TAXONOMY OF *LUPINUS LEUCOPHYLLUS* DOUGL.

A Thesis
Presented to
the Faculty of the Graduate School
University of Missouri

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

by

Michael P. Currier

Dr. David B. Dunn Thesis Supervisor

August, 1988

The undersigned, appointed by the Dean of the Graduate Faculty,
have examined a thesis entitled:

THE TAXONOMY OF LUPINUS LEUCOPHYLLUS DOUGL.

presented by MICHAEL P. CURRIER

a candidate for the degree of MASTER OF ARTS

and hereby certify that in their opinion it is worthy of acceptance.

[redacted]

DEDICATION

This thesis is dedicated to the memory of my father, Edward P. Currier, who died in February of 1987.

Many people knowingly and unknowingly helped with encouragement or assistance. I am indebted to Dr. David B. Dunn for his generosity and timely advice. Kathy Robertson got me started in Plant Taxonomy. Ruth Dalke always helped me find answers to even obscure questions. I appreciate the help Rick Klann and Dr. Les Uhazy provided with Principal Components Analysis and computer time. Dr. George Rottinghaus in the Veterinary Medicine Diagnostic Lab offered his assistance. I am grateful to Dr. Louis Sherman and the Biological Sciences Department at U.M.C. for being patient and giving me the freedom and resources to pursue my interests. The opportunity to teach Plant Taxonomy was invaluable and rewarding. Dr. John David, as graduate advisor, was helpful when problems arose. I thank the members of my committee: Dr. Bill Cumbie, Dr. Clair Kucera, and Dr. Vic Lambeth for their interest in my project. Through their instruction both in and out of class, my interest in botany and plant sciences grew. I am grateful to many professors both in Biological Sciences and Horticulture, too numerous to name. I am pleased to have had the opportunity to associate with Robin Kennedy and respect her for taking on difficult tasks. Sonja Smedley worked hard to keep the Herbarium operative. I appreciate the interest she had in the work I was doing and it was wonderful to share our plant interests. I am thankful for the kindness and support given by Susan Morse and Ellis Birch (my housemates); and Claire Stutesman, Jim Whitehill, Gary Schnurbusch, Susan and Rita and others. I am really lucky to have such friends.

The Taxonomy of Lupinus leucophyllus Dougl.

by

Michael P. Currier

Dr. David B. Dunn Thesis Supervisor

ABSTRACT

The objective of this thesis is to clarify the taxonomy of Lupinus leucophyllus Dougl. The morphological variability observed, has historically rendered the group as difficult, although the species has clear diagnostic characters and is easily recognizable today. Morphological analysis is combined with thin-layer chromatography of alkaloids and geographic information to arrive at a feasable taxonomic treatment at the subspecific level. Principal Components Analysis of 34 morphological attributes resulted in clusters of similiar morphological affinity.

Three subspecies are named based on the evidence: *ssp. leucophyllus*, *ssp. retrorsus*, and *ssp. erectus*. They are characterized by distinct morphological attributes, ecological adaptation, and geographic distribution. The *subspecies leucophyllus* contains three varieties. Varietal designations acknowledge morphologically distinct variants with broad distribution.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF FIGURES

I. INTRODUCTION

The angiosperm genus called Lupinus L. has roots in antiquity. Many authors wrote about this group including Hippocrates (460-377 B.C.), Theophrastus (287 B.C.), Cato (200 B.C.), Pliny (23-79 A.D.), and Crescentius (1300 A.D.). In ancient Rome roasted lupine seeds were considered a delicacy. Containing potentially toxic alkaloids, a special vessel was used ('labrum lupiniarum'), in which lupine seeds were soaked in the debittering process. Pliny recognized 35 uses for the plants, with the primary medicinal value as a cathartic and vermifuge.

Gross (1982) traces the agricultural history of lupines back to 4,000 to 3,000 B.C. in both the Mediterranean and South American (Andean) region. The Chinese use of soybean, Glycine max (L.) Merrill, is compared to the use of lupins (Lupinus albus, L. mutabilis Sweet) in both the Old and New Worlds. These leguminous plants were highly regarded for the beneficial effects ascribed to the root and nodules. It is of no wonder that lupine seeds were found in the tombs of Pharaohs, predating 2000 B.C., a valuable talisman to take to the life beyond.

Differing opinion is held concerning the origin of lupine cultivation for forage and food. Belteky and Kovacz (1984) support the theory that cultivated varieties were introduced into Egypt after 330 B.C. The more commonly accepted theory supports Egypt as the area of origin (Gladstones, 1974, Planchuelo, 1982). Gross (1982) theorizes, based on etymology, that the origins of cultivation may date back to before 2200-2500 B.C.

Through the centuries lupines have been known by a variety of names. The Greeks recognized 'Thermos', while the Egyptians used 'Thermes'. In Arabic and Indian dialects 'Turmus' described what are now refered to as Lupinus in the botanical realm. Among the Inca of Peru 'Tarhui' was the traditional name. In other South American localities the influence of the Spanish is observed as the name 'chocho' is commonly used. The Spanish associated this name to what we now recognize as L. albus L.. Gladstone (1974) and

Gross (1982) both recognize the widespread use of lupines in ancient cultures.

With the decline of the Roman Empire, and growing Indo-European influence, the agricultural use of lupine declined. In the Peruvian Highlands the Incas were practicing crop rotation utilizing 'Tarhui' when the Spanish Conquistadores arrived in the sixteenth century. In Europe, lupines were associated with peasant use and had low agricultural status. This is reflected in the name *lupinus* which, according to source, is derived either from the latin 'lupi' (wolves) or the Greek 'lype' (meaning pain, sadness, bitterness). (Pizzetti, 1968). Lupines were described as bitter, were primarily utilized by the poor, and generally disregarded by agricultural planners within the sphere of Indo- European influence.

Renewed interest in crop lupines resulted from the discovery of low alkaloid forms of Lupinus angustifolius L. and L. luteus L., made possible by the development of a fast method to quantify alkaloid content by Von Sengbusch in 1928. Within several years low alkaloid (sweet) forms of L. albus, L. polyphyllus Lindl., and L. perenis L. were established. Sweet lupines contain only .01 - 0.5% alkaloid compared to the 1.0-2.5% in the parental species (Bogler, 1985). These developments are indirectly responsible for the elevated economic status the genus Lupinus has today. Through continued breeding work, lupines have become more refined as agricultural crops; with production now occurring in the Soviet Union, Poland, Australia, New Zealand, South Africa, and Chile.

II. TAXONOMIC HISTORY OF LUPINUS

The genus name Lupinus L., first used as a botanical reference by Tournefort, is taxonomically recognized because of it's inclusion in Carolus' Linneus' important publication *Species Plantarum*, dated 1753. Among the six species described therein, L. perennis L. was a New World entity from Virginia. L. albus, L., the type species of the genus Lupinus, is the Old World annual described as economically important in Egyptian, Greek, and Roman civilizations.

Dziekanowski (1971) provides an eloquent account of the history of Lupinus classification. As exploration of the New World continued, the number of described species increased greatly. The discovery, description, and transportation of new plants to the Old World Botanical Centers was an activity of political and economic significance.

In 1825, De Candolle in his publication, *Prodromus*, recognized thirty-six species of lupines, separated into annuals and perennials. The first and only worldwide monograph (to date) of the genus, *Synopsis Generis Lupini*, was published in 1835 by Jacob Georg Agardh, Swedish botanist and professor of botany at Lund. This remarkable work separates the genus into 5 groups of annuals and 7 groups of perennials. Seven other species are delegated to "Species Inquirende" of uncertain affinity; for a total of 83 entities described. Twelve groups were circumscribed based on both technical vegetative and floral characteristics. Placed in the tribe *Sericei*, Lupinus leucophyllus Dougl. and Lupinus plumosus Dougl. share affinity with other Great Basin perennials: L. arridus Dougl., L. laxiflorus Dougl. and L. sericeus Pursh.

Torrey and Gray (1838-1840), in the *Flora of North America*, published an artificial key with a section on Lupinus which generally follows Agardh's delineation; recognizing 45 species and 5 varieties, based on the collections of Douglas, Wyeth, and Nuttall. L. leucophyllus and L. plumosus, were grouped along with the previously mentioned Great Basin perennials. Comments included attest to the confusion in properly discriminating between L. leucophyllus and L. plumosus as labeled in the garden of the London

Horticultural Society. Nonetheless they were recognized as two distinct species by Torrey and Gray.

About thirty years later, Sereno Watson published a monographic revision of the extra tropical species of the genus Lupinus. Subdivisions include: *Lupinus proper* subdivided into unifoliolate perennials, palmately compound multifoliolate perennials and annuals; *Platycarpos*, annuals with ovate pods and two ovules; and *Lupinellus*, consisting of a single species, L. uncialis, Watson. These subdivisions were based on differences in habit and number of ovules. Watson's treatment placed L. plumosus in synonomy with L. leucophyllus.

From 1875 until the publication of the *Flora of the Rocky Mountains and Adjacent Plaines,* by Swedish botanist Per Axel Rydberg (1917), approxiamtely 300 names (including varieties) were published in North America and Mexico. During the later half of the 19th century, many European and American botanists collected in Mexico including Cyrus Pringle, Dr. Edward Palmer, and Joseph Rose (Bogler, 1985).

Studies after 1900 were focused on regional treatments. Charles Vancover Piper, professor of botany at Pullman, Washington and Benjamin Lincoln Robinson, assistant to Sereno Watson at the Gray Herbarium, in the *Flora of Washington* (1906), established as subgenera Watson's *Lupinus* proper and *Platycarpos*. They also described sections based on the supraspecific groupings of Agardh, though these were reinterpreted and added to in the process. Piper and Robinson recognized 28 species, 4 subspecies, and one form of Lupinus for the state of Washington. (Phillips, 1955).

Interest in Lupinus in the Pacific Northwest resulted in a number of new names. Though L. leucophyllus continued to be grouped with *Sect Sericei*, it was now associated with Lupinus canescens Howell, described by the Oregon botanist in 1893.

Several additional names became associated with L. leucophyllus during the next fifteen years. L. retrorsus Henderson (described in 1900); L. macrostachys Rydberg (1907); L. jonesii Rydberg (1903); L. barbiger Watson (1873); and L. ramosus Nelson (1899) were placed along with L. leucophyllus and L. plumosus in *Sect. Leucophylli* of

Rydberg's Flora. In all nineteen sections were recognized based on vegetative and floral distinctions. Names presently recognized as allied to L. leucophyllus were placed in other sections: L. erectus Henderson (1900) in *Sect. Lepidi;* and L. tenuispicus A. Nels. (1912) in *Sect. Ornati.*

Charles Piper Smith, a Canadian born botanist, studied at both Purdue and Stanford Universities. Working in a number of capacities during his life, he became a central figure in the study of Lupinus.

Collecting extensively in North and South America and Mexico, he privately funded the publication, *Species Lupinorum* (1938-1953), as a vehicle to coordinate his efforts to study the genus. He knew of the presence of alkaloids in Lupinus, but relied on morphological characters in his treatment. Apparently, in his zeal to resolve a difficult group, C.P. Smith created a large number of new names, many based on a single specimen. In addition, many of his descriptions are not clear and some keys simply do not work very well. (Bogler, 1985).

His contribution to the flora of North America is valuable. In Abrams, *Illustrated Flora of the Pacific States* (1944), C.P. Smith provided a regional key for lupines. Nineteen sections are recognized, with L. leucophyllus and L. Peirsonii H.L. Mason (1928) comprising *Sect. Leucophylli.* Several names are reduced to synonomy including L. plumosus and several varieties appeared including *var. Belliae* C.P. Smith, *var. canescens* (Howell) C.P. Smith, and *var. tenuispicus* (Nels.) C.P. Smith These were all published in 1924. Varietal designations were based on flower size and pubescence type. Among C.P. Smith's publications, *A Distributional Catalogue of the Lupines of Oregon* (1927), remains as a valuable work. During the early 1900's, several botanists were active in the study of Lupinus including: Alice Eastwood, L.E. Detling, Harold St. John and A.A. Heller. In 1955, Lyle L. Phillips published, *A Revision of the Perennial Species of* Lupinus *of North America, exclusive of Southwestern United States and Mexico.* In discussing the nomenclatural confusion of the genus (800-900 epithets for North America), he recognized that in the past the effort was to categorize and describe variability rather than study evolutionary relationships. More specifically, he described L. leucophyllus as having considerable natural variation. He regarded the variability observed in the species to be independently segregating characters without morphological or geographical distinctness.

Phillips primary contribution was the determination of chromosome numbers for all taxa of northwestern United States Lupinus. Several taxa, including L. leucophyllus, were observed to contain morphologically indistinguishable 'chromosome races' of 48 and 96, (octoploid and sixteen-ploid from a base number of six). He also added another morphological attribute to the growing list, 'the banner index', which later was used in the comprehensive floristic treatment, *Vascular Plants of the Pacific Northwest*, by C. Leo Hitchcock and Arthur Cronquist (1961).

In recent times the greatest contribution toward understanding the genus Lupinus has resulted from the efforts of Dr. David B. Dunn and his students: Hess,1969; Fleak,1971; Dziekanowski,1971; Christian,1971; Cox,1972; Schaller,1972; Harmon,1972; Rafail, 1976; Kenney,1977; Bennett, 1978; Planchuelo,1978; Conrad,1980; and Bogler,1985.

Dr. Dunn initiated breeding and experimental growth studies in the1940's and elucidated pollination mechanisms in *The Breeding Systems of Lupinus, Group Micranthi* (1956). Using an integrated biosystematic approach, both genetic and evolutionary relationships were considered in the treatments of various complexes. Breeding and chemical studies were utilized along with ecological and geographical information to support determinations based on morphological evidence. This approach allowed for the incorporation of new techniques to elucidate relationships including study of: alkaloids, flavonoids, gel electophoresis of proteins and computer based analysis.

Additional information supplied through molecular techniques and the use of more sophisticated computer software will add to the pool of information; to help clarify taxonomic relationships presently and in the future.

III. EVOLUTION OF LUPINUS

Bentham (1865), in *Genera Plantarum,* segregated the subfamily Papilionoideae of Leguminosae into eleven tribes based on habit, leaf characters, degree of staminal fusion, and fruit attributes. Lupinus L. was placed within the tribe *Genisteae.* To date, several modifications of this classical interpretation have been offered, but the overall delineation remains intact.

"If the tribes and subtribes of the classical system of Bentham are made equivalent in rank, the effect of more recent work has not had an extensive effect on generic groupings." (Polhill, 1985).

Taubert (1893) and successors, in *Pflanzenfamilien,* introduced changes in tribal order to indicate evolutionary trends and added genera. Hutchinson (1964), largely due to the increase in the number of described tropical genera, both elevated Bentham's subtribes to tribal level and added to them.

Genisteae was separated into nine tribes including *Lupineae*, into which Lupinus and Argyrolobium Eckl. & Zeyh. were placed. The primary criticism of Hutchinson's treatment is directed towards it's artificiality (Polhill,1981).

Modern interpretations have relied on cryptic features (chromosomes, anatomical features, and phytochemistry) to provide a greater understanding of evolutionary patterns.

Polhill (1981) subdivided Bentham's *Genisteae* into four tribes including: *Bossiaeae*, comprised of ten Australian genera; *Crotalarieae*, sixteen genera, mostly African; *Liparieae*, five genera restricted to the Cape Province of South Africa; and *Genisteae*, mostly European, including Lupinus.

Bisby (1981) further separates *Genisteae* into subtribes *Lupininae* and *Genistinae*. *Lupininae* contains the single genus Lupinus, mostly New World herbs with bluish flowers; while *Genistinae*, with nineteen genera, is mostly European shrubs and trees with yellow flowers.

While Lupinus is a distinct taxon based on morphology, pollination features, root-nodule characters, geographic data and seed protein serology; the Genista-Cytisus Complex has historically been a difficult group. Lupinus ranks as the largest genus of the tribe, although it's number of species is unresolved. Dunn (1984) estimates that out of 1700 described epithets, only 500 will remain as valid species or subspecies names.

Two theories describe the phylogeny of Lupinus. Dunn (1984) supports an origin from a Crotalariod ancester. Crotalaria L., with primitive traits including: shrubby habit, simple leaves, widespread subtropical distribution, and mostly diploid chromosome numbers, exhibits floral morphology very simliar to Lupinus (Dunn and Planchuelo,1981). Dunn and Gillett (1966) report a base number of six chromosomes for the genus. A reasonable evolutionary sequence would make the early species of Lupinus an aneuploid derivative of a simple leaved Crotalaria (N=7) ancestor.

Argyrolobium and Adenocarpus DC., traditionally placed in *Genisteae*, also appear to share affinity with members of *Crotalarieae*, perhaps providing another connecting link (Bisby,1981).

Polhill (1981) supports the derivation of *Genisteae* from woody *Thermopsideae*. With *Sophoreae* as a base group, the elaboration of lupine-type quinolizidine alkaloids provides a link from *Thermopsideae* to *Genisteae* (Figure 1).

By association, drawn from the derivation of Baptisia Vent. from Thermopsis R. Br.; Lupinus is said to have evolved from a Thermopsoid ancestor, arriving in the New World at a later date. *Crotalarieae*, with both quinolizidine and pyrrolizidine alkaloids, is considered an advanced tribe based on chemical attributes (Turner, 1981). Due to conflicting information, the phylogeny of Lupinus is left open to discussion and interpretation.

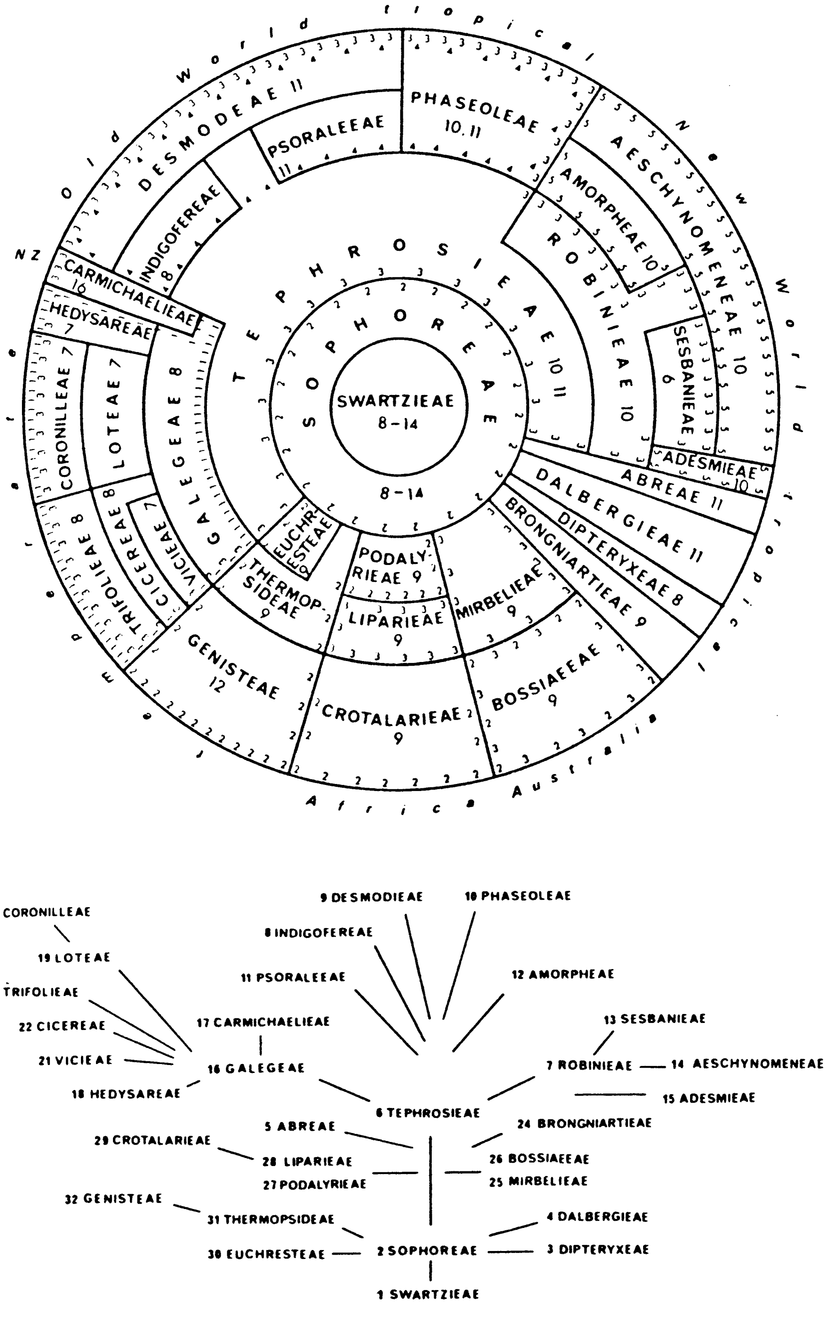


Figure 1. Simplified representation of the supposed relationship of tribes of Papilionoideae. 1- Dormer's epulvinate series minus Thermopsideae; 2- quinolizidine alkaloids; 3- canavanine; 4- marked thickening of endexine; 5- marked reduction of endexine. From Polhill, 1981.

III. EVOLUTION AND ADAPTATION

The origin of the family Leguminosae is placed at the end of the Cretaceous Period, 70 million years ago. (Raven In Arroyo, 1981). Described by Polhill, Raven, and Stirton (1981), the evolutionary development of Papilionoideae can be divided into three phases. Initially, basic types of foliation patterns, nectaries, and seed structures were established. In the second phase elaboration of flowers, development of chemical and biological defense systems, symbiotic relationships with Rhizobium and ectotrophic mycorrhizae, and elaborate mechanisms to make and break dormancy evolved. Those genera entering into the third phase are characterized by migration into savannas and geologically recent habitats; with abrupt diversification away from established patterns.

Lupinus, considering a Crotalaroid ancestry to be valid, evolved from a simple-leaved arborescent perennial. Originating in S.E. Brazil, the group radiated into the highlands of South America, spreading northward along mountain ranges during a series of glacial and interglacial periods. Modifications of vegetative parts including the development of palmately compound leaves, pubescence, and the ability to conduplicate offered an advantage in water-stressed alpine habitats.

Stebbins (1974) provides a summary of modifications favored in a deteriorating environment. Features facilitating the adaptation to alpine conditions include: reduction in growth; the development of hormonal mechanisms and specialized organs providing dormancy and renewal; physiological modifications and the development of protective coverings as well as various internal substances to provide protection against dessication, cold, and the attack of herbivores.

As open, disturbed habitats greatly expanded from the Miocene onward, Lupinus moved into the niche available to pioneering species. (Polhill,1981). The ability to generate polyploids, the presence of herbivore detering quinolizidine alkaloids, and symbiotic association with Rhizobium and other mycorrhizae offered selective advantage.

The cytological ability to form amphiploids enabled Lupinus to perpetuate an adaptively valuable hybrid through sexual reproduction. Amphiploidy is promoted in "long-lived perennials that exhibit a common occurrence of natural interspecific hybridization, accompanied by chromosome repatterning" (Grant,1981). Amphiploids, due to multiple sets of genes, have a greater capacity to adapt to the exigencies of the environment.

"Amphiploids exhibit superior vigor, homeostatic buffering and adaptability as compared to diploid relatives." (Grant, 1981).

Lupinus was able to colonize habitats left open by Pleistocene climatic and topographic changes. (Stebbins,1971). Love and Love (1949) correlate the occurrence of polyploidy to habitats characterized by disturbance or stress.

The genetic system, although offering advantage under certain conditions, imposes limitations on evolutionary potential by acting as a buffer against mutations and the generation of new variability. As such, there is infinite variability on an old theme. (Grant, 1981).

Lupinus exhibits a range of floral characteristics that promote reproductive success. Canalization of floral morphlogy maximizes the efficiency of flower-pollinator interaction providing an adaptive link between plant and pollinating agent. Lupine floral design is specialized for the protection of energetic resources; floral modification generally reflects coevolution with Hymenoptera. (Arroyo,1981).

Described by Dunn (1956), Lupinus possesses the typical papilionaceous flower, with certain modifications. Flowers are generally blue, yellow, or cream (all bee colors), and mostly lack nectar and scent. The banner at anthesis is reflexed and may include a light colored spot which serves to orient the pollinator and signal hidden rewards. Ultra-violet color guides are sometimes observed; L. nanus Dougl. (Jones and Buchanan, 1974). A dimorphic monadelphous androecium and modified gynoecium are enclosed within the distally fused incurved keel. At the acumen, a pore occurs through which the pollen reward is ultimately presented. Prior to anthesis, the upper anthers dehisce, forming a large pollen mass (Figure 2). The foraging pollinator, lands on the flower and mechanically 'trips' a piston mechanism whereby the lower stamens push the pollen mass past the stigma and through the distal pore. A ring of long, stiff hairs restricts self-pollination; acting as a physical isolating barrier. Sculpturing of the wing petals (Stirton,1981), facilitates the lever action induced by the pollinator as the keel is raised and lowered. Through this manipulation the pollinator retrieves pollen, while pollinating the exposed stigma. Cross-pollination depends on contact between a receptive stigma and pollen from another flower.

Lupinus, with a racemose inflorescence and a phenotypically controlled capabilitiy for inflorescence development, provides nutritive resources over an extended period of time. In some species after pollination, (24-36 hours), the banner spot changes color, indicating pollen resources are exhausted. This phenomena is described as 'post-pollination phenomena' (Gore,1983). Agents exhibiting pollinator constancy are presented with a reliable food source over an extended period of time. Constancy is mediated by visual signals (color guides) and an abundant energy (pollen) resource. Selection on the male component of plant fitness is the primary impetus for color change. (Gori, 1983).

The size of the flower may influence the identity of the pollinator (Dunn,1956) or indicate the potential for selfing (Harding, Mankinen, & Elliott,1974). Small flowered lupines, like L. bicolor Lindl., are wholly self-fertilized (autofertility). Other species, like L. nanus Dougl., show variability from population to population.

Symbiotic associations with Rhizobium Lupini Schroeter mycorrhizae give Lupinus the capacity to inhabit and modify nitrogen-deficient habitats. Lupines are characterized by high efficiency in the mobilization of nitrogen to fruits during seed filling (Pate, 1984). The capacity to fix nitrogen has obvious adaptive advantages. Biologically, the interaction between Rhizobium and Lupinus is influenced both by characteristics of the soil and climate. Some lupine nodules lack leghaemoglobin, indicating that not all strains are capable of nitrogen-fixation (Corby, 1981). Rhizobium spp. are sensitive to soil pH, with different strains active within different ranges. Tolerance limits in Rhizobium growth may

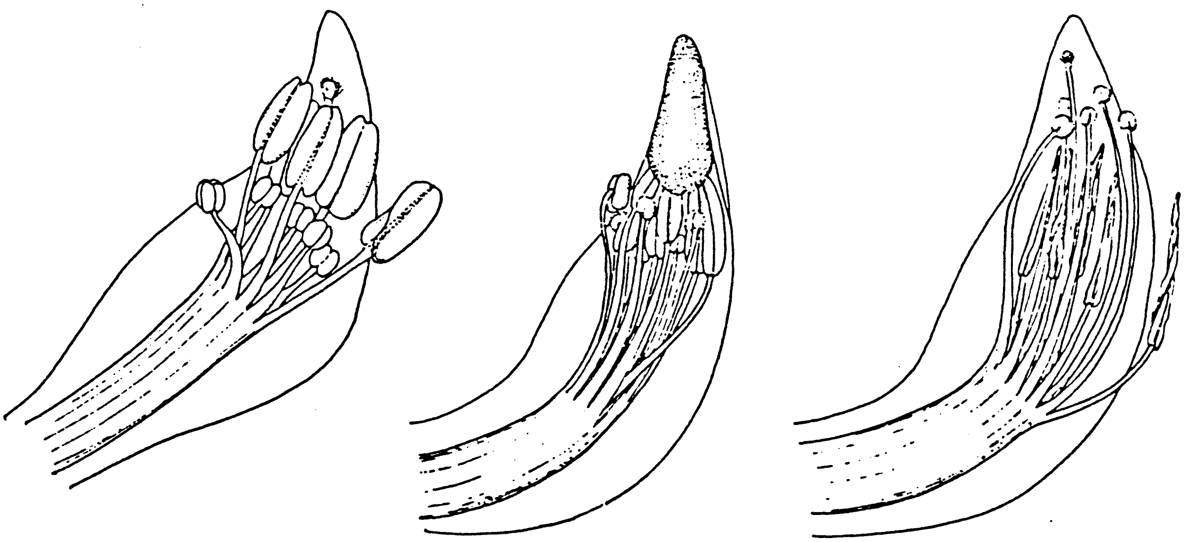


Figure 2. Three stages of pollen presentation in flowers of L. pilosus. (from left to right). From Plitmann, Heyn, and Hazy, 1979.

determine where host lupines occur since the pH range tolerated by the legumes is usually wider than the range for suitable nodulation and successful establishment (Mishustin and Shil'nikova, 1971). Also, in a nitrogen enriched habitat, nitrogen fixation is generally diminished.

Quinolizidine alkaloids, generally recognized as chemical feeding deterrants, function as an integrated adaptive system responsive to selective pressures at the population level. A study of alkaloid variability based on predation patterns of a small flower-feeding butterfly, *Glaucopsyche lygadamus (Doubleday)*, reveals a direct correlation between predation levels and amounts, kinds and individual variability of alkaloids produced in several populations of L. caudatus Kell. (Dolinger and Ehrlich,1973).

Extensive variability is exhibited throughout the range of the annual, L. nanus. (Mankinen and Harding,1975). All populations showed quantitative variability for at least two alkaloids. Polymorphism increases the efficiency and use of resources (Dobzhansky, 1957).

In L. amplus Greene, the selective response to lycaenid attack on flowers resulted in an advancement of flowering time to offset anthesis from lycaenid activity; the strategy being avoidance. (Breedlove and Ehrlich, 1968).

Downey and Dunn (1964) described a host specific symbiotic relationship between the blue butterfly (*Plebejus icarioides (Boisduval)*) and various lupine species. Specialized in feeding, the lycaenid is in this case tolerant of usually toxic alkaloids. Eggs are deposited on the undersides of densely pubescent, preferentially younger, leaves. The larvae are protected and cared for by ants in return for a sweet exudate produced by the abdominal glands of the larvae. Interestingly, the tendency of lupines to hybridize and introgress broadens the range of host-plant specificity; extending the range of the blue butterfly.

Fruit dispersal mechanisms influence the patterns of plant distribution. L. dedeckerae Munz and Dunn exhibits schizochory, explosive seed dispersal (Taylor,1981). Fruit development occurs on the lowermost whorls, proceeding upward as environmental conditions permit. With the drying and twisting of valves (autochorus dispersal), seeds are propelled a distance of 1-2 meters; with 1-2 seeds remaining to fall at the base of the mother plant. Mast year phenomena is implied. Perennial lupines, such as L. dedeckerae, show distribution in clusters and patches; ideal for bee foraging patterns.

Seeds are variable in color, size, weight, chemical content, and thickness of testa. Exhibiting cryptic colors and patterns, seeds are camouflaged to escape predation. In Artemisia-Purshia communities the deer mouse (Peromiscus maniculatus) is a primary granivore.

The hardness and thickness of the testa, which functions in inbibition, germination and protection, provides the seed with long viability.

Seeds of Lupinus arcticus S. Wats., found stored in lemming caverns beneath permanently frozen tundra in Upper Yukon, Alaska, exhibited the capacity to germinate after a period of 1500 years. Stored as a viable component of a seed bank, germination occurs when the conditions are favorable (Porsild, 1967).

Two weeks after the erruption of Mt. St. Helens, lupine seed were germinating in the ash along with fireweed and bunchgrass (Findley, 1981).

V. GEOGRAPHICAL DISTRIBUTION

The primary centers of diversity for the genus Lupinus are the Rocky Mountains and Sierras of western North America, and the Andes of South America. The uplands of East Central Brazil in the extensive range known as Serra do Espinhaco is theorized as the center of evolutionary origin. The geology of the area is complex, with a rich endemic flora, including simple-leaved lupines, occurring on isolated upland islands referred to as 'campo rupestre'. Thirteen unifoliolate species, all perennials, occupy the area; which includes a species (L. paraguariensis Chod & Hass) with a mixture of "unifoliolate" and multifoliolate leaves (Montierro and Gibbs, 1986). (Figures 3 and 4).

Long-range seed dispersal is implicated in the migration of the simple-leaved lupines of southeastern Brazil to the southeastern United States; though chromosome counts for the simple-leaved species are not available to substantiate this theory. (Dunn, 1971). Transport by man is one theory explaining the presence of genetically divergent annuals in the Mediterranean and African region. Dunn (1984) describes these species as "transported agronomic selections," based on chromosomal variability, seed size variability, and fruit characters.

The twelve species of Mediterranean and African lupines are all annuals, separated morphologically according to the designation "smooth-seeded" or "rough-seeded".

South American species occur in two ecogeographic regions, subdivided through the 64th parallel, into Andean and Atlantic Coast groups. Andean lupines, with compound leaves, range from stunted alpine forms to 'trees' 20 centimeters (diameter at breast height) and 4.5 meters tall. Perennials, biennials, and annuals are observed. Atlantic Coast species have either simple or palmately compound leaves. North American lupines are concentrated on the West Coast, with several species distributed throughout the Atlantic States, (eg. L. villosus Willd. and L. perennis L.). L. perennis migrated from Mexico after the last glacial period 10,000-11,000 years ago (Dunn, 1984). Advanced perennial complexes occupy alpine or glacial margins. The highest concentration of endemic species

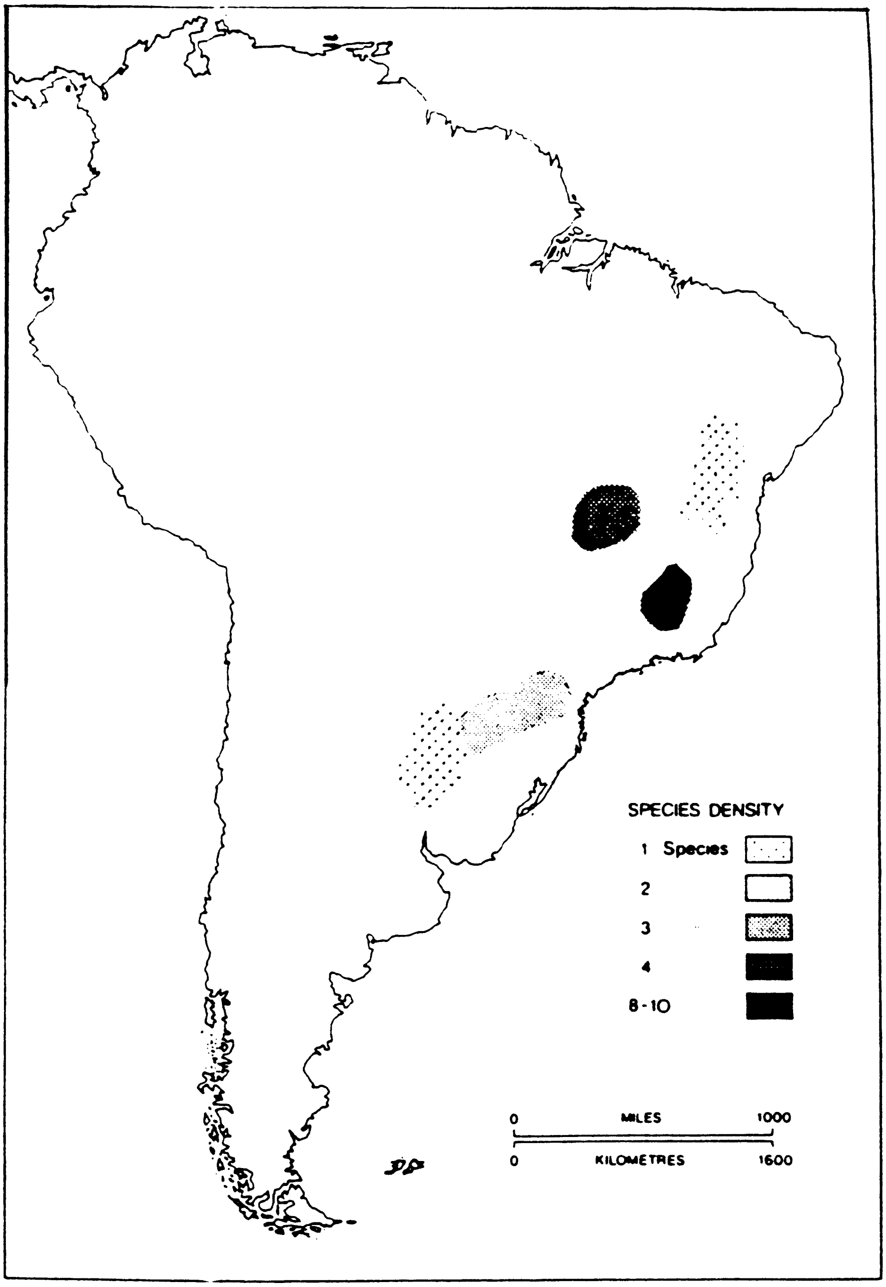


Figure 3. Species density of unifoliolate lupins in Brazil. Area of highest endemism is the region of the Serra do Espinhaco-Serra do Cipo-Diamantina. From Montierro and Gibbs, 1986.

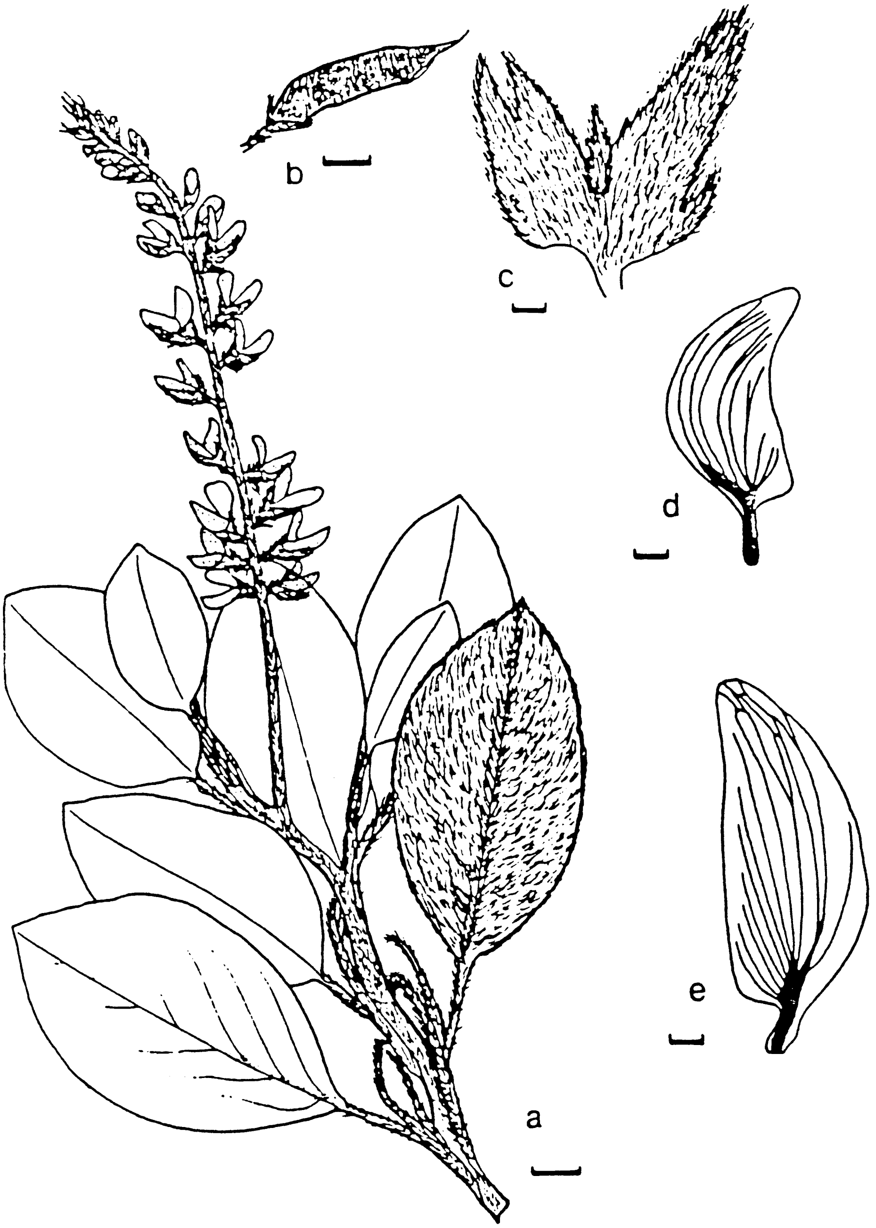


Figure 4. Lupinus crotalarioides: a, habit (pubescence partly omitted); b, legume; c, calyx; d, keel petal; e, wing petal. Scales a & b = 1 cm.; c-e = 1 mm. From Montierro & Gibbs, 1986.

is in California, with adjacent states exhibiting slightly reduced numbers (Harmon, 1968). Results imply that individual lupine species evolved to inhabit relatively narrow environmental niches based on soil acidity, soil texture, and elevation. (O'Leary, 1982).

L. leucophyllus. is a perennial polyploid complex exhibiting wide morphological plasticity (Dunn, 1984). Distributed throughout the Great Basin basaltic region (east of the Cascades), the wooly-leaved lupine prefers an open, well-drained habitat. The wooly pubescence covering stems, leaves, and calyx provide protection from intense radiation, due to properties of reflectance, and helps to reduce evapotranspiration by creating a still air layer around these surfaces. Ranging from 0.3 to 2.0 meters in height, L. leucophyllus is a pioneering species adapted to the arid interior regions of Oregon, Washington, California, Idaho, Montana; and parts of Nevada, Utah, Wyoming and Canada (Figure 5). Chromosome numbers have been determined to be both 2N=48 and 2N=96 (Phillips, 1957) making the species either octoploid or sixteen-ploid. Several species including L. leucophyllus commonly introgress when disturbance brings them together, creating a mosaic of variability which has been a source of taxonomic confusion. Diagnostic characteristics for L. leucophyllus include: ciliated keel; dorsally pubescent banner; banner ratio less than one; long, dense subverticillate raceme; and dense bilayered pubescence on vegetative parts (except L. leucophyllus *var. canescens* (Howell) Smith and L. leucophyllus *subsp. erectus* (Henderson) Harmon.

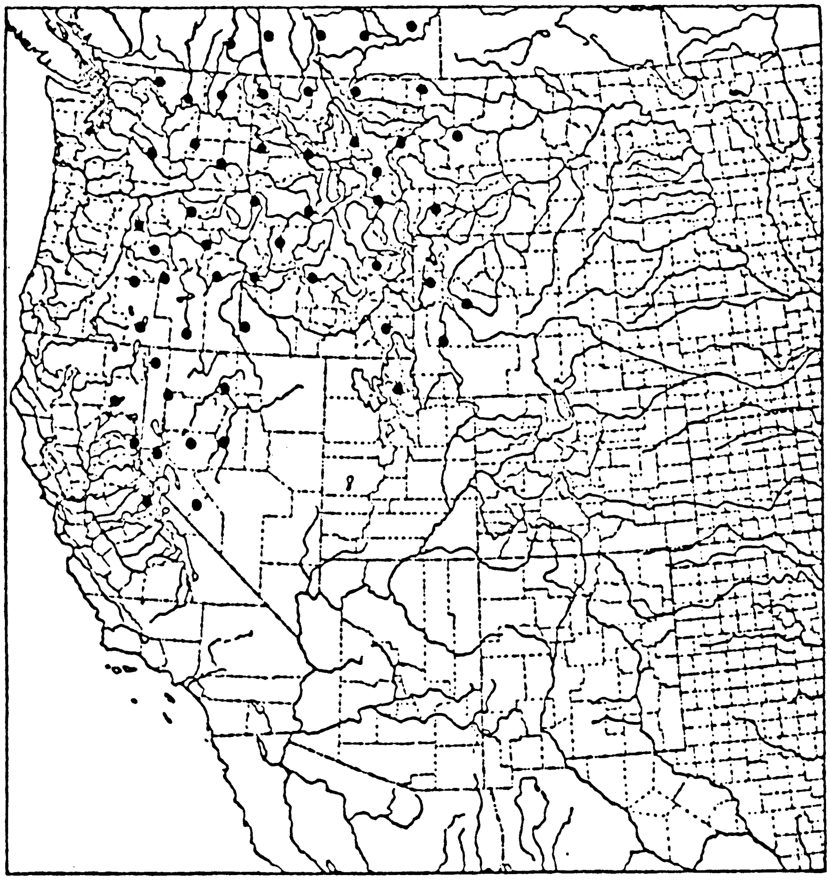


Figure 5. Distribution of Lupinus leucophyllus Dougl. From Phillips, 1955.

VI. GEOLOGY OF THE PACIFIC NORTHWEST

"Spatial and temporal variation in lithospheric complexity has a major control on speciation rates and explains patterns of species diversity." (Cracraft, 1985).

Geomorphological events provide a foundation upon which soil forming processes act; together influencing the character of the vegetation. The Pacific Northwest has a rich geological history.

The area involved in this study includes parts of eight states and Canada. L. leucophyllus shows distribution in Oregon and Washington (east of the Cascades); northern and eastern California (high Sierra Plateau); the northern and western fringes of Nevada; the Watsatch and Shoshone Ranges of Utah; the border counties of western Wyoming; Montana east of the Bitterroot Mountains; western Idaho including the Snake River plains; and southern British Columbia. The states of Oregon and Washington are the primary focus of study.

During the Precambrian Era, the Pacific Northwest was a shallow marine environment. The geologic strata were sedimentary in nature, composed of sandstone, shale, and limestone. The Cordilleran syncline, basically a long linear trough representing a thick succession of stratified sediments, was interbedded with volcanic material during the Paleozoic and Mesozoic Eras. Today, Precambrian strata are mostly hidden except in areas of uplift where more recent strata has eroded away.

From late Mesozoic to early Cenozoic the Cordillera was disrupted by massive folding and faulting, extensive metamorphism, the emplacement of granitic intrusions, and widescale uplift (McKee, 1972).

By the late Cretaceous Period, the ocean east of the present day Cascades had completely disappeared.

The Mesozoic Era featured climates both warmer and moister than observed today. Fossils dated from the Eocene Epoch of the Cenozoic Era reveal the presence of such mammals as rhinoceros, tapir, crocodile, and camel.

The Eocene Epoch featured the beginning of a period of widespread pyroclastic events, resulting from the interaction between converging Pacific (Juan de Fuca) and continental American crustal plates (cf. the theory of Plate tectonics).

In the Miocene Epoch tremendous basaltic flows occurred in the area of the Columbia Plateau and in southeastern Oregon (Steens Mts. and the Owyhee Uplands). The Columbia River basalt flows erupted through many long cracks and fissures, up to 100 miles long. In southeastern Washington, north-central Oregon, and western Idaho, these flows completely buried prebasalt topography. Over time basalt flows reached 100 feet thick, with a maximum depth of 5,000 ft.

"In central Washington and adjacent areas more than 200,000 km^3 of Miocene tholeiitic basalt of the Columbia River basalt group underlies a 160,000 km^2 area". (Graf,1987).

Intervals between flows sometimes were hundreds or thousands of years apart. At times basalt inundated a landscape of rich vegetation, lakes and streams.

In the mid-Miocene fossil evidence indicates a semi-arid grassland zone existed in the area of the Blue Mountains.

Entry into the Pliocene Epoch marked the establishment of two trends: (1) gradual global cooling and (2) initiation of the uplift resulting in the Cascade Mountains. Stream and lake sediment, interlaced with minor basaltic flows, characterized the strata of the late Tertiary Period. Evidence indicates the presence of a large ancestral river in the general area of the present Columbia River as early as the Pliocene (McKee, 1972).

Growth of large strato-volcanoes along the Pacific Coast and N.E. Washington provided a vast high altitude area which became glaciated as global temperatures dropped. The uplift of the Cascade Range and the formation of strato-volcanoes had a great effect on the climate of interior regions. Once warm and moist; a climactic shift resulted in cooler and drier conditions.

During the Tertiary, glaciation became a predominant force in the shaping of the continent. But, it is the Pleistocene Epoch that is usually associated with numerous major glacial and interglacial periods (Porter, 1983). These processes influenced the development of land features through the effects of abrasion and erosion, transport of sediment and glacial erracts, fluvial and aeolian deposition, and the formation of massive inland proglacial and pluvial lakes.

During the Pleistocene, the great Cordilleran Ice Sheet extended in the Pacific Northwest from Mount Adams near the Columbia River in the south to the Alaskan Islands in the Northwest. The major Glacial Periods during this time include (from oldest to most recent): Nebraskan, Kansan, Illinoian, and Wisconsin. Each period lasted thousands of years during which glacial advance and recession was in a state of continual flux. Each period is subdivided to reflect more localized glaciation events. During the Wisconsin Glacial Period, (20,000-12,000 years B.P.), the Cordilleran ice sheet advanced as the Okanogan lobe to the northern edge of the high basalt rim of the Columbia Plateau in central Washington. The Columbia River was displaced successively into more southern valleys until it occupied the course of the Grand Coulee (Porter, 1983). Glacial ice jams created several massive glacial lakes; the largest being Lake Missoula with a maximum volume of 2,500 km^3 (Baker In Graf, 1987). During this time other glacial lakes included Lake Pend Oreille (Northern Idaho), and Lake Spokane (eastern Washington).

The great pluvial lakes of the Great Basin were extremely large during this time. Lake Bonneville (51,640 km^2) occupied western Utah with extensions into southern Idaho and Oregon; Lake Lahontan (22,900 km^2), an interconnected series of long, narrow lakes, extended throughout the western half of Nevada, including parts of southern Oregon and eastern California.

As the southern margin of the Cordilleran Ice Sheet gradually retreated to the Canadian border (12,000 years B.P.), several effects followed.

Voluminous melt waters from glacial margins, carrying sediment down swollen rivers, resulted in massive fluvial deposition in adjacent areas. Wind or aeolian deposition (during several glacial periods) covered much of the Columbia plain with tens of meters of loess. Large ice-damned lakes were suddenly emptied as cataclysmic flooding reshaped the terrain.

Lake Missoula had a series of sixteen 'jokulhaups' (major floods) from 17,000-12,000 B.P. The Channeled Scablands of east-central Washington were carved by these floods. The "Spokane Flood Hypothesis", championed by J. Harlan Bretz initially met much opposition due to the sheer scale of these floods. Lake Missoula, at the height of such a flood, reached a discharge rate of 21 x 10^6 meters per second. (Baker In Graf, 1987). Floodwaters reached a depth of 60 meters.

Large quantities of suspended sediment accompanied the flood waters down the Columbia River. Due to the volume of water, hydrolic flooding completely submerged the Willamette Valley (between the Cascade Mountains and the Coastal Ranges), leaving behind large glacial erratics transported on ice blocks.

Lake Bonneville, 15,000 years B.P., also the source of catastrophic flooding, overflowed the pass at Red Rocks, inundating the Snake River plain. A combination of volcanic, aeolian, and fluvial events combined to shape the topography of southern Idaho.

Also, Lake Coeur D'Alene, in northern Idaho, is a remnant of the large proglacial Lake Pend Oreille; and Lake Missoula extended into Ravalli Co., to the east of the Bitterroot Range in Montana.

By the mid-Holocene, the large pluvial lakes had dried up, except Lake Bonneville (Salt Lake) and Lake Lahotan (Pyramid Lake), as evapotranspiration exceeded rainfall. A dry, warm period (Altithermal Interval) set in (7,500-7,000 years B.P.). Uplift in the Blue Mountains occurred during the late Cenozoic, and the Cascade Range had frequent volcanic activity, forming the western High Lava Plains up to recent times.

The geomorphological events described, form the basis for a discussion of soil genesis and vegetation to follow.

VII. PEDOGENESIS

"Variations in soils, as significant parts of the fabric in the environmental mosaic, operate as agents of natural selection . . . Microevolutionary reaction to soil differences will take the form of degrees of ecotypic differentiation, the development of broad genotypic tolerance, or ecological exclusion." (Kruckeberg, 1969)

With parent material generated by various geomorphological processes, soil genesis occurs due to the influence of several forming factors, resulting in a mosaic of soil-types. Much of the Pacific Northwest steppe exhibits a similiar geologic history; several factors have contributed to local differences in soil character.

The soil forming factors include: parental material, topography (or aspect), climate, biological activity, and time. As will be made apparent, all these factors combine to influence soil formation.

The discussion to follow is derived from soil surveys of Oregon and Idaho, and Fundamentals of Soil Science by Foth (1978).

The Columbia Plateau and other regions included in this study feature parent material derived from volcanic activity. Basalt is a basic igneous rock, more fertile than acidic igneous rock. Nearly all soils in the study area have moderate to high base saturation. In past geologic periods, sedimentary rock such as sandstone, limestone, and shale were deposited but the chemical character of alkalinity reflects more recent volcanic deposition. Modifications on a large scale resulted from parental processes including glacial and periglacial effects, and aeolian deposition of loess and volcanic ash. Glaciolacustrine sedimentation, glacial outwash, and alluviation characterize the study area.

Properties of parent material that influence soil development are texture, mineralogical composition, permeability to water and air, thermal properties, and degree of stratification. Loess, for example, is an unconsolidated, unstratified, porous wind deposited substrate. Composed of feldspar, quartz, calcite, and mica (all easily weathered minerals); it is naturally fertile. If loess is underlain by glaciolacustrine sediment, this would modify soil properties and influence, to a degree, the vegetation supported.

Climate exercises the most important influence on soil development. Temperature and precipitation (including seasonality and diurnal influences), control the rate of pedogenesis, as well as influencing the accumulation of organic matter. Mineralogical weathering occurs through temperature mediated physical and chemical processes. Considerable climatic variability occurs regionally and locally due to differences in elevation and aspect.

The climate of the Pacific Northwest is described as having a maritime influence. The Coastal Ranges and more particularly the Cascades restrict the amount of precipitation occurring in the interior. Generally, the interior regions are arid with seasonal fluctuations. The majority of precipitation falls during the winter, summers are hot and dry. Throughout Oregon and Washington, precipitation increases from south to north, and from lower to higher elevation. Soils on forested mountain slopes are more leached and more acidic than soils at lower elevations.

Parental material is most rapidly weathered under warm, moist conditions; while basalt weathers slowly under arid conditions.

Topography is the factor that most contributes to local soil differences. Separate microenvironments occur on south as compared to north-facing slopes. Topography modifies the effects of climate by influencing the quantity of precipitation absorbed and retained in the soil, and regulating soil temperature, snow cover, and freezing and thawing. The rate of erosion (movement of materials in suspension or solution) is directly correlated to slope, characteristics of the parent material, and amount of precipitation.

Deposition of air-borne materials is deepest on the windward side of a slope. In Gilliam Co., Oregon, north-facing slopes exhibit a thick layer of loess-derived silt loam, while south slopes feature very stony silt loams, from a thin loess mantle. (Hosler, 1984).

Biological activity (plant, animal, invertebrate, and microorganism) affects the accumulation of organic matter in the soil. Organic matter improves soil structure, fertility and water-holding capacity. It increases with moderate temperature and high precipitation, but decreases with high temperature and low moisture. Also the chemical state of the soil influences organic matter accumulation; high pH retards decomposition of organic matter by microorganisms.

Conditions hastening soil development are: a warm, humid climate; permeable, unconsolidated parent material (low in lime); and flat or depression topography with good drainage.

The degree of profile development generally indicates the relative age of the soil. Of special importance is the degree of illuviation, the amount of clay accumulating in the subsoil. While the accumulation of organic matter can occur in a few hundred years, the accumulation of clay may take a thousand years. Time is the determining factor of soil development.

VIII. PHYSIOGRAPHIC AND GEOLOGICAL PROVINCES

Oregon and Washington are subdivided into fifteen physiographic and geological provinces. Four are of particular importance in this study. These include the following: the Columbia Basin, Blue Mountains, High Lava Plain, and Basin and Range Provinces (Figure 6). Coeur D'Alene, Idaho is included as an additional study region.

L. leucophyllus has a range of distribution throughout these areas. This study is restricted to Oregon and Washington, including Coeur D'Alene, Idaho. Here all taxonomically significant variants are found.

East of the Cascades, the climate reflects maritime and continental influences. Mean annual precipitation varies between 20.0 cm. in south-central Washington to 60.0 cm. in the northwestern corner of the state; with 70%-80% occurring between October 1 and March 31 (a high proportion as snow).

In general, winters are colder, summers hotter, and frost-free seasons shorter east of the Cascades (vs. the western slope).

The Blue Mountain Province separates the Columbia Plateau to the north from the Basin and Range Province to the south.

A. The Columbia Basin

The Columbia Basin is the largest physiographic province covering an extensive area south of the Columbia River between the Cascades and the Blue Mountains, and two-thirds of the area east of the Cascades in Washington state. The Columbia plateau is surrounded by the Okanogan Highlands to the north; Clearwater Mountains to the east; Blue Mountains to the south; and the Cascades to the west. The topography was formed by sedimentary strata interbedded with basalt; additionally influenced by lacustrine deposition, wind mediated transport of loess and volcanic ash, alluvial and colluvial effects, and cataclysmic events. Loess deposits in the region of the Palouse River in Washington are up to 45 meters thick, representing many cycles of deposition. Inherently

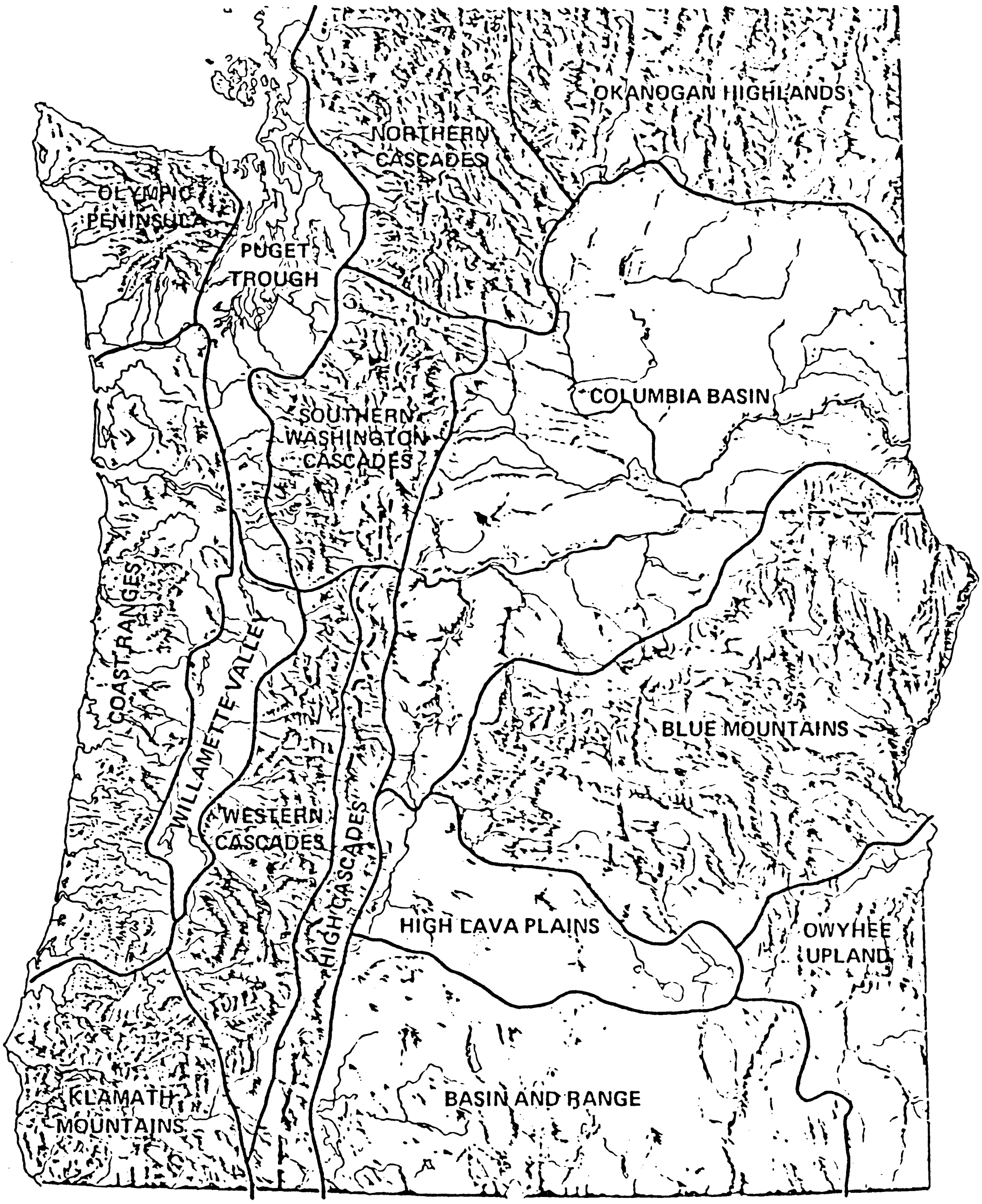


Figure 6. Physiographic Provinces of Oregon and Washington. From Franklin, 1973.

fertile, the Palouse region is noted for it's high wheat productivity.The 'Channeled Scablands' are features specifically correlated to cataclysmic flood events. Occurring in central-eastern Washington, they appear as a series of deeply cut channels in Columbia River basalt that form an extensive drainage network.

When the Columbia Gorge became dammed during the great Pleistocene floods, large lakes formed, fed by glacial melt waters. Lacustrine sediments characterize a large area in Oregon and Washington.

'Biscuit Scablands' are a unique landscape formation of the region, including features described as 'patterned ground'- biscuits, stone nets, and stone stripes. 'Biscuits' are round or elongated, erosion-modified, polygonal mounds that are underlain at a depth of 2-3 feet by basalt. Formed by frost action, and fairly common in the province, they are generally classified under the designation 'periglacial features' (nonglacial processes and features of cold climates on land characterized by intense frost action) (Porter,1983).

The soils of the Columbia Basin have moderate to high base saturation. Most of the differences between soil groups are attributed to precipitation, which is heaviest along the margins of the basin and decreases inward (Figure 7). Temperature ranges from 28.9^{o} C. to 33.5^{o} C. (mean July maximum) and 9.0 ^{o} C. to 5.0^{o} C. (mean January minimum).

Four distinct soil groups are recognized: (1) Argixerolls, formed from loess, with 400-600 millimeters of annual precipitation. This group has maximum profile development with calcium carbonate leached to below the solum layer. The deep, fertile soils of the Palouse region of Washington exemplify Argixerolls. (2) Haploxerolls, derived from loess under 230-400 mm. of annual precipitation. These are characterized by a zone of calcium carbonate in the 'B_3' horizon. With a moderately thick 'A' horizon, these soils are moderately well developed. (3) Haploxerolls, derived from loess and sandier windblown materials with 230-400 mm. of annual precipitation, are recognized as distinct from the above because they are relatively low in organic matter and poorly developed. (4) Camborthids, as desertic soils formed under 100-230 mm. of annual

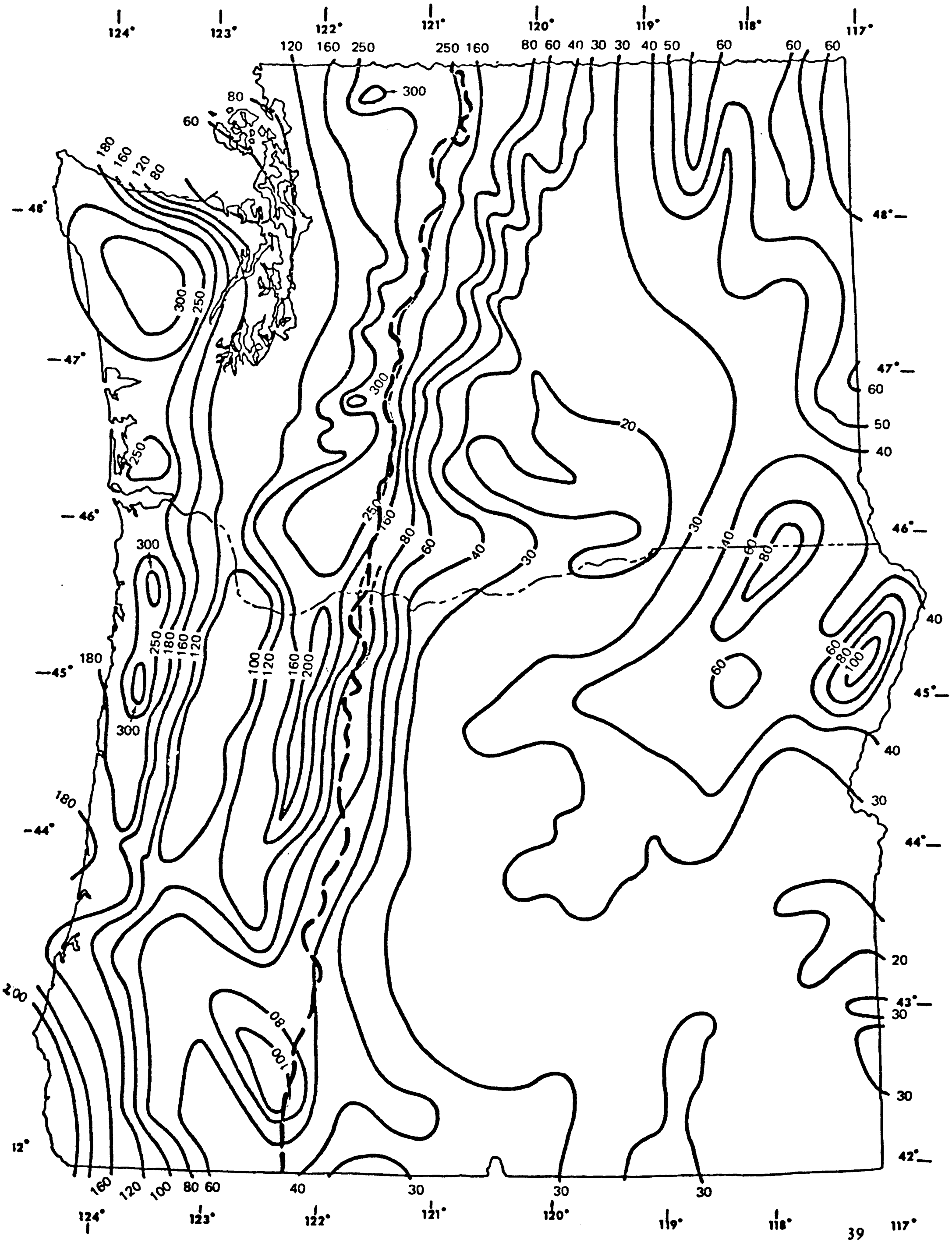


Figure 7. Mean annual precipitation in Oregon and Washington. (U.S. Weather Bureau 1960 a,b). From Franklin, 1978.

precipitation, characterize the depressed bowl-like center of the Columbia Basin. These soils usually have a carbonate enriched horizon, and may be cemented.

The four soil groups mentioned support distinct plant communities and represent the predominant soil groups within this province. They generally show concentric distribution from the desertic bowl-like center. The modifying maritime effects of the Pacific Ocean are felt in areas adjacent to the Columbia River, the major drainage to the ocean.

B. Blue Mountains Province

The Blue Mountains extend from Prineville in central Oregon, east and northeast into western Idaho. Described as a "large asymmetric anticline with a steep north face and a gentle south flank", (McKee,1972); several distinct mountains combine to form this feature. These include: the Elkhorn, Blue and Wallowa mountains to the northeast, and the Strawberry, Ochoco and Aldrich Ranges to the west. Maximum elevations range from 2100 meters in the Ochoco Mountains to 2900 meters in the heavily glaciated Wallowa Mountains in northeast Oregon. Valley elevations range from about 750 meters in the Ochocos to 900 meters in the broad basin between the Blue and Wallowa Mountains near La Grande, Oregon.

The John Day River provides drainage into the Columbia River for the western half of the province; while the east slope drains into the Snake River (Hell's Canyon) forming the eastern boundary.

The region can be subdivided into eastern and western units with an imaginary dividing line drawn just to the east of John Day. The western Blue Mountains feature some of the oldest rock formations in the state of Oregon. Triassic and Jurassic sedimentary formations include conglomerates, sandstone, siltstone, shale, and limestone. Also, Columbia River basalt occupies large areas. The eastern section, spanning a large part of the geologic timescale, includes Triassic limestone and argillaceous beds; granitic stocks, representing extension of the Idaho Batholith; and widespread Columbia River basalt, which forms the bulk of the range between Pendleton and La Grande. Evidence indicates uplift of at least part of the Blue Mountains after the basalt flows of the Miocene Epoch. Volcanic ash and fine pumice subsequently covered the area.

During the Pleistocene, glaciation was widespread in both the Blue and Wallowa Mountains. At times several valleys were inundated by glacial melt waters, including Baker and La Grande valleys. Many upland areas of the eastern Blue Mountains were mantled by loess in the late Pleistocene.

The climate of the region is continental with some maritime influence from the Pacific Ocean. Generally, precipitation is greater and temperatures average lower in mountainous areas as opposed to low basins. At Union, Oregon, July maximum temperatures, over a ten-year period, average 28.8^{O} C. (37.8^{O} C. maximum); while January minimum temperatures average 2.7^{O} C. (-22.8^{O} C. minimum). Precipitation, over a ten-year period averages 35.5 cm. yearly with most falling as snow.

Soils are derived from colluvium, volcanic ash, lacustrine sediments, and loess. Generally, soils were formed at moderate to high elevations under mostly forest vegetation, or at lower elevations under grassland or shrub-grassland influence. In the western section of the province, soils are derived from ancient lake-deposited sediments, and feature a clay-loam surface horizon over a clay-textured subsoil. In the eastern section, soils developed from loess and basic igneous rocks; with a silt-loam surface over clay-loam subsoils.

C. High Lava Plains Province

The High Lava Plains Province of central Oregon is characterized by relatively young lava flows of moderate relief, with scattered cinder cones and lava buttes dispersed throughout the region. Most of the province has a base elevation of 1200 meters above sea level. Precipitation averages 30 cm. per year.

Geological formations originated from Pliocene and Pleistocene lavas, tuffs, and alluvium. Several basins contained large lakes during the Pleistocene. An extensive pumice mantle characterizes the landscape; a result of the eruption of Paulina Peak and

Mount Mazuma, further to the southwest. Soils include Camborthids and Natargids.

Camborthids are derived from pumice and water-laid materials. Never moist for a period as long as three months; they are low in organic matter. Natrargids are deep silty soils with a subsurface horizon of clay with sodium accumulations. They are formed in old lake basins, and typify soils in the general vicinity of Malheur and Harney Lakes.

D. Basin and Range Province

South of the Blue Mountains, the Basin and Range Province is characterized by fault-block mountains enclosing internally drained basins. Virtually all rock in the region date from the Miocene to recent. With only 18.0-30.0 cm. of precipitation annually, many basins feature shallow saline lakes.

Major fault-block features include Winter Ridge, Abert Rim, and the Steens Mountains, all capped with Miocene basalt. Elevations range from 1200 meters in the basins to 2930 meters in the Steens Mts. Evidence suggests extensive glaciation in the Steens Mts.

Soils can be divided into two groups, based on whether they developed under forest or shrub-grassland vegetation. In the western part of the province, soils derived from pumice, Vitrandepts, are slightly acid due to the high porosity of pumice, but have greater water-holding and cation exchange capacities than generally expected. These are developed under forest vegetation. Soils characteristic of shrub-grassland vegetation are Haplargids and Dumargids. Common in the eastern part of the province; Haplargids, derived from basalt, are described as having a very stony loam surface underlain by a clay or stony subsoil. Durargids have a very stony loam surface over a clay subsoil that may feature a silica-cemented hardpan layer. Scattered throughout the province, a number of dry lakebeds occur characterized by deep silty lacustrine deposits. These soils generally have a silica-cemented hardpan within one meter of the surface.

E. Kootenai County, Idaho Region

Kootenai County, Idaho includes the Coeur D'Alene region, where a distinct variant of L. leucophyllus resides. The physiographic units of Kootenai Co. include the Selkirk and Coeur D'Alene Mountains, Rathdrum Prairie, Palouse Hills, and the Coeur D'Alene River valley.

Parental substrate includes loess, volcanic ash, glaciolacustrine sediments, alluvium and basalt. Basalt flows occurred throughout the area in the past. Average annual precipitation for the region ranges from 50.8-66.0 cm.

Lake Coeur D'alene represents a remnant of the once vast glacial lakes known to exist in northern Idaho. Undoubtedly, the Pleistocene floods of Lake Pend Oreille, that inundated Rathdrum Prairie had a large effect in shaping the region. Soils are generally xeric to mesic mollisols.

F. Columbia River Gorge

As a special feature of the study area, the Columbia River Gorge exercises a significant influence on the region. Described as a giant wind tunnel, the gorge is a low point in the Cascade Arch allowing maritime influences to penetrate inland.

During the Pleistocene, evidence indicates that the Columbia River existed. With the gradual uplift of the Cascades, the ancient Columbia River was displaced from a conglomerate base to Columbia River basalt base; maintaining it's Pacific connection. Detling (1958) recognizes the Columbia Gorge as both a migration corridor and a specialized relict habitat.

Climatic features of the gorge include high winds (120 miles per hour recorded on Dec. 20,1935). When atmospheric differentials allow for movement of air masses west through the gorge, a condition known as 'silver thaw' may occur. This is a destructive ice storm accompanied by high winds. In Nov., 1921, 54 inches (137 cm.) of snow fell at the Dalles during a three day period. The combination of ice and wind literally blew vehicles off the road.

IX. VEGETATIVE ASSOCIATIONS

A. Introduction

Combinations of environmental characteristics and their geographic zonation explain patterns of vegetation. Attempts to characterize vegetational patterns date back to the early Greeks. In 1717, Tournefort provided a treatise that related vegetational zonation to environmental factors. Many authors have described the vegetational zones of the Pacific Northwest: Daubenmire (1943,1968,1969), Detling (1966,1968), Heusser (1983), Kuchler (1964), Schofield (1969), and others. The purpose of this chapter is to describe, ultimately, vegetational zones which characterize the distribution of L. leucophyllus.

B. Overview

Hess (1969) describes the vegetational communities of the Rocky Mountains. Drawing on Daubenmire (1943) and Oosting (1956) the following zones were described.

1. *The Alpine Zone* is subdivided into Arctic tundra, Petran tundra, and Sierran tundra based on both latitude and environmental characteristics. The alpine zone supports a varied combination of sedges, grasses, and perennials.

2. *The Subalpine Zone* is described as occurring between 700-1,050 meters, variable according to latitude. Fire is an important ecological modifier which may encourage the development of subclimax stands of Pinus contorta Dougl, Pseudotsuga menziesii (Mirbel) Franco, or Populus tremuloides Michx. Characteristic dominants in the subalpine zone are Abies lasiocarpa, (Hook) Nutt. and Picea engelmanni Parry.

3. *The Montane Zone* includes two climax associations: Pseudotsuga menziesii and Pinus ponderosa Dougl. With a broad range in elevation, and typified by a number of different climactic regimes, several vegetational associations occur. Fire, again plays a significant role; replacement species are Pinus contorta, Larix occidentalis Nutt., or Populus tremuloides Michx., depending on micro-environments.

4. *The Woodland Zone* includes vegetation of the foothills and lower slopes. Two major associations are the Pinyon-Juniper Climax and the Oak-Mountain Mahogany Climax.

5. *The Basal Plains* are depicted as prairie-grasslands or semi-deserts. This zone is characterized by Festuca idahoensis Elmer-Agropyron spicatum (Pursh) Scribn. & Smith or Artemisia tridentata Nutt. associations.

L. leucophyllus Dougl. has a wide range of association; it is described as occurring in subalpine, montane, woodland, and basal plains zones. The sections to follow represent a more detailed study of the vegetation of Oregon and Washington.

Generally, the botanical regions of Oregon and Washington can be subdivided into forests, woodlands, and grasslands or shrub-grass communities. Forest dominates the landscape west of the Cascades, with the exception of interior valleys. East of the Cascades, steppe and shrub-steppe communities predominate as well as woodland and forest associations.

Once described as 'high desert' or 'Great Basin Desert', the Columbia Plateau is more accurately known as 'steppe' or 'shrub-steppe'. Daubenmire (1970) equates this confusion of terms to the combination of hot, dry summers and the presence of rattlesnakes, horned lizards, tarantulas, and Opuntia spp. Geographically, the interior region includes the Columbia Basin, High Lava Plains, and the Basin and Range provinces. Steppe is found primarily around the eastern rim of the Columbia Basin, while shrub-steppe occupies the center and most of central and southeastern Oregon. Bunchgrasses and/or sagebrush are the typical dominant vegetation throughout the area.

C. Vegetational Zones of Oregon and Washington

The vegetational communities of Oregon and Washington, where L. leucophyllus is observed, are described below.

1. Interior Valleys

The Interior Valleys, situated between the Coastal Ranges (or Siskiyou Mountains) and the Cascades, are the warmest and driest areas west of the Cascades. Several infrequently found soil types occur; supporting vegetation characteristic of the region, which includes the Willamette Valley of Oregon. Oak woodlands, coniferous forests, grasslands, sclerophyllous shrub communities, and riparian forests are observed.

Quercus kelloggii Newberry (California Black Oak), Quercus garryana Dougl. (Oregon White Oak), and Arbutus menziesii Pursh (Madrono) form forest stands, groves, and savannahs that typify the interior valleys. The Willamette Valley, a broad almost level alluvial terrain, interrupted by low basalt hills, features four Q. garryana communities distinguished by understory dominants.

Coniferous forests (Pseudotsuga menziesii (Mirbel) Franco, or Abies grandis (Dougl.) Forbes) are distributed throughout the foothills in and around the interior valleys.

Grasslands occupy extensive areas. Danthonia californica Boland (Oatgrass) and Stipa L. spp. (Speargrass) are described as common. Grasslands may be invaded by such species as Rosa eglanteria L., Rhus diversiloba T. & G. (Poison Oak), or succeeded by Q. garryana.

Sclerophyllous shrub communities may include Ceanothus cuneatus (Hook) T. & G. (Common Buckthorn) and Arctostaphylos patula Greene (Manzanita). Forming extensive thickets, fire traditionally helped to maintain these associations.

Populus trichocarpa T. & G. (Black Cottonwood), Fraxinus latifolia Benth. (Ash), and Acer macrophyllum Pursh (Oregon Maple) are trees common to riparian communities. Oregon maple is a climax invasive of Q. garryana forests. L. leucophyllus has been observed in Q. garryana dominated forests in southwest Oregon.

2. Forested Zones of Eastern Washington and Oregon

Eastern Oregon and Washington exhibit climax associations similiar to those described for the Rocky Mountains by Hess (1969). Lacking the maritime influence of western forests; eastern forests are arrayed in zones based on elevation. Four zones are described as pertinent to this study.

A. *Juniperus occidentalis* Hook (Western Juniper) Zone

The region adjacent to the Deschutes, John Day, and Crooked Rivers in east-central Oregon is the most xeric of tree-dominated zones in the Pacific Northwest. Generally described as savannah, this zone occurs in the High Lava Plains Province. *J. occidentalis* is a conspicuous species, able to thrive in pumice derived soils, which are low in organic matter and slightly acidic. The major associations occur between: *J. occidentalis* / *Artemisia tridentata* (Big Sagebrush) / *Agropyron spicatum* (Wheatgrass)-*Festuca idahoensis*; though other species may displace species or come into co-dominanace (eg. *Purshia tridentata* (Pursh) D.C. (Antelope Brush)). The most common perennial forbs observed in this zone include: *Agoseris* Raf. spp. (False Dandelion), *Achillea millefolium* L. (Yarrow), *Eriophyllum lanatum* (Pursh) Forbes (Wooly Sunflower), *Astragalus* L. sp. (Locoweed), *Erigeron linearis* (Hook) Piper (Yellow Daisy), and *Lupinus* spp. *L. leucophyllus* is described from this zone. Western juniper displays sensitivity to burning and gives way to an herb or shrub dominated community under this effect. Grazing by cattle can reduce or eliminate *Agropyron spicatum* and *Festuca idahoensis* due to their palpability.

B. *Pinus ponderosa* Dougl. (Western Yellow Pine) Zone

Western yellow pine occupies a narrow band on the eastern flanks of the Cascade range, much of the high pumice plateau extending from the high Cascades region, and large areas in the Blue Mountains Province. It is the most characteristic species of pine in the Arid Transition Zone (Merriam). With distribution correlated to the supply of available moisture (Daubenmire, 1956), the *P. ponderosa* zone abuts the *Artemisia tridentata* steppe or the open *Juniperus occidentalis*-*A. tridentata* woodland.

Due to the open nature of stands, western yellow pine provides abundant niches for heliophytic species, many of which are typical of steppe and shrub-steppe communities. *P.*

ponderosa may be associated throughout it's range with: J. occidentalis (Western Red Cedar), Populus tremuloides Michx. (Quaking Aspen), Pinus contorta Dougl., or Q. garryana. Community associations vary widely with geographic location, depending on soils, elevation, aspect, and successional status. Fire is very important in shaping vegetation within the zone; other conifers such as Pseudotsuga menziesii being less fire tolerant.

Understory dominants may include Symphoricarpos albus (L.) Blake (Snowberry), Physocarpus malvaceus (Greene) Kuntze (Ninebark), Festuca idahoensis Elmer (Idaho Fescue), Stipa comata Trin. & Rupr. (Speargrass) or Purshia tridentata (Antelope Brush).

In natural forest openings, maintained by periodic moisture saturation, heavy soils, or summer drought the following associations may occur:

1) Artemisia rigida (Nutt.) Gray (Stiff Sagebrush) / Poa sandbergii Vasey with Sitanion hystrix (Nutt.) Smith (Squirreltail) and Trifolium macrocephalum Pursh (Big-head Clover).

2) Artemisia arbuscula Nutt. (Dwarf Sagebrush) / Agropyron spicatum (Wheatgrass) with P. sandbergii and Purshia tridentata.

3) A. arbuscula / F. idahoensis with Phlox caespitosa Nutt. (Clumped Phlox), Balsamorhiza serrata Nels. & Macbr. (Toothed Balsamroot), and P. sandbergii. L. leucophyllus *subsp. erectus* (C.P. Smith) Harmon is also observed.

Community development on pumice soils is correlated with moisture availability. Dominant associations, representing edaphic climaxes, are listed by increasing moisture regimes.

1) Pinus ponderosa / Purshia tridentata

2) P. ponderosa / P. tridentata / F. idahoensis

3) P. ponderosa / P. tridentata - Arctostaphylos patula Greene (Green-leaved Manzanita).

4) P. ponderosa / Ceanothus velutinus Dougl. (Mtn. Balm) - P. tridentata

5) P. ponderosa / C. velutinus / Chimaphila umbellata (L.) Bart (Common Pipsissewa) - Pyrola picta Smith (White-veined Pyrola) or Pterospora

andromedea Nutt. (Pinedrops).

(1-4 are edaphic climaxes due to the immaturity of pumice soils).

In the Blue Mountains Province, western yellow pine is generally associated with Purshia tridentata and / or grasses and sedges.

C. Pinus contorta Dougl. (Lodgepole Pine) Zone

Lodgepole pine occurs on the pumice plateau of central Oregon, where it is widespread. With unusually wide ecologic amplitude, P. contorta is often associated with Purshia tridentata (Antelope Brush). Exhibiting rapid invasion of severly disturbed sites, lodgepole pine forms an edaphic climax in poorly drained soils; and a topoedaphic climax in well-drained frost pocket depressions.

D. Pseudotsuga menziesii (Mirbel) Franco (Douglas Fir) Zone

Douglas Fir is widely distributed: "moist to dry areas, sea level to near timberline in the Rocky Mountains." (Hitchcock & Cronquist, 1973). However, it has the greatest ability to maintain a self-reproducing population at a higher elevation than P. ponderosa. Occupying a more mesic environment, P. menziesii is one of the dominant conifers in eastern Oregon and Washington. It is generally associated with Symphoricarpos albus (L.) Blake (Snowberry) and Calamogostis pubescens Buckl. (Pinegrass), among other species. L. leucophyllus is cited from this zone.

3. Steppe and Shrub-Steppe of the Columbia Basin Province

The Columbia River Basin Province of central and southeastern Washington, including a sizeable portion of the northeast quarter of Oregon, is essentially a large basaltic plateau . The steppe and shrub-steppe communities, although potentially fertile, are only able to support bunchgrass and deep-rooted shrubs, due to limited moisture availability (Daubenmire, 1969). Zonal soils, closely related to patterns of rainfall, influence the composition of the vegetation. Artemisia tridentata (Big Sagebrush) is the dominant shrub species, but others contribute to community diversity. Atriplex spinosa (Hook) Collotei

(Spiny Hopsage), Chrysothamnus nauseosus (Pall.) Britt (Rabbit-brush), C. viscidiflorus (Hook) Nutt. (Green Rabbit-brush), Gutierrezia sarothrae (Pursh) Britt & Rusby (Match-weed), Purshia tridentata, and Tetradymia canescens D.C. (Horse-brush), are all adapted to the hot, dry summers and cold winter climate.

Perennial forbs are conspicuous in both the steppe and shrub-steppe associations; but in inverse ratio to the degree of aridity. Over the past 100 years, man has dramatically changed the native vegetation through cultivation and animal and plant introductions. Bromus tectorum, L. is the most problematic species of drier regions. It's early spring development and long root system enables it to have a competitive advantage in disturbed or overgrazed areas. Native bunchgrasses Agropyron spicatum and Festuca idahoensis rarely recover from grazing by undulates. Also, Artemisia tridentata and Purshia tridentata are fire sensitive; both temporarily eliminated from a site by burning. The combination of over-grazing and burning can result in an annual rangeland dominated by the introduced B. tectorum, in which Chrysothamnus nauseosus may be the only significant shrub.

The following discussion describes the zonal associations. Generally, bunchgrasses occur around the eastern rim of the basin, in areas of more favorable precipitation; while a combination of shrubs and bunchgrasses occur in other areas (Figure 8).

I. *Artemisia tridentata / Agropyron spicatum*

Occupying the arid central region of the basin, in the rain-shadow of the Cascades, several species are mentioned as associates. Antennaria dimorpha (Nutt.) T. & G. (Low Pussytoes), Calochortus macrocarpus Dougl. (Mariposa Lily), Poa cusickii Vasey (Bluegrass), and Poa sandbergii Vasey, were observed by Daubenmire (1970).

II. *Artemisia tridentata / Festuca idahoensis*

The addition of Festuca indicates a more favorable moisture regime. Species also observed include: Achillea millefolium L.(Common Yarrow), Agropyron spicatum (Wheatgrass), Astragalus spaldingii Gray (Locoweed), Festuca microstachys Nutt. (Small Fescue), Holosteum umbellatum L.(Jagged Chickweed), Lupinus leucophyllus Dougl.(

Wooly-leaved Lupine), and Phlox longifolia Nutt.(Long-leaved Phlox), and Poa sandbergii Vasey(Sandberg's Bluegrass).

III. *Agropyron spicatum - Festuca idahoensis*

This association occurring in the Palouse Region indicates again a more favorable moisture regime. L. leucophyllus and P. sandbergii are common.

IV. *Symphoricarpos albus (L.) Blake / Festuca idahoensis*

Termed 'meadow steppe' in the literature, this zone consists of herbaceous and shrubby components. Listed species include: Achillea millefolium, Astragalus arrectus Gray, Balsamorhiza sagittata (Pursh) Nutt.(Balsamroot),

Besseya rubra (Dougl.) Rydb.(Red Besseya), Bromus japonicus Thunb. (Japanese Brome), Calochortus elegans Pursh (Elegant Sego Lily), Castilleja lutescens (Greenm.) Rydb. (Yellow Paintbrush), Geranium viscosissimum F. & M. (Purple Geranium), Geum triflorum Pursh (Prairie Smoke), Helianthella uniflora *var. douglasii* (T. & G.) Weber (Little Sunflower), Hieracium albertinum Farr. (Western hawkweed), Iris missouriensis Nutt. (Western Blue Flag), Lupinus leucophyllus (Wooly-leaved Lupine), L. sericeous Pursh (Silky-leaved Lupine), Microsteris gracilis (Hook) Greene (Pink Microsteris), Myosotis micrantha Pall.(Blue Scorpion-grass), Potentilla gracilis Dougl.(Cinquefoil), Rosa nutkana *var. hispida* Fern., Senecio integerrimus *var. exaltatus* (Nutt.) Cronq. (Western Groundsel) and Zygadenus venenosus *var. gramineus* (Rydb.) Walsh. (Meadow Death-camas).

V. *Artemisia tripartita Rydb. / Festuca idahoensis Elmer*

Species composition differs from the previous association with the addition of Artemisia tripartita Rydb. (Cut-leaved Sagebrush), Chrysothamnus Nutt. spp. (Rabbit-brush), Eriogonum heracleoides Nutt. (Wyeth's Buckwheat), L. leucophyllus, and Tetradymia canescens D.C. (Spineless horse-brush); with L. sericeous, R. nutkana, and S. albus dropping out.

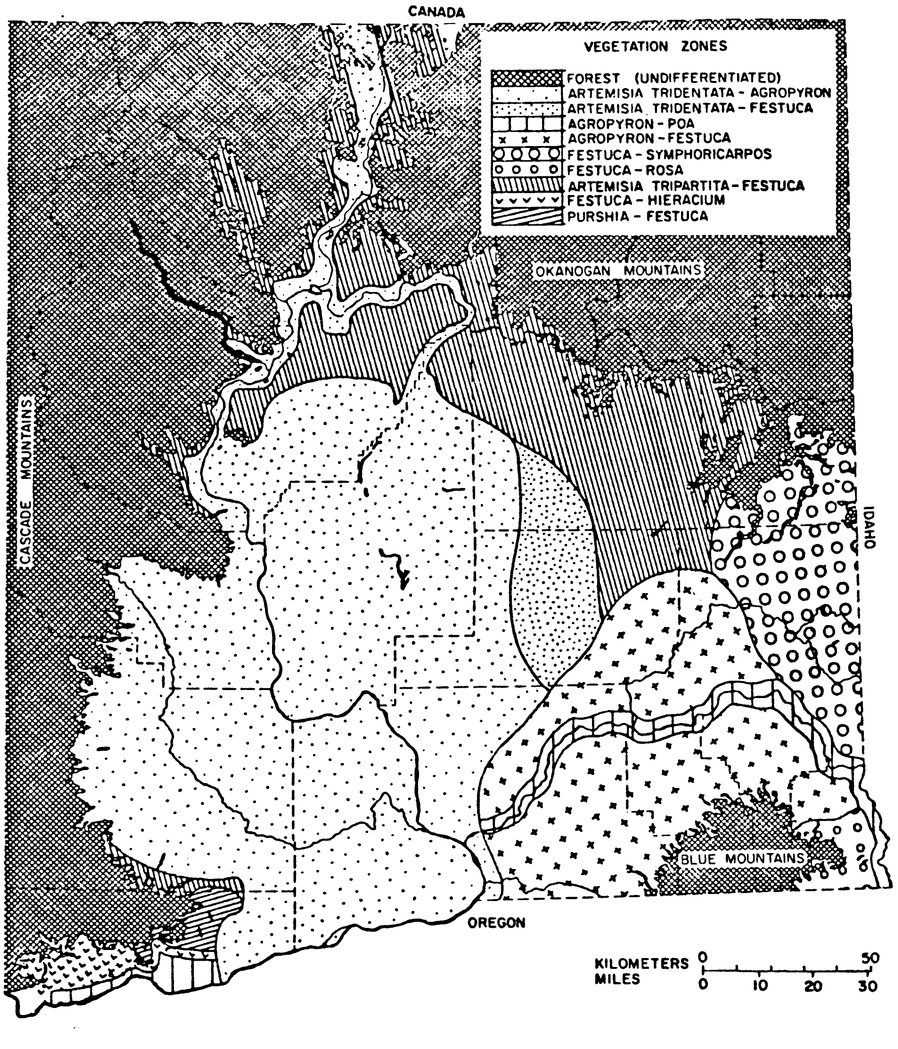


Figure 8. Vegetation zones in the steppe region of eastern Washington. From Daubenmire,1970.

VI. *Purshia tridentata (Pursh.) D. C. / Festuca idahoensis Elmer*

Described as both a topographic climax in the Artemisia tridentata / Agropyron spicatum zone and an edaphic climax alternating with Pinus ponderosa / Purshia tridentata communities in the foothills of the Cascades; this zone is restricted in size. A. spicatum, Balsamorhiza sagittata, Eriogonum heracleoides and Poa sandbergii are mentioned as dominant associates.

VII. Minor Associations:

A. Festuca idahoensis Elmer / Hieracium cynoglossoides Arv-Touv

Localized along the Columbia River in south-central Washington.

B. F. idahoensis / Rosa nutkana.

Restricted to the eastern slope of the Blue Mountains in southeastern Washington.

C. Agropyron spicatum (Pursh) Scribn. & Smitrh - Poa sandbergii Vasey

Observed adjacent to both the Columbia and Snake Rivers. Several more specialized zonal associations could be added to the list.

4. Southeastern Oregon Shrub-Steppe

The environment of southeastern Oregon creates vegetative communities distinct from those of the Columbia Basin. Higher elevation and shallower soils support sagebrush and bunchgrasses; as well as more specialized plants.

The most widespread grouping is Artemisia tridentata / Agropyron spicatum. A. tridentata / Festuca idahoensis is a topographic or topoedaphic climax limited to more mesic sites; while A. tridentata / Elymus cinereus Scribn. & Merr.(Giant Wildrye) occurs along moist alluvial bottomlands. On shallow, stony soils Artemisia rigida (Nutt.) Gray (Stiff Sagebrush) occurs with P. sandbergii. In other situations Purshia tridentata Antelope-brush) or Cercocarpus ledifolius Nutt. (Mountain Mahogany) appear.

Drier alkaline areas of southeastern Oregon feature such species as Atriplex confertifolia

(Torr. & Frem.) Wats. (Shadscale) or Sarcobatus vermiculatus (Hook.) Torr. (Greasewood).

5. The Columbia River

"The Columbia River is the only point between the Fraser River in British Columbia and the Klamath and Pit Rivers in northern California where the Cascade axis has been cut through to the point where it interrupts the continuity of all life zones except the lowest . . . and plant migrations from one side of the range to the other." (Detling, 1966)

Schofield (1969) described a single bryophyte, Desmatodon columbianus Hermann & Lawt as endemic to the Columbia River Gorge as well as seventeen vascular plant species. Saxifraga bronchialis L. *ssp. vespertina (Small) Piper* is cited as an example of divergent evolution after *ssp. austromontana (Wies) Piper* was well established in both the Rockies and the Cascades (Detling, 1966).

X. CHEMOTAXONOMY

A. Introduction

Chemotaxonomy involves the use of chemical evidence, including primary and secondary metabolites, to ascertain phylogenetic relationships between taxa of plants. Historically, plants were grouped according to shared morphological traits or chemical similarities. Techniques, providing greater resolution of chemical components enable a more in depth evaluation of phenetic and phylogenetic relationships. In this section the historical development of chemical taxonomy will be summarized, with reference made of specific chemical attributes utilized in taxonomic studies. Work on Leguminosae, subfamily Papilionoideae, is described to emphasize an integrative approach; comment will be made on the systematic value of chemical analysis.

B. Historical Overview

Early botanical investigation centered around the utilization of various plants by physicians for medicinal use. Plants were recognized according to macro-morphological traits and gross characterization of plant substances or 'vertues'. Nehemiah Grew (1673) and James Petiver (1699) were the first to publish articles concerning the validity of making inferences about plant substances on the basis of recognized morphological groupings. Acknowledged as the beginning of the 'modern' approach to taxonomy, a relationship was recognized between morphological 'class' and plant substance. Petiver (1699) published a paper entitled, "Some *Attempts* made to prove that *Herbs* of the same *Make* or *Class* for the generality, have the like*Vertue* and *Tendency* to work the same *Effects*".

Other investigations such as the work of Camerarius (1699), "De Convenientia Plantarum in Fructificatione et Viribus," expressed similiar views. It is interesting to note that Linnaeus (1756) presented a classification system including descriptors of odor. Plants were classified as: aromatic, fragrant, musk-like, garlic-like, goat-like, foul, or nauseating depending on the nature of the plant juices.

DeCandolle (1804) in "Essai sur les proprietes medicales des Plantes, comparees avec leurs formes exterieures et leur classification naturelle," stresses the taxonomic value of plant secretions. Jasmineae is separated from Oleineae based on the observed ability of insects to discriminate between the groups and grafting incompatability. The juices and fibres were not considered analogous, indicating distinct groups. (Gibbs In Swain,1963).

A pioneer of modern chemotaxonomy, Helen C. de S. Abbott (1886,1887) stated, "There has been comparatively little study of the chemical principles of plants from a purely botanical view. It promises to become a new field of research."

Expressing the scientific interest of the times, an explosion of inquiry occurred during the first half of the twentieth century. Eykman (1888) and Greshoff (1891) did early work on the occurrence of alkaloids in certain plant families. Later Greshoff published a summary of his work at Kew. In his research he studied the occurrence of tannins, cyanogenetic compounds, and saponins in a wide variety of plants. Defining comparative phytochemistry as, "the knowledge of the connection between the natural relationships of plants and their chemical composition," he advocated the inclusion of chemical attributes as part of "every accurate description of a genus or of a new species." (Gibbs In Swain,1961).

Between 1917 and 1945 McNair was a proponent of chemical taxonomy. In separate publications he described: the taxonomic significance of fats and oils; the occurrence of alkaloids in the genus Acontium L.; and angiosperm phylogeny based on a number of chemical attributes. In 1945, he presented a general paper describing chemical ontogeny and phylogeny. McNair's work, evaluated through the perspective of time, exhibited a fallacy not uncommon to new areas of inquiry. He used chemical attributes alone to establish taxonomic relationships. Comparative chemistry, while being useful in taxonomy, has appropriate interpretive value. However, McNair's work did provide a wealth of chemical data and expanded the horizons of plant research.

C. Chemical Attributes

A range of chemical criteria exists to elucidate the relationships between taxa. Cyanogenic glycosides, proteins, terpenoids, alkanes, acetylenic alcohols, anthocyanins, non-protein amino acids, flavonoids and alkaloids are used as taxonomic evidence.

Bogler (1985) provides a concise overview of chemical attributes used in taxonomic work. Recent journal articles indicate the present focus of research.

Coats and Cullis (1987) studied chloroplast-DNA restriction site polymorphism to propose evolutionary relationships between species of Linum L.

Nicholls and Bohn (1983), using a combination of column chromatography and partition chromatography on cellulose identified 56 flavonoid compounds of Lupinus L. by U.V. spectral characteristics. In several groups, flavonoid affinities paralled morphological relationships.

Hart (1979) used a combination of characteristics: geographic distribution, morphological attributes, cytotaxonomic data, hybridization experiments, and flavonoid analysis to clarify the taxonomy of the Bidens ferulaefolia Complex.

D. Literature Review

A comprehensive review of research on legumes is provided by various authors in Advances in Legume Systematics (Polhill and Raven, 1981). In the chapter, "Chemosystematics of the Papilionoideae" by Gomez et. al., the primary literature sources are mentioned.

The Chemotaxonomy of the Leguminosae (Harborne, Boulter and Turner, 1971) includes contributions from several authors. Mears and Mabry (1971) described the 'Alkaloids of the Leguminosae'; 'The Comparative Biochemistry of Non-protein Amino Acids' is provided by Bell (1971); Harborne (1971) authored the 'Distribution of Flavones in the Leguminosae' ; and Boulter and Derbyshire (1971) wrote 'Taxonomic Aspects of the Structure of Legume Proteins'.

Several authors combined to provide detailed information in The Flavonoids, edited by

Harborne and Mabry (1975). Separate articles describe compounds of taxonomic interest.

E. Compounds of Taxonomic Value: Overview

Many chemical compounds are utilized as taxonomically valuable markers. Bell and Lackey (1978) describe the systematic significance of canavanine in the Papillionoideae. Described as a non-protein amino acid, canavanine arose as a secondary defense compound in a group of plants already able to accumulate alkaloids. Found in 93 genera (16 tribes), canavanine serves multiple functions. It is recognized as a feeding deterrant, storage compound and has demonstrated allelopathic properties. The tribes characterized by the occurrence of canavanine are considered relatively advanced.

Enzymes and storage proteins are commonly used to identify parents of F_1 hybrids. Protein band patterns generated by gel electrophoresis are superior to morphological characters in detailing outcrossing (Boulter, 1981).

Protease inhibitors form characteristic bands when run in a disc electrophoresis system. Functioning to inhibit proteolytic enzymes, these compounds also serve as defense compounds and storage proteins (Weder, 1982).

Lectins are described as "plant or animal proteins (glycoproteins) not known to be antibodies, that combine specifically with an antigen to produce a phenomena resembling an immunological reaction." Plant haemmagglutinins are recognized as possible taxonomic markers in the Papilionoideae. As multifunctional substances they act as natural plant antibodies, defense agents against pathogenic bacteria, and as binding agents for symbiotic bacteria (Rhizobium). (Toms, 1981).

Phytoalexins are highly fungitoxic compounds characterized by rapid, localized production. A variety of compounds act as phytoalexins or 'stress metabolites'. Most legumes accumulate various flavonoid and non-flavonoid phytoalexins. These compounds can provide information of systematic and evolutionary value (Ingham, 1982).

Terpenoids including gibberellins, plant resins, and certain carotenoid pigments are valuable. "The variety of terpenoids in different plant parts and indication of tight genetic control of even quantitative composition will entice more systematic and ecological studies of this important group of compounds." (Langenheim, 1982).

Flavonoids, as a diverse group, have a variety of functions. Bogler (1985) provides a good discussion. The anthocyanins yield color expression in flowers and fruits. Other flavonoids provide protection against damaging U.V. radiation. A positive correlation between altitude and leaf flavonoid content is described for Eucalyptus in Tasmania (Swain, 1966). Flavonoids also function as feeding deterrents and phytoalexins.

F. Theory

A divergence of opinion exists concerning the weight chemotaxonomic evidence should receive in resolving taxonomic problems. Several advantages are inherent in the use of chemical evidence. The genetic basis for expression is more easily determined.

"Chemical constituents are genetically controlled, and have the advantage over morphological ones in that they can be very exactly described in terms of definite structural and configurational chemical formulae." (Harborne and Turner, 1984).

Hybridity and introgression can be readily determined from chemical data. Flavonoid arrays enabled the identity of up to four parental species of Baptisia Vent. involved in hybrid swarms; where no more than two could be identified solely on morphological grounds (Alston and Turner, 1963).

Chemical attributes provide information useful in clarifying the taxonomy of various groups. Crawford and Julian (1976) utilized seed protein profiles and flavonoids to clarify the taxonomy of western U.S. narrow-leaved Chenopodium L.

There are also several disadvantages. Chemical characters are affected by environmental variables. It is known for example that alkaloid production is greater in arid environments. Metabolic pathways are difficult to understand with certainty; different interpretation leads to different conclusions. Also, homology between characters can be difficult to define. Particular chemical attributes are valuable markers in some taxa; while less valuable in others. The resources and equipment necessary for the study of chemotaxonomy generally limit such research to specialized institutions. Chemical attributes are not directly useful by field biologists but can provide a more biologically valid system which in turn can be adapted to field use.

XI. ALKALOIDS

A. Background information

The beginnings of alkaloid chemistry date to 1817 when it was recognized that morphine was the principle responsible for the effect of opium. (Swain, 1963). The first report of alkaloids in Lupinus was in 1835 (Fleak, 1971).

Alkaloids are more or less toxic substances which act primarily on the nervous system. Basic in character, they contain heterocyclic nitrogens and are synthesized in chloroplast membranes from amino acids or their immediate derivatives. These are defined as 'true alkaloids'. Other chemically related compounds are described which are considered taxonomically distinct. 'Protoalkaloids' are derived from amino acids but lack a heterocyclic nitrogen ring. Ephedrine, mescaline, and aliphatic quaternary bases such as choline and acetylcholine are examples. When 'protoalkaloids' occur in a genus or family also exhibiting 'true alkaloids', they are recognized as alkaloids if a biogenetic relationship exists. The majority of 'true alkaloids' belong to classes which are structurally related to parent bases such as pyridine, piperidine, isoquinoline, and tropane (Harborne and Turner, 1984; Smith, 1976).

Waller and Nowacki (1978) report in excess of 6,000 known alkaloids. Alkaloid-containing plants are described as accumulating greater than .01% (dry weight) of the crystalline substance. Ten dicot and two monocot families are known to be rich in alkaloids (Figure 9). Within each of these families fifty or more structures are known. Leguminosae is described as having over 450 alkaloids, with quinolizidine alkaloids representing the largest group. Many of these occur in the genus Lupinus; Dunn (1984) estimates over one-hundred. The designation 'lupin alkaloids' generally refers to all quinolizidine alkaloids but is a misnomer. Non-quinolizidine alkaloids occur in Lupinus, and 'lupin alkaloids' occur elsewhere. Genera of Solanaceae accumulate lupin alkaloids. Lupinus accumulates quinolizidine and piperidine alkaloids as well as the indole alkylamine, gramine.

FAMILIES WITH HIGH LEVELS OF ALKALOIDS

DICOTS	MONOCOTS
Apocynaceae	Amaryllidaceae
Compositae	Liliaceae
Leguminosae	
Loganiaceae	
Menispermaceae	
Papaveraceae	
Ranunculaceae	
Rubiaceae	
Rutaceae	
Solanaceae	

Figure 9. (Derived from Harborne & Turner,1984).

B. Evolutionary Considerations

The biosynthetic pathways leading to the production of quinolizidine alkaloids extend back to the divergence of the three subfamilies of Leguminosae, 50-60 million years ago. The South American genus Acosmium Schott In Sprengel is the most primitive legume known to biosynthesize quinolizidine alkaloids. It is identified from Tertiary strata. (Kinghorn and Ballandrin, 1982). The origin of these compounds is described by Ehrlich and Raven (1964,1967). "Angiosperms have through occasional mutations and recombination produced a series of chemical compounds not directly related to their basic metabolic pathways but not inimical to normal growth and development."

Nowacki and Waller (1978) elaborate: "A plant in which a repressor or inhibitor system cannot be switched off, owing to a mutation, will produce more of a given amino acid than required for protein synthesis. When the level of this amino acid passes a certain threshold value, the plant will either perish or the accumulated amino acid will induce enzyme synthesis so further metabolism can occur. Alkaloids are reasonable products for this metabolism. A species that turns to alkaloid production probably escapes certain predators."

Quinolizidine alkaloids occur in ten of the most primitive tribes of Papilionoideae. Salatino and Gottlieb (1980) describe the systematic and ecogeographic significance of quinolizidine alkaloids based on evolutionary advancement parameters (EAP values). Geographically, African genera have low EAP_x values (skeletal complexity); while European, Asiatic, and American genera have increasing EAP_x and decreasing EAP_y (level of oxidation) values.

Genisteae exhibits two distinct evolutionary lines. A primitive ascending line starting with Lupinus and leading to Argyrolobium is characterized by specialization of the oxygenation pattern. A descending pattern beginning with Cytisus L. and leading to Laburnum Medic. represents an advanced line with elaboration of skeletal complexity and progressive blocking of specialized oxygenations.

The alkaloid data on Lupinus suggests an origin in tropical Africa with subsequent radiation into South America and later into North America. Five advanced N. American species are the only taxa that contain the enzyme systems necessary to synthesize cytisine. The alkaloid virgilene is described as a marker indicating affinity between North and South American Lupinus (Dunn, 1984). Quinolizidine accumulating Papilionoideae appear to have radiated from tropical Africa in three directions: along a southern temperate pre-cytisine route; a northern temperate pre-cytisine/ cytisine route; and a tropical ormosamine route with a temperate cytisine branch. (Salatino and Gottlieb, 1980). Ormosia-type quinolizidine alkaloids are considered advanced. (Kinghorn and Balandrin, 1982).

A tribal sequence of evolutionary advancement, based on the biogenesis of alkaloids, indicates the following order: Sophoreae-Genisteae-Thermopsideae (Salatino and Gottlieb, 1980).

C. Biosynthesis of Quinolizidine Alkaloids

Wink and Hartmann (1981) proposed a biosynthetic pathway leading to the production of quinolizidine alkaloids. Lysine decarboxylase catalyzes the conversion of lysine to cadaverine. Then, 17-oxosparteine synthase, a membrane associated, pyruvate dependent, cadaverine-pyruvate transaminating enzyme, acts to form 17-oxosparteine. Through isomerization lupanine is formed, becoming the precursor to: "sparteine, 13-hydroxy lupanine, the saturated tricyclic alkaloids (angustifoline and tetrahydrorhombifoline), as well as esters and the alpha-pyridone alkaloids and their respective tricyclic analogs (rhombifoline and tinctorine). " (Wink, 1984).

Nowacki and Waller (1975), Robinson (1968) support an alternate biogenetic scheme, where lupinine is the precursor to sparteine, with successive oxidations leading to lupanine and hydroxylupanine. Research indicates that labeled sparteine, injected into lupine seedlings, can be converted into lupanine and hydroxylupanine, but the reverse reactions are observed at an insignificant level. The chloroplast stroma was identified as the subcellular site of synthesis. Lysine accumulation in the leaves is light-dependent;

resulting in a diurnal rhythm of alkaloid production. Synthesis is favored at pH 8; the pH-optima of the catalyzing enzymes. (Wink and Hartman, 1981).

Quinolizidine alkaloids are exported through the phloem and unloaded into the peripheral cell layers of stems, including the epidermis and one or two subcellular layers. Late in the season they are transported into seeds, pods, and roots where they serve a protective function. Rowson (1945) established that induced polyploids produce more alkaloids than the original diploids.

Kinghorn and Balandrin (1982) describe the 'dynamic metabolic state of alkaloids.' as they are synthesized, disappear, and reappear depending on stage of development, organ examined, or time of the year. Five amino acids: phenylalanine, tyrosine, tryptophan, lysine, ornithine, and in exceptional cases aspartic acid, were found to be the precursors of a majority of alkaloids. A number are derived from nicotinic acid or from a pyridine nucleotide pool; others from monoterpenes, sesquiterpenes, diterpenes and steroids; and a smaller number from anthranilic acid, purines, and pyrimidine bases. (Nowacki and Waller, 1978). One mutation may cause a conversion of one amino acid into an alkaloid.

"Charles P. Smith, through the work of J. F. Couch (1940) was aware of the presence of alkaloids in selected lupine taxa; however there is little evidence that he placed much confidence in the alkaloids for chemosystematics." (Fleak, 1971).

Schaller (1977) determined through hybridization and breeding studies that parental alkaloids may disappear in the F_1 hybrids, or additional alkaloids not formed in either parent may appear.

Dunn and others (Bogler,1985; Conrad, 1980; Bennett, 1978; Schaller, 1977 Rafaill, 1976; Vaughan, 1975; Cox, 1972; Harmon, 1972; Fleak, 1971) used alkaloids in Lupinus to help assess relationship and delineate between species in large complexes. Alkaloid data was used to validate natural divisions based on morphological characters and geographic distribution.

D. FUNCTION OF ALKALOIDS

The seven major classes of toxins in plants are: alkaloids, cyanogens, non-protein amino acids, iridoids, sesquiterpene lactones, diterpenoids, and triterpenoids (including steroids) (Harborne and Turner, 1984). Alkaloids, as secondary metabolic compounds, are utilized in various ways by higher organisms. The alkaloids of the Columbian arrow-poison frog, Phyllobates aurotaeni, are highly toxic as well as those secreted by the dinoflagellates, Gonyaulax tamarensis and G. catanelli, creating 'Red Tides' (Pelletier,1986). The Texas leaf-cutting ant (Atta texana) uses certain alkaloids as trail pheromones (Pelletier,1986). Many poisonous plants signal their toxic nature through a strongly disagreeable taste. On a Pacific Northwest rangeland, lupines are generally the last plants to be consumed by grazing livestock.

The functions of quinolizidine alkaloids are described by Wink (1985), Wink & Witte (1984), Wink (1984), Harborne & Turner (1984), Kinghorn & Balandrin (1982), Waller & Nowacki (1978) and others. Alkaloids may function as protective defense compounds against herbivores and deleterious micro-organisms, detoxification products of primary metabolism, growth regulators, storage reservoirs of nitrogen, substitutes for minerals, and are implicated in having allelopathic effects. Janzen (1981) described the function of phytochemical compounds: "Natural selection favors parsimonious function; one trait is likely to function in many roles, and one class of traits even more so."

Meeker & Kilgore (1987) described a widely recognized toxicity effect attributed to the alkaloid anagyrine.

"Crooked calf disease is a congenital deformity characterized by twisted or bowed limbs (arthrogryposis), curvature of the spine (scoliosis), twisted neck (torticollis), and/or cleft palate."

Anagyrine represents 86% of the total alkaloid fraction in L. latifolius Agardh and 50% in L. caudatus Kell. The highest occurrence of livestock losses is during seeding stage. Concentrations of lupanine, a primary lupin alkaloid, has a range of 1 to 40 mM./kg. in leaves, stems, and roots, or up to 200 mM./kg. in seeds. (Wink, 1984). The pyridone alkaloids anagyrine and cytisine are more acutely toxic than saturated alkaloids such as sparteine and lupanine (Kinghorn & Balandrin, 1982).

Quantitative variation of alkaloids within a species is well documented. (Harborne & Turner, 1984; Mankinen, Harding, and Elliott, 1975).

"Individual variability in alkaloid profiles would constitute an anti-specialist chemical defense mechanism which impedes or prevents selection of resistant strains of phytophagous insect pests." (Harborne & Turner, 1984).

This is borne out in several species of Lupinus, in studies on the predation patterns of Glaucopsyche lygdamus (Doubleday), the lycaenid larvae (Dolinger, Ehrlich, Fitch, Breedlove, 1973). Lycaenids place a frequency dependent selection pressure on lupine populations.

Plebejus icarioides (Boisvidal), the blue lycaenid butterfly larvae is an obligate feeder on species of Lupinus. Equipped with a quinolizidine alkaloid physiological detoxification system, the blue butterfly occupies a specialized niche. Aphis cytisorum Hartig., Acyrthosiphon spartii (Koch) and a few Lepidoptera are also specialized on quinolizidine producing plants. (Wink, 1984).

Several studies provide insight into the functions of alkaloids. Lupanine, sparteine, lupinine, and 13-hydroxylupanine are reported to be toxic and inhibitive to the growth of the aphid, Acyrthosiphon pisum (Harris) (Kinghorn & Balandrin, 1982). The same aphid is noted to transmit a virus (lupine leaf narrowness) to alkaloid-poor L. luteus.

Lupanine is described as a toxic feeding deterrant and growth inhibitor of the two-striped grasshopper, Melanophus bivittatus (Say) . Also, thrips (Frankiniella Karney) exhibit a preference for 'sweet' or low-alkaloid lupines (Kinghorn & Balandrin, 1982).

Lagopus lagopus, the European hare, has the ability to discriminate between sweet and bitter lupine varieties (Waller & Nowacki, 1978).

Thrips, locusts, bruchid beetles, and aphids are all deterred by quinolizidine alkaloids acting as a 'systemic pesticide' (Wink, 1984). They also act as a general deterrent against herbivores including: molluscs, snails, and slugs. (Wink, 1986; Pate, 1984).

Plant anti-microbial defense systems employ a variety of strategies. At the morphological level the texture and composition of tissues influence the capacity for bacterial or fungal infection. Balanced osmotic pressures (suitable pH) discourage bacterial growth. Also, secondary chemical compounds provide protection from bacterial or fungal attack. Wink (1984) reported that the growth of bacteria was completely inhibited if the medium contained sparteine at a concentration of 20 mM. In addition, the growth of six phytopathogenic fungi was inhibited at a sparteine concentration of 15 mM/kg. It is also stated that quinolizidine alkaloids are less effective antifungal compounds than isoflavones and pterocarpanes.

Research indicates that quinolizidine alkaloids act as detoxification products, nitrogen storage compounds, allelopathic compounds, and growth regulators.

Waller & Nowacki (1978) describe detoxification products as "removing active molecules from sites where they could unbalance the metabolism."

Wink and Witte (1984) indicate that alkaloids are a potential nitrogen source in seeds. Alkaloid contents in seeds and seedlings decreased by 20-100% during germination and seedling stages (Wink, 1985).

Describing growth effects, Waller & Nowacki (1978) report that lupinine and hydroxylupanine were slightly stimulating to seedling growth, while sparteine and lupanine were inhibiting. It was noted that some alkaloid precursors are powerful growth stimulators; while others show an inhibitory effect.

Quinolizidine alkaloids inhibit the germination of Lactuca sativa L. and grass seeds implying allelopathic effects. (Pate, 1984).

The most widely recognized function is that of feeding deterrence. The evolution of alkaloids parallels the development of herbivore feeding patterns. The horse is much more susceptible to these toxins than the goat, sheep, or rabbit; reflecting the more recent evolution of the later in the Eocene Era.

Figure 10. Informational pamphlet promoting Tarwi - Lima, Peru.

XII. ECONOMIC BOTANY

Historically, Lupinus has been an economically important genus. Grown primarily as a green manure, lupines were also valued for human consumption, animal fodder, and ornamental purposes. With the continued development of low-alkaloid, disease-resistant strains, lupines have become an important crop. As a pioneer plant, food production is extended into marginal areas. Protein-rich and easily digestible; lupines could well complement the soybean in providing much needed nutrition for the world's hungry. In addition, their ornamental value is considerable as attested to by the breeding work which has resulted in numerous garden varieties.

Lupines have been utilized for 6,000 years. In Greek and Roman civilizations, they were grown as a green manure and recognized for their palatability. A 3-4 day continuous rinsing process eliminated the bitter taste (toxic alkaloids), enabling consumption.

In South America, the Incas utilized lupines as a green manure, for human consumption, animal forage, and for medicinal purposes.

Under Indo-european influence lupines fell out of favor, probably due to their high alkaloid content. In the 1800's consumption of the plants decimated flocks of sheep in Germany. Later, this toxic effect was attributed to the presence of a fungus, Phomopsis leptostromiformis, which produces the highly toxic phomopsin A. 'Lupinosis' is now known as a mycotoxicosis caused by this fungus growing on lupines. Undoubtedly, this condition contributed to the low status of crop lupines. With the discovery of low-alkaloid mutations by Von Sengbusch in 1928; the prospect for agricultural use increased. However, with the availability of agrochemicals, agricultural production turned away from a self maintaining production system, to a monoculture of high yielding crops. The emphasis was on profit maximization in the agrarian sector.

Gross (1984) sees a return to the implementation of integrated, ecologically adapted production systems in which legumes play a predominate role.

"In system theory it is by now common knowledge that the degree of diversification of a biological system determines the efficiency of a buffering system against external influences." (Gross, 1984).

Lupines are frequently used as a green manure and a 'break crop'. Research indicates that 176 kg. of nitrogen per hectare per year is fixed on average in Rhizobium lupini Schroeter nodules. (Rowland, Mason, and Hamblin, 1986). Increases in yields of crops grown after lupines of 30 to more than 100 percent have been recorded when compared to a wheat monoculture. Melzer and Lucke (1984) report a positive beneficial effect of Lupinus polyphyllus Lindl. on the increment of Picea abies (L.) Karst. Improved soil structure is also observed. Deep penetrating roots provide erosion control, while supplying organic matter deep within the subsurface. Nitrogen fixing nodules have been found to a depth of 60 cm. Crops show increased cold resistance, and better grain yield and quality in rotation with lupines. As a 'break crop', disease organisms lack a suitable host. The crops that follow are healthier, more vigorous plants; less vulnerable to disease. In Germany, both L. luteus and L. angustifolius L. are utilized as fertility building crops. However, the removal of soil-derived nitrogen in seed leads to a net reduction of total soil nitrogen. (Rowland, Mason, and Hamblin, 1986).

Cropping of lupines for grain yield is an established practice in Europe, the U.S.S.R., and the highlands of South America. In Australia, production has increased from a few hundred hectares in 1960 to 500,000 hectares in 1984. (Plitmann and Heyn, 1984). Literature available through the International Nutrition and Genetics Corp. compares the potential of crop lupines to soybean. With the ability to grow in a much greater range of soil types; lupines can outyield soybeans by up to 100 percent.

Diseases of lupines cause a severe reduction in acreage planted. Fusarium oxysporum Schlect ex Fr., Griffiths (soil-borne wilt) is a serious problem in Eastern Europe; while Pleiochaeta setosa (Kitchn.) Hughes remains as the most destructive pathogen of winter sown lupins. No resistance is known. (Watkin & Williams, 1986).

Aboriginal use of lupine in South America took many forms. Antunez (1986) estimated a five percent incidence of 'Tarwi' in the diet. L. mutabilis Sweet was found among the offerings for the dead in the tombs of Nazca. 'Tarhui' (pre-Columbian Peru) was consumed on the occasion of the "huaillapa" (time when spirits of the house reigned). In addition, tarhui was used in native pharmacopoeia to promote fertility, cure heart ailments, eliminate intestinal parasites, as an insecticide and more. Many of the medicinal qualities can be attributed to the nutritional value of lupines.

Today, 'sweet' lupine (less than .03 % alkaloids) is recognized as an economical protein and energy source. With negligible amounts of trypsin inhibitors, it is easily digestible. Lupines are non-gassy, containing no raffinose or stachyose; both implicated in causing intestinal flatulence and general digestive stress. In addition, containing the more stable oleic acid, processed lupine has a longer shelf life than soy products. The primary quality is the protein content; ranging between 32-42 percent, depending on variety tested. Protein quality is higher if supplementary methionine is added. High in fiber, lupines are utilized as a food supplement. El Instituto de Nutricion in Lima, Peru has been promoting the use of lupine as a nutritional supplement since the early 1980's. Striving to curb the incidence of malnutrition; the program has focused on making 'Tarwi' publicly acceptable. In feeding trials with children (8-15 years old), results indicate improved protein intake and general acceptability (Hill, 1984).

Lupines also contain a relatively high oil content compared to other pulses, with the exception of soybeans and peanuts. The species most utilized for human consumption include: L. albus , L. angustifolius, L. luteus, and L. mutabilis. Lupine flour is incorporated into pasta, bread, and mixed with other grains.

The primary market for lupine is as animal feed. (Watkin, 1986). It has been added to the diet of chickens, pigs, turkey (Hill,1984), and rainbow trout, (Herrera, 1980), with positive results. Research is focused on creating disease resistance and crop protection. The development of lupin seed with increased levels of methionine is also a priority. Watkin Williams (1984) described the genetic control of alkaloid production in L. mutabilis: "Plants homozygous for the mutant allele 'mutal' are organoleptically sweet."

Concentrations of sparteine and lupanine are reduced.

The area cultivated in lupines is approximately two million hectares divided 50/50 between grain production and green manure. (Bellido, 1984). The U.S.S.R crops 53.3% of the total area, Australia 26.6%, and Poland 15.2%. L. luteus is widely grown in the U.S.S.R. and Poland; while L. angustifolius is used predominantly in Australia, and L. albus in the U.S. In 1983, crop lupines represented 0.8 percent of the world production of grain legumes. Australia has shown an exceptional increase in production over the past few years. Chile and Poland also show signs of an upturn.

Lupinus spp. is also recognized for it's ornamental qualities. Early botanical collectors were looking for exotic plants to take back to ornamental gardens. L. leucophyllus and L. plumosus were first described in Edward's Botanical Register: *Plants and Shrubs Cultivated in British Gardens*. In 1911, George Russell through hybridization and selection created the "Russell hybrids". With 20 cultivars named (eg. 'Torchlight'), these remain as the most popular garden lupines. They are generally considered to be the result of crosses and backcrosses between L. polyphyllus Lindl., L. arboreus Sims., and L. nootkatensis Donn ex Sims. Other species utilized in garden situations include: L. arboreus, L. hartwegii Lindl., L. luteus L., L. mutabilis Sweet., L. nanus Dougl., L. perennis L., and L. polyphyllus. From time to time, natives are recommended for garden use: L. albifrons *var. flumineus* (Schmidt, 1980), and 'Canyon Sunset', a cross between a 'Russell Hybrid' and L. latifolius *var parishii* (Emery, 1977). Physiologically restricted to climates featuring cool summer nights, lupines are a valuable garden addition.

XIII. MORPHOMETRIC METHODS

During the summer of 1984, field investigations of L. leucophyllus involved collection of specimens and observation of the habitat. I am indebted to Dr. David B. Dunn for his encouragement and financial assistance.

Initially, the major herbaria were visited in Montana, Oregon, and Washington with ecological information recorded and distributions mapped. These visits complemented the information Dr. Dunn supplied concerning the distribution of the subtaxa. Specimens were collected in Wyoming, Montana, Idaho, Washington, Oregon, and California; the majority collected in Oregon. These specimens were accessioned to the herbarium of the University of Missouri (UMO).

Herbarium material was borrowed from various institutions for this study (Figure 11). These loans were also greatly appreciated. Twenty-five specimens were selected (when available) from each of seven groups for measurement. Specimens utilized were selected randomly except that those chosen were complete specimens.

Two flowers and a floral bract were removed from each for detailed study. These flowers were fully open, without fruit development. Hopefully, those selected reflected developmental uniformity, without the potential alteration created by pollinator activity or ovary expansion. Slide labels included collector, specimen number and location. The two flowers and floral bract were placed in a 100 ml. beaker, filled with distilled water to which a few drops of saffranin dye was added. The parts were heated to slightly less than boiling on a hot plate until they were pliable enough for dissection (10-15 minutes). Conrad (1980) provides a description of the dissection procedure as outlined by Dunn (1954). A muscilage and distilled water mixture functioned as the binding agent. Applied to the slide prior to dissection, this mix included the addition of a few crystals of phenol as an antifungal agent.

"One flower was mounted whole with the left surface exposed on a 1" by 3" glass microscope slide in the upper left corner. The second flower was dissected as follows:

CAS - California Academy of Sciences, San Francisco
DS - Dudley Herbarium, Stanford University
GH- Gray Herbarium of Harvard, Cambridge, Mass.
HSC- Humboldt State University, Arcata, Calif.
IDS- Idaho State University, Pocatello, Id.
MO- Missouri Botanical Garden, St. Louis, Mo.
MONT- Montana State University, Bozeman, Mt.
MRC- University of Montana, Missoula, Montana
NY- New York Botanical Garden Herbarium, Bronx, N.Y.
ORE- University of Oregon Herbarium, Eugene, Ore.
OSC- Oregon State University Herbarium, Corvallis, Ore.
POM- Pomona College, Claremont, Calif.
RM - Rocky Mountain Herbarium, Laramie, Wyoming
RSA- Rancho Santa Ana Botnaic Garden, Claremont, California
UTC- Utah State University, Logan, Utah
WS - Washington State University, Pullman, Wa.
WTU- University of Washington- Seattle, Wash.

Figure 11. Herbarium Citation

Using a dissecting needle, the left lateral sinus of the calyx was split just beneath the bracteole, and then the calyx lips were reflexed from the petals and severed at it's base from the remainder of the flower so that the petiole was left attached to the calyx. Then the calyx was mounted, with it's inner surface exposed, on the slide to the right of the whole flower. The banner was then separated from the remainder of the flower and carefully mounted with it's dorsal surface exposed on the slide to the right of the calyx. Next, the two wing petals were separated from the base of the keel and with their apices still connate, the claws were spread the maximum distance apart, and then mounted with the lateral surfaces exposed on the slide beneath the banner. The keel was then mounted with the right lateral surface exposed beneath the whole flower. The floral bract was mounted, with it's pubescent abaxial surface exposed, to the left of the keel. As each flower or individual part was placed into position, dissecting needles were used to carefully spread the petals and gently float them on the mounting liquid to avoid any folding of the margins." (Conrad, 1980).

Excess liquid was removed using blotter paper. After a period of drying, slides were painted with a dilute herbarium plastic cement and dried under an incandescent light. (Figure 12). The mixture contained two parts plastic resin to one part toluene; described by Dunn (1954) as Archer's Mix. Periodically, toluene was added to maintain the proper consistency.

Measurements were taken from roughly 200 slides including the type specimens prepared by Dr. Dunn, and other species noted as introgressing with <u>L</u>. <u>leucophyllus</u> (Figure 13).

Three types of data resulted: numerical (measurements recorded in metric units or degrees), ordinal (counted attributes such as the number of undeveloped ovules in the carpel), and qualitative data (descriptions of characteristics - eg. blue flowers).

Figure 14 indicates the floral attributes measured and recorded. Measurements were made to the nearest tenth of a millimeter. Rough assessments of banner and keel angles

Figure 12. Photo. Slides prepared for measurement; a comparison of variability

L. argenteus Pursh.

L. caudatus Kell.

L. christinae Heller

L. holosericeus Nutt.

L. laxiflorus Dougl.

L. oreganus Heller

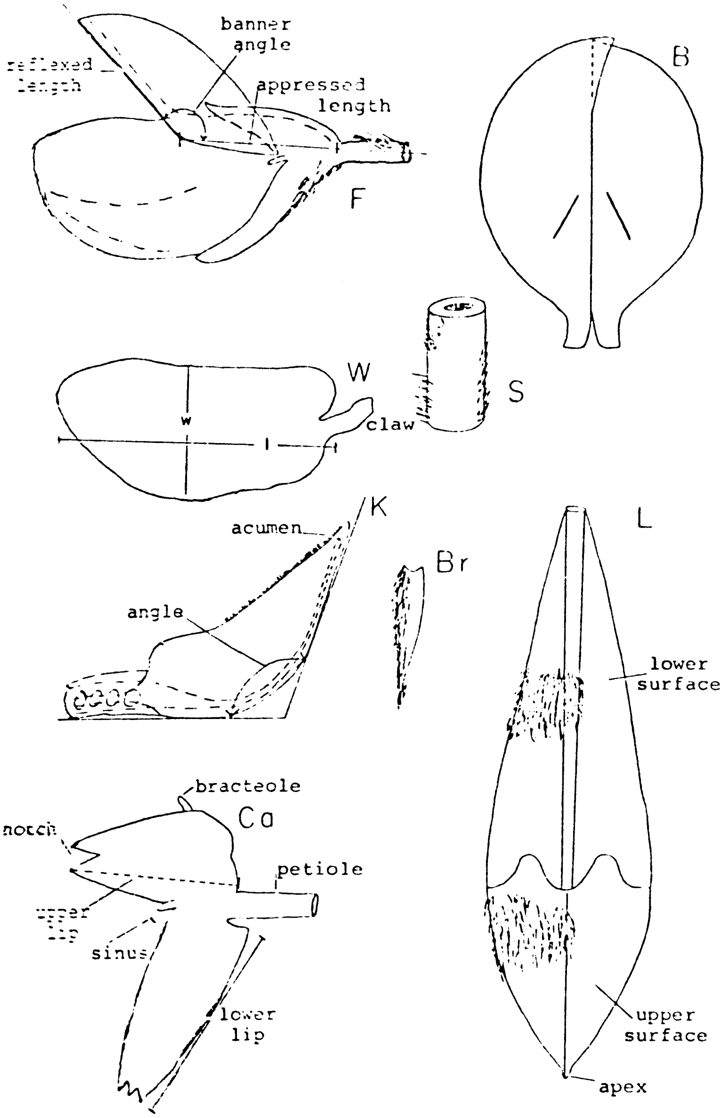
L. polyphyllus Lindl.

L. sericeus Pursh

L. sulphureus Dougl.

L. wyethii Wats.

Figure 13. Species known to introgress with *L. leucophyllus* Dougl.



Diagnostic characters in the Lupinus flower, stem, and leaflet. F - Flower, Left lateral view, B - Banner, Br - Bract, S - Stem, W - Wing, K - Keel, Ca - Calyx inside view, cut open at left lateral sinus, L - Leaflet.

Figure 14. Diagnostic characters of flower, stem, and leaflet.

(From Conrad, 1978)

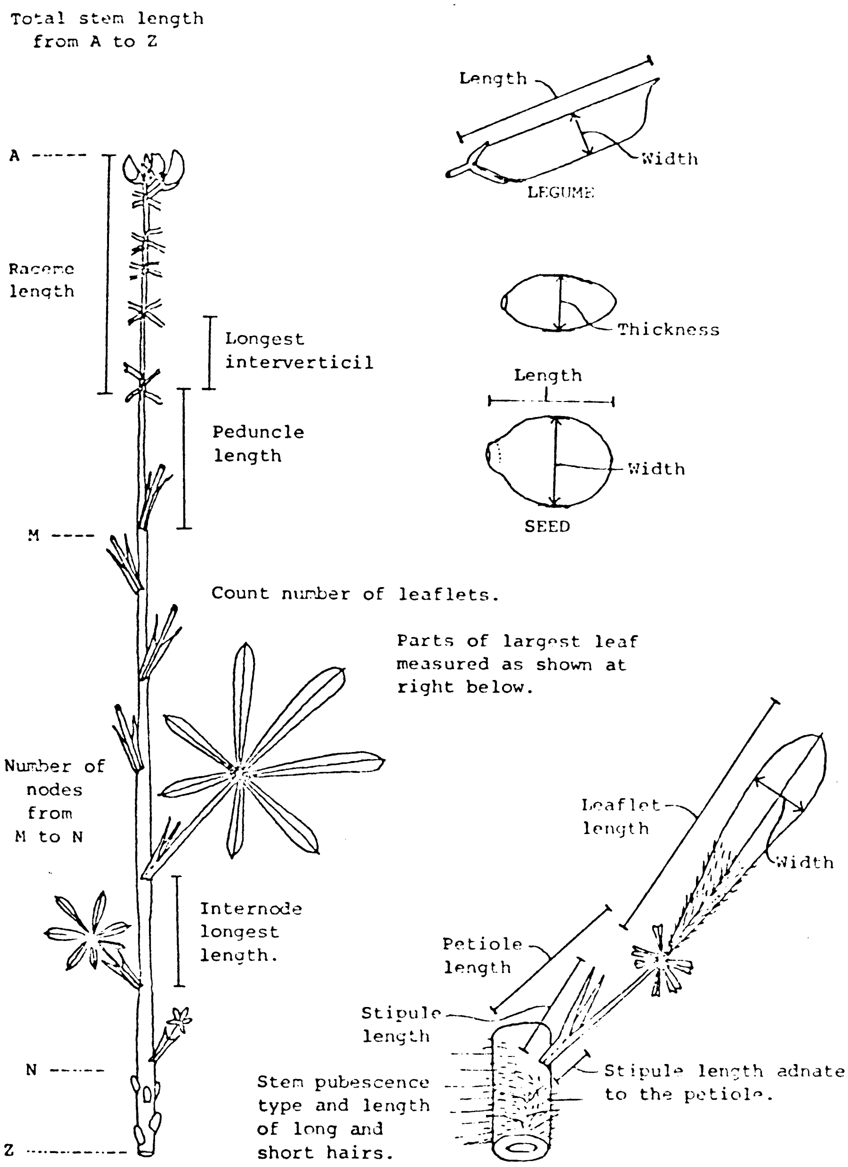


Figure 15. Vegetative, fruit, and seed characteristics . (From Conrad, 1978).

angle from a compass. Angular measurements were recorded to the nearest degree.

Vegetative traits were also measured and recorded (Figure 15). These were made to the nearest tenth of a millimeter, except for ratios, and ordinal or qualitative data.

Information was initially recorded on a data sheet prior to transfer to a matrix and subsequent entry into a computer data base. Standardization of data occurred as part of Principal Components Analysis. Objects lacking data points were automatically eliminated from statistical analysis and graphic representation.

XIV. PRINCIPAL COMPONENTS ANALYSIS

Statistical analysis of data continues to be an integral part of plant systematic work. The availability of statistical software enables a diversity of techniques to be used in assessing variation. Statistical analysis provides an expedient way to test data, identify significant diagnostic characteristics, identify correlated attributes, and obtain groups through clustering techniques. Several types of analysis are illustrated in recent publications: cluster analysis (Takahata and Hinata, 1986); canonical discriminate analysis (Ranker and Schnabel, 1986); multivariate analysis (Furlow, 1987; Adams, 1982); and principal components analysis (Walters, 1988). Romesburg (1984) provides a clear overview of clustering techniques.

For the statistical analysis of data obtained from morphological measurements in the study of L. leucophyllus, principal components analysis was utilized. The Princomp Procedure outlined in the S.A.S. User's Guide, Version Five (S.A.S. Institute, 1985) was used on the University of Missouri mainframe to generate clusters and three-dimensional graphs based on the primary components.

Principal components analysis (P.C.A.) was originated by Pearson (1901) and further developed by Hotelling (1933). Rao (1964) presents details and applications. P.C.A. is a multivariate technique for examining relationships among several variables. In this study it is used to reduce the number of variables in clustering and graphic applications. Each principal component represents a linear combination of the original variables (S.A.S. Institute, 1985). Each combination is a unique set, dependent on the attributes selected for comparison. A series of analyses were run using different combinations of attributes and objects. Of the thirty-four attributes used; four were nominal descriptors of a physical characteristic, designated as either present or absent (1,0). Separate runs depict principal components of vegetative, reproductive, and combined attributes. In one, the qualitative characters were omitted (See Appendix I).

Objects were designated by symbol and group affinity. Herbarium specimens were sorted according to a visual assessment of similarity, which emphasized floral dimensions and pubescence type. Groups were designated A through G. The resulting printouts included two-dimensional graphs with individual herbarium specimens coded by symbol, and a three-dimensional principal components graph with objects recognized by group identity.

Three two-dimensional graphs allowed for adequate separation of 179 objects. These were used to identify outliers and to reinspect groupings of specimens. On several occasions a symbol coded specimen was reinspected and determined to be best placed in another group. A number were maintained in their original groups.

A final analysis based on selected attributes provides a clearer picture of the taxonomic relationships in L. leucophyllus. Group A (*ssp. erectus*) is clearly set apart from the rest of the groups, and is recognized as a distinct subspecies, based on floral and vegetative characteristics. Group B from Coeur d'Alene is set apart based on vegetative characteristcs. Analysis of the remainder of the field, with Group A and B objects deleted reveals a range of variability for the remaining groups.

The printout includes simple statistics (mean, standard deviation, correlations), and eigenvalues for the principal components. The primary components are defined as those which contribute 5% or more to the cumulative variability. By inspection, the variability represented by each principal component can be described. The first component, accounting for the bulk of the variation, emphasizes floral variability; while the second component has more weight on vegetative attributes. (See Appendix II).

XV. CHROMATOGRAPHY

A. Introduction

Chromatography is a method of analysis in which the flow of solvent or gas promotes the separation of substances by differential migration from a narrow initial zone in a porous sorptive medium (Heftmann, 1961).

A crude form of chromatography was practiced by Pliny, who utilized a solvent of ferrous sulfate on a medium of papyrus. The invention of modern chromatography is dated as 1903, when. Mikhail Tswett used the technique to separate carotenoids and chlorophyll. Tswett recognized and correctly interpreted chromatographic processes, and devised laboratory procedures that included the developing of chromatograms by pure solvents.

Thin layer chromatography (T.L.C.) has several advantages over paper chromatography. It offers sharper separations, relatively low limits of detection, and a shorter running time. Quantitative determination is best acheived by high resolution techniques such as gas chromatography / mass spectrometry.

Several factors influence the quality of the TLC plate:

(1) The activity of the adsorbent layer is dependent on the nature and quality of the adsorbent, activation conditions, and atmospheric humidity.

(2) Layer thickness, plate format, and layer preparation combine to effect migration. "For best separation adsorbent grain size should be small and adsorption layer homogeneous." (Heftmann,1961).

(3) Chamber saturation is a prerequisite for good results. R_f values in an unsaturated tank are generally higher than in a saturated tank. (Pataki,1966).

(4) The techniques and procedures of chromatography may influence the results. Procedures should be standardized and carried out with consistency.

(5) The developing distance and the distance of the starting point from the surface of the solvent, influence both the rate of migration and the resulting R_f values.

(6) Quality of the solvent is important.

(7) Good TLC separation depends on sample application. Samples are best applied in equal volumes. (With alkaloids, application of 5-10 ug is ideal.)

(8) The presence of contaminants may influence the results. Reactions can occur between various solvents, or contaminants in solvents and alkaloids. (Svendsen, 1983). Artefacts may form particularly in chloroform solutions when photochemical decomposition occurs. The stability of alkaloids varies widely. Some are sensitive to light, others to pH, and others to various organic solvents. 'Edge-effects', where the solvent front migrates at a faster rate on the edges as compared to the center of the plate, can occur due to an unsaturated developing tank or differences in polarity between the components of the solvent solution.

B. Methods

The intent of the chromatography procedure was to compare the alkaloids of the variants of L. leucophyllus throughout their range to gain insight into the taxonomy. The quantitative or qualitative assessment of alkaloids was not of particular importance. The compounds resolved through TLC include alkaloids, as well as pigments, resins, flavonoids, and others.

"In dried plant material, alkaloids are generally liberated as bases from their salts by the addition of a stronger base, and then saturated with organic solvents." (Heftmann, 1961).

For the extraction of alkaloids, several leaves were selected from each specimen; a combination of younger and older leaflets. These were diced with a dissection razor into smaller pieces and then inserted into a clean, labeled test tube. Between each dicing procedure, all implements including the cutting surface were thoroughly cleaned. The material within the test tube was then pulverized with a glass rod; also cleaned after use. About ten drops of 30% potassium hydroxide (KOH) was added to cover the leaf fragments. The test tubes were gently rotated to insure adequate coverage and set aside for a half hour. Then, 1-2 ml. of chloroform (CH_2CL) was added after which the test tubes were rotated twice, secured with stoppers and placed in a refrigerator over night.

Regardless of the care taken, the concentration of alkaloids in the chloroform layer was variable. The objective was to extract a good concentration of alkaloid without regard to the level of standardization necessary to be able to make a quantitative assessment.

Silica Gel plates, 20cm. by 20cm., with 60u grain size and 250u layer thickness were utilized as the stationary phase. They were marked 2.5 cm. above the base with spots beginning 1.0 cm. from the left edge. Each plate had the capacity for nineteen alkaloid applications (1.0 cm. apart). The distance of migration was set by a line placed 15.0 cm. above the base line. Plates were activated prior to the application of alkaloid samples by being heated in an oven for 4-6 hours at 120° C. Before application, the mobile phase was measured, with the components set aside in separate flasks. Trial runs indicated superior results using a mobile phase of chloroform, methanol, and diethylamine (84:15:1). In retrospect, according to Svendsen (1983), "diethylamine interferes with alkaloid detection when using Dragendorf's or Iodoplatinate reagents." A representative series of plates were developed using chloroform, methanol, and ammonium hydroxide (95:4:1). Also, two-dimensional plates were run using chloroform, methanol, and ammonium hydroxide (95:4:1 and 85:14:1). A total volume of 200 ml. of solution was prepared for each run, providing for a depth of one and a half cms. of solution in the tank. The lateral sides of the tank were lined with chromatography paper and saturated with the developing solution to insure chamber saturation. Application of alkaloid spots occurred under a ventilator hood with additional air flow supplied by a hair dryer to rapidly evaporate the chloroform. A consolidated, concentrated spot resulted. By rotating and tilting each test tube, a microsyringe could retrieve the alkaloid-containing chloroform layer largely without contamination. After each application, the microsyringe was rinsed in chloroform six times. Trial spotting of the last rinse to the plate showed no observable transfer from previous lanes. Each lane corresponded to a recorded collector and specimen number. L. formosus (Dunn, et. al. #7193) was utilized as a standard due to the presence of concentrated sparteine and lupanine. Other pure alkaloid standards were utilized when available. After spotting two plates, they were briefly placed in the oven while the solvents were poured into the chamber and allowed to equilibrate. Plates were placed on metal racks, lowered into the tank, and quickly sealed. Developing usually took an hour and a half to complete. When the solvent front reached the top line; the plates were removed and placed under the hood to dry. Each plate was examined under short-wave and long-wave ultraviolet light; with fluorescing spots lightly traced in pencil. Next, they were sprayed with Dragendorf's Reagent until the fingerprint pattern of compounds became visible as light yellow or orange 'prints'. These were also traced, after which the plates were xeroxed for a permanent record. R_f values, size, shape, and color of each spot was recorded. While the relative size of spots between lanes was of little value; within a lane values were informative. Iodoplatanate reagant was then applied to obtain additional diagnostic information about the alkaloids present (Figure 19).

C. Results

Chromatography did not provide additional diagnostic criteria to aid in assessing the variability of L. leucophyllus. Through it's range of distribution a characteristic pattern of alkaloids is observed with only quantitative variation exhibited. The pattern is consistent and useful as a diagnostic marker for the species (Figure 20). Two-dimensional chromatographs of the groups of variants were remarkably similiar in chemical pattern. The greatest utility of thin-layer chromatography was in the identification of specimens exhibiting introgression or evidently distinct.

The isotype for L. leucophyllus *var. belliae* C.P. Sm., (May Bell Zundel, 09/10/1914), showed an alkaloid pattern distinctly different from that characteristic of the species. Separate plates run with different solvent systems duplicated this discontinuity. As noted previously (Schaller, 1977), introgression may produce a mixed pattern of compounds, entirely different compounds or similiar compounds compared to the original species involved.

Thirteen compounds appeared during the developing process (including application of Dragendorf's and Iodoplatinate reagants). These included alkaloids, pigments, flavonoids, various artefacts, and other substances (Bogler, 1985). Three or possibly four alkaloids were identified through the use of reagents. Dr. William Keeler, a research chemist at the Poisonous Plant Research Laboratory in Logan, Utah (pers. comm.), described two or possibly three alkaloids from his collections of L. leucophyllus. (These specimens were not available for identification.). By mass spectrometry, the primary alkaloid was identified as lamprolobine. Lamprolobine had been previously identified from Lupinus holosericeus Nutt. (Keller, 1980), and prior to that only from the Australian legume Lamprolobium fruiticosum Benth. (Hart, Johns, and Lamberton, 1968). Further chromatography, utilizing various standards supplied by Dr. Keeler indicated that D-hydroxylupanine may be the identity of one of the other alkaloids. Cho and Martin (1971), using a mobile phase of chloroform/ methanol/ concentrated ammonia (95:4:1 - 85:14:1) obtained R_f values of .72 and .05 for these two alkaloids respectively.

Chromatography was also used to ascertain the chemical patterns of reputed introgressing species and known combinations. A gradient study showed the effects of increased spot concentrations to the resolution of chemical compounds. In another study, floral parts were the subject of chromatography. Lacking the pigments of vegetative tissues, the corolla exhibited a smaller number of compounds. These ancillary procedures contributed to the understanding of alkaloids. (See Appendix III. for Chromatography Plates Xeroxes).

Dragendorff's Reagent

Solution A

Bismuth Nitrate	1.7 g
20% Acetic Acid	100.0 ml

Solution B

Potassium Iodide	40.0 g
Distilled Water	100.0 ml

(Equal parts of Solution A are mixed with Solution B).

Potassium Iodoplatinate Reagant

Chloroplatinic Acid (10% solution)	3 ml
Distilled Water	97 ml
6% Potassium Iodide (6 g KI in 94 ml water)	100 ml

Store in an amber bottle.

Figure 16.

R f values, Thin-layer Chromatography
Lupinus leucophyllus Dougl.
(*Cotton 1552*, Dalles, Wa.)

Rf-value and Description

.76	(large, oval, light under short wave UV-light)
.71	(oval, light blue- iodoplatinate)
.68	(oval, dark under short wave UV-light)
.67	(alkaloid, large, oval shape, dark blue- iodoplatinate)
.63	(oval, light blue- iodoplatinate)
.59	" "
.56	" "
.51	" "
.46	" "
.32	" "
.25	(alkaloid, large, parabolic shape, dark blue- iodoplatinate)
.23	(alkaloid, parabolic shape, dark blue- iodoplatinate)

Figure 17.

XVI. TAXONOMY

A. The Lupinus leucophyllus Complex

Plants perennial, erect, 3.0-10.5 dm. tall; stems fistulose, clumped, arising from a woody caudex; *stems with a mixed vesture of longer and shorter pubescence, villous / tomentose, spreading to retrorse , or appressed tomentose to puberulent pubescence, white to buff to tawny*; longest internode 2.2-11.7 cm.; leaves palmately compound, basal and cauline, with basal petioles to 25.0 cm., cauline to 16.8; ratio longest cauline leaflet to cauline petiole .40-1.6; leaflets 7-10, oblanceolate to lanceolate, conduplicate, apex acute with a small mucro; 1.3-10.8 cm. long, .23-1.9 cm. wide; appressed to spreading, (wooly) villous / tomemtose on both surfaces or scattered; stipules linear, 4.5-42.5 mm. (including 1.5-16.0 mm connate to petiole); flowers in primary and secondary racemes; peduncle 1.35-12.5 cm. long; *racemes dense, subverticellate* 5.6-40.0 cm. long; distance between subverticels 4.0-17.0 mm.; flowers 6-15 mm. long, white, pale lavender to lilac or blue; pedicel length 1.4-3.0 mm.; banner obovate to orbicular, 6-13 mm. long, 5-13 mm. wide, *dorsally villous*; banner angle averaging 125^0, *banner ratio averaging .62*; wing petals fused distally, 5.5-11 mm. long, 3.5-7 mm. wide, glabrous; wing claws 2.0-3.5 mm. long; keel 1.7-4.0 mm. wide, *ciliate along length of upper edge*; length from keel claw to acumen 5.5-12.0 mm.; keel angle averaging 88^0; calyx gibbous, two-lipped; three sepals fused into an entire or tridentate lower lip, the upper-lip notched, formed by the fusion of 2 sepals, wooly; lower lip 4.5-11.5 mm. long, 2.0-4.0 mm. wide; upper lip 3.0-9.3 mm. long, 2.2-6.5 mm. wide; notch depth averaging 0.5 mm.; calyx connate distance averaging 2.1 mm.; bracteole length 0.5-3.5 mm.; floral bract , 3.4-22.5 mm. long, averaging 1.4 mm. wide, wooly; pod 16.5-33.5 mm. long, 4.0-8.0 mm. wide, vesture wooly-tomentose appressed to spreading; ovules averaging 4 per legumen.

Several names were associated with L. leucophyllus by C.P. Smith for which no specimens are available for observation.

L. pureriae C.P. Smith, Sp. Lup. 192. 1940. (Type: *E.A. Purer 7748*, near Coles Corner, Chelan Co., Wash., Aug. 9, 1938). Identity problematic.

L. agropyrophilus C.P. Smith, Sp. Lup. 728. 1952. (Type: *W.A. McDowell 1321*, Peck Mt. Weiser Forest, Adams Co., Ida., Aug., 1928. "Certainly related to L. leucophyllus, but we deem it too far removed to be called even a variety of same".

L. lysichitophilus C.P. Smith, Sp. Lup. 729. 1952. (Type: *V.M. Brewer 78*, Smith Mt., Weiser Forest, Adams Co., Ida., Aug. 17, 1929). "Labelled L. leucophyllus and certainly a Leucophylloid." Sounds like *var. belliae*.

L. salicisocius. C.P. Smith, Sp. Lup. 747. 1952. (Type: *M. Anderson 119*, Trail Station, Blackfoot R., Caribou Forest, Caribou Co., Ida., Sept. 7, 1913). "Labeled L. leucophyllus." Due to late collection date a difficult determination.

L. andersonianus C.P. Smith, Sp. Lup. 748. 1952. (Type: *M. Anderson 119a*, Diamond Creek, Caribou Forest, Caribou Co., Ida., July 20, 1913). "Labeled L. leucophyllus." Sounds like *var. belliae* or hybrid, but two names are based on one specimen.

L. falsoerectus C.P. Smith, Sp. Lup. 749. 1952. (C. F. Korstian 44, Mink Creek Planting Area, Cache Forest, Franklin Co., Ida., Aug. 4, 1917). "Labeled L. leucophyllus Douglas."

Figure 18. Photo, original illustration of type collected by Douglas in 1829.

B. Key to the Lupinus leucophyllus Complex.

Flowers 6-8 mm., pubescence appressed, single-layered; Blue Mountains and other montane or subalpine habitats.

.... L. leucophyllus *ssp. erectus*

Flowers 8-15 mm.

Flowers 8-11 mm., pubescence appressed or ascending.

Pubescence appressed, single-layered; scattered throughout range.

............................L. leucophyllus *ssp. leucophyllus var. canescens*

Pubescence ascending to spreading, bi-layered; wide distribution.

............................L. leucophyllus *ssp. leucophyllus var. belliae*

Flowers 10-15 mm., pubescence retrorse or ascending.

Pubescence retrorse; larger leaflets sparsely pubescent on upper surface; plants from vicinity of Lake Coeur D'Alene, Idaho.

.............................L. leucophyllus *ssp. retrorsus*

Pubescence spreading or ascending; leaflets wooly (both surfaces).

Floral bract 12.0-22.5 mm. long; isolated population above the Walla-Walla River near Milton-Freewater, Oregon.

.............................L. leucophyllus *ssp. leucophyllus var. leucophyllus*

(Syn. *L. plumosus Dougl. ex Lindl.*, *L. leucophyllus ssp. plumosus Robins. ex Piper*).

Floral bract 4.0-12.0 mm. long.

Flowers mostly blue, scattered throughout range.

............................L. leucophyllus *ssp. leucophyllus var. leucophyllus*

Flowers white; restricted population along the Columbia River near the Dalles.

...............................L. leucophyllus *ssp. leucophyllus var. leucophyllus*

(small, contiguous white flowered population; not random white flowered mutations.)

1a. Lupinus leucophyllus Dougl. ex Lindl. *ssp. leucophyllus var. leucophyllus*
Bot. Reg. 13: pl. 1124. 1827.

Type: *Douglas*, "Great Falls of the River Columbia in North America to the source of the Missouri among the Rocky Mountains".

Synonyms: *L. plumosus* Dougl. ex Lindl. Bot. Reg. 15:pl. 1217. 1829.
L leucophyllus ssp. plumosus Robins. ex Piper, Contr. U.S. Nat. Herb. 11:354. 1906. (Type: *Douglas*, "In North California, lat. 45^{o}, growing in gravelly soil, at the sources of the Wallahwallah river, near the Blue Mountains".)
L. forslingii C.P. Smith, Species Lupinorum 743. 1952. (Type: *Clarence L. Forsling S-27*; h 70 (44539), U.S. Sheep Experiment Station, along road northeast of Paddock).

Plants perennial, erect, *3.0-10.1 dm. tall*; *stem diameter 3.3-10.5 mm., with a bilayered vesture of longer and shorter hairs, spreading to retrorse, villous (wooly) / tomentose , 1.4-4.5 mm.;* white to buff to tawny; leaves basal and cauline, basal petiole 4.6-15.8 cm., mid-cauline petiole 2.5-11.5 cm.; leaflets 7-10, oblanceolate to lanceolate, conduplicate; 1.3-7.8 cm. long, 0.4-1.0 cm. wide; appressed to spreading (wooly) villous / tomentose on both surfaces, or sparse on upper surface; stipules 5.8-22.0 mm. (1.8-11.5 mm. connate); peduncle 1.6-6.7 cm. long; racemes dense, subverticellate; *flowers 8-12 mm. long;* white, pale lavender to lilac or blue; banner 8.2-13.0 mm. long, 6.0-13.0 mm. wide, dorsally villous; wing petals fused distally, 8.3-13.0 mm. long, 4.5-7.5 mm. wide; keel 2.7-4.5 mm. wide; length from keel claw to acumen 8.6-12.0 mm.; calyx gibbous, two-lipped; lower lip 6.5-11.5 mm. long, 2.0-4.0 mm. wide; upper lip 5.5-9.3 mm. long, 3.2-5.8 mm. wide; bracteole 0.5-3.0 mm. long; floral bract 5.0-22.5 mm. long, wooly.

The ssp. *leucophyllus var. leucophyllus* includes several variants localized to specific regions throughout it's range. Plants with long floral bracts (12.0-22.5 mm. long) are only known from above the Walla-Walla River, southeast of Milton-Freewater; and a contiguous white-flowered population, first collected in 1829, grows along the Columbia River near the Dalles, Oregon. These are not significant enough morphologically or geographically to merit recognition as varieties. By virtue of the large flower size (11-15 mm.) and vegetative dimensions *var. leucophyllus* is recognized as distinct from *var. belliae*. In distribution, *var. leucophyllus* appears to be localized throughout the range, but particularly evident in Wasco Co., Oregon. While Principal Components Analysis suggests a clinal grade in morphological features, field observation indicates localized populations. It is plausible that bee foraging patterns maintain these populations although this is open to investigation. It would be interesting to determine the ploidy levels of *var. leucophyllus* and *var. belliae* as Phillips (1955), reports both octoploid and sixteen-ploid numbers for the species.

PARTIAL CITATION

CALIFORNIA:

Lassen Co.: 12 miles north of Fall River Mills, elev. 4500 feet, *Hitchcock 6614* (POM).

Modoc Co.: 1 mile south of Adin along Hwy. 139, *Huber 102* (MO).

Shasta Co.: Burney, *Eastwood 703* (CAS).

Siskiyou Co.: Yreka, *Greene 799* (MO).

IDAHO:

Latah Co.: 15 miles north of Moscow along Hwy. 15, *Currier 043* (UMO); bluffs above Lewiston, 3,000 ft., arid transition zone, *Benson 1646* (MO).

Lemhi Co.: 12 miles west of Lemhi, *Hitchcock and Muhlick 9209* (NY).

Nez Perce Co.: Open ground along the banks of the Clearwater River east of Lewiston, *Baker 5800* (NY); Lewiston Hill, 1300' elev., *Hitchcock and Samuel 2528* (RSA).

MONTANA:

Gallatin Co.: Belgrade, *E. J. Moore 06/25/1900* (GH).

Ravalli Co.: 10 miles south of Florence on Hwy. 93, *Currier 021* (UMO); 3-4 miles south of Stevensville on Hwy 93, *Currier 025* (UMO).

OREGON:

Baker Co.: Frequent, 10 miles northwest of Baker, *Maguire and Holmgren 26676* (NY); Between Pleasant Valley and Durkee: *Hitchcock 13837* (WS).

Benton Co.: Artemisia tridentata and Agropyron spicatum, *Daubenmire 59135* (WS).

Gilliam Co.: Dry sandy desert, Blalock, *Thompson 4712* (MO); 3 miles south of Olex, sagebrush canyon, *Hitchcock 19219* (NY).

Jefferson Co.: 9 miles west of Cove Palisades State Park, *Currier 073* (UMO); 2 miles from Hwy. 20 along forest route 1430 toward Camp Sherman, *Currier 062*

(UMO).

Sherman Co.: dry slope 1 mile south of Biggs, *Peck 13760* (OSC).

Umatilla Co.: Shady bank in the valley of the Walla Walla River, 7 miles southeast of Milton-Freewater, *Hitchcock and Muhlick 20974* (NY); between Milton -Freewater and Tollgate, back-road, *Currier 153* (UMO); Pendleton, *Heller 10128* (GH); 3 miles south of Milton-Freewater on route 11, on roadbank, *Fleak, Cox, and Dunn 1575* (UMO); Weston, *Booth May 14, 1942* (WS).

Wasco Co.: Sagebrush on rocky basalt, 24.9 miles south of the Dalles on Hwy. 197, *Conrad, Dunn, and Kenney 6820* (UMO); 5-8 miles west of Hwy. 216 and 197, *Currier 086* (UMO); Dalles, *A.A. Heller 10081* (MO); Crates Point, between Rowena and the Dalles, *Currier 100* (UMO); 1 mile north of Tygh Grade Summit, in sagebrush and grass community along Hwy. 197. East-facing slope, *Currier 094* (UMO); high, dry plateau south of Maupin, *Benson 1679* (POM); dry, rocky roadside north of Maupin, *Thompson 4958* (MO); barren soil, west of the Dalles, *Thompson 4317* (MO).

Wheeler Co.: Rocky knolls in scattered Pinus ponderosa forest, halfway between Fossil and Service Crk., *Hitchcock and Muhlick 22468* (WS); grassy hillsides below Fossil on Butte Crk, *Henderson 5381* (GH); rocky knolls in scattered Pinus ponderosa forest about halfway between Fossil and Service Creek, *Hitchcock and Muhlick 22466* (WS).

WASHINGTON:

Benton Co.: north slope of Rattlesnake Mtn., Artemisia tridentata, Agropyron stand, *Daubenmire 63119* (WS); Rattlesnake Mtn., east end, *Daubenmire 59135* (WS).

Columbia Co.: open, overgrazed field 1/2 mile north of Delaney, *Hitchcock and Muhlick 8257* (GH).

Klickitat Co.: 1/2 mile east of Lyle, Hwy. 97, *Currier 097* (UMO); sagebrush slopes at North Dalles, *Thompson 11 117* (GH); near Goldendale, chromosome number n = 24, *Phillips 658* (WS); 10 miles east of Lyle, all plants white flowered, *Hitchcock*

13769 (NY); 1 mile east of Murdock along Hwy. 12, in dry, sandy soil blown over basalt, very dry grassland, *Dunn, Ledoux, and Kenney 20612* (UMO); midway between Columbia River and lower slope of hills, sandy soil in abandoned field, *Christ and Smith, 15418* (NY); inside the circle of Stonehenge, high terraces above the Columbia River, *Christ and Smith 15414* (NY); near Rockford, *Suksdorf 5087* (WS,GH); hillsides N.E. of the Dalles, *Cotton 1552* (WS).

Spokane Co.: grasslands along roadside near Freeman, *Gleason 502* (WS).

Whitman Co.: 45 miles west of Lewiston, along north bank of the Snake River, *Hitchcock and Muhlick 21843* (WS); moist draw above Palouse Falls, *St. John and Pickett 6187* (WS).

Yakima Co.: Rattlesnake Mts. *J.S. Cotton 692* (MO).

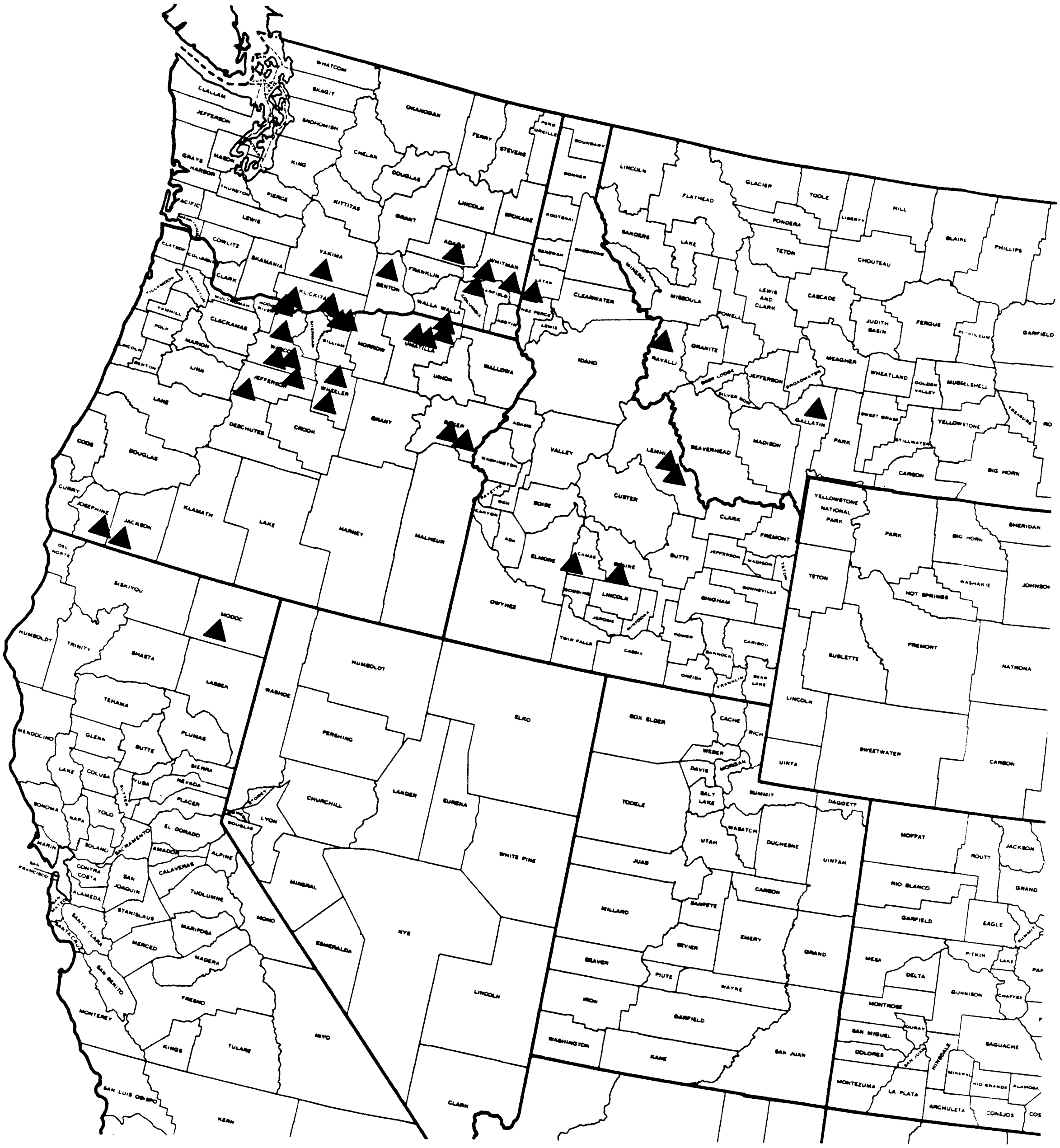


Figure 19. Map of the distribution of L. leucophyllus *ssp. leucophyllus var. leucophyllus.*

Figure 20. Photo of type specimen, *L. leucophyllus ssp. leucophyllus var. leucophyllus.*

Figure 21. Photo of plumose variant of *var. leucophyllus* from Milton-Freewater, Ore.

Figure 22. Photo of typical specimen (*ssp. leucophyllus var. leucophyllus*).

Figure 23. Photo of floral dissection (*ssp. leucophyllus var. leucophyllus*).

1b. Lupinus leucophyllus *ssp. leucophyllus var. belliae* C. P. Smith, Bull. Torrey Club 41:305. 1924.

Type: *M. B. Zundel, Sept., 1914* Crystal Cr., Power Co., Ida..

Synonym: *L. cyaneus* Rydb. Bull. Torrey Club 28:35. 1901. (Type: *E. V. Wilcox 446*, in Mont., in 1900).

L. sulphureus var. echlerianus C.P. Smith in St. John, Fl. S.E. Wash. 229. 1937. (Type: *Smith & St. John 415*, Echler Mt., Columbia Co., Wash., July 5, 1927).

(Appears as *ssp. leucophyllus var. leucophyllus* X *ssp. erectus*).

L. enodatus C.P. Smith, Sp. Lup., 1951. (Type: Lyman Benson 1646, High bluffs north of Lewiston, overlooking the mouth of the Clearwater River, 1500 ft. Jun. 16, 1929).

Plants perennial, erect, *3.5-7.2 dm. tall; stem diameter 2.3-7.3 mm., with a bilayered vesture of longer and shorter spreading hairs, villous (wooly) / tomentose , 1.5-5.2mm.;* white to buff to tawny; leaves basal and cauline, basal petiole 4.4-22.5 cm., mid-cauline petiole 2.8-10.8 cm.; leaflets 7-10, oblanceolate to lanceolate, conduplicate; 2.0-7.5 cm. long, 0.2-12.2 cm. wide; appressed to spreading (wooly) villous / tomentose on both surfaces; stipules 6.8-22.0 mm. (1.4-6.8 mm. connate); peduncle 1.3-7.2 cm. long; racemes dense, subverticellate; *flowers 8-11.2 mm. long;* white, pale lavender to lilac or blue; banner 7.3-11.2 mm. long, 4.8-10.0 mm. wide, dorsally villous; wing petals fused distally, 6.5-10.0 mm. long, 3.5-5.6 mm. wide; keel 2.2-4.2 mm. wide; length from keel claw to acumen 7.1-10.2 mm.; calyx gibbous, two-lipped; lower lip 5.4-9.3 mm. long, 2.0-3.3 mm. wide; upper lip 4.2-8.1 mm. long, 2.8-5.0 mm. wide; bracteole 0.3-2.3 mm. long; floral bract 4.2-10.6 mm. long, wooly.

The *var. belliae* differs from *var. leucophyllus* in flower size and general vegetative dimensions. Both are distributed throughout the Columbia Basin, but *var.belliae* shows greater representation in the Palouse Region and the Okanogan Highlands of Washington; and the central and eastern parts of the Columbia Basin and High Lava Plains in Oregon. Characteristics of the soil and climate may influence distribution. The *var. belliae* extends into Idaho, Montana, Utah, Nevada, California and Wyoming where it is the most characteristic variant of the species observed. Dr. Dunn (pers. comm.) suggested there may be different species of Apis or Bombus predominantly active with *var. belliae* as compared to *var. leucophyllus*. The type specimen for *var. belliae* (*M. B. Zundel, Sept., 1914* Crystal Cr., Power Co., Ida..), exhibits an alkaloidal pattern that is distinctly different from that characteristic of the species. Morphologically, however, it is typical. It is not thought necessary at this point to invalidate the name based on alkaloidal characteristics, although certainly hybridization or mutation occurred.

PARTIAL CITATION

CALIFORNIA:

Modoc Co.: *Milo S. Baker June 20, 1893* (GH).

Trinity Co.: along state route 3 1/4 mile east of Hayfork Serpentine, *Nelson and Nelson 4231* (UMO).

IDAHO:

Adams Co.: 12 miles west of Council, on granitic sandy hillside, *Christ and Christ 16617* (NY).

Clearwater Co.: 2 1/2 miles west of Weippe, Idaho on rolling plateau with yellow pine and Douglas fir, *Christ and Smith 15391* (NY).

Idaho Co.: 4 miles north of Cottonwood, Idaho on rolling prairie, *Christ and Smith 15341* (NY).

Latah Co.: open pine woods, one mile east of Viola, *Dillon and Dillon 740* (WS); dry woodland near Grizzley Camp, *Parker 512* (WS).

Lincoln Co.: Shoshone, *Nelson and Macbride 1185* (GH).

Nez Perce.: one mile south of Genesee, Idaho, *Christ and Smith 15321* (NY).

MONTANA:

Granite Co.: grassy slope, near summit of grade between Phillipsburg & Rock Creek, *Hitchcock 14719* (WS); at Eagle Creek, Rock Creek Canyon, Hitchcock 14424 (WS).

Ravalli Co.: Forest access road to Como Lake Recreation area. Flowers white, *Currier 028* (UMO); west from Bitterroot ca. 8 miles south of Alta, *Hitchcock 14374* (WS).

OREGON:

Baker Co.: moist, deep, loamy meadow near mine at Cornucopia, Hitchcock 19697 (OSC).

Crook Co.: just northwest of Fossil, Hwy. 19, *Currier 107* (UMO); in open Pinus ponderosa-Pseudotsuga- Larix Forest, *Kruckeberg 2143* (WS).

Jackson Co.: Siskiyou Mountains, near the summit of Mount Ashland, *Baker 6218* (WS); alpine slopes of Red Mtn., Siskiyou Mts., alt. 7500 feet, *Thompson 12 371* (GH).

Jefferson Co.: 5 miles northwest of Warm Springs, *Currier 075* (UMO); Camp Sherman, *Currier 066* (UMO).

Gilliam Co.: 10-15 miles south of Arlington on Hwy. 19, *Currier 102* (UMO).

Grant Co.: swales in basaltic area 12 miles east of John Day, elev. 4000 feet, *Cronquist 7317* (NY); sandy sagebrush plains near Quincy, *Thompson 9090* (NY).

Lake Co.: 1 mile south of Charlo, edge of pothole, *Hitchcock 17772* (WS).

Umatilla Co.: plains, Pendleton, *Henderson 1006* (GH); coll. 90 miles north of Mt. Vernon, north of Battle Mtn. Summit, *Currier 147* (UMO); Bingham Springs Station, 530 meter, *Eggleston 12826* (MO).

Union Co.: Le Grande, *C.P. Smith 3612* (RM); 1-2 miles east of Oregon State Park at Miniam R. along Hwy. 82, *Currier 158* (UMO); coll. 4-5 miles southwest of Union along Hwy. 203, *Currier 165* (UMO).

Walla Walla Co.: *Booth 06/17/1942* (WS).

Wasco Co.: above the Dalles, *Henderson 1006* (GH).

Wheeler Co.: open hillsides, about Fossil, *Henderson 5377* (GH).

WASHINGTON:

Adams Co.: sagebrush slopes near Washtuckna, *Thompson 17377* (MONT); in near virgin stand of Agropyron spicatum/ Poa secunda., *Daubenmire 5478* (WS).

Benton Co.: 10 miles southwest of Kennewick, *St. John and Muller 8266* (WS).

Chelan Co.: dry plains north of Wenatchee, *Thompson 6890* (MO).

Columbia Co.: *Smith & St. John 4146.* (WS).

Douglas Co.: near Wilson Creek, *Sandberg and Leiberg 305* (GH).

Franklin Co.: 12 miles northeast of Pasco, *Ownbey and Ownbey 2341* (MO); sand hills, Upper Sonoran, 8 miles north of Kahlotus, *Constance and McMurray 1149* (WS).

Garfield Co.: 5 miles northeast of Central Ferry, *St. John, Davison, and Scheibe 3413* (RM).

Lincoln Co.: near Sprague, *Sandberg and Leiberg 179* (MO).

Okanogan Co.: in virgin stand of Artemisia tripartita and Agropyron spicatum-Festuca idahoensis near Disautel, *Daubenmire 5535* (WS).

Spokane Co.: low ground southeast of Spangle, *Suksdorf 8733* (WS); dry field, Spokane, *Muenscher & Muenscher 11565* (GH); Plaza, flowers purple, *Otis 1955* (WS).

Stevens Co.: 5 miles east of Fruitland, *Spiegelberg 6-14-73* (RM).

Walla Walla Co.: College Place, *Booth June 17, 1942* (WS).

Whitman Co.: Pullman, *Henderson 2341* (GH); grassy hillside W.S.C. campus, *Pickett 576* (WS); Pullman, *Elmer 1698* (MO).

Yakima Co.: Upper Wenas River, *Henderson 2336* (WS); north Yakima, *Henderson 2326* (GH).

WYOMING:

Lincoln Co.: flowers white to pale lavender, in lodgepole pine woods along Grey's River at Moose flats, 6800 ft., *Porter 5192a* (MO).

Sublette Co.: sagebrush flat, 20 miles west of Big Piney, *Payson & Payson 2604* (GH,MO).

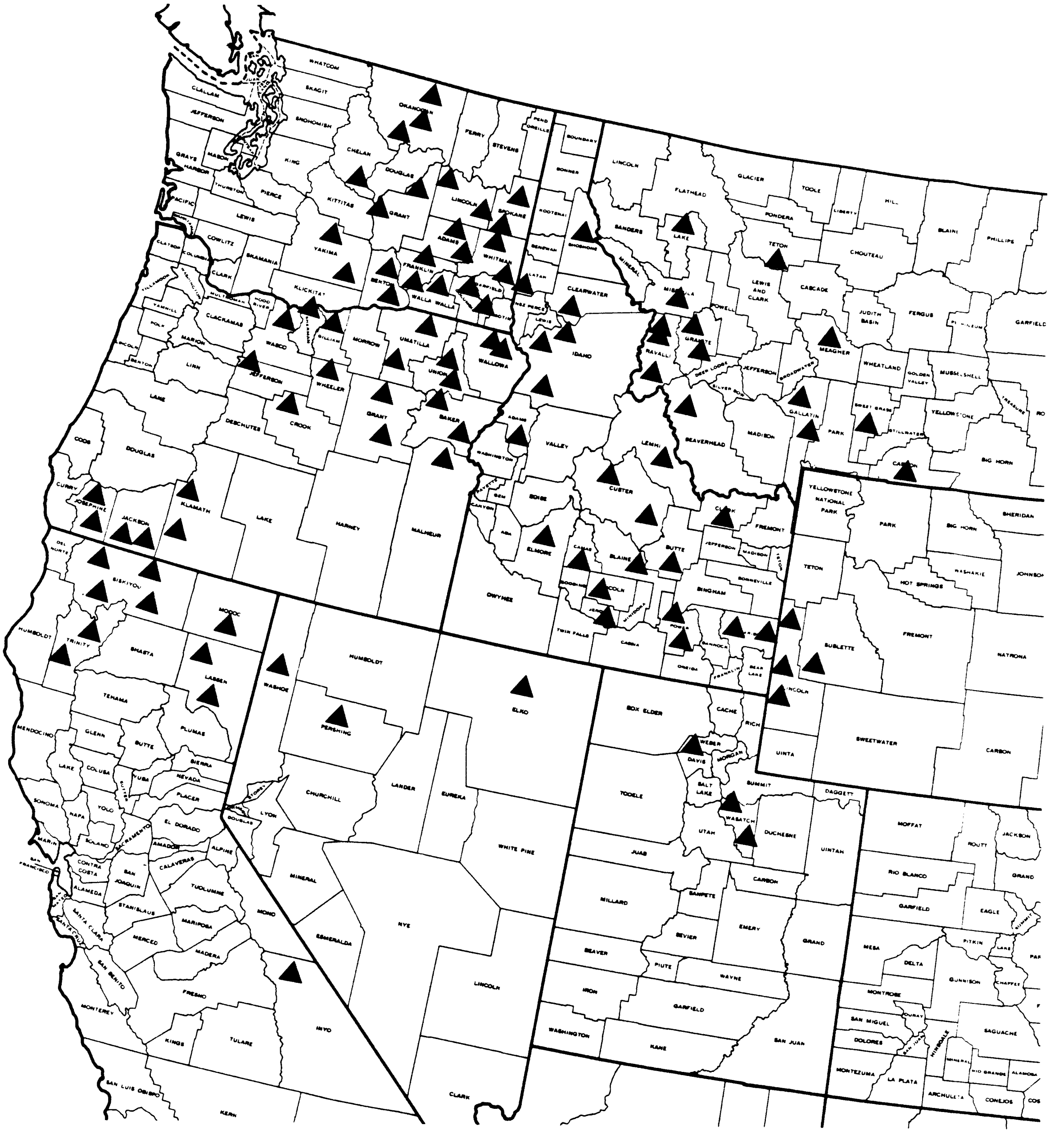


Figure 24. Map of distribution, *ssp. leucophyllus var. belliae.*

Figure 25. Photo of typical specimen, *ssp. leucophyllus var belliae.*

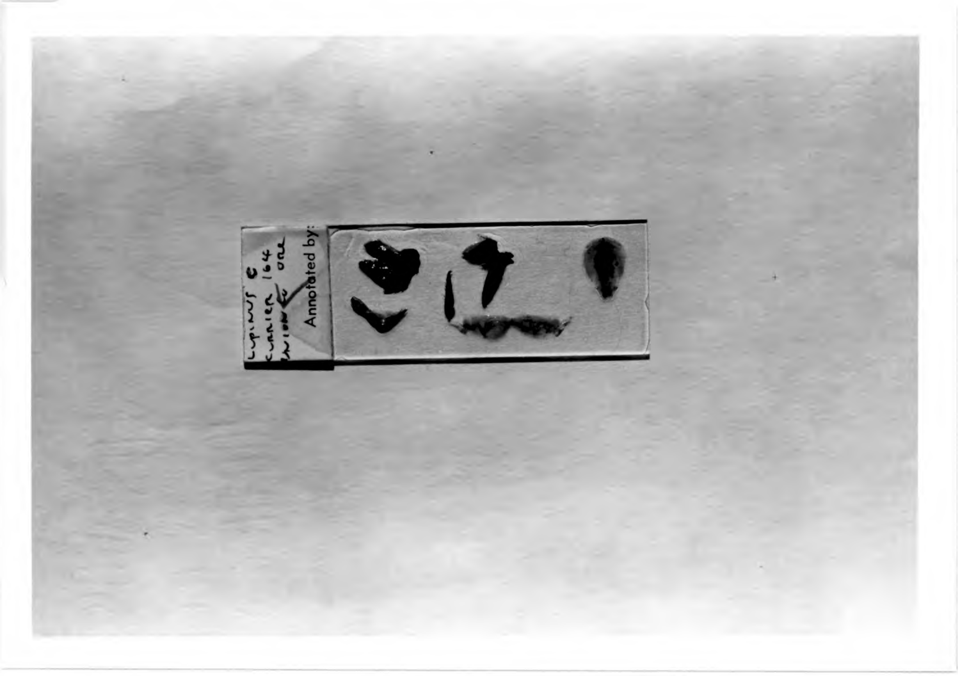


Figure 26. Photo of floral dissection, *ssp. leucophyllus var. belliae.*

1c. Lupinus leucophyllus Dougl. *ssp.leucophyllus var. canescens* (Howell) C.P. Smith, Bull. Torrey Club 51:306. 1924.

Type: *T. Howell 787*, Buck's Mt., Blue Mts., Harney Co., Ore., June, 1885.

Synonym: *L. canescens* Howell, Erythea 1:110. 1893.

L. canescens ssp. amblyophyllus Robins. ex Piper, Contr. U.S. Nat. Herb. 11:354. 1906. *L. holosericeus var. amblyophyllus* C.P.Smith, Bull. Torrey Club 51:304. 1924.

(Type: *Sandberg & Leiberg 402*, Egbert Spgs., Douglas Co., Wash., July 5, 1893).

Scattered throughout the range of the species, *var. canescens* shares many characteristics with *var. belliae*. It differs in having single-layered, appressed tomentose to puberulent pubescence (1.0-3.5 mm. long). Floral dimensions and geographic distribution are similiar. The alkaloid profile obtained for *var. canescens* consistently parallels the distinctive pattern of the species making it unlikely, based on alkaloidal evidence, that *var. canescens* is a result of introgression with another species. Cronquist and Hitchcock mention the possibility that *var. canescens* is a product of introgression with L. laxiflorus Dougl. No apparent ecological explanation is evident to explain the distribution of *var. canescens*. It seems like a particular genetic expression that occurs randomnly throughout the range of *ssp. leucophyllus*.

PARTIAL CITATION

IDAHO:

Caribou Co.: Jct. of Blackfoot River Rd. and road to Georgetown, rolling sagebrush hills, *Harmon 1403* (UMO).

MONTANA:

Gallatin Co.: Bozeman, Montana, *Koch 6736* (MONT); Gallatin Co.: 2 miles west of Bozeman, *Hitchcock & Muhlick 12475* (WS).

OREGON:

Crook Co.: meadow, Big Summit Prairie, Ochoco Forest, *Peck 17205* (OSC).

Deschutes Co.: 3 miles from Camp Sherman, @ 2 miles northeast of Hwy. 20, *Isley & Isley 11294* (UMO).

Harney Co.: dry ground along Silvies River, *Peck 5449* (OSC); frequent stony sagebrush slopes, 1 1/2 miles east of Fish Lake, Steens Mts., *Maguire and Holmgren 26816* (WS).

Jackson Co.: Steinman, 08/04/1922, *Smith 3710* (OSC).

Jefferson Co.: 3 miles from Camp Sherman, ca. 2 miles northwest U.S. 20, alt. 3300 ft., *Isley and Isley 11294* (UMO).

Klamath Co.: 2 miles north of Modoc Point, *C.P. Smith 35121* (UMO); dry woods, 15 mi. west of Keno along Ashland-Klamath Falls road, *Peck 9308* (GH).

Lake Co.: Fremont forest, road to Drake's Lookout at Squaw Butte, *Ingram 3017* (RM).

Umatilla Co.: Kamela, *A.A. Heller 10133* (GH); dry gravelly soil, Ukaih, Blue Mts., *Cusick 3446* (UMO); coll. 80 miles north of Mt. Vernon, along Hwy. 395, *Currier 146* (UMO); on flats above Trail Ridge, Walla Walla River Trail, 1 mile north of Target Meadows, *Kruckeberg 2492* (WS).

Union Co.: Hwy 84 and Hwy. 203, grade of bridge, *Currier 163* (UMO); La Grande, *Smith 3615* (OSC).

Wheeler Co.: Ochoco National Forest, northeast of Ochoco Divide Campground, *Currier 110* (UMO); dry hillsides, Big Butte Crk, *Leiberg 4369* (US).

WASHINGTON:

Klickitat Co.: *Howell June, 1879* (GH).

Whitman Co.: 18 miles north of Ewan, *Pickett 1654* (WS); Rock Lake, *Lake & Hull 432* (WS).

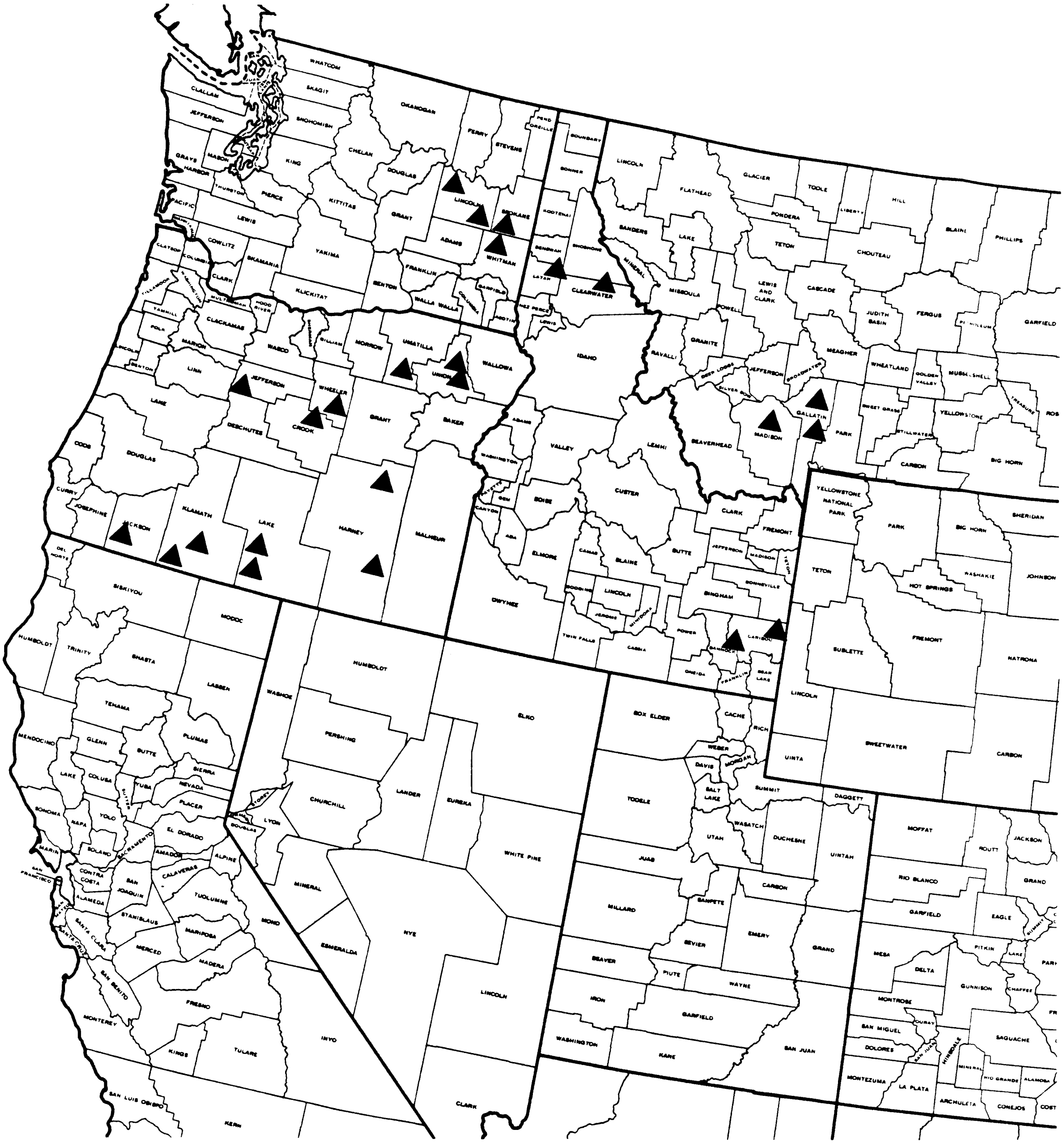


Figure 27. Map of distribution, *ssp. leucophyllus var. canescens.*

Figure 28. Photo of type specimen, *ssp. leucophyllus var. canescens.*

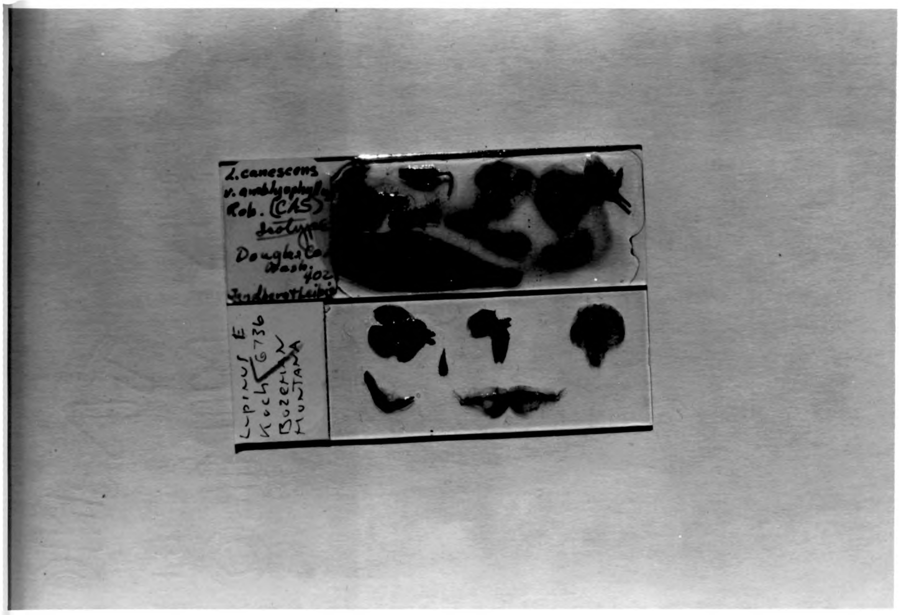


Figure 29. Photo of floral dissection, *ssp. leucophyllus var.* canescens.

2. Lupinus leucophyllus ssp. *retrorsus* (Henderson) Currier comb. nov.

Type: *Henderson 4608*, Coeur d'Alene Lake near Harrison, Kootenai Co., Ida., Aug. 7, 1898.

Synonym: *L. retrorsus* Henderson, Bull. Torrey Club 27:344. 1900. *L. leucophyllus var. retrorsus* Smith ex St. John, Fl. S.E. Wash. 228. 1937.

L. macrostachys Rydb. Bull. Torrey Club 34:44. 1907. (Type: *D.T. MacDougal 253*, Jocko Cr., Missoula Co., Mont., in 1901.)

Plants perennial, erect, *4.0-10.5 dm. tall*; *stem diameter 3.8-10.5 mm., with a bilayered vesture of longer and shorter hairs, retrorse, villous (wooly) / tomentose , 3.2-5.9 mm.;* white to buff to tawny; leaves basal and cauline, basal petiole 5.8-15.2 cm., mid-cauline petiole 3.3-16.8 cm.; leaflets 7-10, oblanceolate to lanceolate, conduplicate; 3.1-10.8 cm. long, 0.6-1.9 cm. wide; appressed to spreading, sparsely villous, greenish on upper surface; stipules 8.0-43.0 mm. (2.0-12.0 mm. connate); peduncle 2.5-12.0 cm. long; racemes dense, subverticellate; *flowers 10-13 mm. long;* lilac or blue; banner 8.2-13.0 mm. long, 8.0-11.7 mm. wide, dorsally villous; wing petals fused distally, 7.4-10.0 mm. long, 4.0-6.3 mm. wide; keel 2.5-3.8 mm. wide; length from keel claw to acumen 6.2-12.0 mm.; calyx gibbous, two-lipped; lower lip 7.0-9.6 mm. long, 2.0-3.5 mm. wide; upper lip 6.0-7.6 mm. long, 3.5-6.5 mm. wide; bracteole 0.5-3.8 mm. long; floral bract 4.0-24.0 mm. long, wooly.

The ssp. *retrorsus* is readily distinguished by it's retrorse stem vesture and large, sparsely pubescent cauline leaves. Inhabiting an area once characterized by large Pleistocene glacier-fed lakes, ssp. *retrorsus* represents a remnant population. The Coeur d'Alene region, with a greater annual precipitation (50.8-66.0 cm. annually) supports spruce-fir forest. The leaflets do not require the dense pubescence advantageous to plants in the Columbia Basin and present a larger surface for photosynthesis. Retrorse pubescence suggests a physical defense perhaps against slugs or other crawling herbivore insects. The alkaloid patterns and floral characteristics of ssp. *retrorsus* place it well within L. leucophyllus.

PARTIAL CITATION

IDAHO:

Clearwater Co.: Ahsahka, on Hwy. 12, along Clearwater River bank in rocky, sandy soil, rather acidic, *Cox, Dunn, & Fleak 1819* (UMO).

Kootenai Co.: Chatcolet, 4 miles west, *C.P. Smith 4146* (UMO); Ford, two miles north, *Smith and St, John 4142* (ORE); Coeur d'Alene, north end of lake, C.P. Smith 4139 (UMO); Chatcolet, Idaho, Jun. 1901, *Cozier 38716* (WS); holotype, low grassy places along dried hills above Lake Coeur d'Alene in pine woods, *Henderson 4608* ( ).

Latah Co.: south side of Gold Hill, *St. John 9625* (WS).

Lemhi Co.: along stream 3 miles northeast of Tendoy, *Hitchcock 23852* (DS).

Lewis Co.: 2 miles north of Winchester, on prairie, *Christ and Smith 15338* (NY).

Nez Perce Co.: yellow pine woods, Lake Winchester, *Jones 4968* (GH).

MONTANA:

Ravalli Co.: Middle Temperate Life Zone, at 3,000 ft., *Jones 07/14/1909* (GH).

WASHINGTON:

Stevens Co.: moist woods near Cheweloh, *Sprague 6-25-23* (WS).

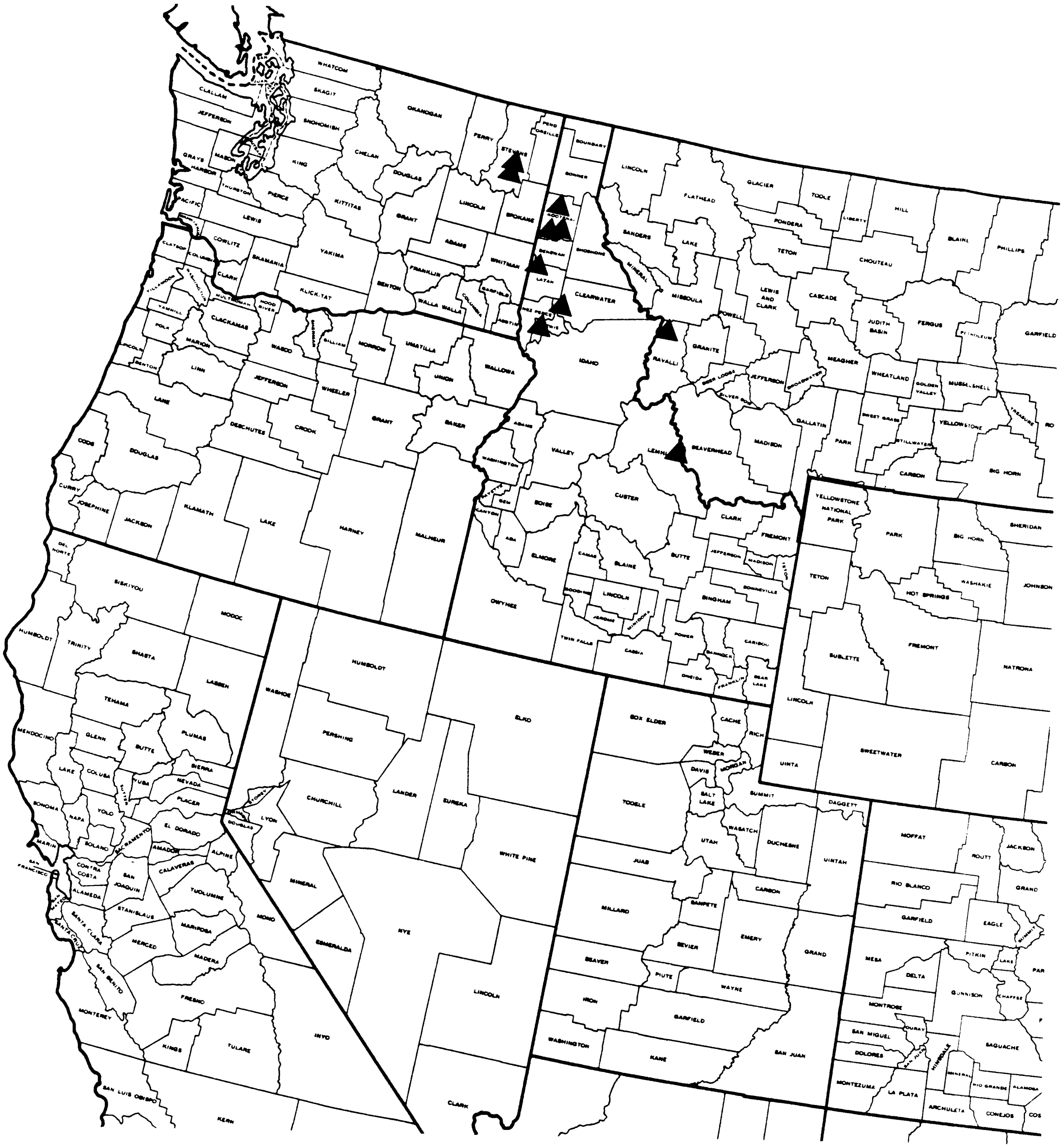


Figure 30. Map of distribution, ssp. *retrorsus*.

Figure 31. Photo of typical specimen, ssp. *retrorsus*.

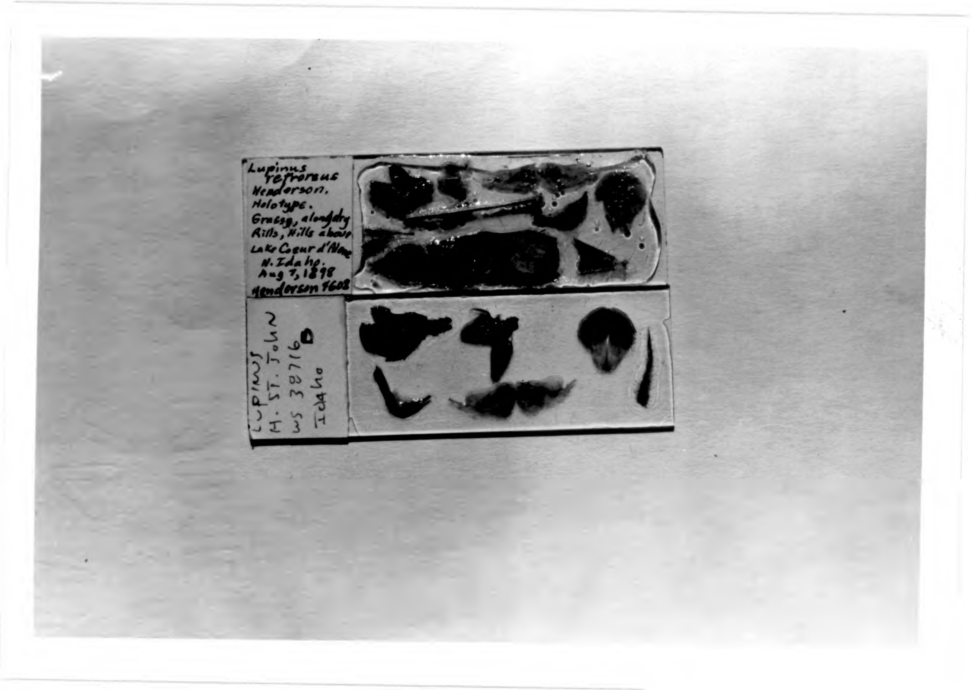


Figure 32. Photo of floral dissection, ssp. *retrorsus*.

3. Lupinus leucophyllus Dougl. ssp. *erectus* (Henderson) Harmon, Trans. Missouri Acad. Sci. 6: 163. 1973.

Type: *Henderson: July 4, 1895,* Long Valley, Boise Co., Idaho.

Synonyms: *L. erectus* Henderson, Bull. Torrey Club 27:343. 1900.

L. tenuispicus A. Nels., Bot. Gaz. 54:410. 1912. *L. leucophyllus var. tenuispicus* C.P. Smith, Bull Torrey Club 51:306. 1924. (Type: *J. Clark 203*, Washington Co., Ida., Aug. 8, 1911).

Plants perennial, erect, *3.4-6.2 dm. tall*; *stem diameter 1.4-5.5 mm., with a vesture of appressed tomentose to puberulent pubescence, 0.5-2.7 mm. long*; white to buff to tawny; leaves basal and cauline, basal petiole 2.8-17.0 cm., mid-cauline petiole 3.2-10.5 cm.; leaflets 8, oblanceolate to lanceolate, conduplicate; 1.4-5.6 cm. long, 0.4-1.1 cm. wide; appressed to spreading, villous / tomentose on both surfaces; stipules 5.2-16.0 mm. (1.8-8.5 mm. connate); peduncle 1.8-7.9 cm. long; racemes dense, subverticellate; *flowers 6.0-8.0 mm. long*; white, pale lavender to lilac or blue; banner 6.0-8.4 mm. long, 5.0-8.5 mm. wide, dorsally villous; wing petals fused distally, 5.3-7.5 mm. long, 2.0-5.0 mm. wide; keel 1.7-3.0 wide; length from keel claw to acumen 5.5-9.0 mm.; calyx gibbous, two-lipped; lower lip 4.5-7.0 mm. long, 1.8-4.0 mm. wide; upper lip 3.1-6.8 mm. long, 2.2-4.0 mm. wide; bracteole 0.5-2.2 mm. long; floral bract 3.5-7.1 mm. long.

The ssp. *erectus* is distinct morphologically and geographically from ssp. *leucophyllus*. Principal Components Analysis yields a clear separation based on morphological attributes; this is backed by geographic distribution. The ssp. *erectus* occurs on alpine slopes, open pine forests, and open mountain meadows from 750-2500 meters. Observed in the major mountainous regions east of the Cascades in Oregon, it extends into northern California, Washington, Idaho, and Montana. Ecologically, the habitats occupied are markedly distinct from conditions in the Columbia Basin (eg. climate, soil type, length of growing season, and vegetative association). The ssp. *erectus* is morphologically distinct with flowers 6-8 mm. in length; appressed, single-layered pubescence; and generally smaller vegetative dimensions. The flower size would seem to indicate a smaller pollinator.

A plausible theory explaining the generation of variability would characterize L. leucophyllus Dougl. as endemic to the Columbia Basin of Oregon and Washington, subsequently radiating into similiar habitats and introgressing with sympatric species. After the basaltic flows of the Miocene and the uplift of the Blue Mountains, ssp. *erectus* evolved as a selectively advantageous variant, becoming established throughout open mountain habitats.

PARTIAL CITATION

IDAHO:

Adams Co.: low gravelly clay hills, north of town, New Meadows, *Ripley and Barneby 10705* (RSA); 4 miles north of New Meadows, Barr 190 (ASU).

Washington Co.: Tamarack, west slope, 4,200 ft. elev., *Clark 203* (isotype) (MO); salmon meadows, *Jones 6252* (UMO).

OREGON:

Grant Co.: yellow pine slopes 10 miles north of Seneca, *Thompson 11 950* (GH); Strawberry Creek, below lake, *Peck 10303* (MO); yellow pine forest south of John Day, in canyon, deep shade by a stream, 15.8 miles north of Grant Co. line, *Dunn 11927* (RSA); frequent, deep loan soil under yellow pine, Logan Valley, Malheur Natl. Forest, Blue Mts., *Maguire and Holmgren 26899* (GH,WS); coll. 35 miles north of Mt. Vernon, *Currier 142* (UMO); coll. 18 miles north of Mt. Vernon, along Hwy. 395, *Currier 136* (UMO).

Harney Co.: 26 mile from Burns on Izey road, *Henderson 8134* (CAS); open mountain meadow near Fish Lake, Steens Mts, *Thompson 12 160* (GH); Steens Mountain, near road by Whorehouse Meadow, elev. 7500 ft, *Wright 1425* (OSC); yellow pine slopes, 25 miles north of Burns, *Thompson 13290* (MO); frequent in moist meadow, 14.5 miles northwest of Suntex maintenance station, *Holmgren and Tillett 9649* (GH); frequent, stony slopes, sagebrush, 1.5 miles east of Fish Lake, Steens Mts., *Maguire and Holmgren* 26816 (GH).

Jackson Co.: alpine slopes of Red Mt., Siskiyou Mts., *Thompson 12 371* (MO).

Umatilla Co.: one mile east of Tollgate on Hwy. 204, along roadcut, *Currier 156* (UMO); along side of road, *Currier 145* (UMO); near Tollgate in the Blue Mts., southeast of Milton-Freewater, coll. about 20 miles east of Jct. Hwy. 11 and 204 along roadcut, *Dunn 12276* (UMO); dry ground, *Peck 3990* (GH); coll. in vicinity of woodward campground in Spruce, Larch, Lodgepole, & Fir, just west of Tollgate, *Bennett, Dunn, & Dziekanowski 541* (UMO).

Union Co.: 8-12 miles east of Elgin, pasture at side of Hwy 82, *Currier 157* (UMO); 17 miles northwest of Le Grande, on top of Blue Mts. plateau, shallow basaltic soils with lodgepole pine, *Christ and Christ 18312* (NY); dry, open ground near summit of Blue Mts. between Pendleton and Le Grande, Ore., *Thompson 4884* (MO).

Wallowa Co.: 15 miles northeast of Tollgate, opening in spruce-fir forest, *Harmon 1109* (UMO); moist hillside along Lick Creek, 25 miles southeast of Joseph, *Ownbey and Ownbey 1825* (POM); open pine forests, S. Blue Mts, *Cusick 2048* (MO,GH).

WASHINGTON:

Lincoln Co.: sagebrush slopes near Almira, *Thompson 11 671* (GH).

Figure 33. Map of distribution, *ssp. erectus*.

Figure 34. Photo of type specimen, *ssp. erectus.*

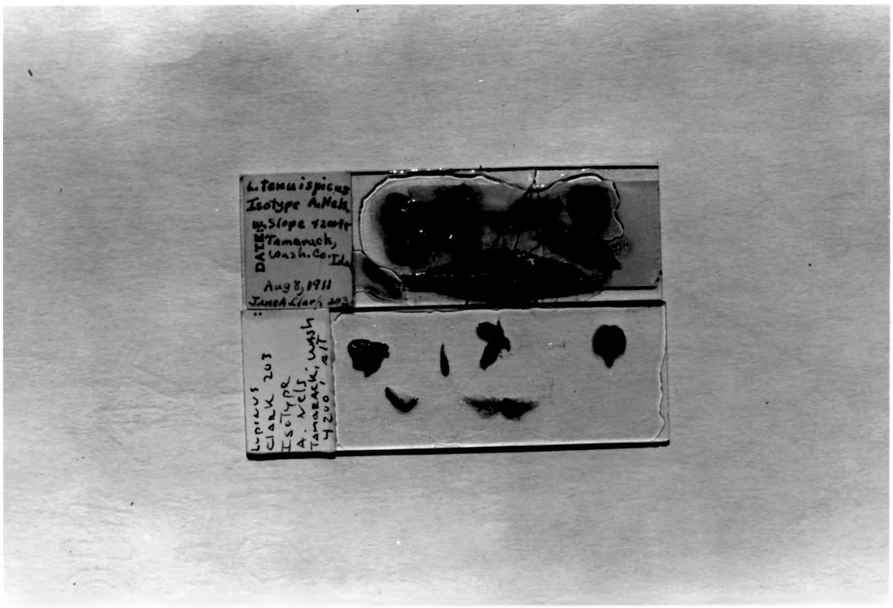


Figure 35. Photo of floral dissection, *ssp. erectus*.

XVII. SUMMARY AND CONCLUSIONS

The objective of this study is to clarify the taxonomy of Lupinus leucophyllus Dougl., an octoploid and sixteen-ploid species which is known to hybridize and introgress with several other Pacific Northwest species. Historically the wide range of morphological variation was difficult to handle taxonomically. Although the diagnostic criteria for the species is clear and distinct today, different treatments mark subspecific designations. L. leucophyllus is either lumped together as a species with two subspecies (*ssp. leucophyllus* and *ssp. erectus*) (Hitchcock and Cronquist, 1973) or is split into a number of varieties (Smith, 1924).

Morphological analysis is combined with thin-layer chromatography of alkaloids and geographic information to arrive at a feasable taxonomic treatment. The results of chromatography indicate little more than the integrity of the species. Principal Components Analysis using 34 morphological attributes provides linear expressions of variability which are used to group objects into clusters of similiar morphological affinity.

Three subspecies are named based on the evidence. The subspecies *retrorsus* from Lake Coeur d'Alene in Idaho, extending into Stevens Co., Washington possesses distinct vegetative attributes while occupying a habitat with marked ecological differences. The subspecies *erectus,* from the Blue Mountains, Oregon and similiar habitats of relatively recent origin, is characterized by smaller floral and vegetative dimensions. The subspecies *leucophyllus*, with three varieties, occupies the arid Columbia Basin and similiar basaltic basins throughout the range of the species. Varietal designations acknowledge morphologically distinct variants with broad distribution. Included in the text of the thesis is a discussion of geologic history of the Pacific Northwest, climate and soil forming factors, and vegetative and physiographic provinces of the Pacific Northwest.

LITERATURE CITED

Adams, Robert P. A comparison of multivariate methods for the detection of hybridization. Taxon 31(4): 646-661; 1982.

Agardh, J. G. Synopsis Generis Lupini. Lundae, Berlin; 1835.

Allen, J. G. Lupinosis, a review. In: Proc. 4th Intern. Lupin Conf. Geraldton, W. Australia; 1986: 173-187

Alston, R.E. and Turner B. L. New techniques in analysis of complex natural hybridization. Proc. Natl. Acad. Sci. 48: 130-137; 1962.

Anderson, E. Introgressive hybridization. John Wiley and Sons, N.Y.; 1949.

Antunez de Mayolo, S. "Tarwi" in ancient Peru. In: R. Gross and E. S. Bunting (eds.) Agricultural and Nutritional Aspects of Lupines. Proc. First Intern. Lupine Workshop. Lima-Cuzco, Peru.; 1980.

Arroyo, K. Breeding systems and pollination biology. In: Polhill, R.M. and P. H. Raven (eds.) Advances in Legume Systematics, Part 1, pp. 723-770. Roy. Bot. Gard., Kew.; 1981.

Baldwin, Ewart M. Geology of the Columbia River Gorge. Northw. Sci., 40: (4); 1984.

Bell, E. A. Non-protein amino acids in the Leguminosae. In: Polhill, R. M. and P. H. Raven (eds.), Advances in Legume Systematics, Part 2; pp. 489-500. Roy. Bot. Gard., Kew.; 1981.

Bellido, Luis Lopez. World report on lupin. In: Proc. 3rd Intern. Lupin Conf., La Rochelle, France; 1984: 465-510.

Belteky, Bela and Istvan Kovacs. Lupin, the new break, Panagri Ltd.; 1984.

Bentham, G. In Bentham, G. and J.P. Hooker, Genera Plantarum, Vol. 1. Reeve, Vienna; 1865.

Bisby, F. A. Genisteae. In Polhill, R. M. and P. H. Raven (eds.) Advances in legume systematics, Part 1. Roy. Bot. Gard. Kew.; 1981: 409-425.

Bogler, David Monograph of the Lupinus aschenbornii Schauer, Complex of the central highlands of Mexico. Univ. of Missouri-Columbia; 1985. 194 p. Thesis.

Booth, W. E. and J. C. Wright . Flora of Montana. Montana State University Press.; [1959] 1966.

Boulter, D. Proteins of legumes. In: Polhill, R. M. and P. H. Raven (eds.), Advances in legume systematics, Part 2. Roy. Bot. Gard. Kew.; 1981: 501-512.

Chavez, E. and P. Untied. Program for lupine, 'tarwi' or 'chocho' production in the Peruvian Highlands: Outlook and limitations. In: R. Gross and E. S. Bunting (eds.), Agricultural and nutritional aspects of lupines. Proc. First Intern. Lupine Workshop. Lima-Cuzco, Peru.; 1980.

Cho, Y.D. and R.O. Martin. Resolution and unambiguous identification of microgram amounts of 22 lupin alkaloids by sequential use of thin-layer chromatography and mass spectrometry. Analytical Biochem. 44: 49-57; 1971.

Coats, David and Christopher A. Cullis. Chloroplast D.N.A. variability among Linum species. Amer. J. Bot. 74(2): 260-268; 1987.

Cole, David N. Vegetation of two drainages in Eagle Cap Wilderness, Wallowa Mountains, Oregon. (Res. Pap.) INT-288. Intermountain Forest and Range Experiment Station, Ogdon, Utah; 1982.

Conrad, M. L. Monograph of the Lupinus albicaules-L. formosus complex. Univ. of Missouri-Columbia; 1980. 369 p. Dissertation.

Couch, J.F. Lupine studies. XV. The alkaloids of L. sericeus Pursh. J. Amer. Chem. Soc. 62: 554; 1940.

Cox, B. J. Biosystematics of Lupinus lepidus-L. caespitosus complex. Univ. of Missouri-Columba.; 1972. 343 p. Dissertation.

Cox, B. J. A monograph of the Lupinus ornatus complex. Univ. Missouri-Columbia; 1970. 133 p. Thesis.

Cranmer M. F. and B. L. Turner. Systematic significance of lupine alkaloids with particular reference to Baptisia (Leguminosae). Evolution 21: 508-517; 1967.

Crawford, Daniel J. and Edward A. Julian. Seed protein profiles in the narrow-leaved species of Chenopodium of the western U. S.: Taxonomic value and comparison with distribution of flavonoids. Amer. J. Bot. 63(3): 302-308; 1976.

Cracraft, Joel . Biological diversification and it's causes. 1985.

Cronquist, A. On the taxonomic significance of secondary metabolites in angiosperms. Plant Syst. Evol., Suppl. 1: 179-189; 1977.

Cullvenor, C. C. J., and D. S. Petterson. Lupin toxins- alkaloids and phomopsins. In: Proc. 4th Intern. Lupin Conf. Geraldton, W. Austalia; 1986: 188-198.

Daubenmire, R.F. Ecologic plant geography of the Pacific Northwest. Madrono 20 (3): 111-128; 1969.

Daubenmire, R.F. On the vegetational zonation in the Rocky Mountains. Bot. Rev. 9: 325-393; 1943.

Davies, R. A. Index Kewensis. Clarendon Press, Oxford; 1978.

Davis, R. J. Flora of Idaho. Brigham Young Univ. Press. Provo, Utah; 1952.

Detling, Leroy E. Peculiarities of the Columbia River Gorge flora. Madrono: 14: 160-171; 1958.

Detling, Leroy E. The flora of the Columbia River Gorge. Northwest Sci. 4(4): 133-137; 1966.

Dobzhansky, T. Genetics and the origin of species. Columbia Univ. Press. N.Y., N.Y.; 1957.

Dolinger, Peter M., P. R. Ehrlich, W. L. Fitch and D. E. Breedlove. Alkaloid and predation patterns in Colorado lupine populations. Oecologia (Berl.) 13, pp. 191-204; 1973.

Dorn, Robert D. Manual of the vascular plants of Wyoming. Garland Publishing Co., N.Y., N.Y.; 1977.

Downey, John C. and David B. Dunn. Variation in the lycaenid butterfly, Plebejus icarioides III. Additional data on food-plant specificity. Ecology 45(1): 172-178; 1964.

Dunn, D. B. Cytotaxonomy and distribution of New World lupin species. In: Proc. 3rd Intern. Lupin Conf. La Rochelle, France; 1984: 67-86.

Dunn, D. B. A case of long range dispersal and rapid speciation in Lupinus. Trans. Missouri Acad. Sci. 5: 26-38; 1971.

Dunn, D. B. The lupines collected by David Douglas from 1825-1827; their type localities and nomenclature. Leafl. West. Bot. 8: 47-54; 1956 a.

Dunn, D. B. The breeding systems of Lupinus, group *Micranthi*. Amer. Midl. Nat. 55(2): 443-472; 1956 b.

Dunn, D. B. A method of mounting pressed flowers for study and preservation. Rhodora 56(671): 258-260; 1954.

Dyksterhuis, Eugene L. and C.T. Hugh. Soil Survey of Union County, Oregon. U.S.D.A. Soil Conservation Service. U. S. Government Printing Office; 1985

Dyksterhuis, Eugene L. Soil Survey of Grant County, Oregon. U.S.D.A. Soil Conservation Service. U.S. Government Printing Office; 1981.

Dziekanowski , C. Biosystematics of the Lupinus sparsiflorus complex. Univ. Missouri-Columbia; 1971. 213 p.

Edwards, Sydenham, et. al. Botanical register: Consisting of coloured figures of exotic plants in British gardens. vol. 13. London; 1827. 14 vols.

Elliott, Mathilde, J. Harding and C.B. Mankinen. Genetics of Lupinus VI. Reproductive barriers in the *Nanus* Group. Taxon, 23(4): 585-594; 1974.

Emery, Dara E. Lupinus 'Canyon Sunset', a new perennial for the garden. Leafl. of the Santa Barbara Bot. Gard. 2(1): 3-7; 1977.

Erdtman, H. Some aspects of chemotaxonomy. In: T. Swain (ed.), Chemical plant taxonomy. Academic Press, London; 1963.

Findley, Rowe. Mount St. Helens: The day the sky fell. Natl. Geogr. 159: 50-65; 1981.

Fleak, S. Biosystematics of the Lupinus sericeus complex. Univ. Missouri-Columbia; 1971. 209 p. Dissertation.

Foth, Henry D. Fundamentals of soil science, 6th ed. John Wiley and Sons, N.Y., N.Y.; 1978.

Franklin, Jerry F. Natural vegetation of Oregon and Washington, U.S.D.A. Forest Service Tech. Rept. PNW-8, Portland, Oregon.; 1973.

Furlow, John J. The Carpinus caroliniana complex in North America I. A multivariate analysis of geographical variation. Syst. Bot. 12(1): 21-40; 1987.

Gibbs, R. D. Chemotaxonomy of flowering plants, Vol. 1. Montreal: McGill-Queen's Univ. Press; 1974.

Gibbs, R. D. History of chemical taxonomy. In: T. Swain (ed.), Chemical plant taxonomy. Bellflower, CA: Academic Press; 1963.

Gori, David F. Post-pollination phenomena and adaptive floral changes. In: Jones, Eugene C. and R. John Little (eds.), Handbook of experimental pollination biology. New York: Van Nostrand Reinhold Co. Inc.; 1983: 31-49.

Graf, W. L. (ed.), Geomorphic systems of North America. Geological Society of North America; 1987.

Grant, Verne. Speciation. New York: Columbia Univ. Press; 1981.

Green, George L. Soil Survey of Wasco County, Oregon. U.S.D.A. Soil Conservation Service. U.S. Government Printing Office; 1982.

Gross, R. Lupins in the old and new world: A biological cultural coevolution. In: Proc. 4th Intern. Lupin Conf. Geraldton, W. Australia. 1986; 244-277.

Harborne, J. B. and B. L. Turner. Plant chemosystematics. Bellflower, CA: Academic Press; 1984.

Harding, James, C. B. Mankinen, and M. H. Elliott. Genetics of Lupinus VII: Outcrossing, autofertility, and variability in natural populations of the *Nanus* Group. Taxon 23(5/6): 729-738; 1974.

Harding, James. Genetics of Lupinus II: The selective disadvantage of the pink flower color mutant in Lupinus nanus. Evolution 24: 120-127; 1970.

Harding, James and C. B. Mankinen Genetics of Lupinus I: Variations in flower color from natural populations of Lupinus nanus. Canad. J. Bot. 45: 1831-1836; 1967.

Harmon, W. E. 1972. Comparative study of Lupinus parviflorus complex. Univ. Missouri-Columbia; 1972. 194 p. Dissertation.

Harmon, W. E. A study of Lupinus endemic to the United States. Univ. Missouri-Columbia; 1968. Thesis.

Hart, Charles R. 1979. The systematics of Bidens ferulaefolia complex (Compositae). Syst. Bot. 4(2): 130-147; 1979.

Hart, N.K., S.R. Johns, and J.A. Lamberton. Alkaloids Of Lamprolobium fruticosum Benth. Aust. J. Chem. 21: 1619; 1968.

Heftmann, Erich Chromatography. New York: Van Nostrand Reinhold Co. Inc.; 1961.

Herrera, H. and H. Nunez. Feeding of rainbow trout using lupine. In: R. Gross and E. S. Bunting (eds.), Agricultural and nutritional aspects of lupines. Proc. First Intern. Lupine Workshop, Lima-Cuzco, Peru; 1980.

Hess, L. 1969. Biosystematics of the Lupinus argenteus complex. Univ. Missouri-Columbia; 1969. 230 p. Dissertation.

Heusser, Calvin J. Vegetational history of the northwest including Alaska, In Stephen Porter (ed.), Late quaternary environments of the United States, Vol. 1, Minneapolis, MN: Univ. Minn. Press; 1983.

Heyn, C. C. and Uzi Plitmann. Population analysis of wild leguminous species in the Middle East with reference to germination and establishment. Israel Binational Science Foundation, P.O.B. 7677; 1979.

Hill, G. D. Recent developments in the use of lupins in animal and human nutrition. In: Proc. 4th Intern'l Lupine Conf. Geraldton, W. Australia; 1986.

Hitchcock, C. L. and A. Cronquist. Flora of the pacific northwest. Seattle, WA. : Univ. Washington Press; 1973.

Holmgren, P. K. and W. Keuken. Index Herbariorum, Part 1: The Herbaria of the World, 6th ed. Oosthoek, Scheitema, Holkema and Emmalaan; 1974.

Hosler, Richard E. Soil Survey of Gilliam County, Oregon. U.S.D.A. Soil Conservation Service., U.S. Government Printing Office: 1984.

Hotelling, H. Analysis of a complex of statistical variables into principal components. J. Ed. Psych., 24: 417-441, 498-520; 1933.

Hutchinson, J. The genera of flowering plants, Vol. 1. Oxford; 1964: 297-489.

Ingham, J. L. Phytoalexin induction and it's taxonomic significance in the Leguminosae (subfamily Papilionoideae). In: Polhill R. M. and P. H. Raven (eds.), Advances in legume systematics. Roy. Bot. Gard. Kew.; 1981.

Janzen, D. H. Community structure of secondary compounds in plants. In: Chemistry in evolution and systematics. New York: Academic Press; 1972.

Jones, C.E. and S.L. Buchanan. Ultraviolet floral patterns as functional orientation cues in hymenopterous pollination systems. Anim. Behav. 22: 481-485; 1974.

Karlsson, E. M. Quantitative analysis of lupine alkaloids by fluorescence measurements. In: R. Gross and E. S. Bunting (eds.), Agricultural and nutritional aspects of lupines. Proc. First Intern. Lupine Workshop. Lima-Cuzco, Peru; 1980.

Kartesz, J. T. and Kartesz, R. A checklist of plants of North America and Greenland, Chapel Hill, NC.: Univ. N. Carolina Press; 1981.

Keller, William J. Lamprolobine and other quinolizidine derivatives from Lupinus holosericeus. Phytochem. 19: 2233-2234; 1980.

Kevan, Peter G. Floral colors through the insect eye: What they are and what they mean. In: Jones, Eugene C. and R. John Little (eds.), Handbook of experimental pollination biology, New York:Van Nostrand Reinhold Co. Inc.; 1983: 3-30.

Kinghorn, A. D. and S. J. Smolenski 1981. Alkaloids of the Papilionoideae. In: Polhill, R. M. and P. H. Raven (eds.), Advances in legume systematics, Part 2; 1981: 585-598.

Kinghorn, A. D., Mohamed A. Selim, and S. J. Smolenski. Alkaloid distribution in some new world Lupinus species. Phytochemistry 19: 1705-1710; 1980.

Kreig, R. Sweet lupines in poultry rations. In: R. Gross and E. S. Bunting (eds.), Agricultural and nutritional aspects of lupines. Proc. First Intern. Lupine Workshop, Lima-Cuzco, Peru; 1980.

Kruckeberg, Arthur R. An essay: The stimulus of unusual geologies for plant speciation. Syst. Bot. 11(3): 455-463; 1986.

Langenheim, J. H. Terpenoids of the Leguminosae. In: Polhill, R. M. and P. H. Raven (eds.), Advances in legume systematics, Part 2. Roy. Bot. Gard. Kew.; 1981: 627-656.

Lawrence, G.H.M. Taxonomy of Vascular Plants. New York: MacMillan Co.; 1951.

Lopez de Roma, Guillermo. Lupin in human nutrition. In: Proc. 3rd Intern. Lupin Conf., La Rochelle, France; 1984.

Love, A. and Love, D. The geobotanical significance of polyploidy. Proc. Sixth Int. Grassl. Congress, St. Coll. 1953: 240-246.

Lynott, Robert E. Weather and climate of the Columbia Gorge. Northw. Sci. 40(4): 129-132; 1966.

Mabry, T. J. and J. A. Mears. Alkaloids and plant systematics. In: S. W. Pelletier (ed.), Chemistry of the alkaloids. New York:Van Nostrand Reinhold Co. Inc.; 1970.

Mankinen, C.B., J. Harding, and M. Eliott. Genetics of Lupinus VIII: Variations in the occurrence of alkaloids in natural populations of Lupinus nanus. Taxon 24(4): 415-429; 1975.

Manske, R.H.F. and H. L. Holmes. The alkaloids: Chemistry and physiology, Vol. 3. Bellflower, CA: Academic Press; 1953.

Marsh, C. D. Lupines as poisonous plants. U.S.D.A. Bull., 405; 1916.

Mason, Georgia. Guide to the plants of the Wallowa Mts. of northeastern Oregon. Univ. of Oregon, Museum of Natural History Eugene, Oregon; 1975.

Mayers, Leo R. Soil Survey of Sherman County, Oregon. U.S.D.A. Soil Conservation Service. U.S. Government Printing Office; 1964.

McGee, Bates. Cascadia, The geologic evolution of the Pacific Northwest. New York: McGraw-Hill; 1972.

McKenzie, B. A. and G. D. Hill. Use of lupins to maintain soil fertility in cropping cycles. In: Proc. 3rd Inter. Lupin Conf. LaRochelle, France; 1984: 574-575.

Mears, J. A. and T. J. Mabry. Alkaloids in the Leguminosae. In: J.B. Boulter, D. Boulter, and B.L. Turner (eds.), Chemotaxonomy of the Leguminosae. Bellflower, CA: Academic Press; 1971.

Meeker, James E. and Wendell W. Kilgore. Identification and quantification of the alkaloids of Lupinus latifolius. J. Agri. Food Chem. 35: 431-433; 1987.

Melzer, Erhart Walter. The effect of Lupinus polyphyllus (Lindl.) on Picea alba (L.) Karst. in forest ecosystems. In: Proc. 3rd Inter. Lupin Conf., LaRochelle, France; 1984: 227-236.

Mermoud, J. F., C.D. Sepulveda, et. al. Incorporation of sweet lupine flour in the food of a group of school children. In: R. Gross and E. S. Bunting (eds.), Agricultural and nutritional aspects of lupines. Proc. First Inter. Lupine Workshop. Lima-Cuzco, Peru; 1980.

Mishustin and Shil'nikova Biological fixation of atmospheric nitrogen. University Park: Pennsylvania State Univ. Press; 1971.

Monteiro, R. and P.E. Gibbs. A taxonomic revision of the unifoliolate species of Lupinus (Leguminosae) in Brazil. Notes Roy. Bot. Gard. Edinb. 44(1):71-104; 1986.

Nicholls, K. W. and B.A. Bohm. Flavonoids and affinities of some North American lupines. Canad. J. Bot., 61: 708-730; 1983.

Nowacki, Edmund K. and George R. Waller. Use of metabolic grid to explain the metabolism of quinolizidine alkaloids in Leguminosae. Phytochemistry 14: 165-171; 1975.

Nowacki, Edmund K. and D.B. Dunn. Shrubby California lupines and relationships suggested by alkaloid content. Genetica Polonica 5(1): 47-55; 1964.

O'Leary, John F. Habitat preferences of Lupinus (Fabaceae) in the western transverse ranges of Southern California. Southwest. Nat. 27(4): 369-397; 1982.

Oosting, H.J. The Study of Plant Communities. San Francisco: W.H. Freeman & Co.; 1956.

Pataki, Gyorgy. Techniques of thin-layer chromatography in amino acids and peptide chemistry. Belzoni, MS: Humphrey Science Publishers Inc.; 1966.

Pate, J.S. Nitrogen metabolism of lupins. In: Proc. 3rd Inter. Lupin Conf., LaRochelle, France; 1984: 289-310.

Peck, Morton E. A manual of the higher plants of Oregon, Portland, OR.: Binfords and Mort.; 1961. 2nd ed.

Pelletier, S.W. Alkaloids: Chemical and biological perspectives, Vol. 4. New York: John Wiley and Sons; 1986.

Phillips, Lyle L. A revision of the perennial species of Lupinus of North America exclusive of southwestern United States and Mexico. Res. Stud. of the State College of Washington, 23(3); 1955.

Piper, Charles V. and R. K. Beattie. Flora of southeastern Washington and adjacent Idaho. Weaverville, CA.: New Era Printing Co.; 1914.

Piper, Charles V. Flora of the state of Washington. Washington Government Printing Office; 1906.

Pizzetti and Cocker. Flowers: A Guide for your garden. New York: Abrams Press; 1975.

Plitmann, U. and C.C. Heyn. Old world Lupinus: taxonomy, evolutionary relationships, and links with New World species. In: Proc. 3rd Inter. Lupine Conf., LaRochelle, France; 1984: 55-66.

Plitmann, U., C.C. Heyn, and B. Pazy. Biological flora of Israel 7: Lupinus Palaestinus Boiss. and L. pilosus Murr. Israel J. Bot. 28: 108-130; 1979.

Plitmann, U. and C.C. Heyn. The genus Lupinus in the old world: Problems and prospects. Israel J. Bot. 27:30-31; 1978.

Polhill, R. M. *Genisteae* (Adans.) Benth. and related tribes (Leguminosae). Bitabucak Syst., 1: 143-368; 1976.

Porsild, et. al. Lupinus arcticus Wats. grown from seeds of the pleistocene age. Science 158: 113-114; 1967.

Porter, Stephen C. Late quaternary environments of the United States, Vol. 1: Late pleistocene. Minneapolis, MN.: Univ. Minnesota Press; 1983.

Radford Albert E. Fundamentals of plant systematics, Chap. 9: Variation and speciation in plants. New York:Harper and Row; 1986.

Radford, Albert.E., W.C. Dickison, J.R. Massey, C.R. Bell. Vascular plant systematics. New York: Harper and Row; 1974.

Ranker, Thomas A. and A. F. Schnabel. Allozymic and morphological evidence for a progenitor-derivative species pair in Camassia (Liliaceae). Syst. Bot. 11(3): 433-445; 1986.

Rao, C. R. The use and interpretation of principal component analysis in applied research. Sankya A, 26: 329-358; 1964.

Raven, Peter H. Systematics and plant population biology. Plant Syst. 1(3): 284-306; 1976.

Reveal, James and E. Styer. Miscellaneous chromosome counts of western American plants - II. Great Basin Naturalist 33(1): 19-24; 1973.

Reyes, J. and U. Gross. Experiments on the use of Lupinus mutabilis for human consumption. In: R. Gross and E.S. Bunting (eds.), Agricultural and nutritional aspects of lupines. Proc. First Inter. Lupine Workshop. Lima-Cuzco, Peru; 1980.

Robinson, Trevor. The organic constituents of higher plants. Edina, MN.: Burgess Publ. Co.; 1963.

Romesburg, Charles H. Cluster analysis for researchers. Belmont, CA.: Wadsworth Inc; 1984.

Rowland, I.C., M.G. Mason, and J. Hamblin. Effects of lupins on soil fertility. In: Proc. 4th Inter. Lupin Conf. Geraldton, W. Australia; 1986.

Rowson, J.M. Increased alkaloid contents of induced polyploids of Datura, Atropa, and Hysocyamus. Q. J. Pharm. Pharmac. 18: 175-193; 1945.

Rydberg, P. A. Flora of the rocky mountains and adjacent plains. New York Bot. Gard., New York; [1922] 1954.

Salatino, Antonio and O. R. Gottlieb. Quinolizidine alkaloids as systematic markers of the Papilionoideae. Biochem. Syst. and Ecol. 8: 133-147; 1980.

S.A.S. Institute Inc. S.A.S. User's Guide: Basics Version 5 Edition. Cary, NC.; 1985. 1290 p.

Schmidt, Marjorie G. Natives for your garden. Fremontia 8(3): 23-24; 1980.

Schaller, Leandra. A study of the progeny of hybridization in Lupinus. 1977. Dissertation.

Schofield, W.B. Phytogeography of northwestern North America: Bryophytes and vascular plants. Madrono 20(3): 155-207; 1966.

Scoggan, H.J. The flora of Canada, Part 3. Ottawa: H.J. Scoggan; 1978.

Silberglied, Robert E. Communication in the ultraviolet. Ann. Rev. Ecol. Syst. 10: 373-398; 1979.

Simpson, George. Tempo and mode of evolution. New York: Columbia University Press; 1944.

Smith, Philip M. The chemotaxonomy of plants. New York: Edward Arnold Press; 1976.

Smith, C.P. 1938-1953. Species Lupinorum, Saratoga, FL. 3 vols.

Smith, C.P. A distributional catalogue of the lupines of Oregon. Stanford,CA.: Stanford University Press; 1927.

Smolenski, Stanislaus J., A. D. Kinghorn, and M. F. Balandrin. Toxic constituents of legume forage plants. Econ. Bot. 35(3): 321-355; 1981.

Sokal, Robert W. and F. J. Rohlf. Introduction to biostatistics. San Francisco, CA.: W.H. Freeman; 1973.

St. John, Harold. Flora of southeastern Washington and adjacent Idaho. Escondido, CA.: Outdoor Pictures; 1963. 3 ed.

St. John, Harold and G. N. Jones. An annotated catalogue of the vascular plants of Benton Co., Washington. Washington Northwest Science Association; 1928.

Stafleu, F.A. and R.S. Cowan. Taxonomic literature. Bohn, Scheltema, and Holkema:Utrecht; 1976. 2nd ed.

Stanton, Maureen L. Reproductive biology of petal color variants in wild populations of Raphanus sativus II. Factors limiting seed production. Amer. J. Bot. 74(2): 188-196; 1987.

Stebbins, G.L. Aridity as a stimulus to plant evolution. Amer. Nat. 86(826): 33-44; 1952.

Stebbins, G. L. Polyploidy, hybridization, and the invasion of new habitats. Ann. Missouri Bot. Gard. 72: 824-832; 1985.

Steele, Robert and R. D. Pfister, et. al. Forest habitat types of central Idaho. Intermountain Forest and Range Experiment Station, Gen.Tech. Rept. INT-114, Ogdon, Utah; 1981.

Stirton, C.H. Petal sculpturing in papilionoid legumes. In: Polhill, R.M. and P.H. Raven (eds.), Advances in legume systematics, Part 1. Roy. Bot. Gard. Kew.; 1981: 771-788

Svendson, A.B. and R. Verpoorte. Chromatography of alkaloids. New York: Elsevier Sci. Publ. Co.; 1983.

Swain, T. Chemical plant taxonomy. Bellflower, CA.: Academic Press; 1963.

Takahata, Yoshihito and K. Hinata. A consideration of the species relationships in subtribe *Brassicinae* (Cruciferae) in view of cluster analysis of morphological characters. Pl. Species Bio. 1: 79-88; 1986.

Taylor, Dean W. Endangerment status of Lupinus dedeckerae in the Inyo National Forest, California. Contr. Mono Basin Research Group; 1981.

Toms, G.C. Lectins in Leguminosae. In: Polhill R.M. and P.H. Raven (eds.), Advances in legume systematics. Roy. Bot. Gard. Kew.; 1981.

Torrey, R. and A. Gray 1838-1840. Flora of North America. New York: Wiley and Putnam.

Vaughan, Patricia. The Lupinus latifolius complex. Univ. Missouri-Coluimbia; 1975. 105 p. Thesis.

Waller, George R. and E. K. Nowacki. Alkaloid biology and metabolism in plants. New York: Plenum Press; 1978.

Watson, S. Revision of the extra-tropical North American species of the genus Lupinus. Proc. Amer. Acad. Arts and Sci. 8: 517-548; 1873.

Weimer, J.L. Diseases of cultivated lupines of the southeast. U.S.D.A. Farm. Bull., 2053; 1952

Weisel, Charles D. Soil Survey of Kootenai County area, Idaho. U.S.D.A. Nat'l Coop. Soil Survey. U.S. Government Printing Office; 1981.

Welsh, et al. A Utah flora. Mem. Great Basin Nat. 9. Provo, Utah: Brigham Young Univ. Press; 1987.

Whittaker, Robert H. Evolution of diversity in ecological systems. In: G.M. Woodwell and H.H. Smith (eds.), Diversity and stability in ecological systems. Brookhaven Symp. Bio.: 22, U.S. Dept. of Commerce, Springfield, VA.; 1969.

Williams, Watkin. The current status of crop lupins. In: Proc. 4th Inter. Lupin Conf. Geraldton, W. Australia; 1986.

Williams, Watkin, J. M. Harrison and S. J. Asekera. Genetical control of alkaloid production in Lupinus mutabilis and the effect of a mutant allele 'mutal' isolated following chemical mutagenesis. Euphytica 33: 811-817; 1984.

Wink, M. Storage of Quinolizidine alkaloids in epidermal tissues. Z. Naturforsch. Sect. C Biosci. 41(4): 375-380; 1986.

Wink, M. and L. Witte. Quinolizidine alkaloids as nitrogen source for lupine seedlings and cell cultures. Z. Naturforsch Sect. C Biosci. 40(11/12): 767-775; 1985.

Wink, M. Quinolizidine alkaloids as nitrogen source for lupin seedlings and cell cultures. Z. Naturforsch 40c: 767-775; 1985.

Wink, M. 1984a. Biochemistry and chemical ecology of lupin alkaloids. In: Proc. 3rd Inter. Lupin Conf., LaRochelle, France; 1984: 325-344.

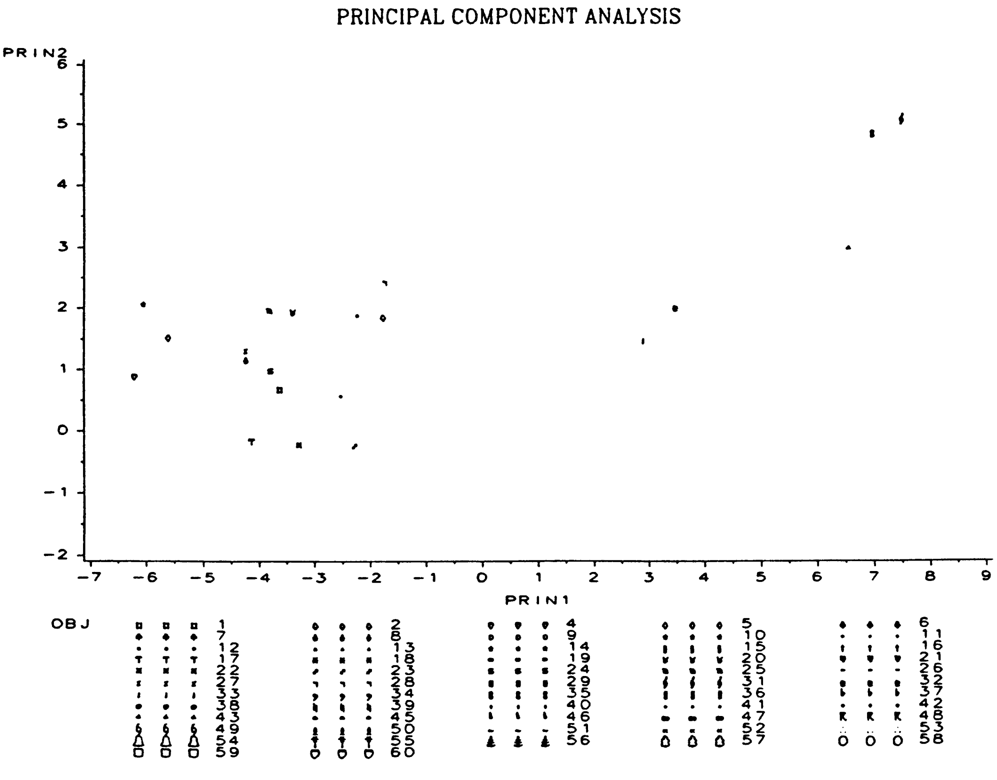
Wink, M. Chemical defense of Leguminosae. Are quinolizidine alkaloids part of the antimicrobial defense system of lupins? Z. Naturforsch 39c: 548-552; 1984.

Wink, M. Chemical defense of lupins. Mollusc-repellent properties of quinolizidine alkaloids. Z. Naturforsch. 39c: 553-558; 1984.

Wink, M and Witte, L. Turnover and transport of quinolizidine alkaloids. Diurnal fluctuations of lupanine in the phloem sap, leaves, and fruit of Lupinus albus L. Natl. Museum of Canada; 1983.

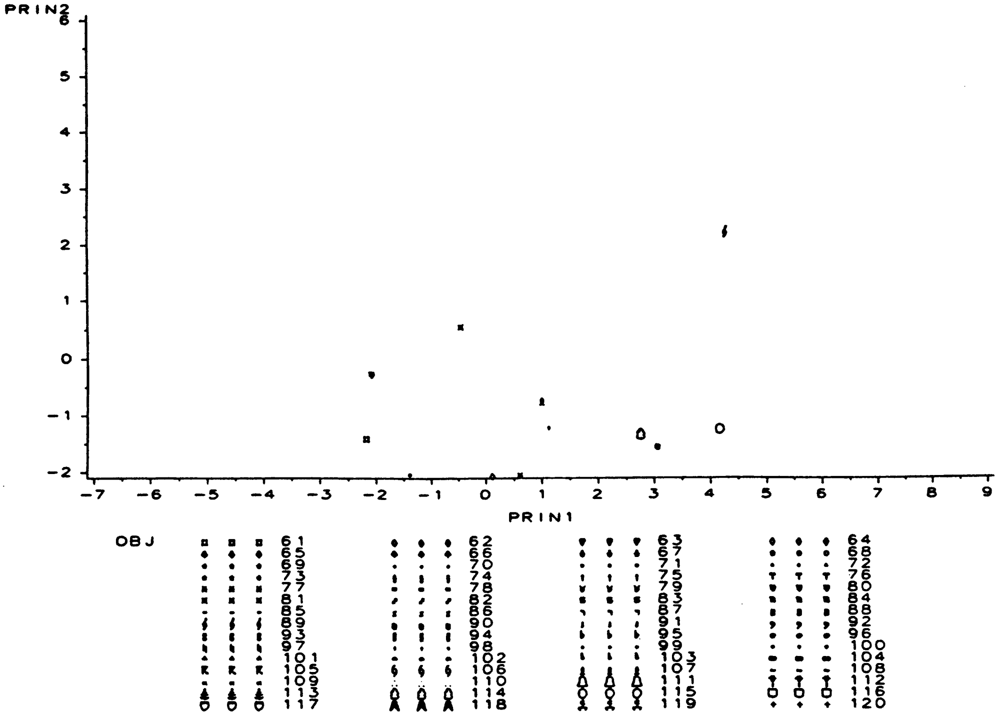
Wink, M. and T. Hartmann 1981. The alkaloid patterns of cell suspension cultures and differentiated plants of Baptisia australis and their biogenetic implications. J. Nat. Prod.44, (1); 1981.

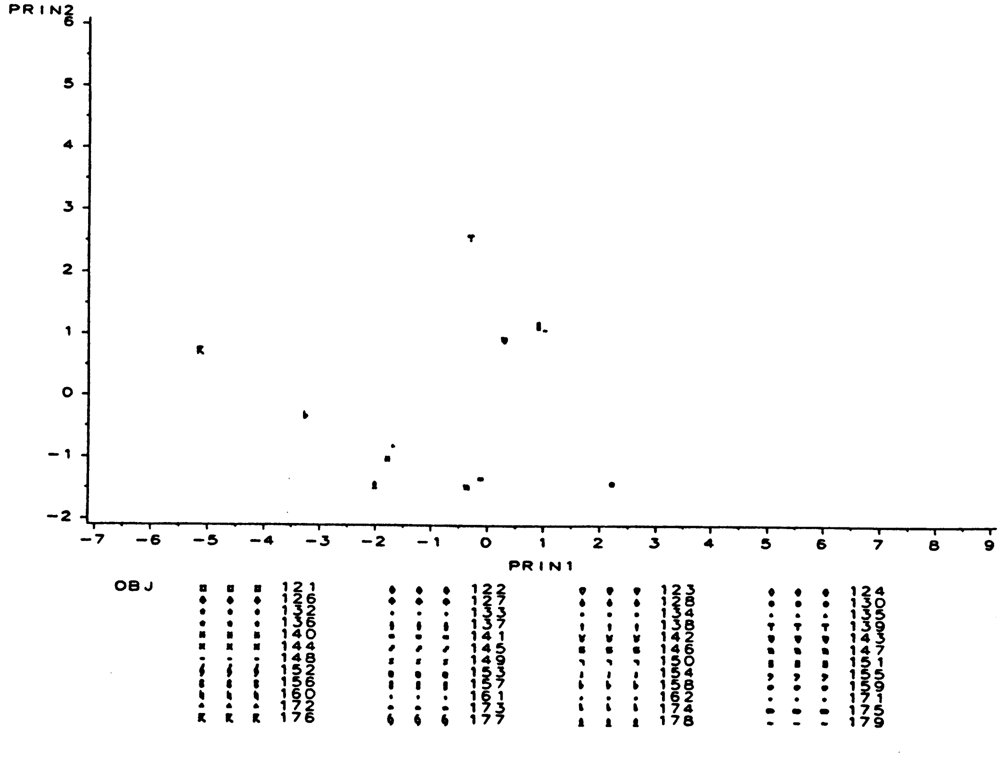
APPENDIX I

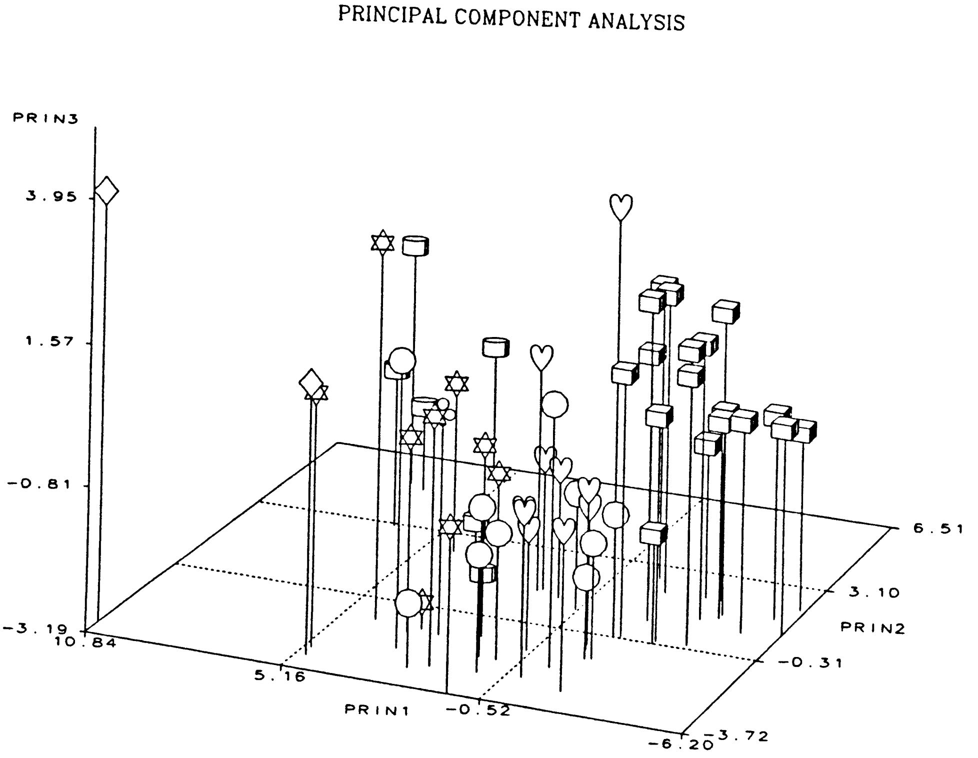


Principal Components Analysis: Graphs based on the two major principal components. Thirty-four attributes are used. Symbols correlate to a single specimen (object). Obj. 1-30 = *ssp. erectus*; obj. 31-39 = *ssp. retrorsus* from Coeur d'Alene, Idaho; obj. 48-84 = small flowered *var. belliae* from the Columbia Basin; obj. 87-107 = large flowered *var. leucophyllus* from the Columbia Basin; obj. 108-112 & 116-128 = *var. leucophyllus* from near the Dalles, Oregon; obj. 129-133 = *var. leucophyllus* from above the Walla Walla River near Milton-Freewater, Oregon; obj. 135-157 = *var. canescens*; obj. 164-178 = various types and species known to hybridize with L. leucophyllus.

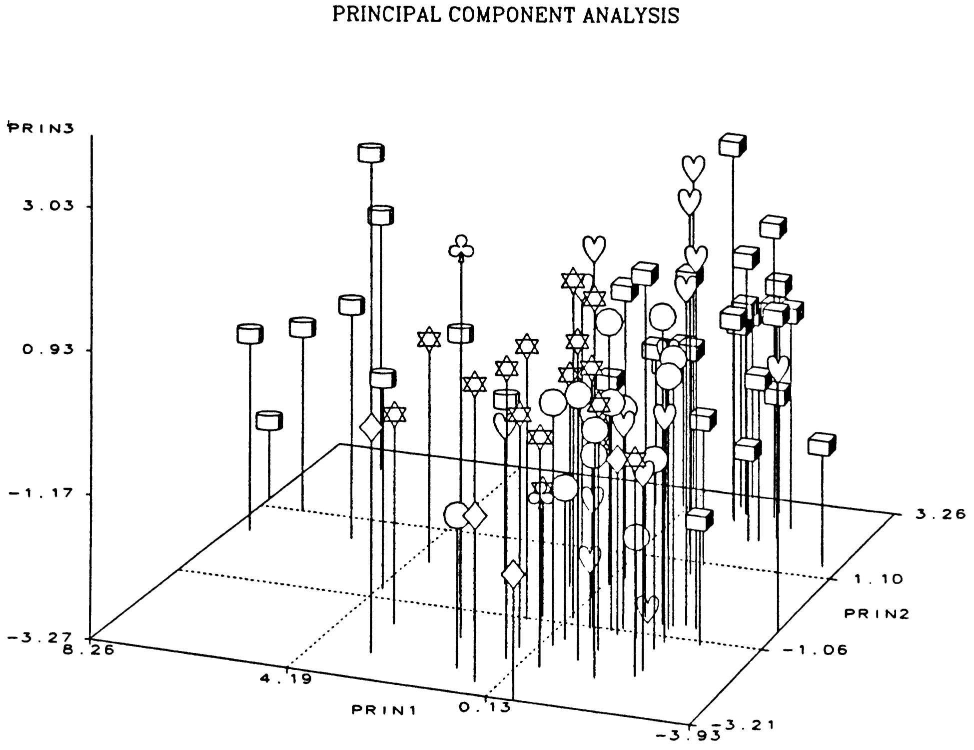
PRINCIPAL COMPONENT ANALYSIS







Principal Components Analysis: Three-dimensional graph based on the three primary components. Cubes = group A, *ssp. erectus*; cylinders = group B, *ssp. retrorsus* from Coeur d'Alene, Idaho; balloons = group C, *var. belliae* from the Columbia Basin; star = group D, *var. leucophyllus* from the Columbia Basin; heart = group E, *var. canescens*; club= group F, *var. leucophyllus* from above the Walla Walla River, near Milton-Freewater, Oregon; diamond = group G, *var. leucophyllus* from near the Dalles, Oregon. Only objects with complete data sets are plotted.



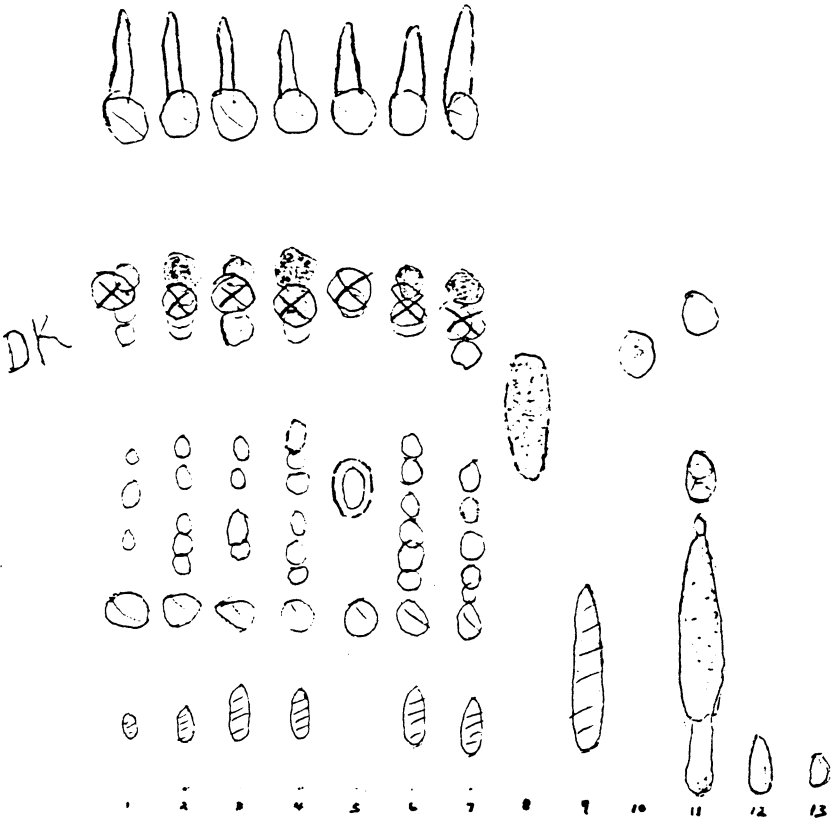
Principal Componments Analysis: clusters based on 15 vegetative attributes. (Right to Left) Cubes= ssp. erectus; hearts= var. canescens; balloons= var. belliae; stars= var. leucophyllus; club= var. leucophyllus from above the Walla Walla River near Milton-Freewater; diamonds= var. leucophyllus from the Dalles, Oregon; cylinders= ssp. retrorsus.

```
                                  SAS

                      PRINCIPAL COMPONENT ANALYSIS

              EIGENVALUE      DIFFERENCE      PROPORTION      CUMULATIVE

PRIN1            13.7966         8.83073        0.405781         0.40578
PRIN2             4.9658         2.79838        0.146054         0.55184
PRIN3             2.1675         0.63485        0.063749         0.61558
PRIN4             1.5326         0.14302        0.045077         0.66066
PRIN5             1.3896         0.17095        0.040870         0.70153
PRIN6             1.2186         0.09622        0.035842         0.73737
PRIN7             1.1224         0.16180        0.033012         0.77039
PRIN8             0.9606         0.09587        0.028253         0.79864
PRIN9             0.8647         0.07983        0.025433         0.82407
PRIN10            0.7849         0.07938        0.023086         0.84716
PRIN11            0.7055         0.12491        0.020751         0.86791
PRIN12            0.5806         0.02924        0.017077         0.88499
PRIN13            0.5514         0.04688        0.016217         0.90120
PRIN14            0.5045         0.04946        0.014838         0.91604
PRIN15            0.4550         0.09257        0.013383         0.92942
PRIN16            0.3625         0.05214        0.010661         0.94009
PRIN17            0.3103         0.04936        0.009127         0.94921
PRIN18            0.2610         0.03804        0.007676         0.95689
PRIN19            0.2229         0.02053        0.006557         0.96345
PRIN20            0.2024         0.01035        0.005953         0.96940
PRIN21            0.1921         0.03677        0.005649         0.97505
PRIN22            0.1553         0.02716        0.004567         0.97961
PRIN23            0.1281         0.00735        0.003768         0.98338
PRIN24            0.1208         0.02193        0.003552         0.98693
PRIN25            0.0988         0.01517        0.002907         0.98984
PRIN26            0.0837         0.01923        0.002461         0.99230
PRIN27            0.0644         0.00721        0.001895         0.99420
PRIN28            0.0572         0.01263        0.001683         0.99588
PRIN29            0.0446         0.00817        0.001312         0.99719
PRIN30            0.0364         0.01032        0.001071         0.99826
PRIN31            0.0261         0.00454        0.000768         0.99903
PRIN32            0.0216         0.01023        0.000634         0.99967
PRIN33            0.0113         0.01134        0.000334         1.00000
PRIN34            0.0000                        0.000000         1.00000
```

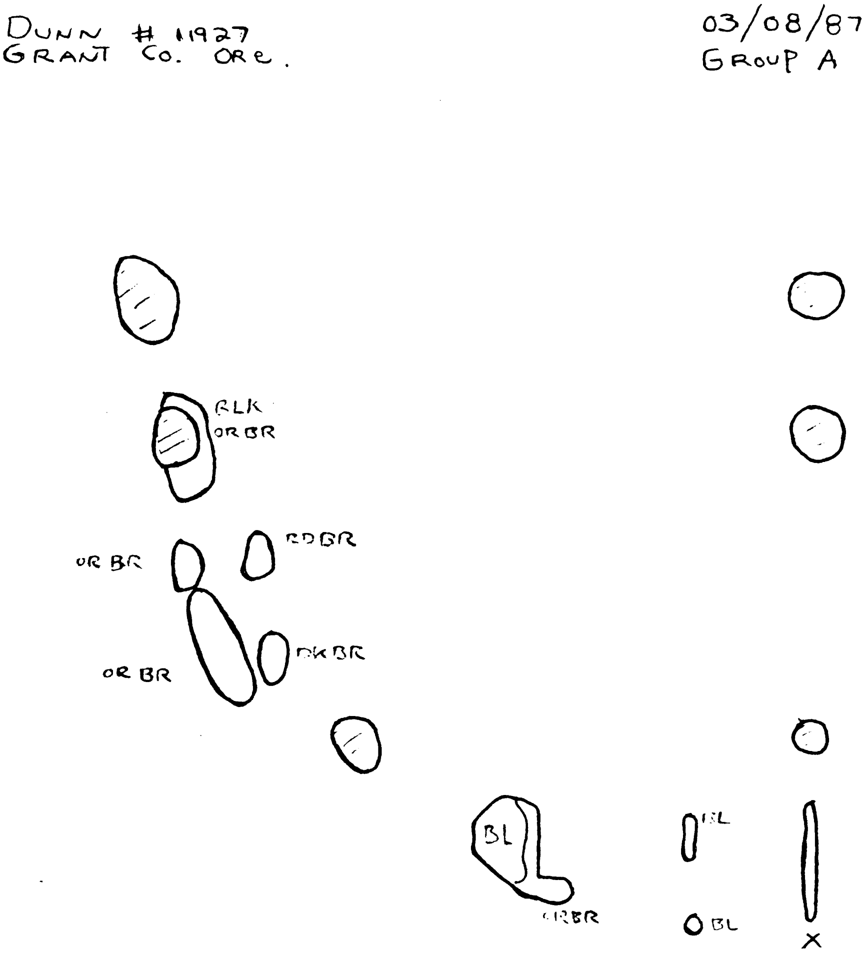
```
              PRIN1       PRIN2       PRIN3

FLRCOL     0.076186    -.102504    0.256704
FLRL       0.249344    -.096136    0.005816
BANL       0.244072    0.005925    0.009537
BANAP      0.248407    -.008852    -.066979
BANRE      0.202157    -.112359    -.092136
WINGL      0.229884    -.156026    0.091274
WINGW      0.212152    -.136563    0.000092
WINGCL     0.208577    -.061729    0.074424
KEELW      0.209188    -.041676    0.085676
KLL        0.216636    -.136629    0.210273
KUL        0.219260    -.096895    0.207969
KUW        0.216227    0.040324    0.054454
KCON       0.162661    0.013229    0.219351
BTEOL      0.113279    0.009134    0.369799
BRACL      0.150280    -.022643    0.181748
PEDUNL     0.047554    0.149651    0.054650
RACL       0.158085    0.170525    -.359144
ACUMEN     0.204390    -.115147    -.063363
INTERL     0.112293    -.035040    -.245277
VHT        0.176688    0.173278    -.161556
VVESTL     0.185801    0.081154    -.283176
VDIA       0.214423    0.036917    0.057151
VINOD      0.187667    -.197786    -.030464
VLINODL    -.028137    0.303964    0.038746
VBSPTL     -.019561    0.175280    0.324516
VCAPTL     0.090538    0.232130    0.030215
VLEAFTS    0.076755    0.207499    0.008573
VLLEAFL    0.150464    0.303557    0.036782
VLLEAFW    0.145639    0.253507    0.075803
VSLEAFL    0.124856    0.321115    -.018389
VSLEAFW    0.137428    0.238390    0.054607
VVSRETR    0.141206    0.268612    -.212846
VVSAPR     -.179926    0.170214    0.309742
VVSSPR     0.081548    -.328518    -.159284
```

Principal Component Analysis statistics: eigenvalues and principal components.

APPENDIX II



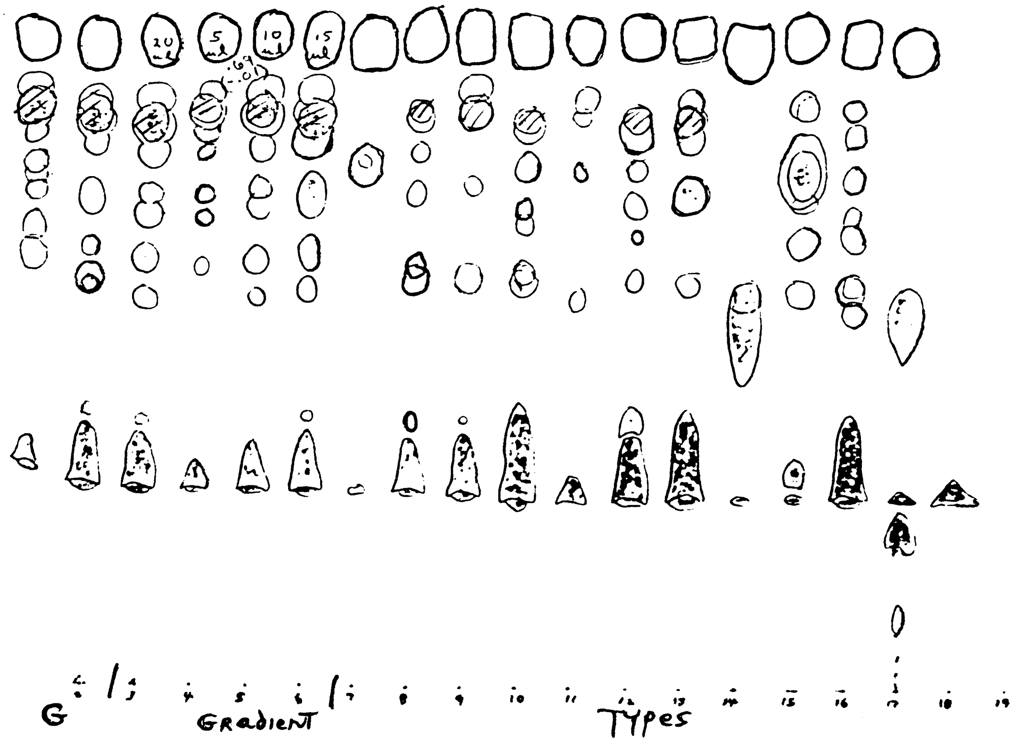
Chromatography plate comparing L. leucophyllus alkaloids to known standards. Lane 1 = typical *var. leucophyllus* from above Walla Walla River near Milton-Freewater, Oregon; lane 2 = *var. leucophyllus* from near the Dalles Oregon; lane 3 = *ssp. erectus* from the Blue Mountains, Oregon; lane 4 = *ssp. retrorsus* from Coeur d'Alene, Idaho; lane 5 = another species or a hybrid with another species;lane 6 = *var. belliae* from the Columbia Basin; lane 7 = *var. canescens*; lane 8 = D-lupanine perchlorate; lane 9 = D-hydroxylupanine; lane 10 = D- isolupanine perchlorate; lane11 = sparteine; lane 12 = (13)-B-hydroxy sparteine; Note: D-hydroxylupanine appears to match alkaloids in L. leucophyllus.



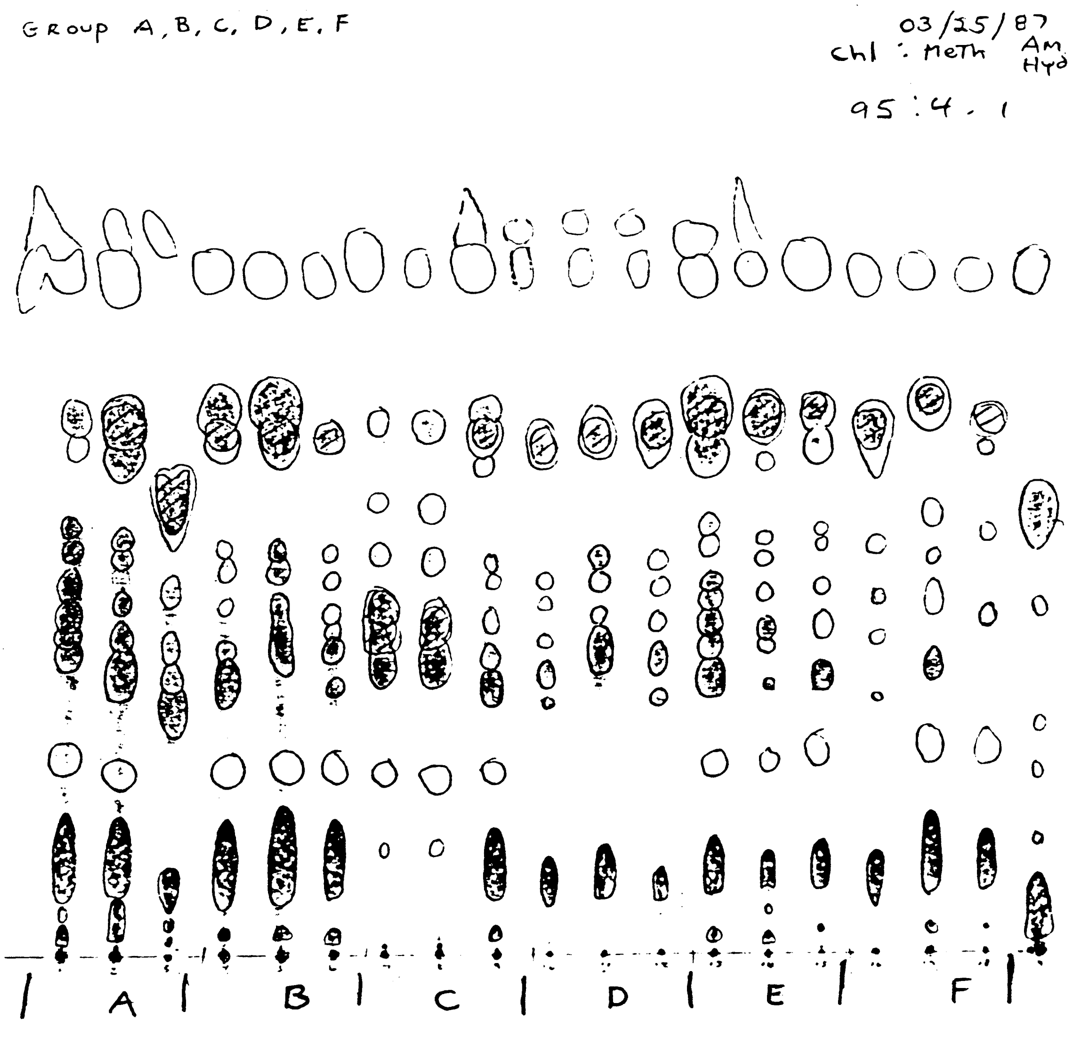
Chromatography plate run in two dimensions. Typical for speciesCompares group D, *var. leucophyllus* from the Columbia Basin and group E, *var. canescens*; and group F, *var. leucophyllus* from above the Walla Walla River near Milton-Freewater; and group G, *var. leucophyllus* from near the Dalles. Mobile phase = chloroform: methanol: diethylamine.

Group G & Gradient Study

03/26/87
Types
165 : 30 : 2



Chromatography plate comparing type specimens. Lane 7 = L. macrostachys Jones 07/14/1909, holotype); lane 8 = L.ornatus (Henderson 3088, isotype); lane 9 = L. plumosus (type); lane 10 = L. canescens (Sandberg and Leiberg 402, type); lane 11 = L. wyethii (Henderson 4608, type); lane 12 = L. tenuispicus (Clark 203, isotype); lane 13 = L. canescens (Howell 787, holotype); lane 14 = L. leucophyllus *var. belliae* (May Bell Zundel 09/10/1914, isotype); lane 15 = L. enodatus (Benson 1646, type); lane 16 = L. leucophyllus (Douglas 1826, type); lane 17= L. formosus *proximus* (Dunn et. al. 7139, standards); lane 18 = lupanine; lane 19 = chloroform control.



Chromatography plate using mobile phase of chloroform: methanol: ammonium hydroxide. Group A = *ssp. erectus*; group B = *ssp. retrorsus* from Coeur d'Alene, Idaho; group C = *var. belliae* from Columbia Basin; group D = *var. leucophyllus* from Columbia Basin; group E = *var. canescens*; group F = *var. leucophyllus* from above the Walla Walla River near Milton-Freewater.

APPENDIX III

PRINCIPAL COMPONENTS: FLORAL CHARACTERISTICS

1. Maturity (flower or fruit) = MATUR
2. Flower Color = FLRCOL
3. Flower length = FLRL
4. Banner length = BANL
5. Banner width = BANW
6. Banner appressed length = BANAP
7. Banner reflexed length = BANRE
8. Banner ratio = BANRA
9. Banner angle = BANANG
10. Wing length = WINGL
11. Wing width = WINGW
12. Wing claw length = WINGCL
13. Keel width = KEELW
14. Keel angle = KEANG
15. Lower calyx length = KLL
16. Lower calyx width = KLW
17. Upper calyx length = KUL
18. Upper calyx width = KUW
19. Distance calyx connate = KCON
20. Bracteole length = BTEOL
21. Bract length = BRACL
22. Pedicel length = PEDL
23. Peduncle length = PEDUNL
24. Racene length = RACL
25. Distance from claw to acumen = ACUMEN
26. Length longest interverticel = INTERL

PRINCIPAL COMPONENTS: VEGETATIVE CHARACTERISTICS

1. Vegetative height = VHT
2. Stem vesture length = VVESTL
3. Diameter of stem = VDIA
4. Number of internodes = VINOD
5. Length longest internode = VLINODL
6. Length basal petiole = VBSPTL
7. Length cauline petiole = VCAPTL
8. Number leaflets =VLEAFTS
9. Length longest leaflet = VLLEAFL
10. Width longest leaflet = VLLEAFW
11. Length shortest leaflet = VSLEAFL
12. Width shortest leaflet = VSLEAFW
13. Ratio longest cauline leaflet to petiole = VRALFPT
14. Length stipule = VSTPL
15. Length stipule connate = VSTPCON
16. Fistulose diameter = VFISDIA
17. Pubescence retrorse = VVSRETR
18. Pubescence appressed = VVSAPR
19. Pubescence spreading = VVSSPR

OTHER CHARACTERS OBSERVED

1. Banner pubescence
2. Number of undeveloped ovules
3. Keel ciliation
4. Calyx notch depth
5. Distance calyx lips connate
6. Bract width
7. Bract shape
8. Flower arrangement
9. Pod width
10. Pod length
11. Pod vesture
12. Number of developed seeds
13. Seed diameter
14. Seed thickness
15. Banner shape
16. Calyx vesture
17. Shape upper calyx lip
18. Shape lower calyx lip
19. Branching
20. Stem vesture length
21. Shape leaflet
22. Shape leaflet apex
23. Leaflet folding
24. Leaf vesture above
25. Leaf vesture below
26. Flower color

University Libraries
University of Missouri

Digitization Information Page

Local identifier	Currier1988

Source information

Format	Book
Content type	Text
Source ID	Gift copy from department; not added to MU collection.
Notes	

Capture information

Date captured	January 2024
Scanner manufacturer	Fujitsu
Scanner model	fi-7460
Scanning system software	ScandAll Pro v. 2.1.5 Premium
Optical resolution	600 dpi
Color settings	8 bit grayscale
File types	tiff
Notes	

Derivatives - Access copy

Compression	Tiff: LZW compression
Editing software	Adobe Photoshop
Resolution	600 dpi
Color	grayscale
File types	pdf created from tiffs
Notes	Images cropped, straightened, brightened. p0069, p0085, p0095, p0096, p0115, are missing images that are supposed to be included in the text. This reflects the appearance of physical thesis.