 Ir-3-0-Rut, 7-0-G	"	Ir-3-0-Rut, Glucose
9 Kp-3-0-Rut, 7-0-G	"	Kp-3-0-Rut, Glucose
10 Lc-3-0-Rut, 7-0-G	"	Lc-3-0-Rut, Glucose
11 Qu-3-0-Rut, 7-0-G	"	Qu-3-0-Rut, Glucose
12 My-3-0-Rut, 7-0-G	"	My-3-0-Rut, Glucose
13 Sg-3-0-Rut, 7-0-Rut	0.1 N HCl	Sg, Sg-7-0-G, Sg-7-0-Rut
14 Ir-3-0-Rut, 7-0-Rut	"	Ir, Ir-7-0-G, Ir-7-0-Rut
15 Kp-3-0-Rut, 7-0-Rut	"	Kp, Kp-7-0-G, Kp-7-0-Rut
16 Lc-3-0-Rut, 7-0-Rut	"	Lc, Lc-7-0-G, Lc-7-0-Rut
17 Qu-3-0-Rut, 7-0-Rut	"	Qu, Qu-7-0-G, Qu-7-0-Rut
13 Sg-3-0-Rut, 7-0-Rut	Emulsin	No Alteration
14 Ir-3-0-Rut, 7-0-Rut	"	"
15 Kp-3-0-Rut, 7-0-Rut	"	"
16 Lc-3-0-Rut, 7-0-Rut	"	"
17 Qu-3-0-Rut, 7-0-Rut	"	"

TABLE VII

UV Absorption Maxima of Flavonoids of the Limnanthaceae¹

COMPOUND	MeOH			MeOH & NaOMe			MeOH & AlCl ₃			MeOH & AlCl ₃ & HCl			MeOH & NaOAc			MeOH & NaOAc & H ₃ BO ₃		
	II	M	I	II	M	I	II	M	I	II	M	I	II	M	I	II	M	I
Lc-3-O-G	255,266s	303	361	268	327	419	272	301s	433	274	309	371,406	272	325	414	262	302s	383
Sg-3-O-G	252,262s	309s	359	266	329	427	273	310	370s,406	273	310	366,405	270	325	422	251,264	306s	364
My-3-O-Rut	261	306	362	266	322	409	273	318s	423	274	320s	374s,408	269	328	409	261	306s	383
Qu-3-O-Rut	258,268s	299	359	266	322	418	276	306s	435	271	299s	366,404	274	328	409	264	295s	382
Lc-3-O-Rut	255,266s	301	361	266,286s	329s	431	274	306s	439	273	302s	367,407	270	322	423	267	302s	384
Km-3-O-Rut	266		350	266	324	405	275	306s	354,397	275	306s	350,394	275	306s	339	268		355
Ir-3-O-Rut	255,268s	300	356	270	332	411	269	298s	373s,407	269	298	364,406	274	323	409	255,268s		361
Sg-3-O-Rut	254,268s	300	361	266	330	426	272	307	380s,410	274	307	369,407	265	323	427	266s	308	365
Qu-3-O-GG	257,268s	298	357	270	322s	409	277	302s	434	271	298s	365,406	271	324	395	262	302s	374
Km-3-O-GG	268		349	270	322	401	275	302s	352,400	275	302s	349,396	275	302s	375	269	302s	351
Sg-3-O-GG	254,265s	303	362	266,288s	358	429	272	310	381s,410	274	310s	370,403	266	322	430	264s	302	365
My-3-O-GRR	255,266s	297	357	266,285s	326s	412	274	310s	426	273	304s	370,407	270	318s	390	263s		366
Qu-3-O-GRR	257,267s	305s	356	270	319	408	274	306s	437	270	301s	368,405	271	326	383	262	310s	374
Lc-3-O-GRR	255,266s	300	359	266	324	417	274	302s	441	275	309s	367,408	270	320	412	260		375
Km-3-O-GRR	267	297s	349	274	324	395	275	305	355,403	276	303	348,399	274	313	383	267		352
Ir-3-O-GRR	255,268s	302s	355	266	326	409	270	301s	371s,409	269	301s	363,404	273	323	404	255,269s		358
Sg-3-O-GRR	254,268s	303	359	266	328	418	274	306s	368s,411	275	306s	371,407	272	316	388	268s		360
Lc-3-O-Rut,7-O-G	258,266	294	358	269		403	273	296s	362s,432	273	296s	368s,403	265	296s	403	261	292s	373

1) Expressed as m μ

TABLE VIII

Proton Chemical Shift Values¹ of TMS Ethers of
Six Flavonol β -D-Rutinosides of the Limnanthaceae

Compound	A-Ring		B-Ring				Carbohydrate			
	6	8	2'	6'	3'	5'	Glc-1-H	Rhm-1-H	Rhm-Me	Other
Kp-3-O-Rut	6.08 d,J2.5 ²	5.36 d,J2.5 ²	(7.68) 2H,d,J8.5		(6.78) 2H,d,J8.5		5.80 ₃ d,J7 ³	4.19 ₄ d,J1 ⁴	0.71	3.26-3.83
Qu-3-O-Rut	6.08	6.36	7.30 d,J2	7.34 q,J2,9.5	-	6.78 d,J9.5	5.75	4.20	0.81	3.29-3.80
Ir-3-O-Rut	6.08	6.38	7.45 d,J2	7.27 q,J2,9	3.84 ₅ 3H,s ⁵	6.77 d,J9	5.84	4.20	0.72	3.25-3.74
My-3-O-Rut	6.06	6.32	(7.08) 2H,s		-	-	5.81	4.22	1.00	3.29-3.85
Lc-3-O-Rut	6.09	6.37	7.20 d,J2	6.90 d,J2	-	3.85 ₅ 3H,s ⁵	5.89	4.23	0.81	3.28-3.76
Sg-3-O-Rut	6.10	6.41	(7.12) 2H,s		(3.84) ₅ 6H,s ⁵		5.93	4.22	0.73	3.25-3.75

1 -- Expressed as δ in ppm relative to tetramethylsilane (0.00) in CCl₄. 2 -- Consistent values for H-6 & H-8.

3 -- Consistent values for glucose-1-H. 4 -- Consistent values for rhamnose-1-H. 5 -- O-methyl groups

aglycone type (e.g., U,V,W, ... etc.) that were resolved after development in the organic solvent.

Only three of the many glycosylation classes occurring in the Limnathaceae are present in relatively great amounts. The most prominent group among these, one that is present in all taxa, consists of the six compounds designated U,V,W,X,Y and Z. The identification of these six flavonoids was crucial to the entire investigation as they were often obtained as hydrolysis products from other flavonoid glycosides, and from these the six aglycone types were identified.

Rfs in aqueous solvents of the compounds U-Z suggested that they were diglycosides. Upon hydrolysis each yielded a different aglycone, and the sugars, glucose and rhamnose (Table V). Four of the six aglycones were kaempferol, quercetin, isorhamnetin and myricetin. UV and NMR spectral data of the corresponding glycosides (Tables VII & VIII) agreed completely with published data for 3-O- β -D-rutinosides. The remaining two rutinosides were new. One (U) had Rf and UV behaviour similar to isorhamnetin 3-O-rutinoside (V) which suggested the presence of an O-methylated B-ring. The NMR spectrum of the TMS derivative, indeed showed the presence of two equivalent O-methyl groups, 3.84 δ , and a singlet at 7.12 δ which integrated for two protons. This singlet indicates equivalent protons on the B-ring. These data indicate that the B-ring is a syringyl-type. Signals at 6.10 δ and 6.14 δ showed the expected meta splitting of the 6-H and 8-H. The aglycone is, therefore, myricetin 3',5'-dimethyl ether, or syringetin.

The second new rutinoside (X) was similar to rutin (Y) in its Rf and UV behaviour (Figure III, Table VII). The NMR spectrum of the TMS derivative showed four aromatic protons, as in the case of syringetin rutinoside, but only one O-methyl group (Table VIII). The B-ring protons appeared as doublets at 7.20 δ and 6.90 δ and displayed meta coupling. These results allow the

assignment of the structure myricetin 3'-methyl ether to the aglycone, or laricytrin as it has recently been named by Tyukavkin et al. (1974).

The acetate derivatives were prepared of syringetin 3-O-rutinoside, laricytrin 3-O-rutinoside and quercetin 3-O-rutinoside, and their melting points determined. The melting points of the three acetate derivatives are: quercetin rutinoside -- 116-118°, laricytrin rutinoside -- 124-126°, and syringetin rutinoside -- 130-132°.

The second major group of glycosides investigated, termed 1,2,3,4,5 and 6, moved further in aqueous solvents than did the rutinosides (Figure III), suggesting a greater degree of glycosylation. Their UV spectra showed them to be 3-O-glycosides (Table VII). Total hydrolyses gave the same six aglycones already described and, in each case, two equivalents of rhamnose and one of glucose (Table V). Partial acid hydrolysis of these triglycosides gave, in each case, the corresponding 3-O-rutinoside and 3-O-glucoside (Table VI). The point of substitution of the terminal rhamnose has not been determined, but the six triglycosides are clearly 3-O-rhamnosyl rutinosides.

The third major group of glycosides, consisting of the six components 7,8,9,10,11 and 12, had higher R_fs in aqueous solvents than the two preceding groups (Figure III). All attempts to fractionate these glycosides into six components by preparative TLC, as was done for the rutinosides and rhamnosyl-rutinosides, were unsuccessful. However, one of these six compounds was isolated from the rest by crystallization from MeOH, and its UV spectrum corresponded to a 3,7-diglycoide (Compound 10, Table VII). Since separation could not be achieved, hydrolysis experiments were performed on all six glycosides simultaneously. Total hydrolysis gave the six aglycones present in the other groups, two equivalents of glucose and one equivalent of rhamnose (Table V). Partial acid hydrolysis yielded flavonol derivatives with R_fs and UV spectral characteristics typical of 7-O-monosides. Treatment with β -gluco-

sidase (emulsin) produced the six 3-O-rutinosides and glucose (Table VI). Therefore, these compounds must be the flavonol 3-O- β -D-rutinoside, 7-O- β -D-glucosides.

The 18 flavonol glycosides described above are ubiquitous in the Limnanthaceae, occurring in high concentrations in nearly every taxon investigated. The other groups of glycosides encountered in this work occur sporadically throughout the family, but in lesser concentrations than the three common groups.

The group of glycosides consisting of compounds I, J, K, L, M and N had Rfs in aqueous solvents indicating that they were biosides. Rfs in the organic solvent and colours after spraying indicated that these six glycosides were based on the six aglycones already encountered. Of the six, only I, K and M were isolated in quantities sufficient for spectral and hydrolytic analysis. These three had UV spectra corresponding to 3-O-glycosides (Table VII), and total acid hydrolysis gave the aglycones syringetin, kaempferol and quercetin, and the sugar glucose (Table V). This information allows the conclusion that the three are flavonol 3-O-diglucosides. Although the compounds J, L and M occur only in trace amounts, and were not isolated in quantity, they are almost certainly the 3-O-diglucoside analogues of isorhamnetin, laricytrin and myricetin, respectively.

The glycosylation series containing the six compounds O, P, Q, R, S and T had Rfs in aqueous solvents characteristic of flavonol monosides (Figure III). Their UV spectra were those of flavonol 3-O-glycosides (Table VII). Total acid hydrolysis yielded each of the six aglycones and only glucose (Table V). Therefore, these compounds are flavonol 3-O- β -D-glucosides.

A series of five compounds, 13, 14, 15, 16 and 17, had Rfs in aqueous solvents even higher than those of the 3,7-branched triosides (Figure III), suggesting a higher degree of glycosylation. Attempts to resolve this group

into its five components by preparative TLC were unsuccessful. Therefore, it was necessary to hydrolyze these compounds as a group. Total hydrolysis yielded the aglycones, syringetin, isorhamnetin, kaempferol, laricytrin and quercetin, and two sugars, glucose and rhamnose, both in apparently equal proportions (Table V). Attempts to hydrolyze these compounds with emulsin were unsuccessful, indicating that glucose does not occur in a terminal position on these compounds. Partial acid hydrolysis gave three series of five compounds each, one series consisting of the five aglycones, and the other two with Rfs and colours characteristic of 7-O-monosides and 7-O-biosides. Insufficient material and time prevented further experiments on these five glycosides, but all of the information gathered is consistent with the assignments of flavonol 3-O-rutinoside, 7-O-rutinosides.

All other flavonoid compounds present in the Limnanthaceae with the exceptions of H, A and B occurred in concentrations too low for isolation and identification. Compound H was found only in Floerkea, of which only small amounts of material were available for study. Its colour characteristics and low Rf in the organic solvent suggest that this is a kaempferol 3-O-monoside (Figure III & Table IV). Its extremely low mobility in the aqueous direction suggests that this is an acylated derivative. Compounds A and B have colour characteristics and RFs which suggest that they are 7-O-biosides of kaempferol and quercetin. Problems in obtaining sufficient amounts of these two compounds, which will be discussed in a later section, have not allowed complete identification, but they are probably flavonol 7-O-rutinosides.

Identification of the three remaining glycosylation series can only be speculated upon based on Rfs. Compounds D and E have Rfs that correspond to 7-O-monosides and are probably the 7-O-glucosides of kaempferol and quercetin. Compounds F and G also have Rfs in the organic solvent indicative of glycosylation at the 7-position of the flavonol nucleus. This fact, taken

together with their mobility in the aqueous direction, suggests that they are 3,7-dimonosides; if this is true, they are probably the 3-O-glucoside, 7-O-glucosides of kaempferol and quercetin. Compounds 19, 20, 21, 22 and 23 remain unknown. Although clearly derivatives of syringetin, isorhamnetin, kaempferol, laricytrin and quercetin, their R_fs in the aqueous solvent, midway between the 3-O-glucosides and 3-O-rutinosides (Figure III), makes the extent of glycosylation unclear. These compounds are either 3-O-monosides or biosides. 3-O-Dirhamnosides would probably have R_fs similar to these compounds, but further information is required before their identity can be established.

SIGNIFICANCE OF THE FLAVONOIDS OF THE LIMNANTHACEAE

A. Aglycones

Although anthocyanins occur in trace amounts in some taxa, flavonols were the major type of flavonoid encountered in the present investigation. Six flavonol aglycone types are made and accumulated by every member of the Limnanthaceae. These include the common flavonol types: kaempferol, quercetin, isorhamnetin and myricetin, the relatively rare syringetin, and the extremely rare laricytrin, or 3'-methyl myricetin. These aglycones differ only in their B-rings, and together they comprise a series of derivatives made by successive hydroxylation and methoxylation additions to this ring. This stepwise elaboration of the B-ring, from kaempferol to syringetin, is shown in Figure IV.

Syringetin has rarely been reported previously. Besides Limnanthes (Parker & Bohm, 1975) and Floerkea, this aglycone has been reported from Lathyrus (Harborne, 1965), Larix (Niemann, 1972; 1973; Tyukavkin et al., 1974), Soymida (Parkhasaradhi & Sidhu, 1972), and Philydrum (Bohm & Collins, 1975).

Laricytrin has previously been reported only from Larix. Its occurrence was suspected by Niemann (1972, 1973) and was verified by Tyukavkin and

FIGURE IV

Stepwise Elaboration of the B-Ring of Flavonols of the Limnanthaceae

coworkers (1974). However, it is probable that laricytrin has a much wider distribution than these data indicate, since this compound is not easily separated from quercetin. If laricytrin is not separated from quercetin, its presence will go undetected since these compounds possess very similar physical properties. TLC chromatography on polyamide DC 6.6 with organic solvents appears to be the only way presently known to separate these two aglycone types. When this technique is more widely adopted, several additional reports of laricytrin will probably be made. The recent discovery of laricytrin in Heuchera (C.K. Wilkins, personal communication) tends to support this observation.

B. Glycosides

A total of twelve glycosylation patterns was discovered in the course of this investigation. Although monosides do occur in various members of the Limnanthaceae, the majority of the flavonoids of Limnanthes and Floerkea are glycosylated with two or more sugars. All of the identified flavonoid glycosylation patterns are based on different combinations of glucose and rhamnose.

Relatively few triosides and tetrasides have been reported, with the exception of 3-O-rutinoside, 7-O-glucosides. To the best of my knowledge, there have been no previous reports of 3-O-rhamnosyl rutinosides, nor have there been any reports of 3-O-rutinoside, 7-O-rutinosides.

THE CONTRIBUTION TO VISIBLE FLOWER COLOUR BY SYRINGETIN DERIVATIVES

Harborne (1967) has reported that syringetin acts as a yellow pigment in the flowers of Lathyrus. Although certain Limnanthes taxa have flowers that contain bright yellow pigments, including two varieties of L. douglasii, syringetin apparently does not contribute to this phenomenon. This was

concluded since high concentrations of syringetin glycosides occur in the petals of most Limnanthes taxa, the majority of which have white flowers. Furthermore, the yellow pigment present in Limnanthes flowers is probably a carotenoid(s) since it is readily extracted with chloroform.

All the white flowered forms of Limnanthes have petals that are tinged with a pale yellow colour, particularly toward their bases. This effect is accentuated by drying specimens. It is quite probable that syringetin glycosides, as well as other flavonol glycosides, contribute to this yellowing.

All syringetin glycosides isolated during this investigation share an obvious property; they are all extremely sensitive to base. When fumed with NH_3 these compounds instantly fluoresce an intense pale yellow colour under UV light. In fact, occasionally syringetin derivatives were detected only by this technique, being invisible under UV following spraying but prior to fuming. After fuming, these compounds appear yellow in visible light and remain this way for a short period (normally they appear a very pale yellow when present in high concentration, and are invisible when present in lower concentration). This observation indicates that slightly basic conditions in a flower might easily cause syringetin glycosides to serve as yellow pigments in visible light.

ENVIRONMENTAL MODIFICATION OF FLAVONOID COMPOSITION

Taxonomic studies using flavonoids as characters routinely assume that flavonoid compositions do not vary with changes in the environment. (This statement is not meant to include the small amounts of flavonoid hydrolysis that occur as plants age.) However, the presence of two flavonoid compounds of Limnanthes, A and B (probable 7-O-rutinosides of kaempferol and quercetin) apparently depends upon environmental factors.

Germination of all Limnanthes seed drops to a low level as soil

temperatures increase from spring to summer. Since seed was germinated outside, this phenomenon caused an acute problem in some instances. On the first attempt, germination of OTUs 12 and 15, L. alba var. alba and L. gracilis var. gracilis, was 1-2%, producing only two plants of each taxon. The pots containing these plants were not transferred to the greenhouse following the standard practice, but were left in cold frames pending additional germination. No further germination occurred, and eventually the two plants of each OTU were harvested, and the flavonoid compositions of petals and whole plant material were determined. Both of these taxa contained A and B in reasonably high concentration. However, insufficient plant material made it impossible to identify these flavonoids.

An additional flat of L. alba var. alba was grown at a cooler time of year, and germination was much improved. However, at this later date the plants were transferred to the greenhouse following the usual practice. Neither the flowers nor whole plant material contained compounds A and B, although otherwise they were chemically identical to the plants grown in the cold frames. The presence of A and B was not detected in any other members of the Limnanthaceae including those taxa represented by dried material.

Presence of A and B in plant material grown in the cold frame is assumed to be environmentally induced rather than a genetic peculiarity of a few plants, since the phenomenon was observed for two different taxa. The nature of the inducing factor is not known; however, it seems unlikely that the compounds A and B are breakdown products since neither L. alba nor L. gracilis contains any 3,7-dirutinosides, presumably the only class of compounds encountered in any taxa of Limnanthes which could possibly break down to yield A or B. At any rate these two compounds have been eliminated from the comparative analysis, since their presence is variable and they are, therefore, unsuitable for taxonomic comparisons within the Limnanthaceae.

COMPARATIVE FLAVONOID DATA

Flavonoid complements of all taxa were determined from two-dimensional thin layer chromatograms. Figures V and VI are colour photographs of an actual two-dimensional map of the whole plant flavonoids of L. montana, OTU 17, taken after spraying and marking. Figure V shows the chromatogram under UV light. Exposure details are: 2 minutes at F 3.5, using a Wratten 2E filter (Kodak) and High Speed Ektachrome film. Figure VI shows the same chromatogram taken in daylight at 1/25 second at F 3.5. Two points are noteworthy about the picture made with UV light. 1) The compounds are correctly shown as fluorescent spots after spraying, but the colours are not accurately represented. Actual differences in colours between compounds are greater than they appear in this picture. 2) The photographic film is more sensitive than the human eye. Compounds which appeared at the limits of detection are clearly visible in this picture, and certain features of the photograph were invisible to the naked eye.

The pictures of the L. montana chromatogram exemplify the experimental results upon which the conclusions of this thesis are based. Photographs of other chromatograms have not been included; however, tracings of labeled and marked chromatograms of petal and whole plant material are included as appendices (Appendices III-L). In both the photographs and the tracings, compounds visible before spraying are marked by solid lines, while compounds visible only after spraying are marked by broken lines.

Table IX lists the flavonoids present in whole plant material of all 30 OTUs included in this study. Compounds present in relatively great concentration are indicated by "Xs", while compounds that became visible only after spraying are indicated by "ts". The compounds are grouped according to glycosylation type with lines drawn between these groups. The groups are distinguished in this fashion so that occurrence of a glycosylation class can

FIGURE V

TLC Map of Whole Plant Flavonoids of L. montana
Taken in Ultraviolet Light

FIGURE VI

TLC Map of Whole Plant Flavonoids of L. montana
Taken in Visible Light

TABLE IX

- 67 -

be readily assessed for each OTU.

Table X lists the flavonoids present in fresh petals of 18 OTUs of Limnanthes. The format is the same as that described for Table IX.

Taxa have been arranged manually in Tables IX and X to place OTUs with the greatest flavonoid similarities next to each other. Although the resulting arrangements in both tables are somewhat subjective, it is possible to see a certain amount of clustering between OTUs.

FLAVONOID DIFFERENCES BETWEEN PETAL AND WHOLE PLANT MATERIAL

Relative concentrations of certain flavonoids often differed markedly between petal and whole plant material. In the varieties of L. douglasii, L. floccosa and L. gracilis, the 3-O-rutinoside, 7-O-glucosides occur in high concentration in leaf material, but are nearly absent from the flowers. The most extreme examples are found in L. douglasii vars. rosea and nivea which possess no detectable amounts of these compounds in their petals. Conversely, the 3-O-rhamnosyl rutinosides appear in higher concentrations in petals than whole plant material, although the differences are not as extreme.

A comparison of Tables IX and X reveals that occasionally compounds occur in petal material but not in whole plant material of some OTUs. Theoretically, the petal flavonoids should always be a subset of the whole plant flavonoids since whole plant material consistently contained flowers. However, this situation has not been realized, presumably because certain petal flavonoids are present in concentrations too low to detect once the petals become a minor component of the total tissue analyzed.

NUMERICAL TAXONOMIC RESULTS

The data contained in Tables IX and X were analyzed by computer using several taximetric techniques described in the previous chapter. Figures

TABLE X

PETAL FLAVONOIDS OF 18 OTUS OF LIMNANTHES

OTU		COMPOUND: 3-O-Rut						3-O-GRR						3-O-Rut,7-O-G						3-O-Rut,7-O-Rut						3-O-G						3-O-GG						D	E	H	A	B										
		U	V	W	X	Y	Z	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	O	P	Q	R	S	T	19	20	21	22	23	F	G						C	I	J	K	L	M	N			
7	<u>bakeri</u>	X	X	X	X	X	X	X	X	X	X	t	X	X	X	X	X								X			X	X	X		X	X	t	X	X	t	t				t			X	t						
8	<u>striata</u>	X	X	X	X	X	X	t	X	X	X	X		X	X		X	X							X	X	X	X	X	X					t	t		X			X		X									
2	<u>doug. doug.</u>	X	X	X	X	X	X		X	X	X	X	X	X		X		X	X	X																						X										
5	<u>doug. sulph.</u>	X	X	X	X	X	X	X	X	X	X	X	X	X	t	t	X	X			X		X																		X		t	X								
3	<u>doug. nivea</u>	X	X	X	X	X	X	X	X	X	X	X	X																													X			X							
4	<u>doug. rosea</u>	X	X	X	X	X	X	X	X	X	X	X	X																																							
10	<u>macounii</u>	X	X	X	X	X	X	X	X	X	X	X		X	X	t	X	X		X	t		t		t			t	t	t													X									
1	<u>doug. doug.</u>	X	X	X	X	X	X	X	X	X	X	X	X	t				t	t					t				t	t												X	X	X	X	X							
9	<u>macounii</u>	X	X	t	X	X	t	X	X	X	X	X	t	X	X	X	X	X																										X								
18	<u>flec. bell.</u>	X	t	t	X	X	X	t	t	X		X							t					t				t	t													X		X								
16	<u>grac. parish.</u>	X	X	X	X	X	X	t	X	X	t	X																																	X							
13	<u>alba versic.</u>	t	t	t	t	X	X			X		X							X					t				t	t																							
17	<u>montana</u>	t	t	t	t	X	X					X						X							t			t	t																							
11	<u>alba alba</u>	X	X	X	X	X	X	t		X		X		t				X						X			X	X	t														t									
12	<u>alba alba</u>	X	X	X	X	X	X	t		X		X		t				X						X			X	X																X								
15	<u>grac. grac.</u>	t	t	X	X	X	X			X		X						t						t				t	t																							
14	<u>grac. grac.</u>	X	X	X	X	X	X	t		X		X		X	X		X	X						X	t	t	X	X	X																							
20	<u>flec. pumila</u>	X	X	X	X	X	X	t				t		X				X						t	t	t	X	X	t																							

VII to X are the results of cluster analysis by the weighted pair group method based on whole plant flavonoids. The figures are dendrograms showing degrees of phenetic similarity between all 30 OTUs based on 46 flavonoids. Figures VII and VIII show phenetic similarities based only on compounds occurring in higher concentration (the Xs in Table IX), while Figures IX and X show affinities based on flavonoids present in all concentrations (the Xs and ts of Table IX). The first of each pair of dendrograms (Figures VII and IX) is calculated using the coefficient of Jaccard which does not consider negative matches, while the second of each pair (Figures VIII and X) is based on the simple matching coefficient which does consider negative matches.

Figures XI to XIV are dendrograms expressing phenetic similarities between 18 OTUs based on the petal flavonoid data contained in Table X. As for the whole plant flavonoid cluster analyses, two of the dendrograms consider all flavonoid occurrences (Figures XIII and XIV), while two only consider compounds present in relatively higher concentration (Figure XI and XII). Also, two are calculated using the coefficient of Jaccard (Figures XI and XIII), while two are based on the simple matching coefficient (Figures XII and XIV). The matrices of similarity coefficients used to produce the eight dendrograms have been attached as Appendices LVII to LXIV. These matrices are arranged in the same order as their corresponding dendrograms.

Figures XV and XVI are representations of three-dimensional plots of 30 OTUs using Principal Components Analysis and Varimax Factor Analysis of 31 whole plant flavonoids. Figures XVII and XVIII are representations of three-dimensional plots of 18 OTUs using 36 petal flavonoids. In these four figures the positions of OTUs in space are represented by the locations of the end sections of cones. These cones have been drawn, either with points downward to indicate a position above the plane determined by the two horizontal vectors, or with points upward to indicate a position below this plane. When the

FIGURE VII

Weighted Pair Group Clustering of 30 OTUs by
Jaccard Coefficient: High Concentrations of Whole Plant Flavonoids .

FIGURE VIII

Weighted Pair Group Clustering of 30 OTUs by
Simple Match Coefficient: High Concentrations of Whole Plant Flavonoids

FIGURE IX

Weighted Pair Group Clustering of 30 OTUs by
Jaccard Coefficient: All Concentrations of Whole Plant Flavonoids

FIGURE X

Weighted Pair Group Clustering of 30 OTUs by
Simple Match Coefficient: All Concentrations of Whole Plant Flavonoids

FIGURE XI

Weighted Pair Group Clustering of 18 OTUs by
Jaccard Coefficient: High Concentrations of Petal Flavonoids

FIGURE XII

Weighted Pair Group Clustering of 18 OTUs by
Simple Match Coefficient: High Concentrations of Petal Flavonoids

FIGURE XIII

Weighted Pair Group Clustering of 18 OTUs by
Jaccard Coefficient: All Concentrations of Petal Flavonoids

FIGURE XIV

Weighted Pair Group Clustering of 18 OTUs by
Simple Match Coefficient: All Concentrations of Petal Flavonoids

FIGURE XV

Principal Components Analysis of 30 OTUs
Based on Whole Plant Flavonoids

FIGURE XVI

Varimax Factor Analysis of 30 OTUs
Based on Whole Plant Flavonoids

FIGURE XVII

Principal Components Analysis of 18 OTUs
Based on Petal Flavonoids

FIGURE XVIII

Varimax Factor Analysis of 18 OTUs
Based on Petal Flavonoids

position of an OTU was obscured by the presence of another in front of it, their positions in the drawings were separated slightly for clarity. The position of L. bakeri, OTU 7, has been moved in somewhat closer to the other OTUs than was determined by the computer analysis. This was done so that all of the taxa would be included in the drawings. The actual coordinates of the OTUs determined by computer which were used to make the four drawings are presented in Appendices LXV-LXVIII.

COMPARISONS OF OTUS BY FLAVONOIDS

A. Occurrence Tables

The majority of the flavonoids discovered in the Limnanthaceae are unusual, and many have not been previously reported. A perusal of Tables IX and X, which list flavonoid occurrences by OTUs, reveals that all OTUs of Limnanthes and Floerkea possess rare derivatives of syringetin and laricytrin. Also, rhamnosyl rutinosides are found in all OTUs of Limnanthes and in some of the OTUs of Floerkea. No other plants are known which share these characteristics. This indicates two things: 1) the OTUs of Floerkea and Limnanthes constitute a natural grouping, and 2) no affinities to other families are indicated on the basis of flavonoid similarities. However, the flavonoid compositions of families with suspected affinities to the Limnanthaceae are poorly known. The future discovery of Limnanthes-type flavonoids in any of these families would be significant and might help clarify relationships above the family level.

The most striking feature of Tables IX and X is the overall similarities in flavonoid composition shown by all the taxa investigated. These similarities are not surprising in view of existing information, all of which indicates that members of the Limnanthaceae are closely related.

The results presented in Table IX indicate that Floerkea is distin-

guished from Limnanthes by two differences: 1) three of the four OTUs of Floerkea contain compound H (possibly an acylated monoside of kaempferol), which is not found in any OTUs of Limnanthes, and 2) the flavonoid patterns of the four OTUs of Floerkea are generally simpler than those of most Limnanthes. However, L. floccosa ssp. pumila, OTU 21, has a pattern of comparable simplicity. Also the rare aglycone types are not present as derivatives in most glycoside series, or if present, occur in trace amounts.

It is significant that OTUs of Limnanthes are separated in Tables IX and X by certain differences, corresponding to the sectional division proposed by Mason. OTUs described as members of the section Reflexae usually possess more derivatives of sytingetin, isorhamnetin, laricytrin and myricetin in certain glycosylation series. This observation is particularly true for the petal data.

OTUs belonging to the same species have usually fallen in adjacent rows in Tables IX and X, although there are notable exceptions: L. douglasii var. douglasii, OTU 1, from Europe, falls between the two OTUs of L. macounii; L. gracilis var. parishii, OTU 16, more closely resembles L. alba var. versicolor and L. floccosa than OTUs of its sibling variety; and subspecies of L. floccosa do not lie together, but instead form subgroupings.

B. Cluster Analyses

The dendrograms are classifications of the Limnanthaceae based purely on similarities in flavonoid composition. All flavonoid characters were given equal weight, and cluster development was not prejudiced by previous taxonomic assessments. Computation of the dendrograms varied according to the way similarities were determined, what concentrations of flavonoids were considered, and whether whole plant or petal material was analyzed. The resulting diagrams reflect these differences.

Although the classifications of OTUs presented in the dendrograms are different, all eight have certain gross features in common. The features of these dendrograms roughly coincide with the recognized taxonomic scheme of the family. In three of the four dendrograms based on whole plant flavonoids, Floerkea is separated from Limnanthes. However, L. floccosa ssp. pumila, OTU 21, is also distinct from the remainder of Limnanthes taxa and clusters with Floerkea in two of these three dendrograms. OTUs belonging to the same section and species generally have clustered together in both petal and whole plant analyses. However, as was observed for the manual arrangement of OTUs in the occurrence tables, certain OTUs fall out of place.

Tables XI and XII are attempts to summarize the information contained in the eight dendrograms. Table XI is a synopsis of the results of the four cluster analyses based on whole plant flavonoids of 30 OTUs, and Table XII is a synopsis of the results of the four cluster analyses based on petal flavonoids of 18 OTUs. The OTUs analyzed from dried material have been underscored with dotted lines in Table XI.

OTUs are grouped together in the left hand columns of Tables XI and XII if they clustered together in each of the four appropriate cluster analyses. OTUs that did not cluster consistently with other taxa, but had variable affinities dependent on the conditions of analysis, are listed in the right hand columns of the tables. The affinities of these "floating" OTUs are indicated by dotted lines drawn to the clusters at the left or to other floating taxa where appropriate. It is important to note that no set similarity coefficient value was used to separate floaters from consistent clusters, or to separate the clusters themselves. Therefore, the consistent clusters shown in these tables are somewhat arbitrary, and they are not directly comparable.

In both Tables XI and XII the compositions of consistent clusters

TABLE XI

Synopsis of the Results of Four Cluster Analyses
Based on Whole Plant Flavonoids of 30 OTUs¹

Taxa with Consistent Affinities		Taxa with Variable Affinities ²	
Cluster	OTU		OTU
1	7 <u>bakeri</u>		
2	8 <u>striata</u>		
	18 <u>floc. ssp. belling.</u>		1 <u>douglasii</u> var. <u>douglasii</u>
3	14 <u>grac. var. gracilis</u>		20 <u>floc. ssp. pumila</u>
	23 <u>floc. ssp. floccosa</u>		
4	9 <u>macounii</u>		
	10 <u>macounii</u>		
5	4 <u>doug. var. rosea</u>		
	5 <u>doug. var. sulph.</u>		
	6 <u>vinculans</u>		
6	2 <u>doug. var. doug.</u>		
	3 <u>doug. var. nivea</u>		16 <u>grac. var. parishii</u>
7	15 <u>grac. var. gracilis</u>		13 <u>alba</u> var. <u>versicolor</u>
	22 <u>floc. var. grandiflora</u>		19 <u>floc. ssp. bellingeriana</u>
	26 <u>floc. var. californica</u>		
8	11 <u>alba</u> var. <u>alba</u>		24 <u>floccosa</u> ssp. <u>floccosa</u>
	12 <u>alba</u> var. <u>alba</u>		25 <u>floccosa</u> ssp. <u>floccosa</u>
	17 <u>montana</u>		21 <u>floccosa</u> ssp. <u>pumila</u>
9	27 <u>Floerkea</u>		
	30 <u>Floerkea</u>		
10	28 <u>Floerkea</u>		
	29 <u>Floerkea</u>		

1 -- The numbers of OTUs analyzed from dried material are underscored with dotted lines.

2 -- Variable affinities are indicated with broken lines drawn between columns and OTUs where appropriate.

TABLE XII

Synopsis of the Results of Four Cluster Analyses
Based on Petal Flavonoids of 18 OTUs

Taxa with Consistent Affinities		Taxa with Variable Affinities ¹	
Cluster	OTU		OTU
1	11 <u>alba</u> var. <u>alba</u>		
	12 <u>alba</u> var. <u>alba</u>		
	14 <u>grac.</u> var. <u>gracilis</u>		
	20 <u>floc</u> ssp. <u>pumila</u>		
2	13 <u>alba</u> var. <u>versicolor</u>		
	15 <u>grac.</u> var. <u>gracilis</u>		
	17 <u>montana</u>		
		18 <u>floc.</u> ssp. <u>bellingeri</u>	
		1 <u>douglasii</u> var. <u>douglasii</u>	
3	3 <u>doug.</u> var. <u>nivea</u>		
	4 <u>doug.</u> var. <u>rosea</u>		
		16 <u>gracilis</u> var. <u>parishii</u>	
4	2 <u>doug.</u> var. <u>douglasii</u>		
	5 <u>doug.</u> var. <u>sulphurea</u>		
	9 <u>macounii</u>		
		10 <u>macounii</u>	
5	7 <u>bakeri</u>		
	8 <u>striata</u>		

1 -- Variable affinities are indicated with broken lines drawn between columns and OTUs where appropriate.

approximately follow the recognized family taxonomy: Floerkea is distinct; OTUs of the Inflexae are not grouped with OTUs of the Reflexae, with the exception that L. striata and L. floccosa ssp. bellingeringiana, OTU 18, group together in the whole plant flavonoid analyses; and usually OTUs of the same species fall into the same group. But there are numerous exceptions to this last observation. OTUs of L. floccosa tend to group with OTUs of L. gracilis, rather than together, and the converse is also true. Limnanthes alba var. versicolor, OTU 13, groups with L. montana and L. gracilis rather than its sibling varieties.

There are eight floating OTUs in Table XI and four floating OTUs in Table XII. It is notable that L. douglasii var. douglasii, OTU 1, and L. gracilis var. parishii, OTU 16, which have variable affinities on the basis of either whole plant or petal flavonoids, were also singled out as anomalies in the preceding section after visual arrangement of OTUs in Tables IX and X.

C. Factor Analyses

The arrangement of OTUs in Figures XV and XVI, the drawings of three-dimensional plots based on whole plant flavonoids, generally agrees with the results of cluster analysis. However, the factor analyses provide a much better representation of the magnitude and direction of variation between OTUs, and between clusters of OTUs. The striking feature in these diagrams is that the Limnanthaceae is unexpectedly divided into three distinct parts: Floerkea, OTUs 27-30, L. bakeri, OTU 7, and the remaining Limnanthes taxa. Three subgroups of taxa can be distinguished within the main cluster of Limnanthes OTUs. These subgroups do not follow the recognized taxonomy of the genus, but generally correlate with the type of breeding system; the most highly evolved outcrossers form a group at one extreme, while many of the autogamous taxa fall into the group at the other extreme.

The features of Figures XV and XVI differ by the rotation of the array

of OTUs in space. The differences between these figures is comparable to the difference that would be detected if the array of OTUs were viewed from two different points.

Figures XVII and XVIII pictorialize the results of the factor analysis of petal flavonoids. As above, the two diagrams differ mainly in perspective. Again, L. bakeri is sharply separated from the remainder of the OTUs of Limnanthes (flower material of Floerkea was unavailable for this analysis). The remaining OTUs of Limnanthes form a long continuous grouping. There are several important features of this grouping: 1) L. striata lies at one extreme, and shows a greater affinity to L. bakeri than any shown by the other OTUs; 2) all the varieties of L. douglasii (OTUs 1-5) and L. macounii (OTUs 9 and 10) form a reasonably tight cluster near L. striata, but below it; and 3) the OTUs belonging to the Section Inflexae are all distributed in a long cluster linked to L. striata by L. gracilis var. gracilis, OTU 14, and to a lesser extent, by variety parishii, OTU 16.

The drawings of factor analyses of petal flavonoids show the section Inflexae and a subgroup of section Reflexae to be non-overlapping groups. However, these groups are joined by affinities to L. striata and, to a smaller extent, L. gracilis. For these features to become apparent, it is necessary to visualize these two figures in all three dimensions, since the links to L. striata depend on the affinities shown by the dimension of variation perpendicular to the plane formed by the two horizontal vectors.

D. Glycosylation Classes

Differences in glycosylation pattern between taxa are probably more taxonomically significant than simple differences in aglycone types within a single glycosylation class, providing that the missing aglycones are present as derivatives of other classes. This is because the presence or ab-

sence of the synthetic machinery necessary to attach an additional sugar at a new location may reflect greater differences between taxa than does the presence or absence of aglycone types within a glycosylation series. Since all OTUs of the Limnanthaceae contain the six flavonol aglycone types, differences in glycosylation between these taxa may deserve special consideration.

Compounds listed in Tables IX and X have been grouped so that presence or absence of each glycosylation type is easily assessed visually for each taxon. In this fashion OTUs are compared on the basis of 12 characters, or the number of glycosylation classes. Although there are differences between OTUs according to glycosylation, these differences are surprisingly few, and there is little variation between taxa. In fact, an attempt was made to cluster OTUs using the 12 glycosylation classes as characters. This attempt was unsuccessful, due to lack of variation.

Certain OTUs in both Tables IX and X are set apart on the basis of possessing an infrequently occurring glycosylation type. As previously indicated, three of the four OTUs of Floerkea fall into this category, since they each possess compound H.

Limnanthes striata, L. bakeri, and L. douglasii var. nivea are all linked by the common occurrence of two glycosylation types, consisting of F and G, and 19,20,21,22 and 23. Limnanthes striata, L. bakeri, L. douglasii vars. douglasii (OTU 2, but not 1), sulphurea and rosea, and L. vinculans and L. macounii (OTU 10, but not 9) all contain at least one of the 3,7-dirutinosides.

The glycosylation class consisting of the single compound C is found only in whole plant material of three OTUs of the section Inflexae; i.e., L. gracilis var. parishii, OTU 16, L. alba var. versicolor, OTU 13, and L. alba var. alba, OTU 11, but not 12. The only other restricted glycoside class, composed of compounds D and E, links L. macounii, OTU 10, with L. alba var.

alba, OTUs 11 and 12, L. floccosa ssp. grandiflora and L. gracilis var. gracilis, OTU 14.

Unfortunately, most compounds belonging to the glycosylation classes with restricted distributions occurred in trace amounts. Consistent presence in trace amounts introduces a problem of accurately determining presence or absence, a problem that is directly related to the inability to completely identify these compounds. Furthermore, compounds consistently appearing in trace amounts may be overlooked in some taxa, if they occur in concentrations too low for detection.

DISCUSSION

METHOD AND VALIDITY OF USING FLAVONOIDS AS TAXONOMIC CHARACTERS

A. Flavonoids as Taximetric Characters

i. Introduction

Both the application of taximetric techniques and the utilization of flavonoids as characters are relatively new to plant taxonomy. The mating of these two techniques is more recent and has left unresolved certain technical and philosophical questions associated with this process. To help resolve these questions, the flavonoid data gathered in this investigation were analyzed using a variety of methods to assess similarities between taxa.

ii. Inclusion of Negative Matches

The question of whether the mutual absence of a compound contributes to similarity between taxa is one that has not been discussed in reference to flavonoid taximetrics. However, this question has been treated by various authors in relation to other types of characters. Davis and Heywood (1963) say that mutual absence does not constitute similarity, while Sokal and Sneath (1963) say that it does in some cases. The negative argument says that all organisms possess an infinite number of mutually absent characters, and that similarities based on such absences are irrelevant and reflect no taxonomic relationships. The positive argument says that, as long as one of the taxa under consideration exhibits a particular character, the other taxa, not possessing this character, are made more similar by the common absence.

In at least two instances, the consideration of negative matches in comparisons based on flavonoid characters may result in the misplacement of

emphasis, and thus lead to unwarranted taxonomic conclusions. If the absence of a flavonoid compound always results in the presence of another, or the converse is true, then the presence or absence of these compounds should not be considered independently in taxonomic comparisons. Similarities based on the mutual occurrence of such compounds should not be increased further by the absence of the alternate compounds, since this practice would introduce redundant information into the comparison. Instead, presence or absence of both compounds should be treated as a single character state. It is possible that two flavonoids might occur in this linked, either-or fashion, if they each provide an identical function in different plants. However, whether this situation actually exists for flavonoids cannot be determined on an a priori basis, since the functions of most of these compounds are unknown.

Unnatural emphasis is also placed on mutual absence, if a compound occurs in none of the taxa being compared. This is the objection of Davis and Heywood, and it is nearly axiomatic, since there is no taxonomic information content in such characters, just as there is none for characters that are consistently present within a group of taxa. However, providing that a flavonoid does occur in at least one of the taxa under consideration, and that the taxa are of parallel rank and are being compared simultaneously, the mutual absence of that flavonoid does indicate a similarity with possible taxonomic significance.

Since resolution of the above arguments is not possible without experimental evidence, taxa were clustered using both Jaccard's similarity coefficient, which does not consider negative matches, and the simple matching coefficient, which does. It was suspected that the results of the simple matching coefficient might possess the greater taxonomic significance for two reasons: 1) certain OTUs of the Limnanthaceae such as L. bakeri contain nearly every flavonoid encountered, which eliminates the possibility of any of

these compounds occurring on an either-or basis; and 2) certain OTUs have much reduced flavonoid complements that are very similar; thus, similarities between these taxa are higher if calculated considering negative matches.

Interestingly, the dendrogram pairs, differing in type of similarity coefficient, are quite similar in each of the four cases. It is possible to select two dendrograms, one from the four based on whole plant material and one from the four based on petal material, which best agree with the accepted taxonomic scheme of the Limnanthaceae. For whole plant material the choice is Figure VII calculated by the Jaccard coefficient, and for petal the choice is Figure XII calculated with the simple matching coefficient. These observations indicate that the results obtained using either coefficient are roughly comparable, and in this case, the choice of which to use is arbitrary.

iii. Flavonoid Concentration

In comparisons of taxa based on flavonoid compositions, it is natural to make comparisons using as many compounds as possible to contribute the maximum information input to the process. However, at the risk of losing some information, perhaps some compounds are best excluded from consideration if they occur in amounts which make detection and/or identification uncertain, the reason being that the partial loss of information is preferable to the inclusion of misleading or incorrect information.

Various flavonoids occurring in the Limnanthaceae consistently were found in trace amounts. Although detection of these compounds was enhanced by the use of a sensitive spray reagent, the determination of compounds made visible by this spray is difficult. Because of this difficulty and because of the possibility that compounds consistently occurring in trace amounts might be overlooked in some taxa, the flavonoid data was analyzed in two ways: 1) considering all occurrences in a taxon regardless of concentration,

and, 2) considering only the compounds occurring in sufficient concentration to be visible under UV light before spraying.

The results of cluster analysis are variable depending on which concentration of flavonoids was used. Dendrograms based on flavonoids present in higher concentration more closely approximate the accepted taxonomy of the family. Providing that this taxonomy is basically correct, this result indicates that the data based on higher concentrations have greater taxonomic significance than do the data based on all concentrations. However, there is no reason to suspect that compounds occurring in trace amounts are inherently less significant. Therefore, the results themselves probably account for this phenomenon, presumably because incorrect or incomplete information was introduced into the analysis by including the trace-amount data.

Since the flavonoid data based on higher concentrations have greater taxonomic significance to the Limnanthaceae, the factor analyses were performed only on these data. The amount of time involved in the computation of factor analyses also influenced the decision to omit further analyses including trace-amount data.

iv. Lack of Variation among Characters.

Each cluster analysis by the weighted pair group method was based on 46 flavonoid characters. However, in each of these analyses, the number of variable characters was less than 46 depending upon the conditions of analysis. Therefore, a certain number of nonvarying characters (either positive or negative) were included in each analysis which increased similarities between taxa. Although the resulting increases are uniform and the cluster composition does not vary, this procedure is not completely acceptable from a systematic point of view. The justification for this procedure is that the disadvantage realized by uniformly increasing similarity coefficients was outweighed by the

convenience provided to the computer operator.

Invariant characters cannot be used in factor analyses. Therefore, the whole plant factor analyses were performed on 31 variable flavonoids, and the petal factor analyses on 36 variable compounds.

B. Use of Dry versus Fresh Plant Material

Whenever possible OTUs of the Limnanthaceae were analyzed using fresh material. Unfortunately, such material was not available for all taxa, and the remainder were necessarily analyzed from dried material. This procedure introduced some problems of interpreting comparative results. The first involved breakdown in dried material. Fortunately, the flavonol glycosides present in Limnanthes and Floerkea are comparatively stable types, so breakdown effects were probably negligible.

A more serious problem arising from the lack of fresh material was the inability to complete the studies of petal flavonoids and UV flower photography. However, the 18 OTUs grown from seed are a good representation of the species and sections of Limnanthes. Fresh material of Floerkea would have been desirable to balance the investigations.

The third problem related to lack of fresh material was the possibility that individuals of a given taxon may vary in their flavonoid compositions. The flavonoid patterns listed for OTUs grown fresh represent the compounds present in many plants, whereas the flavonoids determined from dried material represent the composition of only one or a few available plants. If individual variation is extensive, results based on single plants could be taxonomically misleading.

It is not possible on the basis of the available data to completely evaluate the effects of comparing individuals with groups in some instances. However, no discrepancies were noted for the OTUs analyzed from a single, or

few, dried plants. Apparently the effects are minimal, since these OTUs cluster consistently in roughly the same frequencies as the other OTUs (Table XI) and according to the accepted taxonomic pattern.

C. Petal versus Whole Plant Results

In an earlier chapter the suggestion was made that flavonoids are directly responsible for both the nectar guides of L. douglasii made visible under UV light and the uniformly high absorption characteristics of the flowers of other species. This assumption implies that flower flavonoids serve a useful function in outcrossing plants; i.e., attracting and guiding pollinators. Therefore, in outcrossing taxa of Limnanthes these flower pigments will be selected for, and eventually, linkage groups determining these and other outcrossing traits may be formed. If such linkage groups are formed, certain traits associated with outcrossing will likely persist for a time after the outcrossing habit has been lost.

The presence in the flowers of autogamous Limnanthes taxa of pigments with UV absorption characteristics comparable to those observed in the outcrossing taxa indicates that the flower flavonoids are conserved in the genus. This conservation of the petal flavonoids has taxonomic significance which applies directly to the results of this study.

If the petal flavonoids have been selectively conserved by evolution, it is probable that these compounds will be better indicators of taxonomic relationships in Limnanthes than will the whole plant flavonoids unless these compounds also have been conserved for some reason. Stebbins (1974) has suggested that flavonoids help discourage herbivores and insects by making vegetation unpalatable. However, there is little evidence to support this suggestion in the context of Limnanthes.

Cluster and factor analyses based on the petal data more closely

agree with many points of the existing taxonomy of Limnanthes than do the analyses based on the whole plant flavonoids. Providing that the existing classification is basically sound, this observation supports the hypothesis that the petal flavonoids are indeed better taxonomic indicators in this genus.

D. Factor Analysis versus Conventional Cluster Analysis

Because it should be possible to produce an accurate and natural taxonomic scheme for a group of plants using different methods and different types of significant information, the recurring features of the taximetric analyses of the Limnanthaceae possess special significance. Affinities that are consistently demonstrated between taxa by these methods likely reflect natural relationships.

The consistent clusters of OTUs (Tables XI and XII) produced by the weighted pair group method demonstrate close similarities in flavonoid compositions. These similarities undoubtedly reflect natural affinities between OTUs in these clusters. However, many OTUs do not fall into consistent clusters. In the whole-plant flavonoid analyses, L. bakeri never clusters with other taxa and has been treated as a separate group. Other nonclustering OTUs float between consistent clusters depending on the method of data analysis. What is the significance of this floating phenomenon?

There are at least two possible explanations for the floating phenomenon: 1) the evidence used to cluster taxa is invalid or insignificant; 2) the flavonoid compositions of the floating OTUs are intermediate, and these OTUs serve as links between clusters. Thus, as the conditions of analysis change, OTUs with intermediate compositions show variable affinities. On the basis of the dendrograms alone, it is impossible to determine which of these explanations is correct.

Because the factor analyses ordinate taxa by arranging them in three-dimensional space (in this study) at distances that are proportional to variation between them, these techniques make it possible to estimate superimposed, branched relationships between OTUs. Such multi-branched configurations can be explained if groups have repeatedly diverged from an ancestral line. This pattern of evolution has apparently taken place in the Limnanthaceae, and it is for this reason that some taxa were consistent floaters. They are indeed links between divergent groups.

Although Sokal and Sneath (1963) reported that there is generally good agreement between the results of factor analysis and clustering by the weighted pair group method, a comparison of the results obtained by these two techniques in the present investigation indicates that this consistency depends on how the variation between taxa is expressed. If there is more than one uncorrelated direction of variation expressed between taxa, a one-dimensional clustering cannot adequately convey the relationships between them. Therefore, since most of the variation present in the Limnanthaceae cannot be accounted for in one dimension, relationships cannot be properly assessed within this group unless a multi-dimensional clustering technique is used.

E. Flavonoid Differences between Duplicate Taxa

In many instances two or more OTUs were analyzed that were identified as the same species, subspecies or variety. By doing so, it was possible to estimate the approximate flavonoid variation between populations or recognized taxonomic entities of Limnanthes and Floerkea.

The two OTUs of L. macounii group together as would be predicted. The same was true for the two OTUs of L. alba var. alba. OTUs of L. floccosa generally grouped together, if somewhat diffusely, in the factor analyses but not in the cluster analyses. Although the similarities were not as great between

the four OTUs of F. proserpinacoides, these taxa also grouped together in three out of four cluster analyses and the factor analyses. Since these four OTUs of Floerkea were chosen to represent diverse geographic elements of the species, elements that have probably been isolated for some time, a certain degree of variation was expected attributable to divergence caused by genetic drift or different selection pressures.

Differences in flavonoid patterns of the duplicate OTUs of L. douglasii and L. gracilis are both significant. Limnianthes douglasii is unusual in two respects. First, OTU 1 only shows affinities to its replicate, OTU 2, in the petal factor analyses. In the whole-plant cluster analyses and whole-plant factor analyses it often clusters with more or less distantly related autogamous derivatives. This single observation provides further evidence that the petal flavonoids are conserved to a greater degree than the whole plant flavonoids.

It is possible to partially explain the strange affinities of OTU 1. These plants may be descendants of plants taken to Europe by David Douglas in the early 1830s and have since been maintained as a horticultural variety. Therefore, over the interval of about 140 years (probably somewhat fewer generations), the flavonoid composition of this OTU has differentiated from other members of this variety that naturally occur in the western United States, even though the two remain very similar morphologically. This differentiation may have resulted from the processes of inbreeding, genetic selection, or drift. Because of the magnitude of the differences between OTU 1 and the naturally occurring OTUs of L. douglasii, which cluster consistently with each other, it is probable that inbreeding or some unknown form of artificial selection has played a greater role than random drift in causing this phenomenon. Because OTU 1 clusters with autogamous plants in the whole plant taximetric analyses, it is possible that inbreeding may lead to directed

changes in whole plant flavonoid composition. Why there should be such a direction in flavonoid change associated with autogamy or inbreeding is not apparent.

The results of the cluster analyses and factor analyses indicate that there is great variation in the flavonoid patterns within both L. gracilis and L. douglasii. The observed variation in both of these species is much greater than that observed within other species of Limnanthes, with the possible exception of L. floccosa. Providing that the divergence in flavonoid compositions between segments of a species (or any related group of plants) results mainly from the random process of genetic drift or from comparable selection pressures, the extent of the divergence between elements of the species douglasii, gracilis and floccosa has implications with regard to both the relative ages of these species, and to ranks of the components of these species.

TAXONOMY OF THE LIMNANTHACEAE

A. Introduction

The currently accepted taxonomic scheme of the Limnanthaceae is a slightly expanded form of the system proposed by Mason in 1952 (Table I). There are three primary features of this scheme. 1) Floerkea is distinguished from Limnanthes by numbers of floral parts and by having hypogeal cotyledons. 2) The genus Limnanthes is split into two sections, Reflexae and Inflexae, based on petal position after pollination. 3) Various Limnanthes taxa, including some formerly recognized as distinct species with clearcut morphological and geographical differences, are reduced to varietal or sub-specific rank largely on the basis of interfertility in artificial crosses.

Plant taxonomists have been reluctant to revise classification schemes arrived at by biosystematic methods when conflicting evidence is produced by

phenetic analyses. The type of evidence on which the phenetic analysis is based, whether morphological or chemical, does not seem to matter; the feeling is that biosystematic studies more clearly indicate phylogenetic relationships.

Mason's treatment of Limnanthes, based on morphological and cytological comparisons, and the results of interbreeding trials, is generally considered to be an accurate phylogenetic arrangement of the genus. However, not all of the available evidence supports this conclusion. Of the three types of evidence considered by Mason, his conclusions depend most heavily on the results of artificial crosses. His other results do not necessarily support his arrangement of the genus. Since there was no variation in karyotype morphology of the genus, Mason's revision of Limnanthes was not influenced by his cytological results. Other morphological treatments and studies of the genus conflict with Mason's conclusions. Previous authors (Howell, 1897; Rydberg, 1910; Jepson, 1936) considered several of Mason's varieties to be distinct species based on morphological differences. In addition, the phenetic analysis of Ornduff and Crovello (1968), based on morphological characters, generally does not support all the details of Mason's taxonomic scheme. Therefore, the net effect has been to attach special significance to the results of hybridization trials in Limnanthes.

Three additional observations suggest that the phylogenetic significance of experimental crossing trials in Limnanthes may need reevaluation.

- 1) Ornduff (1971) has reported that genetic isolating mechanisms apparently have arisen between Limnanthes taxa; not on the basis of distance of relationship, but rather on a purely spatial basis; the more geographically distinct two taxa are, the more likely that no genetic barrier to crossing will have developed between them.
- 2) UV flower photography demonstrates that an isolating mechanism of an ecological nature may exist between varieties

of L. douglasii, and that artificial interfertility does not preclude the existence of such a mechanism. 3) The flavonoid data consistently disagrees with certain key features of the existing classification.

There is evidence indicating that the taxonomic status of Floerkea requires reevaluation. 1) Since the original description of the Limnanthaceae, there has been disagreement regarding whether the morphological differences in the family are great enough or discontinuous enough to warrant recognition of two genera. 2) The phenetic analysis of Ornduff and Crovello (1968) does not support the maintenance of separate genera. 3) The cytological characters of Floerkea do not distinguish it from Limnanthes (Ornduff, 1971). 4) The flavonoid data does not support the separation of Floerkea and Limnanthes.

The phenetic similarities between OTUs based on flavonoids provide new evidence that may be applied to the systematics of the Limnanthaceae. Because the flavonoid evidence stands independently of the results of other taxonomic treatments, this evidence provides the basis for a new classification of the family. But a key question must be answered regarding the significance of the flavonoid evidence: do the results of the flavonoid analysis warrant any new conclusions, or require a reinterpretation of relationships within the family?

The demonstration of flavonoid affinities does justify the revision of a classification providing that two conditions are met: 1) other available evidence supports such a revision, and 2) the flavonoid results are unequivocal. Both of these conditions are fulfilled in the context of the Limnanthaceae.

A comparison of the flavonoid evidence with the relevant evidence from all other sources has led to a reconstruction of a hypothetical evolutionary history of the Limnanthaceae and a new proposed classification of the family. In the following sections, the proposed history and classification will be

presented first, followed by a discussion of the evidence for and against these proposals. Finally, the need for taxonomic revision will be discussed, and a synopsis of the family will be presented including the necessary nomenclatural changes.

B. Hypothetical Evolutionary History of the Limnanthaceae

The information presented in this thesis, taken together with the available information regarding distributions and ecological requirements of members of the Limnanthaceae, has many implications concerning the family's history. These implications have made it possible to reconstruct a hypothetical history which is presented below. However, this history is purely speculative since its evaluation requires nonexistent fossil information.

In the early Tertiary, Limnanthes probably closely resembled some of its modern descendents. It was a predominantly outcrossing spring annual, with a haploid number of five, and had medium sized white flowers and compound leaves with slightly divided leaflets. Since the grassland sites occupied by most modern counterparts had not yet appeared, Limnanthes was probably a streamside herb of the woodlands, occurring at moderate elevations, and producing a determinate number of relatively large nutlets. Its distribution was probably reasonably extensive in western North America, and perhaps it extended across the continent. With the uplift of the coastal mountain ranges, the population of Limnanthes probably was split in two.

It is likely that occasional trends to autogamy were ongoing in early elements of Limnanthes, as they are today. One such element, genetically isolated by autogamy, and better adapted to the cooling trend of the Tertiary, was probably able to persist further north than the parent population. This autogamous Limnanthes rapidly evolved reduced flowers with accompanying traits such as cleistogamy. Its dissemination was probably somewhat improved, and

it may have become slightly weedy.

Three modern members of the Limnanthaceae most closely resemble the three postulated ancestral elements. The autogamous derivative is represented by F. proserpinacoides which still occupies the deciduous woodland habitat of its ancestor. Since the selection pressures associated with this habitat have probably stayed fairly constant, this species probably closely resembles the ancestor split off millions of years ago. The second major element, isolated to the east of the coast range, is represented today by L. striata, a species which may still occupy moderate elevation streamside sites. The western element persisting from the split is represented today only by L. bakeri, a narrow endemic of California associated with moderately high elevation vernal pools.

With the gradual cooling that occurred during the Miocene Epoch, each of the three ancestral elements of Limnanthes was pushed southward. In its southward migration, the population represented today by L. striata was divided by the central valley of California, one part proceeding down the west side of the newly uplifted Sierra Nevadas, and the other down the east side of the coast range. Elements of this migration apparently persist today. Although the main range of L. striata is east of the central valley in the Sierra Nevadas, Ornduff (Ornduff & Crovello, 1968; 1971) has recently discovered a variant western element of this species in the northern California coastal mountains, which he at first thought was a new species and referred to as "trinity" (from Trinity County).

It is likely that Limnanthes and Floerkea persisted virtually unchanged as woodland plants through the Tertiary until the comparatively recent development of grasslands in the Pleiocene Epoch. However, with the availability of these new sites, it is conceivable that elements of L. striata invaded and rapidly evolved adaptations appropriate to the new life style. In coastal

areas, the grassland invaders were probably the forerunners of the Reflexae, characterized by the L. douglasii type, while the eastern invaders were probably the ancestors of the Inflexae, characterized by the L. gracilis type. The oldest western elements occupying the range west of the coast mountains were apparently unable to compete successfully in the new sites and have largely disappeared with the exception of L. bakeri.

In the comparatively modern Pleistocene, three repeating processes may have taken place which account for both the distributions and diversity of extant forms of Limnanthes. 1) All populations, including Floerkea, probably have advanced and retreated north and south with vegetational changes accompanying periods of warming and cooling associated with glaciation. During the cool periods, continuous populations may have been split into many groups. 2) Geographically isolated populations may have become genetically distinct due to rapid adaptations to the differing niches made available by the new grassland habitats. In this fashion populations of the sections Reflexae and Inflexae may have diverged on an ecological basis and according to altitudinal and latitudinal zonation. This process could have resulted in various new species, subspecies or varieties. 3) The recurring trend to autogamy in Limnanthes, coupled with the generally better tolerance of these derivatives of slightly cooler conditions, probably has led to the present day persistence of these plants in sites marginal for most outcrossing taxa of Limnanthes. If so, these plants can be interpreted as relics of earlier northward migrations..

It is likely that rapidly occurring adaptations to the modern grassland habitats in the Reflexae have led to the creation of L. vinculans and the four varieties of L. douglasii. Similar differentiation in the Inflexae may have led to the creation of L. montana, the varieties of L. gracilis, the varieties of L. alba and the varieties of L. floccosa. The persistent auto-

gamous relics of the northward migrations are L. macounii, probably derived from an ancestor resembling L. douglasii, and the subspecies of L. floccosa, derived from an ancestral form of this species.

Figure XIX, the hypothetical phylogenetic tree of the Limnanthaceae, summarizes the history of the family. No attempt has been made to indicate which of the species, L. bakeri or F. proserpinacoides, first diverged from the main line of Limnanthes. The amount of divergence from this main line is roughly comparable for both, and it is probable that the split happened at approximately the same time. Sectional divisions have been made in Figure XIX, and these will be discussed below.

C. Assumptions that Led to the Hypothetical History

Processes of evolution in Limnanthes have made it difficult to understand relationships in this group. Certain features have been strongly conserved such as karyotype, fruit type, and basic floral structure. However, other features may have changed rapidly including number of floral parts, flower and plant size, breeding system, and leaflet morphology. These changes result either from adaptations to new niches, or from the loss of the outcrossing habit. Because of evolutionary convergence, taxa possessing common traits of the sort easily modified by evolution may not necessarily have natural affinities.

The use of flavonoid characters fills an obvious gap in evaluating the taxonomy of the Limnanthaceae. Conflicts in previous taxonomic conclusions result from different interpretations of incomplete or insubstantial information. The flavonoid data presented in this thesis is taxonomically useful because it basically supports the taxonomy of the group, as far as it is presently known, and simultaneously, provides additional information by which the present system can be reevaluated and refined.

FIGURE XIX

Hypothetical Phylogenetic Tree of the Limnanthaceae

The taxonomic implications of the flavonoid data are all related to amount of divergence in flavonoid patterns between taxa. Several assumptions must be made regarding this divergence. The first is that divergence in flavonoid pattern from that of a common ancestor is a random process that arises from genetic drift; or, if the changes are caused by selection pressures, these pressures will be much the same for all taxa. This assumption probably cannot be made for horticultural varieties which are the products of artificial selection.

The second assumption is that the rate of divergence of flavonoid composition is approximately uniform for closely related outcrossing annuals, such as most taxa of Limnanthes. After conversion to autogamy, it is probable that the rate of divergence will slow down with the accompanying loss of heterozygosity. However, substantial amounts of variation were detected in all four completely autogamous members of the family. This variation indicates that divergence does occur in autogamous plants, and that the conversion to autogamy does not preclude the retention of some variability. However, whether variation continues to increase indefinitely at the same rate in these autogamous taxa is impossible to determine on the basis of the available data.

The third assumption is closely related to the second. Divergence in flavonoid composition proceeds at a slower rate in the petals than in the rest of the plant. This conservation in the petals results directly from the ability of these compounds to attract pollinators.

Once the above three assumptions are accepted, four conclusions can be drawn which suggest trends in the evolution of Limnanthes. The first is that the amount of divergence between two taxa is roughly proportional to the length of time that has passed since these taxa were derived from a common ancestor. On this basis, F. proserpinacoides and L. bakeri each separated from Limnanthes long before the creation of any of the remaining taxa, with

the possible exception of L. striata.

The second conclusion is that taxa with much internal flavonoid variation are more ancient than those with little variation. For instance, the amount of variation in L. gracilis indicates that this taxon is relatively more ancient than L. alba. Likewise, the roughly comparable total variation present in members of the sections Inflexae and Reflexae (excluding the species striata and bakeri) indicates that these supraspecific taxa are of approximately the same age.

The third conclusion is that divergence in autogamous derivatives of the same magnitude as that present in outcrossers probably indicates a more ancient origin of the autogamous plants. For this reason the variation in the populations of Floerkea, an amount nearly as great as that observed for all taxa of Limnanthes (excluding L. bakeri), indicates that F. proserpinacoides has been a distinct taxon since well before the evolution of most other family members.

The fourth conclusion is that the implications of petal flavonoids are more taxonomically significant than those of the whole plant flavonoids, and thus suggest a more natural classification system. This conclusion is supported by the separation of the section Inflexae from the Reflexae (without L. striata and L. bakeri) by the flavonoid analyses of petals but not of whole plant material. Although the whole plant flavonoid analyses do not separate the two sections recognized by Mason, they clearly distinguish Floerkea and the single Limnanthes species bakeri from the main family line.

Like the flavonoid evidence, the present day distributions of all members of the Limnanthaceae support the hypothetical history of the group and were useful in formulating this theory. It is true that distributions may change rapidly and thus suggest misleading evolutionary conclusions. However, the close agreement between flavonoid and distributional evidence

suggests that present day locations are significant clues useful in tracing the family history.

Great emphasis has been placed on the driving force for evolutionary change created by relocation in new grassland niches. Most modern taxa of Limnanthes can be regarded as recent derivatives moulded by this force. Conversely, certain extant family members have retained the more ancient streamside or woodland habitat and exist today probably little changed for millions of years. Here, the forces of natural selection have probably stayed relatively constant, and changes are largely the result of genetic drift.

Several pieces of evidence suggest that L. striata probably most closely resembles the ancestral Limnanthes type: 1) the whole-plant flavonoid factor analysis shows this species centrally located between Floerkea and L. bakeri; 2) the petal flavonoid factor analysis shows L. striata forming a common link between the section Inflexae and a reduced version of the section Reflexae, while simultaneously showing a greater affinity to L. bakeri than any other taxon; 3) L. striata is the only family member, except F. proserpinacoides and L. montana, which may occupy a streamside habitat rather than the newer grassland sites. As discussed above the streamside habitat probably represents the ancestral condition. The continued presence of a species in this type of site might tend to minimize evolutionary change. 4) The discovery of a far removed, morphologically varied disjunct of this species suggests that it indeed has a comparatively ancient origin.

D. Comparison of the Proposed Classification to the Existing Classification

i. Above the Species Level

The classification presented in Figure XIX is largely the product of factor and cluster analyses of flavonoid data. However, this scheme was devised to provide the best fit utilizing all the available evidence, including

that presented by Mason, Ornduff and Arroyo. Since all of the available information was used, and all family members were considered simultaneously, the new classification probably more closely approximates actual relationships in the Limnanthaceae than did the old scheme.

The hypothetical history and classification of the Limnanthaceae depart from the accepted taxonomy of the family in certain key points. However, both systems share many features, and the new scheme is actually a refinement of the old. The major differences arise because of differing interpretations of group ranks. This presents a problem, since in light of new information, taxa with comparable affinities should be adjusted so that they are of parallel rank.

The proposed classification of the Limnanthaceae has been drawn with five sectional divisions. An alternate, but less desirable, choice would have been division of the family into three sections or three genera consisting of Floerkea, Limnanthes bakeri, and the remaining taxa of Limnanthes.

From the flavonoid evidence there is little doubt that both L. bakeri and F. proserpinacoides have diverged from the remaining family members to a comparatively great extent. This parallel divergence leads to a necessary conclusion; if Floerkea is recognized as a genus apart from Limnanthes, then L. bakeri should also be so recognized. However, the morphological differences between the three groups are relatively trivial, and L. bakeri has been demonstrated to be interfertile with various other members of Limnanthes. This interfertility makes a three generic split of the family untenable. Therefore, the family consists of only one genus.

Although the accepted classification of the Limnanthaceae recognizes two genera, it is unlikely that many taxonomists will object to the inclusion of Floerkea in the same genus with Limnanthes. All the hard data suggests that they are not separate genera. The morphological differences, including

loss of flower parts, are easily accounted for by the conversion to autogamy, particularly since parallel changes have taken place in L. macounii. Only one good morphological trait separates Floerkea from Limnanthes; Floerkea is hypogeous, while Limnanthes is epigeous. Ornduff and Crovello (1968) have suggested that this trait alone does not constitute sufficient grounds for the maintenance of two genera, and I agree. Although extensive interbreeding trials have been made between all Limnanthes taxa, apparently no attempts have been made to cross Floerkea with Limnanthes. If such a cross produced fertile hybrids, this would further support the joining of these two taxa into a single genus. Unfortunately, the tiny cleistogamous flowers of F. proserpinacoides would make artificial cross pollinations extremely difficult.

Since the family Limnanthaceae has probably evolved from three distinct phyletic lines of approximately equal rank, the decision to recognize five sections instead of three was based on convenience and tradition. The two sections, Inflexae and Reflexae, recognized by Mason are most probably natural groupings pending the removal of L. striata and L. bakeri from the Reflexae. Since Mason's two groups have been well accepted, and contain all but three of the species in the family, it is inappropriate to merge these two sections: a merger that would be necessary, if the family were to be divided into three groups of parallel evolutionary rank.

Having made the decision to preserve Mason's sectional division to the maximum extent allowed by the new evidence, it is necessary to erect a section in addition to the Inflexae and Reflexae consisting only of L. striata. This section is named Limnanthes, since it is probable that L. striata most closely resembles the ancestral family type. This division into three sections keeps the Reflexae, Inflexae and Limnanthes parallel and is a necessary step since these three probably evolved from a common ancestor some time after the separation of sections Floerkea (containing F. proserpinacoides) and Bakera

(containing L. bakeri). Since the five groups diverged at different times, they are not completely parallel in rank. However, in view of the circumstances, a division of the family into five sections is the most desirable among the available alternatives.

The old, two-sectional division of Limnanthes is supported by two pieces of evidence: 1) petal position after pollination, and 2) the presence of an intersectional barrier to hybridization. This evidence can equally well be interpreted to support the proposed five-section division.

Petal position after pollination is by itself a trivial character, probably controlled by one or a few alleles. It is possible that inflexion of petals might have resulted from a single mutation of an ancestor with the reflexed petal type. Whether such a mutation might have happened more than once is hard to determine, but the available evidence indicates that it probably has not. The following is a hypothesis attempting to explain why there may be a selection pressure for maintenance of the inflexed flower type in grassland-inhabiting Limnanthes taxa.

It is possible that the petal inflexion, which tends to retain nutlets in the flower, was selected for and maintained in the grassland invading species of Limnanthes since this trait might aid dissemination of nutlets by herbivores or other animals. If this hypothesis is correct, L. floccosa is the most highly evolved member of the section Inflexae in this regard since this species has also evolved inflexing sepals, and most subspecies possess an abscission layer below the calyx which tends to cause dispersal of the flower as a unit.

Dispersal of nutlets by animals, either by ingestion or by the flowers clinging to fur, may overcome one problem of dissemination in Limnanthes. The comparatively large nutlets of this genus are of a size normally associated with woodland plants (Salisbury, 1942; Stebbins, 1974). Although apparently

necessary for competition in shady sites, grassland invaders would be put at a disadvantage by this trait, and thus compensating mechanisms might evolve.

Besides inflexion of flower parts, natural selection probably has favored increased production of seed by grassland inhabitants to provide the potential for rapid colonization of these newer sites. In this regard, it is significant that Higgins et al. (1971) found that L. striata and L. bakeri were more determinate in their flowering than other Limnanthes taxa. This shared character implies that both of these plants are more primitive in this respect than other members of the genus.

How well do the results of artificial crosses support division of the Limnanthaceae into the five sections, Floerkea, Bakera, Limnanthes, Reflexae and Inflexae? Because of barriers to crossing, the Inflexae is distinct. Since no interbreeding trials have been conducted using Floerkea as a parent, no conclusions can be drawn regarding this section. The sections Bakera, Reflexae and Limnanthes are partially interfertile. However, the successful crosses between L. macounii, L. vinculans and the varieties of L. douglasii generally produce more fertile hybrids than those between these taxa and L. striata (Ornduff, 1971). Also, hybrids between L. bakeri and the previous taxa showed the lowest fertility.

On the basis of flavonoid evidence a strong case can be made for treating the sections Limnanthes and Bakera as groups distinct from the Reflexae. In addition, other evidence does not contradict this separation. One additional piece of evidence, presented earlier in this thesis, also supports such a separation. Limnanthes macounii and the varieties of L. douglasii have each evolved UV-visible floral patterning. This character holds these five taxa together, just as it separates L. bakeri and L. striata from this group.

ii. Species Level and Below

Three of the five newly proposed sections, Bakera, Limnanthes and Floerkea, are monotypic, containing the species L. bakeri, L. striata and F. proserpinacoides, respectively. Each of these three taxa has traditionally been regarded as a distinct species. Certain elements of doubt, either have been, or will now be expressed concerning the accepted status and rank of the remaining Limnanthes taxa comprising the sections Inflexae and Reflexae.

In the intervening years since Mason published his scheme, one of his conclusions that has been questioned involves relationships between the three species L. montana, L. alba and L. gracilis. Mason hypothesized that the taxa, L. gracilis vars. gracilis and parishii, and L. montana, once were part of a continuous population of a single species which became discontinuous and diverged into the three distinct taxa. Mason accorded L. montana specific status since it was not interfertile with the other two taxa which he recognized as varieties of L. gracilis. Gentry and Miller (1965) thought that this was an inconsistent treatment and suggested that the varieties of L. gracilis should also be designated as separate species. Ornduff and Crovello (1968) concluded that the three taxa should all be recognized as either varieties or species, since either treatment would be consistent with Mason's hypothesis which they considered correct. Interestingly, the results of Ornduff and Crovello support a different combination: that L. alba var. versicolor, L. gracilis var. parishii and L. montana, are all varieties of one species, and that L. alba var. alba and L. gracilis var. gracilis should be raised to the rank of species. However, they chose not to follow these results which they interpreted to be taxonomically insignificant.

After consideration of the evidence presented by the above authors, and analysis of the five taxa, the best solution is probably to recognize each of the two varieties of L. gracilis, the two varieties of L. alba and L. montana

at the same rank. At any rate, L. montana should not be maintained at a higher rank than the other four taxa, since the flavonoid evidence indicates a very recent divergence of this taxon from the variety versicolor.

The available data support the hypothesis that the putative ancestor of L. montana and the varieties gracilis, parishii, alba and versicolor occupied a continuous range east of the coastal mountains from southern California to central Oregon. Since elements of L. gracilis have the most diverse flavonoid patterns and share affinities with L. striata and the section Reflexae, it is probable that the putative ancestor of the five taxa resembled L. gracilis. At some comparatively recent time this continuous ancestral population was split into three parts. Variety gracilis was derived from the northern population and variety parishii from the southern population. But the central population rapidly diverged in a more radical fashion, creating three distinct taxa, L. montana and the varieties alba and versicolor, following their successful invasions into differing habitats. This hypothesis suggests that the five taxa should be divided into three species and three varieties; i.e., the varieties gracilis, parishii and alba become species, and the hypothetical new species of L. alba consists of the three varieties alba, versicolor and montana. However, this is an impractical solution, since the morphological differences distinguishing the varieties would be of a greater magnitude than those separating the species.

When all the factors are considered, there is little but confusion to be gained by recognizing any of the above five taxa at different ranks. By this criterion, the five might be considered as varieties of one geographically diverse, polymorphic species. However, in view of the pronounced ecological, geographical, and morphological differences between these taxa, and the lack of natural hybridization even where barriers to crossing are incomplete, treatment as five varieties is unsatisfactory. Therefore, the most

reasonable way to treat this group is to elevate each of the varieties of L. alba and L. gracilis to specific status along with L. montana, thus creating five distinct species.

Past taxonomic treatments of the L. floccosa group have drawn conflicting conclusions. Mason (1952) recognized three varieties of L. floccosa, including floccosa, and the rare endemics pumila and bellingermaniana. His recognition of these taxa at varietal status was slightly inconsistent, since he was unable to demonstrate interfertility between the three, and each was formerly recognized as a distinct species. Mason's judgement in this case rested on the morphological similarities and distributions of the three. Since Mason's work, Ornduff (1971) has produced hybrids between floccosa and bellingermaniana. Arroyo (1973a) also has indicated that a certain amount of interfertility exists between some unidentified elements of the L. floccosa group.

Recently, Arroyo (1973a) has changed the treatment of L. floccosa, raising each of Mason's three varieties to subspecies and designating two additional subspecies, grandiflora and californica. This further division was based on the results of a taximetric analysis of plants grown under uniform conditions, considering various floral and vegetative characters.

Although Arroyo divided L. floccosa into five taxa, her results, as she presents them, indicate that a two-way split would have been more appropriate. By her analysis, the subspecies grandiflora, californica and pumila form a discrete grouping distinct from a second group consisting of the subspecies floccosa and bellingermaniana. Arroyo explains that the two groups are separated since each represents one of two discontinuous levels of autogamy in the species.

One of Arroyo's major premises is that L. floccosa recently evolved as an autogamous derivative of L. alba (1973a, 1973b). It is upon this pre-

mise that she bases, at least in part, all of her conclusions about evolution in L. floccosa. This premise is an interesting one and worthy of consideration here.

Although Arroyo does not present her rationale, presumably she sees the highly autogamous forms of L. floccosa linked to L. alba by the subspecies grandiflora, californica and pumila, which are more or less intermediate in flower size, degree of pubescence, and supposedly, level of autogamy. This hypothesis is not consistent with the other available information.

Ornduff and Crovello (1968) found L. floccosa distinct from all other taxa of Limnanthes in three out of four of their phenetic analyses. This consistency was shared by only one other group consisting of Floerkea and L. macounii. In the fourth analysis, only one subspecies, pumila, showed any affinity to L. alba, while the subspecies floccosa and bellingieriana formed a distinct, separate cluster. (The subspecies grandiflora and californica had not yet been described.)

There are few morphological characters which possess much taxonomic utility in distinguishing members of the Limnanthaceae. Generally, the characters that are strongly conserved by evolution are all uniform between taxa, while the plastic characters have evolved rapidly many times in different ancestral lines, often creating similarities by convergence. Interestingly enough, the L. floccosa group is well distinguished from other taxa of Limnanthes by what appear to be excellent taxonomic characters. These distinguishing characters are: the sepals have a characteristic apiculate tip, the sepals become valve-like, infolding as the nutlets mature, and an abscission layer is formed to some degree between receptacle and pedicel, promoting dispersal of the nutlet-containing flower as a unit. The presence of these characteristics in L. floccosa suggests that this species diverged from other Limnanthes taxa in comparatively ancient times. Certainly, these morpho-

logical differences do not support a close relationship with any other members of the section Inflexae, including L. alba.

Mason (1952) placed L. floccosa in the Inflexae on the basis of its petal position after pollination. The flavonoid data clearly support this placement since in the petal factor analyses the subspecies bellingneriana and pumila occur within the grouping formed by other members of the section (Figures XVII & XVIII).

Most of the subspecies of L. floccosa were analyzed from dried material, which included several of Arroyo's collections. Since insufficient petal material was available from these collections, it was, therefore, only possible to perform whole plant flavonoid analyses on the entire L. floccosa group. These analyses have several taxonomically significant implications.

Figure XX is an expanded view of the Principal Components Analysis of whole plant flavonoids of the OTUs of L. floccosa. The three OTUs of subspecies floccosa (23,24,25) form a diffuse grouping. Both of the subspecies pumila (20 & 21) and bellingneriana (18 & 19), analyzed on the basis of fresh and dried material, form two distinct clusters separated cleanly by the approximate total variation present within the entire species. However, the subspecies of californica (22) and grandiflora (26) fall very close to the OTUs of subspecies bellingneriana indicating that these four OTUs have very similar flavonoid compositions.

The flavonoid data support the following hypothesis. Since the divergence in the subspecies floccosa is equivalent in magnitude to the total divergence within the whole species complex, and all other subspecific taxa fall within the limits of this variability, subspecies floccosa most closely approximates the ancestral type. The large discrepancy between pumila and the cluster formed by the remaining subspecies grandiflora, californica and bellingneriana, suggests that these two groups represent separate lines of

FIGURE XX

Principal Components Analysis of 9 OTUs of
L. floccosa Based on Whole Plant Flavonoids

divergence from the ancestral form of L. floccosa.

Because of the presence of two morphological characters, it is probable that subspecies pumila was the first to diverge from the L. floccosa group. These characters are: 1) like other taxa of Limnanthes, the stigmatic branches of subspecies pumila are split only part way down the style, while they are split all the way down in the other four subspecies; 2) the abscission layer that forms beneath the receptacle does not develop to as great an extent in pumila as it does in the remaining subspecies. Therefore, the flowers of pumila do not readily disarticulate as a unit (Arroyo, 1973a).

Before a decision can be made regarding whether the floccosa group requires taxonomic revision, it is necessary to consider the validity of the subspecific erections of Arroyo. Although her taximetric treatment was based on 42 characters, by her own admission the emphasis is largely on floral characters, thus emphasizing the taxonomic significance of autogamy. Since autogamy has evolved many times in different lines of the Limnanthaceae, taxonomic conclusions based on traits associated with selfing probably will be erroneous, or at best misleading.

Besides the weighting of characters linked to autogamy, Arroyo's analysis is further prejudiced on the basis of two traits, pubescence and stature. Of the 42 characters determined, 7 of these concern pubescence of plant parts. The character used by Mason to distinguish two of the varieties of L. floccosa, rows of hairs at the petal bases, is included in her analysis as an eighth character. Also, of the 42 characters, 16 or almost 40%, relate strictly to physical stature, being measurements of lengths and widths of various plant parts. Besides these redundancies, additional character overlap is present. For instance, "fruiting calyx abscission zone" and "mode of nutlet dehiscence" are presented as separate characters, when in fact they are 100% correlated.

Because of the unjustified weighting of certain characters in her analysis, Arroyo's taxonomic conclusions are probably of little value. Even her interpretation of her own results appears inconsistent with her data. Instead of her two principal groups being separated by discontinuous levels of autogamy, it is probable that these two groups were separated simply on the basis of size; one group has larger component parts than the other.

After consideration of the available information, it is clear that certain changes should be made in the classification of the L. floccosa group. Since the evidence indicates that the subspecies pumila diverged from L. floccosa at an earlier time than the other taxa, that it is well differentiated morphologically and chemically from the related taxa, and that it is apparently not crossfertile with the other subspecies, this taxon should be reinstated as a separate species.

The subspecies bellingermaniana, grandiflora and californica all appear to be closely related and probably can be considered as varieties or L. floccosa, or a species distinct from L. floccosa. Of the three subspecies, bellingermaniana is morphologically distinct from grandiflora and californica, which are very close in their morphological and ecological characteristics. However, these latter two subspecies are distinguished by a wide discontinuity in their ranges, and each lies at a different extremity of the range of subspecies floccosa. Since Arroyo has reported that there is a certain amount of natural genetic exchange within the L. floccosa group, it is probably best to include the taxa bellingermaniana, grandiflora, californica and floccosa into one species. The differences between grandiflora and californica are probably not great enough to preserve these as separate entities, and they should be merged into one taxon.

It is suggested that members of the species L. floccosa should be reinstated at varietal rank, rather than at the subspecies level chosen by Arroyo.

This is because: 1) a certain amount of genetic exchange occurs between these taxa, as demonstrated by intergrading of characters in some areas; and 2) Mason's choice of rank is adequate, and there was no apparent reason for her decision to change the taxa to subspecies from varieties.

The Reflexae has been regarded as a natural grouping of species containing L. bakeri, L. striata, L. macounii, L. douglasii, L. vinculans, and perhaps Floerkea proserpinacoides (Ornduff & Crovello, 1968). For the reasons already presented, L. bakeri and L. striata should be deleted from this section, and become the monotypic members of the sections Bakera and Limnanthes.

Limnanthes macounii, L. vinculans, and the four varieties of L. douglasii together form a group encompassing a great range of morphological diversity. In fact these taxa are held together largely on the basis of reflexed petal position after pollination and demonstrated interfertility. However, all of these taxa clustered tightly together in one cluster analysis based on "vegetative characters" (Ornduff & Crovello, 1968).

Mason did not have fresh material of L. macounii when he revised the taxonomy of Limnanthes. Therefore, on the basis of dried material he determined that this taxon was a separate species, and if it belonged to the genus Limnanthes (instead of Floerkea), its reflexed petals placed it in the section Reflexae. With the rediscovery of this taxon in nature, both of these judgments have been upheld.

After having grown fresh material of L. macounii from two populations, it is evident that this species is more closely linked to the Reflexae than is apparent if it is collected from its natural habitat. Although all Limnanthes taxa are phenotypically plastic, L. macounii is particularly so. As it is found in nature, this species is very inconspicuous, being totally procumbent, or if upright, no taller than the surrounding herb elements, and usually no larger than a few centimetres. When grown in the greenhouse under

normal conditions, this species attains an unnaturally large size approximating that of the other Limnanthes taxa. Its foliage resembles that of L. douglasii vars. sulphurea or douglasii, and it not infrequently produces totally, or partially, 5-merous flowers. The size of its flowers, however, remains small.

The evolutionary consequences of autogamy have been extreme in L. macounii, including loss of floral parts and reduction in flower size. These changes notwithstanding, it is apparent that the overall diminutive size of this plant can be attributed largely to the marginal conditions under which it grows naturally. It is almost certain that this plant persists only as a relic in the warmest areas of Vancouver Island, cut off by the Strait of Georgia from some receding population of douglasii-like plants pushed southward by a period of cooling. This hypothesis is supported both by the absence of L. macounii in more southern regions and the present-day disjunctive distribution of L. douglasii along the west coast of the United States (Figure I). It is likely that the ability of L. macounii to persist far to the north of the remaining Limnanthes taxa is made possible largely by its conversion to autogamy (Mosquin, 1966). If so, this situation parallels the ability of the totally autogamous Floerkea to grow in more northern areas. Although L. macounii appears to be in little danger of immediate extinction, several unusually cool, or late springs might decimate what remains of this taxon.

The petal flavonoid data clearly link L. macounii with the varieties of L. douglasii. Furthermore, the two species are linked by similarities in UV-visible flower patterning. The two OTUs of L. macounii, which cluster together in both whole plant and petal analyses, exhibit a fairly small amount of divergence in flavonoid composition. This relatively small amount of divergence supports the hypothesis that L. macounii has only recently diverged from the main Reflexae line providing that there has been the retention of

some variability by this species.

Although two were formerly considered separate species, Mason merged the taxa sulphurea, nivea, rosea and douglasii as varieties of the species L. douglasii. However, these varieties have morphological differences of the same magnitude as those separating other species of Limnanthes. With the exception of partial intergrading of varieties rosea and nivea in an area of overlap, natural hybrids apparently are not formed between these four varieties. However, all four are experimentally interfertile, particularly when allopatric populations serve as parents. Therefore, Mason recognized the four as varieties since the biological species concept was uppermost in Mason's taxonomic treatment. With the evidence made available here, this treatment requires reconsideration.

All the varieties of L. douglasii are predominantly bee-pollinated outcrossers. UV floral photography discloses differences between the varieties that may lead to isolation resulting from ethological, rather than genetic factors.

The flavonoid factor analyses indicate that most varieties of L. douglasii are as distinct from one another as most of the recognized species in the family. The exceptions are the varieties rosea and nivea which fall closely together in all the factor analyses.

If the taxa rosea, nivea, sulphurea and douglasii are to be varieties of one species, it is apparent that equally distant taxa have been assigned different ranks. The divergence in flavonoid composition of these four varieties is no less than the divergence of L. macounii, a distinct species, from these varieties. A similar situation also exists for the recently described species L. vinculans.

When Ornduff described L. vinculans (1969), he cited leaflet characteristics that he interpreted to show that this species was intermediate

between L. bakeri and L. douglasii. However, the similarities between the leaflets of L. bakeri and L. vinculans probably result from different evolutionary processes. Although the effect on the morphology is similar, it is probable that the leaflets of L. vinculans have become entire by loss of laterally divided segments, while the leaflets of L. bakeri remain mostly undivided. This hypothesis is supported by the leaf morphology of the seedlings of L. vinculans, since at this stage it is the only species of the Limnanthaceae having non-compound linear leaves.

Similarities in leaflet margins between L. bakeri and L. vinculans probably result from analogous adaptations caused by comparable selection pressures characteristic of the coastal habitats of these species. In other characters L. vinculans closely resembles the L. douglasii group, and probably shares its closest natural affinity with variety nivea. This affinity is indicated by the similarities in floral characters and by high interfertility between these two taxa (Ornduff, 1971), although hybrids are only formed between allopatric populations.

Although fresh material of L. vinculans was not available for this investigation, its whole plant flavonoids were determined from dried material and compared to the other taxa. In the cluster and factor analyses, L. vinculans clustered with OTUs of L. douglasii indicating a close relationship. In addition, the distance separating L. vinculans from the varieties of L. douglasii is no greater than the distance separating the varieties themselves.

On the basis of the available information, it is possible to hypothesize how evolution proceeded in the Reflexae. In a process parallel to that hypothesized for the Inflexae line, elements of a douglasii-type ancestral population became isolated geographically and/or ecologically and diverged into distinct entities as a result of different selection pressures in the

various new habitats. Six of these divergent entities exist today, the four varieties of L. douglasii, and the species L. macounii and L. vinculans. Probably all six diverged comparatively recently. However, among these six taxa are some of the most highly adapted forms in the family.

There is little justification for maintaining the six taxa of the section Reflexae at different ranks, with the exceptions of the varieties nivea and rosea. Since these taxa have been found to intergrade in a region of sympatry, perhaps they should be recognized as varieties of one species. Since L. macounii and L. vinculans have been recognized and maintained as separate species, and there are good distinguishing morphological, distributional and ecological characters separating the varieties douglasii, sulphurea and rosea, it would probably be most consistent to elevate these latter three taxa to the rank of species. The proposed species rosea would consist of the two varieties nivea and rosea.

E. Summary of Proposals for Revision of the Limnanthaceae

i. Introduction

After consideration of the available evidence regarding relationships in the Limnanthaceae, it is apparent that the family requires internal taxonomic revision at every level. Some researchers will insist that a family should not be revised solely on the basis of chemical characters; I agree, and this is not what is being proposed. It is true that the flavonoids provide a set of characters of great taxonomic utility in the Limnanthaceae, and therefore were depended on heavily in formulating a new classification. However, in no instance does the other available evidence conflict with the flavonoid information. Although interpretations of data may vary, it is improbable that different interpretations would greatly alter the proposed family classification, at least until new information from other sources is

brought to bear on the problem.

ii. Genus Level

Floerkea and Limnanthes should be merged into one genus. The dissimilarities between these two genera are simply not great enough, or discontinuous enough, to preserve them as separate genera. On this issue, the evidence is conclusive.

With regard to the merit of maintaining Floerkea as a genus distinct from Limnanthes on traditional grounds, the following passage from Jepson (1951, p. 14) has application:

... It is, however, necessary that the limits of genera should, with increase of knowledge of their structure, probable phylogeny, geographic history and ecology, be subject to revision and modification. No genus has any vested rights on account of long usage or approval by the great masters. Continued research, increased knowledge and an enlarged viewpoint must continually find vent in new generic expressions.

The generic name should become Floerkea since this genus was described 32 years before Limnanthes, and therefore takes precedence.

iii. Section Level

The previous classification, which recognized Floerkea and two sections of Limnanthes should be amended to a single genus containing five sections: Bakera, Floerkea, Inflexae, Reflexae and Limnanthes. The primary reason for this alteration is to provide internal consistency of rank.

iv. Species and Varietal Levels

With the object of providing uniformity in ranking parallel taxa, it is necessary to revise designations of many taxa in the Limnanthaceae at the species level and below. It is also necessary to revise the concept of species differences as it is applied in this context. The biological species concept

has been found wanting as applied to the Limnanthaceae. Since this criterion was previously assumed to hold the utmost in taxonomic significance, other sorts of evidence were deemphasized in earlier treatments.

The Limnanthaceae should be revised to consist of 15 species, the species rosea containing two varieties and the species floccosa containing three varieties. With one exception, all of the taxa designated by previous authors would remain distinct, but the ranks of many of these taxa would be changed. It is recommended that the subspecies of L. floccosa, grandiflora and californica, as described by Arroyo, be merged into the single taxon floccosa var. grandiflora.

F. Synopsis of the Family Limnanthaceae

A single genus designated Floerkea Willd., Neue Schrift. Gesellschaft Nat. 3:449.1801.
[Limnanthes R. Br., London and Edin. Philos. Mag. III, 2:70. 1833.]

Section Limnanthes

1. F. striata (Jepson) Parker, comb. nov.
[L. striata Jepson, Fl. Calif. 2:411. 1936.]

Section Bakera

2. F. bakeri (J.T. Howell) Parker, comb. nov.
[L. bakeri J.T. Howell, Plantae Occidentales. III. Leaflet. West. Bot. 3:206. 1943.]

Section Floerkea

3. F. proserpinacoides Willd., Neue Schrift, Ges. Nat. 3:449. 1801.
[F. occidentalis Rydberg, Mem. N.Y. Bot. Gard. 1:268. 1900.]

Section Inflexae

4. F. alba Greene, Fl. Fran. 100. 1891.
[L. alba Bentham, Pl. Hartw. 301. 1848.]
[L. alba var. detonsa Jepson, Fl. Calif. 2:411. 1936.]
[L. alba var. alba (Benth.) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]

Section Inflexae -- continued

5. F. floccosa (Howell) var. floccosa Parker, comb. nov.
[L. floccosa Howell, Fl. NW Amer. 1:108. 1897.]
[L. floccosa var. floccosa (Howell) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]
[L. floccosa ssp. floccosa (Howell) Arroyo, Brittonia 25:177. 1973.]
- var. bellingieriana (M.E. Peck) Parker, comb. nov.
[L. bellingieriana M.E. Peck, Proc. Biol. Soc. Wash. 50:93. 1937.]
[L. floccosa var. bellingieriana (M.E. Peck) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]
[L. floccosa ssp. bellingieriana (M.E. Peck) Arroyo, Brittonia 25:177. 1973.]
- var. grandiflora (Arroyo) Parker, comb. nov.
[L. floccosa ssp. grandiflora Arroyo, Brittonia 25:177. 1973.]
[L. floccosa ssp. californica Arroyo, Brittonia 25:177. 1973.]
6. F. gracilis (Howell) Parker, comb. nov.
[L. gracilis Howell, Fl. NW Amer. 1:108. 1897.]
[L. gracilis var. gracilis (Howell) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]
7. F. montana (Jepson) Parker, comb. nov.
[L. montana Jepson, Fl. Calif. 2:412. 1936.]
8. F. parishii (Jepson) Parker, comb. nov.
[L. versicolor var. parishii Jepson, Fl. Calif. 2:411. 1936.]
[L. gracilis var. parishii (Jepson) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]
9. F. pumila (Howell) Parker, comb. nov.
[L. pumila Howell, Fl. NW Amer. 1:108. 1897.]
[L. floccosa var. pumila (Howell) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]
[L. floccosa ssp. pumila (Howell) Arroyo, Brittonia 25:177. 1973.]
10. F. versicolor Greene, Erythea 3:62. 1895.
[L. versicolor (Greene) Rydberg, N. Amer. Fl. 25:99. 1910.]
[L. alba var. versicolor (Greene) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]

Section Reflexae

11. F. douglasii Baillon, Adansonia 10:362. 1871.
[L. douglasii R. Br., London Edin. Philos. Mag. III, 2:70. 1833.]
[L. sulphurea odorata Loud., Enc. Pl. 1543. 1855.]
["L. sulphurea (Loud.)" Rydberg, N. Amer. Fl. 25:98. 1910.]
[L. howelliana Abrams, Madrono 6:27. 1941.]
[L. douglasii var. douglasii (R. Br.) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]
12. F. macounii (Trelease) A. Gray, Syn. Fl. 1:363. 1897.
[F. proserpinacoides Macoun, Cat. Canad. Pl. 1:90. 1883.]
[L. douglasii Macoun, Cat. Canad. Pl. 3:502. 1884.]
[L. macounii Trelease, Mem. Boston Soc. Nat. Hist. 4:85. 1887.]

Section Reflexae -- continued

13. F. rosea Greene var. rosea, Fl. Fran. 100. 1891.
[L. rosea Benth. Pl. Hartw. 302. 1848.]
[L. pulchella Hartweg, Jour. Hort. Soc. London 3:220. 1848.]
[L. rosea var. candida Jepson, Fl. Calif. 2:411. 1936.]
[L. douglasii var. rosea (Benth.) C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]

..... var. nivea (Mason) Parker, comb. nov.
[L. douglasii var. nivea C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952.]
14. F. sulphurea (Mason) Parker, comb. nov.
[L. douglasii var. sulphurea C.T. Mason, Univ. Calif. Publ. Bot. 25:455. 1952]
15. F. vinculans (Ornduff) Parker, comb. nov.
[L. vinculans Ornduff, Brittonia 21:11. 1969.]

CONCLUSIONS

- 1) The flavonoids found in the Limnanthaceae are flavonol glycosides. They are unusual with regard to type of aglycone, including various derivatives of syringetin and laricytrin. These flavonoids are also unusual regarding extent of glycosylation; di-rutinosides and rhamnosyl-rutinosides are found naturally occurring for the first time.
- 2) On the basis of presently known flavonoid similarities, the Limnanthaceae is distinct; no families appear to share any special affinities with it.
- 3) Because of similarities in flavonoid composition between all members of the Limnanthaceae, it is apparent that they are all closely related.
- 4) The application of taximetric techniques to flavonoid taxonomy of the Limnanthaceae indicates that the flavonoids are useful for the clarification of relationships in this family.
- 5) Flavonoids occurring in relatively greater concentrations are better indicators of relationships between members of the Limnanthaceae than those occurring in trace amounts.
- 6) When flavonoid characters are analyzed by conventional clustering techniques (i.e., the weighted pair group method), it makes little difference whether or not mutual absences are considered.

- 7) No taxonomically significant differences were detected due to the use of dried plant material when fresh material was unavailable.
- 8) Classifications based on petal flavonoids more clearly indicate natural relationships between taxa of the Limnanthaceae than do classifications based on whole plant (including flowers) flavonoids.
- 9) The techniques of factor analysis provide clearer indications of relationships between taxa in the Limnanthaceae than do the standard clustering techniques. However, the results derived from the two techniques do not conflict. Apparently, ordination of taxa is required to understand the multiple branching of evolutionary lines and the different ongoing rates of evolution in the family.
- 10) The flavonoid pattern of the horticultural variety of L. douglasii cannot be directly compared with other taxa of Limnanthes in chemosystematic studies because of the effects of artificial selection and/or inbreeding.
- 11) Certain members of the Limnanthaceae have floral patterning visible only under UV light. This character is taxonomically significant and helps to distinguish one supraspecific group from the rest of the family.
- 12) A new classification of the Limnanthaceae is proposed after integration of the flavonoid data and other available information.
- 13) It is apparent from discussion of the taxonomically significant information known for the Limnanthaceae, that this family requires taxonomic revision at all levels.

LITERATURE CITED:

1. Abrams, L. 1941. A new Limnanthes from Oregon. Madrono 6:29.
2. Arroyo, M.T.K. 1973a. A taximetric study of infraspecific variation in autogamous Limnanthes floccosa (Limnanthaceae). Brittonia 25:177-191.
3. 1973b. Chiasma frequency evidence on the evolution of autogamy in Limnanthes floccosa (Limnanthaceae). Evolution 27:679-688.
4. Baillon, H. 1871. Notes sur les Géraniacées et les Linacées. Adansonia 10:360-371.
5. Bate-Smith, E.C. 1962. The phenolic constituents of plants and their taxonomic significance. I. Dicotyledons. J. Linn. Soc. (Bot.) 58:95-173.
6. Bentham, G. 1848. Plantae Hartwegianae [1839-1852]. London.
7. Bohm, B.A. & F.W. Collins. 1975. Flavonoids of Philydrum lanuginosum. Phytochemistry 14:315-316.
8. Brown, R. 1833. Characters and descriptions of Limnanthes. London & Edin. Philos. Mag. III, 2:70.
9. Cole, D.F. 1974. Effects of temperature and light on germination of two accessions of Limnanthes alba seed. Economic Botany 28:155-159.
10. Cronquist, A. 1968. The Evolution and Classification of Flowering Plants. Houghton Mifflin, Boston.
11. Daumer, K. 1958. Blumenfarben, wie sie die Bienen sehen. Zeitschr. Vergl. Physiol. 41:49-110.
12. Davis, P.H. & V.H. Heywood. 1963. Principles of Angiosperm Taxonomy. Van Nostrand, Princeton, N.J.
13. Dement, W.A. & P.H. Raven. 1974. Pigments responsible for ultraviolet patterns in flowers of Oenothera (Onagraceae). Nature 252:705-706.
14. Engler, A. & K. Prantl. 1896. Die natürlichen Pflanzenfamilien, Teil 3 (Abt. 5):136-137. Leipzig.
15. Ettlinger, M.G. & A.J. Lundeen. 1956. The mustard oil of Limnanthes douglasii seed, m-methoxybenzyl isothiocyanate. J.A.C.S. 78:1952-1954.
16. Gentry, H.S. & R.W. Miller. 1965. The search for new industrial crops IV. prospects of Limnanthes. Economic Botany 19:25-32.
17. Gray, A. 1897. Synoptical Flora of North America 1 (part 1, fasc. 2) New York.
18. Greene, E.L. 1891. Flora Franciscana. San Francisco.
19. 1895. Novitates occidentales. XII. Erythea 3:62.

20. Harborne, J.B. 1965. Plant polyphenols XV. Flavonols as yellow flower pigments. *Phytochemistry* 4:647-657.
21. 1967. Comparative Biochemistry of the Flavonoids. Academic Press, London.
22. Hartweg, T. 1848. Journal of a mission to California. *Jour. Hort. Soc London* 3:220.
23. Higgins, J.I., W. Calhoun, B.C. Willingham, D.H. Dinkel, W.L. Raisler & G.A. White. 1971. Agronomic evaluation of prospective crop species II. The American Limnanthes. *Economic Botany* 25:44-54.
24. Horovitz, A. & Y. Cohen. 1972. Ultraviolet reflectance characteristics in flowers of crucifers. *Amer. J. Botany* 59:706-713.
25. Howell, J.T. 1943. *Plantae occidentales* III. Leaflet. *West. Bot.* 3:206.
26. Howell, T. 1897. *Flora of Northwest America*, Vol. 1 (Phanerograms). Portland, Ore.
27. Hutchinson, J. 1926. *The Families of Flowering Plants*. Macmillan & Co., Ltd., London.
28. Jepson, W.L. 1936. *Flora of California* 2:410-412. San Francisco.
29. 1951. *A Manual of the Flowering Plants of California*. Univ. of Calif. Press, Berkeley.
30. Kodak Data Book M-27. 1968. Ultraviolet and fluorescence photography. Eastman Kodak Co., Rochester.
31. Loudon, J.C. 1855. *Encyclopedia of Plants*. London.
32. Mabry, T.J., K.R. Markham & M.B. Thomas. 1970. *The Systematic Identification of Flavonoids*. Springer-Verlag, New York.
33. Macior, L.W. 1971. Co-evolution of plants and animals ---systematic insights from plant-insect interactions. *Taxon* 20:17-28.
34. Macoun, J. 1883-1886. *Catalogue of Canadian plants*. vols. 1,3. Montreal.
35. Maheshwari, P. & B.M. Johri. 1956. The morphology and embryology of Floerkea proserpinacoides Willd. with a discussion on the systematic position of the family Limnanthaceae. *Bot. Mag. Tokyo* 69:410-423.
36. Mason, C.T., JR. 1951. Development of the embryo-sac in the genus Limnanthes. *Amer. J. Bot.* 38:17-22.
37. 1952. A systematic study of the genus Limnanthes R. Br. Univ. Calif. Publ. Bot. 25:455-512.
38. Mathur, N. 1956. The embryology of Limnanthes. *Phytomorphology* 6:41-51.
39. Miller, R.W., M.E. Daxenbichler, F.R. Earle & H.S. Gentry. 1964. Search for new industrial oils. VIII. The genus Limnanthes. *J.A.O.C.S.* 41:167-169.

40. Mosquin, T. 1966. Reproductive specialization as a factor in the evolution of the Canadian flora. In: Taylor, R.L. & R.A. Ludwig (Eds.). The Evolution of Canada's Flora. Univ. of Toronto Press.
41. Munz, P.A. & D.D. Keck. 1963. A California Flora. Univ. of Calif. Press, Berkeley.
42. Niemann, G.J. 1972. Phenolics from Larix needles IV. Constituents of L. laricina. Acta Bot. Neerl. 21:549-552.
43. 1973. Flavonoids from needles of Larix leptolepis. Phytochemistry 12:2056.
44. Ornduff, R. 1969a. Limnanthes vinculans, a new California endemic. Brittonia 21:11-14.
45. 1969b. Reproductive biology in relation to systematics. Taxon 18:121-244.
46. 1971. Systematic studies of Limnanthaceae. Madrono 21:103-111.
47. Ornduff, R. & T.J. Crovello. 1968. Numerical taxonomy of the Limnanthaceae. Amer. J. Bot. 55:173-182.
48. Ornduff, R. & T. Mosquin. 1970. Variation in the spectral qualities of flowers in the Nymphoides indica complex (Menyanthaceae) and its possible adaptive significance. Canad. J. Bot. 48:603-606.
49. Parker, W.H. & B.A. Bohm. 1975. Flavonol glycosides of Limnanthes douglasii. Phytochemistry 14:553-556.
50. Pardhasaradhi, M. & G.S. Sidhu. 1972. Obtusifoliol, syringetin and di-hydrosyringetin from Soyimida febrifuga. Phytochemistry 11:1520-1521.
51. Peck, M.E. 1937. New plants from Oregon. Proc. Biol. Soc. Wash. 50:93-94.
52. Rydberg, P.A. 1900. Catalogue of the flora of Montana and the Yellowstone Park. Memoirs of the N.Y. Bot. Garden 1:1-492.
53. 1910. Limnanthaceae. North American Flora 25:97-100.
54. Salisbury, E.L. 1942. The Reproductive Capacity of Plants. Bell, London.
55. Sokal, R.S. & P.A. Sneath. 1963. Principles of Numerical Taxonomy. W.H. Freeman & Co., San Francisco.
56. Somaroo, B.H., M.L. Thakur & W.F. Grant. 1973. A useful spray reagent to differentiate common phenolic compounds on thin-layer plates and paper chromatograms. J. Chromatography 87:290-293.
57. Stebbins, G.L. 1974. Flowering Plants: Evolution above the Species Level. Belknap (Harvard Univ. Press), Cambridge.
58. Takhtajan, A. 1969. Flowering Plants: Origin and Dispersal. Oliver & Boyd, Edinburgh.

59. Thompson, W.R., J. Meinwald, D. Anashansley & T. Eisner. 1972. Flavonols: pigments responsible for ultraviolet absorption in nectar guide of flower. *Science* 77:528-530.
60. Toy, S.J. & B.C. Willingham. 1966. Effects of temperature on seed germination of ten species and varieties of Limnanthes. *Economic Botany* 20:71-75.
61. 1967. Some studies on secondary dormancy in Limnanthes seed. *Economic Botany* 21:363-366.
62. Trelease, W. 1887. North American Geraniaceae. *Mem. Boston Soc. Nat. Hist.* 4:84-85.
63. Tyukavkin, N.A., S.A. Medvedev & S.Z. Ivanov. 1974. New flavonol glycosides from Larix siberica. *Khim. Prir. Soedin.* 1974(2), 157-160.
64. Von Frisch, K. 1967. *The Dance, Language and Orientation of Bees*. Harvard University Press, Cambridge.
65. Warburg, E.F. 1938. Taxonomy and relationship in the Geraniales in the light of their cytology. *New Phytologist* 37:130-159, 189-209.
66. Willdenow. 1801. *Neue Schrift. Gesellschaft Nat.* 3:449.

APPENDIX I

Mason's Classification of Limnanthes R. Br.¹

Section Inflexae

1. L. floccosa Howell var. pumila (Howell) Mason
" var. bellingeriana (M.E. Peck) Mason
" var. floccosa
2. L. alba Benth. var. alba
" var. versicolor (Greene) Mason
3. L. gracilis Howell var. gracilis
" var. parishii (Jepson) Mason
4. L. montana Jepson

Section Reflexae

5. L. douglasii R. Br. var. douglasii
" var. nivea Mason
" var. sulphurea Mason
" var. rosea (Benth.) Mason
6. L. striata Jepson
7. L. bakeri J.T. Howell
8. L. macounii Trelease

1 -- Mason, C.T. 1952. A systematic study of the genus Limnanthes R. Br.
University of California Publications in Botany 25:455-512.

APPENDIX II

Voucher Specimens of Limnanthes Taxa Grown from Seed¹

OTU	Taxon Name	U.S.D.A. Plant Accession No.	W.H. Parker Collection No.
1	<u>L. douglasii</u> var. <u>douglasii</u>	278170	114,116,138
2	<u>L. douglasii</u> var. <u>douglasii</u>	283708	105,165
3	<u>L. douglasii</u> var. <u>nivea</u>	283713	100,166,117
4	<u>L. douglasii</u> var. <u>rosea</u>	283715	104,169,118
5	<u>L. douglasii</u> var. <u>sulphurea</u>	283718	101,170
7	<u>L. bakeri</u>	283706	102,120
8	<u>L. striata</u>	283727	106,119
9	<u>L. macounii</u>	315048	112
10	<u>L. macounii</u>		128,167
11	<u>L. alba</u> var. <u>alba</u>	283701	111,160
12	<u>L. alba</u> var. <u>alba</u>	B55689	113
13	<u>L. alba</u> var. <u>versicolor</u>	283705	108,157
14	<u>L. gracilis</u> var. <u>gracilis</u>	283722	164,168
15	<u>L. gracilis</u> var. <u>gracilis</u>	283723	107
16	<u>L. gracilis</u> var. <u>parishii</u>	283724	103,159
17	<u>L. montana</u>	283725	109,158,161
18	<u>L. floccosa</u> ssp. <u>bellingneriana</u>	283720	115,162
20	<u>L. floccosa</u> ssp. <u>pumila</u>	283721	110,163

1 -- Voucher specimens are deposited in the Herbarium of the University of British Columbia.

APPENDIX III

TLC Map¹ of Petal² Flavonoids of
L. douglasii var. douglasii, OTU 1

1) See Materials and Methods for details of extractions and developments

APPENDIX IV

TLC Map of Whole Plant Flavonoids of
L. douglasii var. douglasii, OTU 1

APPENDIX V

TLC Map of Petal Flavonoids of
L. douglasii var. douglasii, OTU 2

APPENDIX VI

TLC Map of Whole Plant Flavonoids of

L. douglasii var. douglasii, OTU 2

APPENDIX VII

TLC Map of Petal Flavonoids of
L. douglasii var. nivea, OTU 3

APPENDIX VIII

TLC Map of Whole Plant Flavonoids of

L. douglasii var. nivea, OTU 3

APPENDIX IX

TLC Map of Petal Flavonoids of
L. douglasii var. rosea, OTU 4

APPENDIX X

TLC Map of Whole Plant Flavonoids of

L. douglasii var. rosea, OTU 4

APPENDIX XI

TLC Map of Petal Flavonoids of
L. douglasii var. sulphurea, OTU 5

APPENDIX XII

TLC Map of Whole Plant Flavonoids of
L. douglasii var. sulphurea, OTU 5

APPENDIX XIII

TLC Map of Whole Plant Flavonoids of
L. vinculans, OTU 6

APPENDIX XIV

TLC Map of Petal Flavonoids of
L. bakeri, OTU 7

APPENDIX XV

TLC Map of Whole Plant Flavonoids of
L. bakeri, OTU 7

APPENDIX XVI

TLC Map of Petal Flavonoids of
L. striata, OTU 8

APPENDIX XVII

TLC Map of Whole Plant Flavonoids of
L. striata, OTU 8

APPENDIX XVIII

TLC Map of Petal Flavonoids of

I. macounii, OTU 9

APPENDIX XIX

TLC Map of Whole Plant Flavonoids of
L. macounii, OTU 9

APPENDIX XX

TLC Map of Petal Flavonoids of

L. macounii, OTU 10

APPENDIX XXI

TLC Map of Whole Plant Flavonoids of
L. macounii, OTU 10

APPENDIX XXII

TLC Map of Petal Flavonoids of
L. alba var. alba, OTU 11

APPENDIX XXIII

TLC Map of Whole Plant Flavonoids of

L. alba var. alba, OTU 11

APPENDIX XXIV

TLC Map of Petal Flavonoids of

L. alba var. alba, OTU 12

APPENDIX XXV

TLC Map of Whole Plant Flavonoids of
L. alba var. alba, OTU 12

APPENDIX XXVI

TLC Map of Petal Flavonoids of
L. alba var. versicolor, OTU 13

APPENDIX XXVII

TLC Map of Whole Plant Flavonoids of
L. alba var. versicolor, OTU 13

APPENDIX XXVIII

TLC Map of Petal Flavonoids of
L. gracilis var. gracilis, OTU 14

APPENDIX XXIX

TLC Map of Whole Plant Flavonoids of
L. gracilis var. gracilis, OTU 14

APPENDIX XXX

TLC Map of Petal Flavonoids of
L. gracilis var. gracilis, OTU 15

APPENDIX XXXI

TLC Map of Whole Plant Flavonoids of
L. gracilis var. gracilis, OTU 15

APPENDIX XXXII

TLC Map of Petal Flavonoids of
L. gracilis var. parishii, OTU 16

APPENDIX XXXIII

TLC Map of Whole Plant Flavonoids of
L. gracilis var. parishii, OTU 16

APPENDIX XXXIV

TLC Map of Petal Flavonoids of

L. montana, OTU 17

APPENDIX XXXV

TLC Map of Whole Plant Flavonoids of
L. montana, OTU 17

APPENDIX XXXVI

TLC Map of Petal Flavonoids of
L. floccosa ssp. bellingneriana, OTU 18

APPENDIX XXXVII

TLC Map of Whole Plant Flavonoids of
L. floccosa ssp. bellingermana, OTU 18

APPENDIX XXXVIII

TLC Map of Whole Plant Flavonoids of
L. floccosa ssp. bellingneriana, OTU 19

APPENDIX XXXIX

TLC Map of Petal Flavonoids of
L. floccosa ssp. pumila, OTU 20

APPENDIX XL

TLC Map of Whole Plant Flavonoids of
L. floccosa ssp. pumila, OTU 20

APPENDIX XL I

TLC Map of Whole Plant Flavonoids of

L. floccosa ssp. pumila, OTU 21

APPENDIX XLII

TLC Map of Whole Plant Flavonoids of
L. floccosa ssp. grandiflora, OTU 22

APPENDIX XLIII

TLC Map of Whole Plant Flavonoids of
L. floccosa ssp. floccosa, OTU 23

APPENDIX XLIV

TLC Map of Whole Plant Flavonoids of

L. floccosa ssp. floccosa, OTU 24

APPENDIX XLV

TLC Map of Whole Plant Flavonoids of
L. floccosa ssp. floccosa, OTU 25

APPENDIX XLVI

TLC Map of Whole Plant Flavonoids of
L. floccosa ssp. californica, OTU 26

APPENDIX XLVII

TLC Map of Whole Plant Flavonoids of
F. proserpinacoides, OTU 27

APPENDIX XLVIII

TLC Map of Whole Plant Flavonoids of
F. proserpinacoides, OTU 28

APPENDIX XLIX

TLC Map of Whole Plant Flavonoids of
F. proserpinacoides, OTU 29

APPENDIX L

TLC Map of Whole Plant Flavonoids of
F. proserpinacoides, OTU 30

APPENDIX LI

100 mHz NMR Spectrum of TMS Ether of Kaempferol 3-O- β -D-Rutinoside

- 189 -

APPENDIX LII

100 MHz, NMR Spectrum of TMS Ether of Quercetin 3-O- β -D-Rutinoside

APPENDIX LIII

100 MHz, NMR Spectrum of TMS Ether of Isorhamnetin 3-O- β -D-Rutinoside,

APPENDIX LIV

100 MHz ^1H NMR Spectrum of TMS Ether of Myricetin 3-O- β -D-Rutinoside

APPENDIX LV

100 MHz NMR Spectrum of TMS Ether of Laricytrin 3-O- β -D-Rutinoside

APPENDIX LVI

100 MHz NMR Spectrum of TMS Ether of Syringetin 3-O- β -D-Rutinoside

Xs, 30 OTUs, whole plant, Jaccard

[illegible]

- 197 -

X_s & T_s , 30 OTUs, whole plant, Jaccard

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
2	0.791667								
3	0.730769	0.785714							
4	0.826087	0.740741	0.689655						
5	0.730769	0.785714	0.733333	0.684615					
6	0.666667	0.724138	0.793103	0.814815	0.837143				
7	0.640000	0.586207	0.548327	0.666667	0.600000	0.600000			
8	0.650000	0.680000	0.692398	0.782609	0.692308	0.629630	0.600000		
9	0.736842	0.583333	0.538462	0.603696	0.538462	0.538462	0.636364	0.600000	
10	0.727273	0.653846	0.607143	0.615385	0.607143	0.607143	0.640000	0.608696	0.736842
11	0.666667	0.666667	0.733333	0.580645	0.625000	0.677419	0.454545	0.629630	0.481481
12	0.680000	0.678571	0.750000	0.586207	0.633333	0.689655	0.451613	0.708333	0.480000
13	0.826087	0.807692	0.814815	0.703704	0.750000	0.750000	0.551774	0.708333	0.608696
14	0.666667	0.538462	0.615385	0.625000	0.555556	0.615385	0.583333	0.619048	0.578947
15	0.625000	0.628630	0.703704	0.535714	0.586207	0.642857	0.482276	0.520000	0.478261
16	0.863636	0.769231	0.777778	0.800000	0.777778	0.714286	0.629630	0.818182	0.636364
17	0.708333	0.703704	0.777778	0.607143	0.655172	0.714286	0.666667	0.666667	0.500000
18	0.900000	0.720000	0.730769	0.826087	0.730769	0.730769	0.708333	0.850000	0.736842
19	0.782609	0.762231	0.846154	0.730769	0.777778	0.777778	0.629630	0.737130	0.636364
20	0.789474	0.625000	0.576923	0.652174	0.576923	0.576923	0.608696	0.736842	0.703882
21	0.666667	0.600000	0.615385	0.625000	0.615385	0.555556	0.520000	0.700000	0.500000
22	0.652174	0.653846	0.666667	0.555556	0.607143	0.607143	0.464286	0.541667	0.500000
23	0.636364	0.576923	0.592593	0.538462	0.592593	0.592593	0.500000	0.521739	0.550000
24	0.826087	0.807692	0.814815	0.703704	0.750000	0.750000	0.551774	0.708333	0.608696
25	0.652174	0.653846	0.666667	0.555556	0.607143	0.607143	0.464286	0.541667	0.500000
26	0.652174	0.653846	0.666667	0.555556	0.607143	0.607143	0.464286	0.541667	0.500000
27	0.619048	0.560000	0.518519	0.520000	0.518519	0.464286	0.423077	0.500000	0.450000
28	0.500000	0.461538	0.438571	0.473077	0.428571	0.379310	0.333333	0.573810	0.333333
29	0.400000	0.320000	0.296296	0.333333	0.296296	0.296296	0.291667	0.421053	0.352941
30	0.666667	0.600000	0.615385	0.625000	0.615385	0.555556	0.520000	0.700000	0.500000

Matrix of Similarity Coefficients Calculated for
30 OTUs by Simple Match Coefficient: All Concentrations

Xs & ts, 30 OTUs, Whole Plant, Simple Match

	1 21	2 22	3 23	4 24	5 25	6 26	7 27	8 28	9 29	10 20
2	0.891304									
3	0.847826	0.869565								
4	0.913043	0.847826	0.804348							
5	0.847826	0.869565	0.826087	0.934783						
6	0.804348	0.826087	0.869565	0.891304	0.913043					
7	0.804348	0.739130	0.695652	0.804348	0.739130	0.739130				
8	0.934783	0.826087	0.826087	0.891304	0.826087	0.782609	0.782609			
9	0.891304	0.782609	0.739130	0.804348	0.739130	0.739130	0.826087	0.826087		
10	0.869565	0.804348	0.760870	0.782609	0.760870	0.760870	0.804348	0.804348	0.891304	
11	0.804348	0.782609	0.826087	0.717391	0.739130	0.782609	0.608696	0.782609	0.695652	0.804348
12	0.826087	0.804348	0.847826	0.739130	0.760870	0.804348	0.630435	0.847826	0.717391	0.782609
13	0.913043	0.891304	0.891304	0.826087	0.847826	0.847826	0.717391	0.847826	0.804348	0.826087
14	0.847826	0.739130	0.782609	0.804348	0.739130	0.782609	0.782609	0.826087	0.826087	0.804348
15	0.804348	0.782609	0.826087	0.717391	0.739130	0.782609	0.652174	0.739130	0.739130	0.760870
16	0.934783	0.869565	0.869565	0.891304	0.869565	0.826087	0.782609	0.913043	0.826087	0.847826
17	0.847826	0.826087	0.869565	0.760870	0.782609	0.826087	0.652174	0.826087	0.739130	0.760870
18	0.956522	0.847826	0.847826	0.913043	0.847826	0.847826	0.847826	0.934783	0.891304	0.869565
19	0.891304	0.869565	0.913043	0.847826	0.869565	0.869565	0.782609	0.869565	0.826087	0.847826
20	0.913043	0.804348	0.760870	0.826087	0.760870	0.760870	0.804348	0.891304	0.891304	0.869565
21	0.847826	0.782609	0.826087	0.891304	0.804348	0.847826	0.804348	0.913043	0.847826	
22	0.826087	0.804348	0.804348	0.739130	0.760870	0.760870	0.673913	0.760870	0.760870	0.826087
23	0.826087	0.760870	0.760870	0.739130	0.760870	0.760870	0.717391	0.760870	0.804348	0.782609
24	0.913043	0.891304	0.934783	0.869565	0.891304	0.891304	0.760870	0.891304	0.804348	0.826087
25	0.847826	0.869565	0.956522	0.847826	0.847826	0.934783	0.891304	0.913043	0.978261	0.826087
26	0.826087	0.804348	0.847826	0.739130	0.760870	0.804348	0.673913	0.760870	0.760870	0.782609
27	0.826087	0.760870	0.717391	0.739130	0.717391	0.673913	0.673913	0.760870	0.760870	0.782609
28	0.652174	0.717391	0.717391	0.782609	0.782609	0.739130	0.739130	0.717391	0.739130	0.804348
29	0.739130	0.630435	0.586957	0.652174	0.586957	0.586957	0.630435	0.760870	0.760870	0.695652
30	0.847826	0.782609	0.739130	0.760870	0.739130	0.695652	0.695652	0.782609	0.782609	0.804348

APPENDIX LXI

Matrix of Similarity Coefficients Calculated for
18 OTUs by Jaccard Coefficient: High Concentrations

Xs, 18 OTUs, Petals, Jaccard

	3	8	7	2	5	4	10	1	9	18
	16	13	17	11	12	15	14			
8	0.480000									
7	0.444444	0.655172								
2	0.666667	0.560000	0.518519							
5	0.736842	0.555556	0.517241	0.750000						
4	0.857143	0.400000	0.423677	0.647059	0.631579					
10	0.631579	0.600000	0.615385	0.736842	0.714286	0.611111				
1	0.823529	0.428571	0.400000	0.571429	0.636364	0.705882	0.545455			
9	0.526316	0.520000	0.600000	0.631579	0.619048	0.500000	0.777778	0.454545		
18	0.466667	0.291667	0.269231	0.411765	0.350000	0.428571	0.388889	0.470588	0.352941	
16	0.714286	0.434783	0.400000	0.625000	0.526316	0.692308	0.588235	0.588235	0.470588	0.636364
13	0.266667 0.363636	0.217391	0.200000	0.312500	0.263158	0.307692	0.294118	0.222222	0.250000	0.444444
17	0.200000 0.272727	0.173913 0.800000	0.160000	0.250000	0.210526	0.230769	0.235294	0.166667	0.187500	0.333333
11	0.444444 0.571429	0.521739 0.416667	0.480000 0.333333	0.473684	0.409091	0.500000	0.450000	0.380952	0.350000	0.428571
12	0.500000 0.642857	0.565217 0.384615	0.520000 0.307692	0.576316 0.923077	0.454545	0.470588	0.500000	0.428571	0.400000	0.500000
15	0.428571 0.600000	0.260870 0.571429	0.240000 0.428571	0.375000 0.500000	0.315789 0.461538	0.500000	0.352941	0.352941	0.235294	0.555556
14	0.363636 0.444444	0.695452 0.312500	0.640000 0.250000	0.523810 0.750000	0.458333 0.705882	0.400000 0.375000	0.571429	0.320000	0.476190	0.333333
20	0.333333 0.428571	0.434783 0.250000	0.400000 0.272727	0.444444 0.692308	0.380952 0.642857	0.375000 0.333333	0.421053 0.625000	0.285714	0.315789	0.285714

APPENDIX LXII

Matrix of Similarity Coefficients Calculated for
18 OTUs by Simple Match Coefficient: High Concentrations

Xs, 18 OTUs, Petals, Simple Match

	3 16	8 13	7 17	2 11	5 17	4 15	10 14	1	9	18
8	0.717391									
7	0.673913	0.782609								
2	0.869565	0.760870	0.717391							
5	0.891304	0.739130	0.695652	0.891304						
4	0.956522	0.673913	0.673913	0.869565	0.847826					
10	0.847826	0.782609	0.782609	0.891304	0.869565	0.847826				
1	0.934783	0.652174	0.608696	0.804348	0.826087	0.891304	0.782609			
9	0.804348	0.739130	0.782609	0.847826	0.826087	0.804348	0.913043	0.739130		
18	0.826087	0.630435	0.586957	0.782609	0.717391	0.826087	0.760870	0.804348	0.760870	
16	0.913043	0.717391	0.673913	0.869565	0.804348	0.913043	0.847826	0.847826	0.804348	0.913043
13	0.760870	0.608696	0.565217	0.760870	0.695652	0.804348	0.739130	0.695652	0.739130	0.891304
17	0.739130	0.586957	0.543478	0.739130	0.673913	0.782609	0.717391	0.673913	0.717391	0.869565
11	0.782609	0.760870	0.717391	0.782609	0.717391	0.826087	0.760870	0.717391	0.717391	0.826087
12	0.804348	0.782609	0.739130	0.804348	0.739130	0.804348	0.782609	0.739130	0.739130	0.847826
15	0.826087	0.630435	0.586957	0.782609	0.717391	0.869565	0.760870	0.760870	0.717391	0.913043
14	0.695652	0.847826	0.804348	0.782609	0.717391	0.739130	0.804348	0.630435	0.760870	0.739130
20	0.739130	0.717391	0.673913	0.782609	0.717391	0.782609	0.760870	0.673913	0.717391	0.782609
	0.826087	0.804348	0.826087	0.913043	0.891304	0.826087	0.869565			

APPENDIX LXIII

Matrix of Similarity Coefficients Calculated for
18 OTUs by Jaccard Coefficient: All Concentrations

Xs #ts, 18 OTUs, Petals, Jaccard

	3 16	8 13	7 17	2 11	5 12	4 15	10 14	1	9	18
8	0.481481									
7	0.451613	0.727273								
2	0.666667	0.500000	0.468750							
5	0.636364	0.548387	0.558824	0.652174						
4	0.857143	0.407407	0.387097	0.647059	0.545455					
10	0.461538	0.666667	0.617647	0.538462	0.538462	0.440000				
1	0.608696	0.580645	0.542857	0.625000	0.607143	0.521739	0.566667			
9	0.684211	0.571429	0.580645	0.789474	0.818182	0.666667	0.680000	0.576923		
18	0.578247	0.500000	0.424242	0.523810	0.407407	0.555556	0.538462	0.695652	0.478261	
16	0.750000	0.538462	0.451613	0.666667	0.565217	0.733333	0.583333	0.608696	0.684211	0.666667
13	0.444444	0.461538	0.387097	0.473684	0.360000	0.500000	0.500000	0.521739	0.428571	0.647059
17	0.388889	0.479077	0.354839	0.421053	0.320000	0.437500	0.458333	0.478261	0.380952	0.588235
11	0.409091	0.535714	0.454545	0.434783	0.392857	0.450000	0.576923	0.600000	0.458333	0.650000
12	0.526316	0.576923	0.483871	0.550000	0.480000	0.500000	0.625000	0.652174	0.571429	0.727273
15	0.444444	0.461538	0.387097	0.473684	0.360000	0.500000	0.500000	0.521739	0.428571	0.647059
14	0.360000	0.703704	0.500000	0.440000	0.448276	0.391304	0.629630	0.482759	0.520000	0.500000
20	0.363636	0.615385	0.474242	0.391304	0.357143	0.400000	0.538462	0.500000	0.416667	0.523810

Xsfts, 18 OTUs, Petals, Simple Match

	3	8	7	2	5	4	10	1	9	18
	14	13	17	11	12	15	14			
8	0.695652									
7	0.630435	0.804348								
2	0.869565	0.695652	0.630435							
5	0.826087	0.695652	0.673913	0.826087						
4	0.956522	0.652174	0.586957	0.869565	0.782609					
10	0.695652	0.782609	0.717391	0.739130	0.739130	0.695652				
1	0.804348	0.717391	0.652174	0.804348	0.760870	0.760870	0.717391			
9	0.869565	0.739130	0.717391	0.913043	0.913043	0.869565	0.826087	0.760870		
18	0.826087	0.695652	0.586957	0.782609	0.652174	0.826087	0.739130	0.847826	0.739130	
16	0.913043	0.739130	0.630435	0.869565	0.782609	0.913043	0.782609	0.804348	0.869565	0.869565
13	0.782609	0.695652	0.586957	0.782609	0.652174	0.826087	0.739130	0.760870	0.739130	0.869565
17	0.760870	0.673913	0.565217	0.760870	0.630435	0.804348	0.717391	0.739130	0.717391	0.847826
11	0.717391	0.717391	0.608696	0.717391	0.630435	0.760870	0.760870	0.782609	0.717391	0.847826
12	0.804348	0.760870	0.652174	0.804348	0.717391	0.804348	0.804348	0.826087	0.804348	0.891304
15	0.782609	0.695652	0.586957	0.782609	0.652174	0.826087	0.739130	0.760870	0.739130	0.869565
14	0.652174	0.826087	0.630435	0.695652	0.652174	0.695652	0.782609	0.673913	0.739130	0.739130
20	0.695652	0.782609	0.586957	0.695652	0.608696	0.739130	0.739130	0.717391	0.695652	0.782609

APPENDIX LXV

Coordinates of 30 OTUs Plotted in Figure XV

FULL EVIDENCE 31 CHARACTER 30 TAXON WHOLE PLANTS FACTOR ANALYSIS			
3	5.10472	-1.35043	1.74875
8	2.38190	-1.34949	1.63352
7	12.56763	16.18733	-7.08433
2	2.68327	-1.55822	0.16525
5	6.49256	0.50374	2.37778
6	7.96075	-1.90374	5.16727
4	5.86290	-1.87779	4.02508
10	-1.92433	-2.91848	-0.18532
1	-1.61403	-1.72009	-2.56479
9	-2.77445	-3.32734	-0.51118
21	-3.41676	0.03864	-1.68558
25	-0.89394	-0.97814	-0.47168
19	0.64432	-0.88189	-0.96837
24	2.44781	-1.22541	0.07238
18	1.64478	-1.87544	0.88212
16	4.12720	-1.05214	2.21242
13	2.34465	-1.31800	-0.58141
17	1.47492	-1.09444	-0.49022
11	5.14329	-1.12005	3.25865
12	1.62888	-1.03165	0.26171
15	0.55283	-0.99326	-0.95467
14	-2.27061	-1.93151	-2.63492
22	1.39721	-1.21424	-1.27773
20	-3.11173	-1.81081	-2.40233
26	0.32894	-1.17416	-1.81768
23	-2.39296	-1.80789	-2.28976
29	-16.13764	6.13993	2.13874
28	-8.22529	2.50804	-0.90234
27	-10.99766	5.32908	1.86713
30	-11.02921	4.80788	1.01151

Principal Components Analysis

Whole Plant, 31 flavonoids, 30 OTUs

Coordinates of OTUs

APPENDIX LXVI

Coordinates of 30 OTUs Plotted in Figure XVI

FULL EVIDENCE 31 CHARACTER 30 TAXON WHOLE PLANTS FACTOR ANALYSIS(V			
3	3.21928	0.62447	4.49291
8	1.50211	-0.57834	2.75176
7	2.52730	21.45496	1.86687
2	2.55308	-0.11239	1.76760
5	2.75269	2.56790	5.82146
6	3.68256	0.30971	8.94659
4	2.87302	-0.23809	6.76683
10	0.50069	-3.71428	-1.29330
1	1.21282	-1.27731	-3.00657
9	0.34279	-3.82744	-2.06476
21	-1.40398	-0.95383	-3.41115
25	0.21078	-1.05147	-0.91014
19	1.40729	-0.10109	-0.37409
24	2.25668	0.08534	1.54881
18	1.69776	-1.09127	1.71099
16	2.18008	0.26560	4.26759
13	2.56977	0.18476	0.96680
17	1.83000	-0.05950	0.51094
11	2.35664	0.31730	5.71596
12	1.51780	-0.19365	1.20201
15	1.40422	-0.23906	-0.41786
14	0.94048	-1.72691	-3.45868
22	2.24218	0.07471	-0.16185
20	0.20707	-2.09082	-3.78400
26	1.79377	-0.19609	-1.23886
23	0.61740	-1.79867	-3.25904
29	-15.08429	-3.04708	-8.11638
28	-6.33614	-1.38548	-5.71783
27	-11.13691	-1.27501	-5.21317
30	-10.43195	-1.42650	-5.90945

Varimax Factor Analysis

Whole Plant, 31 flavonoids, 30 OTUs

Coordinates of OTUs

APPENDIX LXVIII

Coordinates of 18 OTUs Plotted in Figure XVIII

FULL (V.F.A.)			
3	2.80535	-1.39534	-1.45724
8	6.46086	2.86507	10.33435
7	7.53850	14.42979	5.31503
2	4.46101	0.30339	0.34355
5	7.40208	0.84145	0.86824
4	1.21041	-1.64758	-1.75707
10	5.14429	1.68514	1.06514
1	5.60514	-1.05767	-0.39649
9	2.44199	2.28316	0.21298
18	-5.45416	-3.24556	-3.29506
16	-1.95039	-2.53121	-2.70632
13	-9.58709	-3.54636	-3.58407
17	-10.97436	-3.74321	-3.82487
11	-2.32462	-1.19221	0.05549
12	-1.54074	-0.96795	0.04995
15	-7.37026	-3.59780	-3.62874
14	0.60115	2.05435	3.27592
20	-4.46916	-1.54248	-0.87129

Varimax Factor Analysis

Petals, 36 flavonoids, 18 OTUs

Coordinates of OTUs