# THE CHEMISTRY OF GEOGRAPHICAL RACES

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## ABSTRACT

Different populations of many, if not all, plant species show some chemical variation in one or more classes of constituent. In some cases, well-defined chemical races have been shown to exist and it has been suggested that these be treated as intraspecific taxa. It is suggested that since many of the natural products are known to be useful in defence against predators, as attractants for pollinators and in several other ways, much of the chemical diversity which is encountered arises from the selection of populations which show an ecological amplitude in a given habitat. Examples are given from lichens, ferns and angiosperms covering a range of diverse chemical compounds and different examples of geographical variation.

## I. INTRODUCTION

As far as is known every plant species produces some unique secondary compounds, often in remarkably large quantities[2a]. Many of these compounds are known to serve as defensive compounds against herbivores and other predators; others regulate biochemical processes and mediate a variety of developmental responses. Some are metabolically active and undergo turnover often in a few hours; thus the quantities in the plant, in these instances at least, represent steady-state concentrations. In connection with the present account of the chemistry of geographical races† (that is, the chemistry of

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† Grant[1] has suggested that population systems be classified in the following way:

(1) Population systems intergrading continuously in morphological or physiological characters, and hence judged to be interbreeding freely: (a) Allopatric—contiguous geographical races; (b) Sympatric—ecological races.

(2) Population systems intergrading discontinuously or partially, and judged to be interbreeding on a restricted scale: (a) Allopatric (i) Differentiated morphologically or physiologically to a moderate degree—disjunct geographical races (ii) Differentiated morphologically or physiologically in a considerable degree—allopatric semispecies; (b) Sympatric (i) Not isolated reproductively—ecological races (ii) Partially isolated reproductively—sympatric semispecies.

(3) Population systems separated by a discontinuity in the pattern of morphological and physiological variations, and evidently not interbreeding: (a) Allopatric (i) Not isolated reproductively—allopatric semispecies (ii) Reproductively isolated—allopatric species; (b) Sympatric (i) Reproductively isolated—sympatric species.

In this review we employ the expression 'geographical races' in a broad sense to include essentially all the above population systems; no new nomenclatural systems are introduced here.

different populations of a given or closely related taxa) it is well known most if not all species have been able to radiate geographically and ecologically through dispersal and adaptations usually exhibiting some sort of ecotypic variation including chemical variation. Some of the ecotypic chemical variation represents only minor qualitative differences while in other instances the variation appears to represent dramatic structural changes as the result of mutational loss: that is, the ability to synthesize certain compounds has been lost.

Chemical agents are recognized[2] to be of major significance in the adaptation of species and organization of communities. Thus, the interactions of plants, animals and microorganisms with each other and with ecological and environmental elements of a given ecosystem will determine much of the secondary chemistry of a plant population. Indeed some investigators believe that most of the natural products chemistry of a particular plant taxon serves as a defensive system against predators. Nevertheless, it is important to recognize that despite even drastic changes in the ecosystem a particular structural theme (regardless of the class of compounds) will almost always continue to persist in all populations of a taxon or populations of closely related taxa. The sort of chemical ecotypic variation that is regularly encountered on going from population to population involves the number of compounds, degree of oxygenation, methylation and glycosylation and such other structural modifications as rearrangements and cyclizations. A portion of such ecotypic variation may result from the fact that the new ecosystem requires a physiologically different organism and this biochemically different plant then produces, secondarily, the various minor modifications in the natural products chemistry just mentioned.

Also, genetic drift, occurring in geographically isolated populations, is a potential source of genetic variation which could lead to chemical races. Major geological and climatic events such as the shifting of land masses, formation of mountain chains and periods of glaciations, have all contributed in various ways to the origin of the chemical races present in today's taxa.

In general, most populational chemical variation will be understood only when the detailed physiological basis for the differentiation is explained; for most of the examples cited here these explanations are either lacking or are incomplete. The present account of the chemistry of geographical races will therefore be restricted, for the most part, to the presentation of only a few examples selected to illustrate the patterns of variation observed for different types of secondary compounds (e.g., alkaloids, terpenes and phenolics) at the infra- and inter-specific levels. Most of the examples of ecotypic variation illustrate on a population to population basis minor changes in a basic structural theme of the natural products chemistry.

Such works as Alston's 1967 review[3] of 'Biochemical Systematics' and Tetenyi's book *Infraspecific Chemical Taxa of Medicinal Plants*[4] have dealt with many additional examples of chemical races and, in addition, set forth some of the major principles associated with their formation. Alston commented on his own views, as well as some of those of Tetenyi's, with respect to chemical variation as follows[3]:
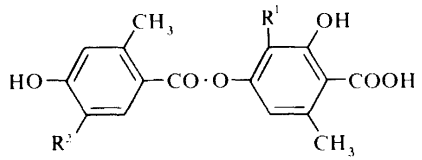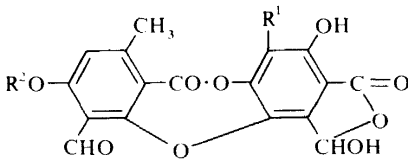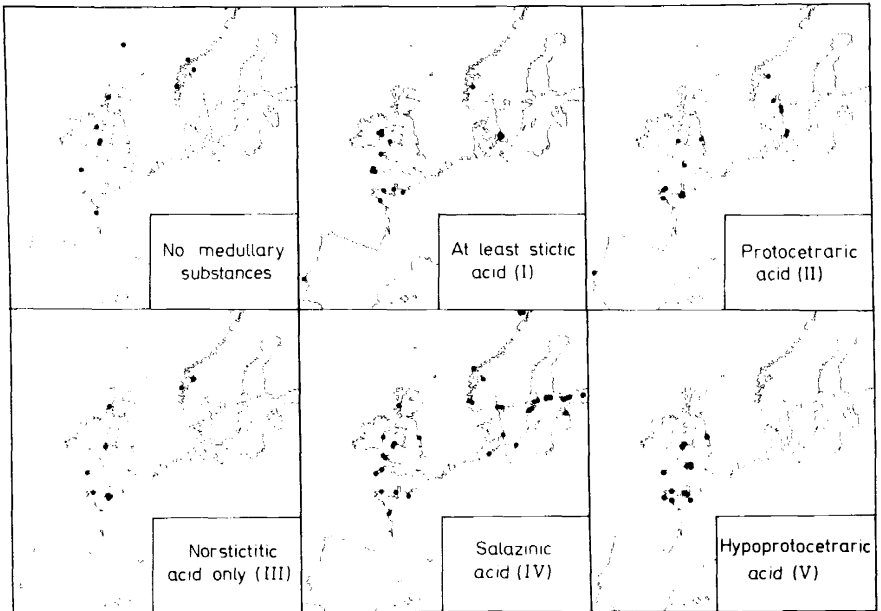
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Chemical variation that is interpopulational and extends significantly beyond the limits of intrapopulational variation is especially interesting from an evolutionary point of view. Sometimes, well-defined chemical races are found to exist and indeed there have been suggestions that authenticated chemical races be given formal recognition. One of the proponents of formal recognition of chemical races, Tetenyi[5], has stated: 'The existence of infra-specific chemical taxa is a fact. Work on their nomenclature is an indispensable task and also a preliminary to the necessary exactness of our scientific field.' Tetenyi has cited the recent emphasis on chemotaxonomy as adding more urgency to the task. If I understand Tetenyi correctly, he does not favor the erection of chemical species (although chemical data may be taken into account along with other data in formulating species concepts in a group). He has clearly emphasized that only the totality of morphological, physiologi-cal, and reproductive data can serve as the basis for the recognition of a species. What Tetenyi wishes to do is to introduce the prefix *chemo-* before the appro-priate infraspecific category, e.g., chemovar, chemosubspecies, chemocultivar, and so forth, followed by an appropriate qualitative statement as to the specific chemical entity involved. He has again called attention to the fact that in *Cinnamomum camphora* there occur chemovars high in borneol, in camphor, in cineole, and so forth. Tetenyi has suggested even the possibility of including a quantitative designation, for example in varieties of *Cinnamomum camphora* one could refer to chemoform 71 per cent linalool and chemoform 85 per cent linalool. There is an obvious advantage in the system recommended by Tetenyi, if it should prove to be practically possible, for pharmacologists and other scientists that have considerable pragmatic interest in chemotaxonomy. How-ever, I believe that the practice of the formal recognition of chemical races by plant systematists would create many difficult problems not immediately apparent to a person primarily interested in cultivated plants. From the point of view of theoretical biology, or evolutionary biology, the existence of chemical races represents a further opportunity for analysis of the taxon with respect to its ecology, its genetic heterogeneity, perhaps its mode and region of origin, incipient speciation, and the like. Although the answers may not be provided to these questions, the important thing is that all variation is potentially explainable and hence informative. It is the biological explanation of such variation which is important. . . .

## II. CHEMICAL RACES IN LICHENS

There are many rather remarkable examples of chemical races in lichens; often, in these plants, the only sure identification involves chemical analysis. For the most part, the chemically different races of lichens occupy different habitats and represent genetically determined physiological races with different ecological tolerances; the accumulation of unique secondary compounds being one type of phenotypic expression.

### Ramalina siliquosa group

The *Ramalina siliquosa* complex is one of the most common maritime lichens of Western Europe; it exhibits six chemical races (*Figure 1*); each of which has a characteristic amplitude of ecologic tolerance which appears to regulate its habitat selection. While many European taxonomists have treated the entire *R. siliquosa* complex as one or two species, Culberson[6–8]

| No medullary substances | At least stictic acid (I) | Protocetraric acid (II) |
| Norstictic acid only (III) | Salazinic acid (IV) | Hypoprotocetraric acid (V) |

I Stictic acid; $R^1$, $R^2 = CH_3$
III Norstictic acid; $R^1 = CH_3$, $R^2 = H$
IV Salazinic acid; $R^1 = CH_2OH$, $R^2 = H$

II Protocetraric acid; $R^1 = CH_2OH$, $R^2 = CH_3$
V Hypoprotocetraric acid; $R^1$, $R^2 = CH_3$

Figure 1. The geographic ranges of the chemical variants in the *Ramalina siliquosa* complex as determined from herbarium specimens.[6]

has interpreted the complex as a set of six species differing essentially by chemical, geographical and ecological traits :[7]

On all northern and western shores of Europe where cliffs of acidic rock are exposed, rock surfaces from above the littoral zone up to where there is enough soil for vascular plants are covered with lichens of the *Ramalina siliquosa* complex. These large fruticose plants lack asexual propagules and have abundant pycnidia and apothecia, indicating prolific sexual reproduction. Mass samples, involving 1042 individuals from four localities in Britain and France, and studies of herbarium specimens showed that the *R. siliquosa* lichens belong to six chemical races. (Five races have different β-orcinol medullary depsidones and one lacks medullary constituents.) The geographic ranges of the races, although extensively sympatric, differ considerably. The

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salazinic acid race is the most northern; it ranges to arctic Norway and Iceland and throughout the Baltic. The race producing norstictic acid and the one lacking medullary substances are restricted to southwestern Norway, western Britain, and the peninsula of Brittany the classic euoceanic life zone of Europe. The hypoprotocetraric acid race, common in western Britain and France, ranges south to Portugal. The protocetraric and stictic acid races, most common in Portugal, go as far north as southern Norway.

All six chemical types are sympatric on the coast of Wales. An analysis of the distribution of the chemical races on a small promontory with a rich *Ramalina* vegetation showed that the range of habitats available grossly exceeded the amplitude of tolerance of every chemical race and that what appears to the eye as a solid stand of *Ramalinae* is in reality a sequence of bands of the chemical races intensively sorted by amplitude of tolerance. . . . The findings from the study in Wales proves not only that environmental conditions can sort chemical races by habitat, but also that environment does not determine the chemical constitution of the individuals.

In summary, it is unlikely that the minor chemical changes observed for the *Ramalina* populations represent selection of chemical types for defensive purposes; rather, it appears that a slightly different ecosystem requires a physiologically modified lichen which, secondarily, exhibits minor structural changes in the natural products chemistry.

### *Cetraria ciliaris* complex

The *Cetraria ciliaris* complex is of interest because in regions of sympatry its two chemical races do not seem to show different ecologies, thus differing from the situation observed for *Ramalina siliquosa*[7].

The *Cetraria ciliaris* complex is differentiated into two races, an olivetoric acid-producing race in the eastern United States and northern Europe and an alectoronic acid-producing race ranging widely through northern North America, Siberia, and northern Europe. In the central Appalachian Mountains, where the chemical races of these common epiphytes are sympatric and about equally abundant, Hale[9] found no habitat preferences (top vs. bottom of trees, bark of *Pinus* vs. bark of *Quercus*, or position of the host tree in the woods) in an analysis of 16,398 individuals. A study of 1896 individuals in Michigan gave similar results. It seems that the different geographic distributions of the two chemical races of this complex may have resulted from historical factors and that there has been no accompanying ecological differentiation. In spite of the evidence of prolific sexual reproduction noted in the populations in both studies, no indication of hybridization was observed.

### Chemical variation in *Parmelia bolliana*

The exclusively North American *Parmelia bolliana* group provides a striking example of morphologically-indistinguishable chemical races based on two biogenetically unrelated compounds, protolichesterinic acid and lecanoric acid[7]. (See *Figure 2*.)

Both races are extensively sympatric in the central United States, but the lecanoric acid race is more western and the protolichesterinic acid race, more eastern. These sexually reproducing, chemically different plants commonly grow crowded together on the same tree trunk in places where their ranges overlap, but an analysis of more than 1000 individuals revealed no indication of hybridization.
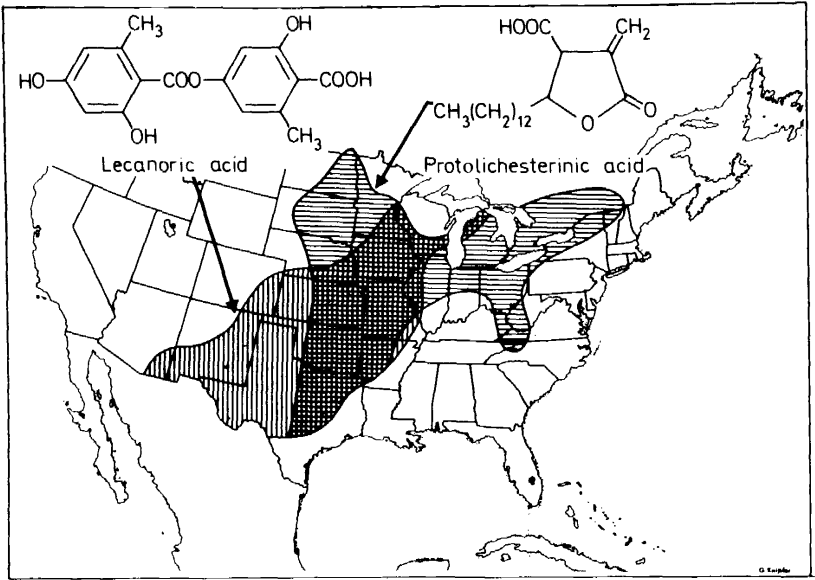
*Figure 2.* Chemical variation in the *Parmelia bolliana* complex in the United States. The eastern race produces the fatty acid lactone protolichesterinic acid and the western race, which extends far south into Mexico, produces the depside lecanoric acid. The chemically dissimilar races are usually considered to be distinct species, *P. bolliana* (protolichesterinic acid) and *P. hypoleucites* (lecanoric acid) (from Culberson[7]).
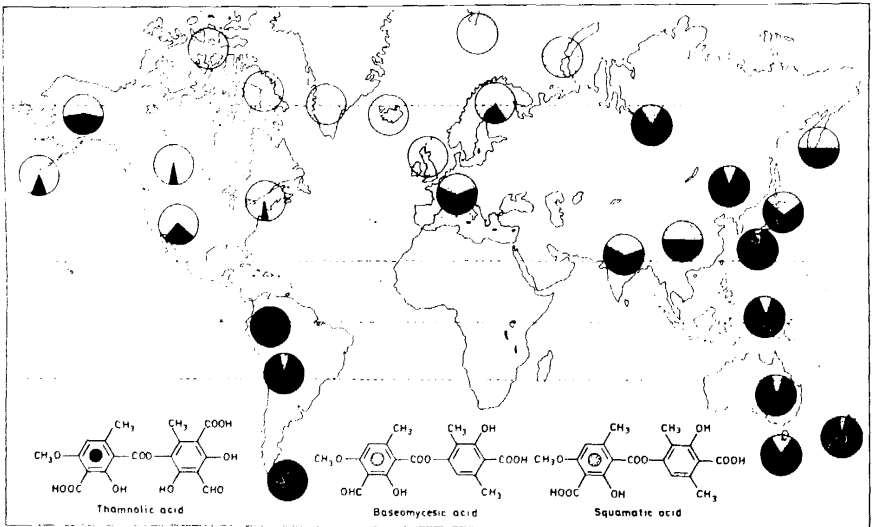


*Figure 3.* The map (from Sato[11] and Culberson[10]) shows the relative abundance of two chemical races in the lichen genus *Thamnolia* based on herbarium specimens (these represent random samples since the two races cannot be distinguished morphologically). Culberson (private communication; May, 1972) suggested that this 'fantastic' distribution might be related to flight pathways of bipolar birds. He emphasized that the absence from Africa is real.

**Chemical races in** *Thamnolia*

Culberson noted (private communication, 1972) 'that most non-licheno-logists respond with greatest interest to what is one of the most enigmatic distributions of chemical races that I know, the one in *Thamnolia*', a genus which is monotypic or ditypic depending upon the weight assigned to chemistry:[10]

> In spite of being totally asexual, *Thamnolia* is one of the most successful of all lichen genera, occurring abundantly on bare soil and among herbs throughout the arctic and alpine regions of the world. Individuals belong to one or the other of two chemical types: they produce either a pair of β-orcinol-type *para*-depsides (baeomycesic and squamatic acids) or a β-orcinol-type *meta*-depside (thamnolic acid). The constituent(s) of a given specimen can be determined only by appropriate chemical analysis, and consequently the abundant representation of the genus in herbaria constitutes a world-wide random sample of the chemical variation. By testing herbarium specimens Sato[11] demonstrated that the *para*-depsides are produced exclusively or more commonly in populations in the northern hemisphere while the *meta*-depside is produced exclusively or more commonly in populations in the southern hemisphere, even though most populations in both hemispheres contain both chemical types (*Figure 3*). Are these differences to be interpreted as coming from an allopatric differentiation in populations that have subse-quently become extensively sympatric? The chemical differences involved are sufficiently complex that they would seem to reflect more than a superficial genetic modification and to require more than a simplistic explanation.

## III. NITROGENOUS COMPOUNDS

**Cyanogenic glucoside races in** *Trifolium repens* **(Leguminosae)**

Wild *Trifolium repens*, now widely distributed over much of Europe and Asia, probably originated in the Mediterranean basin. Today, individual plants in these European and Asian populations either possess or lack two cyanogenic glucosides, lotaustralin and linamarin (*Figure 4*). Furthermore, when plants possess the glucosides they usually contain the enzyme lina-marase which hydrolyzes the glucosides to yield, as one product, HCN[12]. A gradual decrease in the frequencies of the genes for the glucosides and the enzyme were observed as populations were sampled moving from the Mediterranean region to north-eastern Europe. Indeed, the percentage of cyanogenic plants varied from 0–100% depending upon the geographical origin of the population. *Figure 4* shows the distribution for the enzyme linamarase (similar patterns were observed for the glucosides). These patterns appear to be closely correlated with January isotherms; therefore, it has been suggested that decreasing January temperatures, through natural selection, played an important role in the evolution of the decreasing cyanogenic glucoside races in the northern populations of *T. repens*[12]. The lower temperatures may have reduced predator populations thus allowing for the selection of populations of *T. repens* with fewer defensive compounds (one possible role of these cyanogenic glucosides). Or, alternatively, in the regions with lower January temperatures, a race with a different ecological amplitude may have been required and one phenotypic expression of this physiologically distinct race is the observed chemical differences.
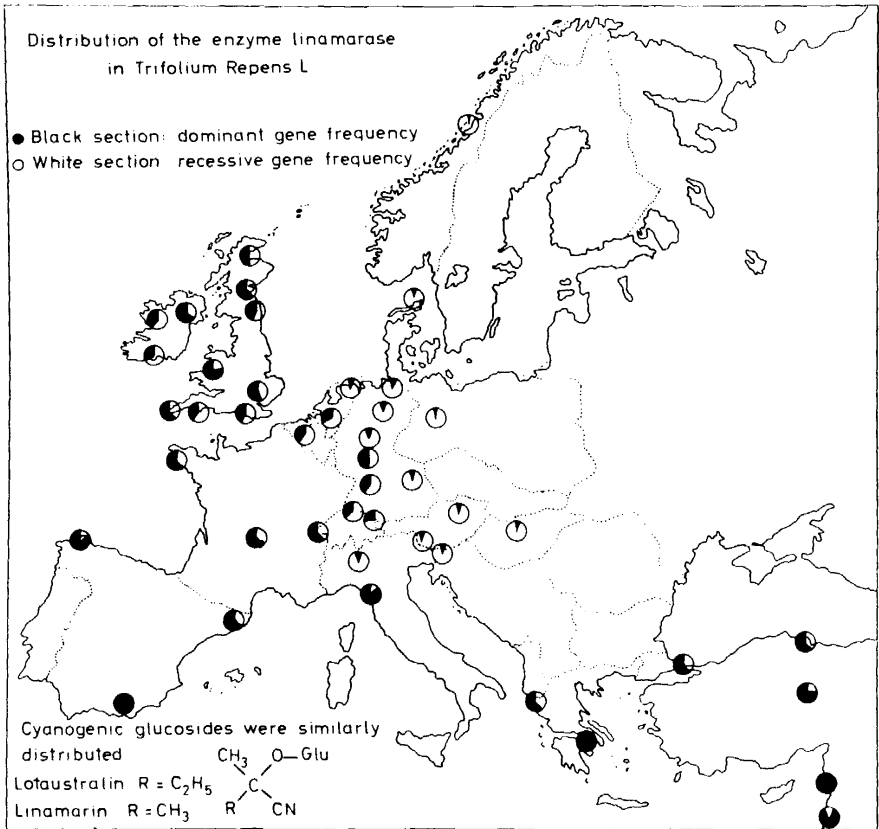
Figure 4. Distribution and frequency of the enzyme linamarase in European and Near Eastern wild populations of *Trifolium repens* L. (adapted from Daday)[12].

## Distribution of lupine alkaloids in the Leguminosae

Although variation for all types of secondary compounds is widespread, it has been our experience that alkaloids tend to exhibit greater variation during development than do some other classes of natural products. If a plant contains a particular type of alkaloid, it will generally produce a dozen or so closely related compounds, and, although individual plants often show considerable qualitative and quantitative variation for some of these alkaloids, a large number of evolutionarily related species and genera usually produce some of the same compounds. Thus, these patterns form interspecific and intergeneric alkaloid races.

Some of the possible sources of variation in the alkaloid content of plants can be illustrated by lupine alkaloid studies in the genus *Baptisia*. This genus, which is a member of the family Leguminosae, consists of sixteen species that occur in the eastern half of North America. Some of the lupine alkaloids that have been detected in *Baptisia*[13] are cytisine, methylcytisine, anagyrine and its steric isomer thermopsine, baptifoline, sparteine, hydroxysparteine, and lupanine (*Figure 5*).

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In order to study the variation of lupine alkaloids during development on a plant-to-plant and population-to-population basis for one *Baptisia* species, the alkaloids of ten separate plants from two physiologically-distinct (based on flowering time, etc.)[3] populations of *Baptisia leucophaea* were sampled at three different stages of development. The results indicated that plants from the two Texas populations which are 120 miles apart were similar in their alkaloid chemistry at the same stage of development, although individual plants at a given developmental stage exhibited considerable quantitative variation.[14, 15] Thus, in this instance, physiological differences between the two populations did not produce any significant differences in the alkaloid content of the plants. In contrast, striking alkaloid variation occurred during development: as the plants matured, the relative amount of cytisine decreased markedly while the relative amount of anagyrine increased, and in most instances, became the dominant alkaloid. Moreover, the percentage of total alkaloid per gram of dry plant material decreased markedly as the plant developed.
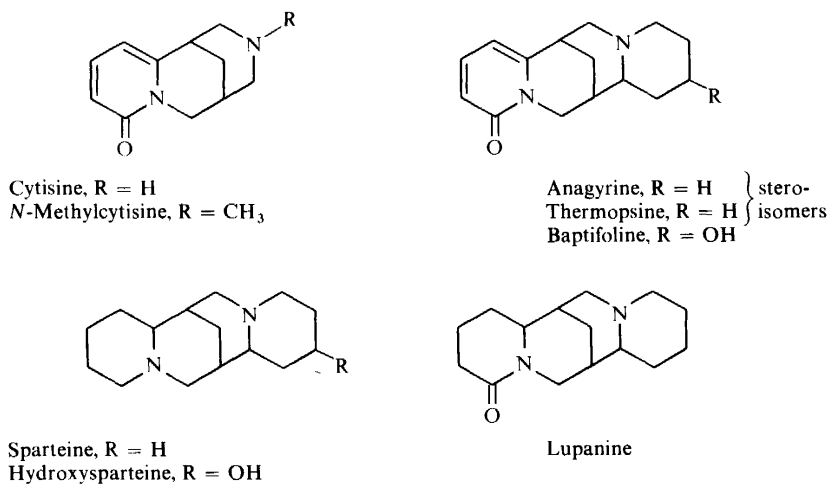


Cytisine, R = H
N-Methylcytisine, R = CH₃

Anagyrine, R = H       } stero-
Thermopsine, R = H     } isomers
Baptifoline, R = OH

Sparteine, R = H
Hydroxysparteine, R = OH

Lupanine

*Figure 5.* Some of the major lupine alkaloids detected in the legume genus *Baptisia*.

Because of these variations during development and because most species of the genus *Baptisia* produce quite similar compounds, the lupine alkaloids were less useful in systematic studies at the specific level than were other secondary compounds[15]. On the other hand, the distribution of these alkaloids does support relationships above the generic level; they occur in a large number of genera and tribes of the Leguminosae which are generally thought to be closely related on other grounds. These latter distributions give rise to intergeneric and intertribal lupine alkaloid races (*Figure 6, Table 1*)[15].

Although the presence of lupine alkaloids in these legume genera is probably associated with their value as defensive compounds against various predators, the particular patterns of minor variations may be associated with the
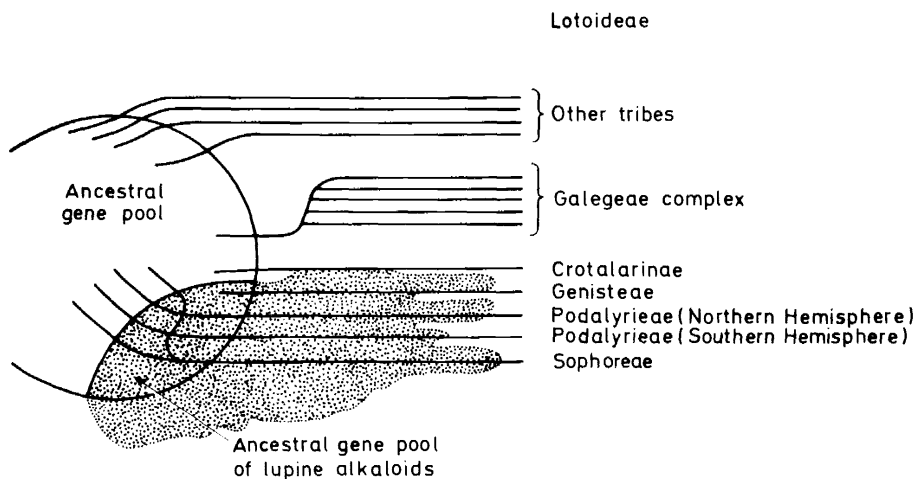
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Lotoideae



*Figure 6.* Tribes of the Leguminosae subfamily Lotoideae are arranged according to possible phyletic affinities; those containing lupine alkaloids form an inter-tribal chemical race.[15]

phenomenon of ecotypic variation involving physiologically different organisms adapted for different ecosystems. Of course, in some instance the particular chemical patterns may have been selected for improved defensive systems.

## IV. FLAVONOIDS AND OTHER PHENOLICS

The occurrence of phenolic and flavonoid chemical races is well known and numerous examples have been summarized elsewhere, therefore only a few examples are discussed here; for others, see Alston[3] (which includes an account of much of the pioneering work with the legume genus *Baptisia*) and Levin, 1971[16]:

One of the most interesting examples of intraspecific variation occurs in *Hymenoxys*[17]. Over 30 flavonoids are present in *H. scaposa, H. acaulis,* and *H. iresiana.* The patterns of distributions of these compounds are correlated more strongly with population positions along an east west gradient extending from Arizona to Texas than with the diagnostic exomorphic features of the species. The biochemical parallelism observed for populations of different species in the same region suggests that the species are engaging similar selective forces. A somewhat similar parallelism has been described in *Eucalyptus*[13]. Several species display a strong association between stilbene concentration and habitat aridity.

East-west differences have also been observed in *Pyrus*[19]. The flavones luteolin and apigenin occur in the leaves of the eastern Asian species, whereas the flavonols kaempferol and quercetin occur in the western Asian and European species. Similarly, Henke[20] found that in *Malus* some flavonoids present in most of the eastern Asian species were absent from those in western Asia,

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*Table 1.* Distribution of lupine alkaloids[a] among legume genera[14,15].

| Genus | Cytisine | Methylcytisine | Anagyrine | Sparteine | Lupanine | Baptifoline | Hydroxysparteine | Thermopsine | No. of other alkaloids reported |
|---|---|---|---|---|---|---|---|---|---|
| **Podalyrieae** | | | | | | | | | |
| *Baptisia* | ++ | ++ | ++ | + | + | + | + | + | 1–2 |
| *Thermopsis* | ++ | ++ | ++ | ++ | + | | | + | 5 |
| *Anagyris* | ++ | + | ++ | ++ | | | | | 0 |
| *Piptanthus* | ++ | | | + | | | | | 2 |
| *Podalyria* | | | | | ++ | | | | 0 |
| **Genisteae** | | | | | | | | | |
| *Genista* | ++ | ++ | ++ | ++ | + | | ++ | | 2–4 |
| *Retama* | + | + | ++ | ++ | + | + | ++ | | 3–4 |
| *Cytisus* | + | ++ | + | ++ | ++ | | | | 4 |
| *Ulex* | ++ | | ++ | + | | | | | 0 |
| *Spartium* | | | + | ++ | | | | | 0 |
| *Sarothamnus* | | + | | ++ | + | | | | 1 |
| *Lupinus* | | | + | ++ | ++ | | | + | 9 |
| *Hovea* | | | | ++ | | | | | 0 |
| *Templetonia* | + | | | ++ | | | | | 0 |
| *Adenocarpus* | | | | | | | | | 0 |
| *Laburnum* | ++ | | | | | | | | 0 |
| **Sophoreae** | | | | | | | | | |
| *Sophora* | + | + | + | + | | + | | | 6 |
| *Ammodendron* | | | + | ++ | | | | | 1 |
| *Ammothamnus* | | | | ++ | | | | | 2 |
| *Virgilia* | | | | | + | | | | 2 |
| *Ormosia* | | | | + | | | | | 3–4 |

[a] If the alkaloid is a major component for the genus as a whole, it is indicated by + + notations; minor alkaloids are denoted by +.

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Europe, and North America. In addition, the North American species contained two flavonoids absent from the other species. *Eucryphia* provides another instance of geographical discontinuity and flavonoid disparity[21]. The two South American species have azaleatin and caryatin; these are lacking in the three Australian species which contain few flavonoids[16].

## Stability of flavonoid patterns in *Spirodela* and *Prosopis* species

The studies of McClure and Alston[22] for *Spirodela oligorhiza* (Lemnaceae) illustrate that, for the most part, flavonoid patterns are under genetic rather than environmental control. *Spirodela oligorhiza*, one of the smallest and morphologically simplest flowering plants, is rich in flavonoids, containing at least 15 different compounds. It was found that the basic flavonoid patterns were essentially unaltered qualitatively when clonal material of *Spirodela oligorhiza* was cultured using more than 50 different sterile media which were varied in nutrient salts, plant hormones, metabolic inhibitors, sucrose, and light and temperature conditions[22]. Clearly secondary chemistry can show remarkable stability regardless of environmental factors when the same genetic stock is involved.

*Table 2.* Flavonoids known from various species of *Prosopis*:



| $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|-------|-------|-------|-------|
| OH | O-glucosyl | H | H |
| OH | O-rhamnosyl | H | H |
| OH | O-arabinosyl | H | H |
| OH | O-rhamnoglucosyl | H | H |
| H | H | C-glycosyl | H |
| H | H | H | C-glycosyl |
| OCH$_3$ | O-glucosyl | H | H |
| OH | OH | H | H |
| OH | OCH$_3$ | H | H |
| OCH$_3$ | H | H | H |
| OCH$_3$ | OCH$_3$ | H | H |

Flavonoid investigations from our laboratory (unpublished data of Neil Carman) of various *Prosopis* species (Leguminosae) which occur in North and South America further document the stability of flavonoid patterns in geographically isolated populations.

*Prosopis alba* (Argentina) and *P. chilensis* (Chile) are essentially identical in their flavonoid chemistry (*Figure 7*) which suggests that they were derived

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from the same ancestral stock involving few mutations; the taxa may represent populations of the same ancestral species which are now geographically isolated by the Andean mountain chain.

Similarly, the two disjunct varieties of *P. reptans* also have nearly identical flavonoid patterns (*Figure 8*). These data are especially noteworthy since one variety (*reptans*) occurs in South America while the North American one, *cinerascens*, is separated from it by thousands of miles. The history of these two populations of *P. reptans* is not known; they may represent introductions from South to North America by long-distance dispersal mechanisms. Nevertheless, it is of considerable interest that the same chemical race of
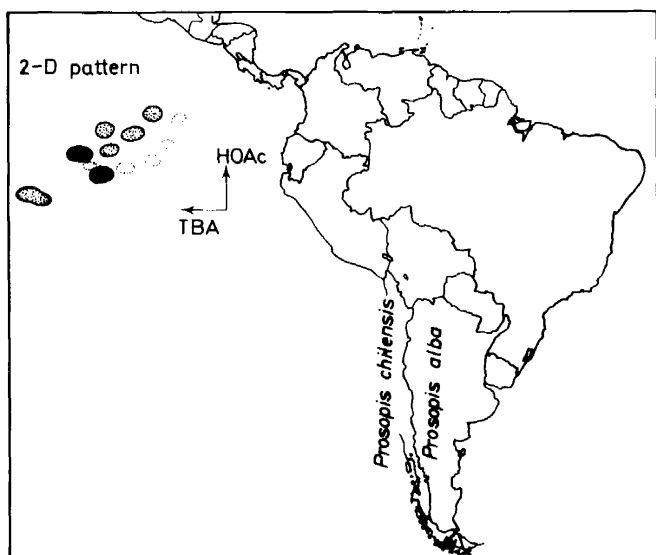


*Figure 7.* Identical flavonoid patterns are observed for the two geographically isolated South American *Prosopis* species, *P. alba* (Argentina) and *P. chilensis* (Chile). (Structures for some of the compounds are given in *Table 2*.)

*P. reptans* continues to persist in the geographically isolated populations occurring in similar ecological environments. These *Prosopis* examples suggest that ecotypic variation may not occur if the disjunct populations occupy ecosystems which do not require a physiologically different organism.

**Phenolic Races in** *Pityrogramma triangularis*

Perhaps no other large group of plants has received as little chemical attention as have the ferns. Nevertheless, the available data[23, 24] do indicate that secondary chemistry can be a valuable aid in their taxonomic treatments.

Possibly the most complex example of chemical races in ferns occurs in the North American *Pityrogramma triangularis* group which extends from British Columbia south to Baja California and eastward into Arizona and
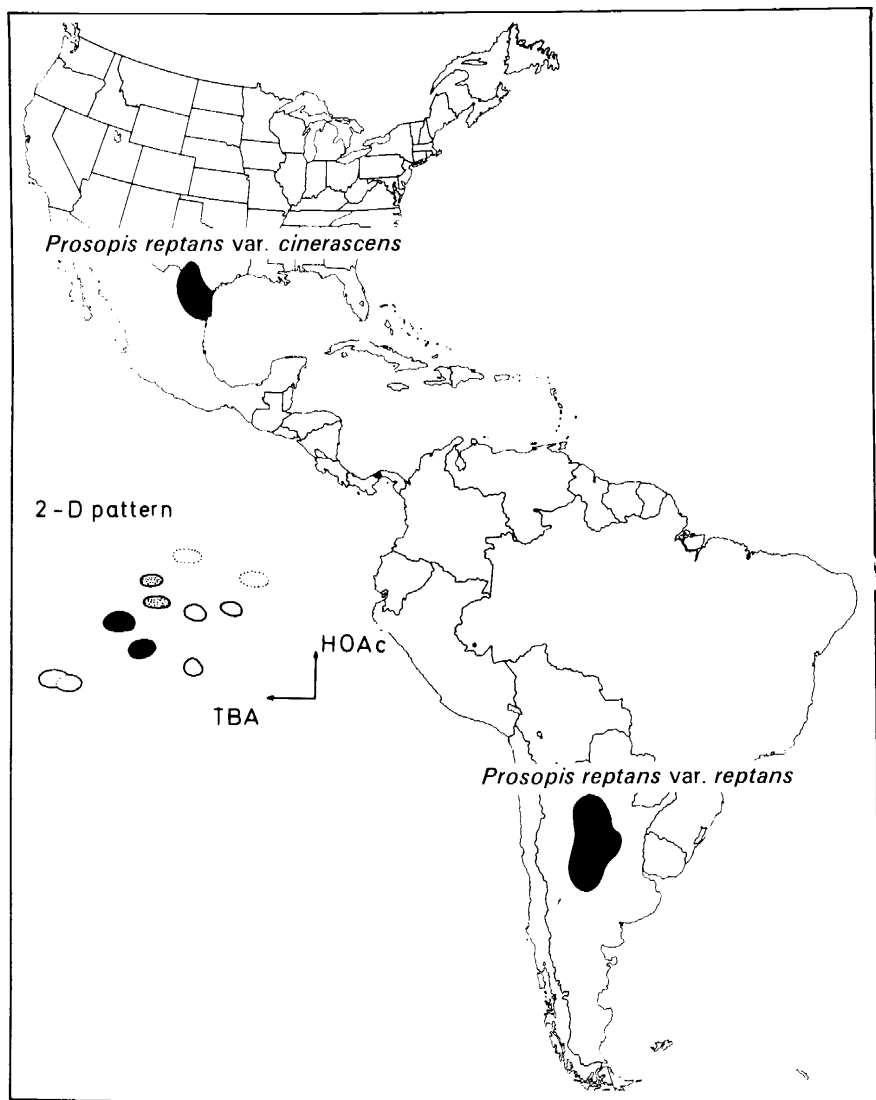
*Figure 8.* The two disjunct populations of *Prosopis reptans* in North and South America exhibit nearly identical flavonoid patterns. (Structures for some of the compounds are given in *Table 2.*)

New Mexico[25]. The lower surfaces (and only rarely the upper surfaces) of the fronds of these ferns are covered with a yellow or white waxy powder thus giving them the gold- and silver-backed appearances which are often used by taxonomists for recognition purposes. However, it is now known that totally different exudate pigments may be responsible for similar yellow coloration (e.g., ceroptin (VI) mixed with pityrogrammin (VII) or kaempferol and kaempferol methyl ethers (VIII and IX)) (*Figure 9*)[24, 26].
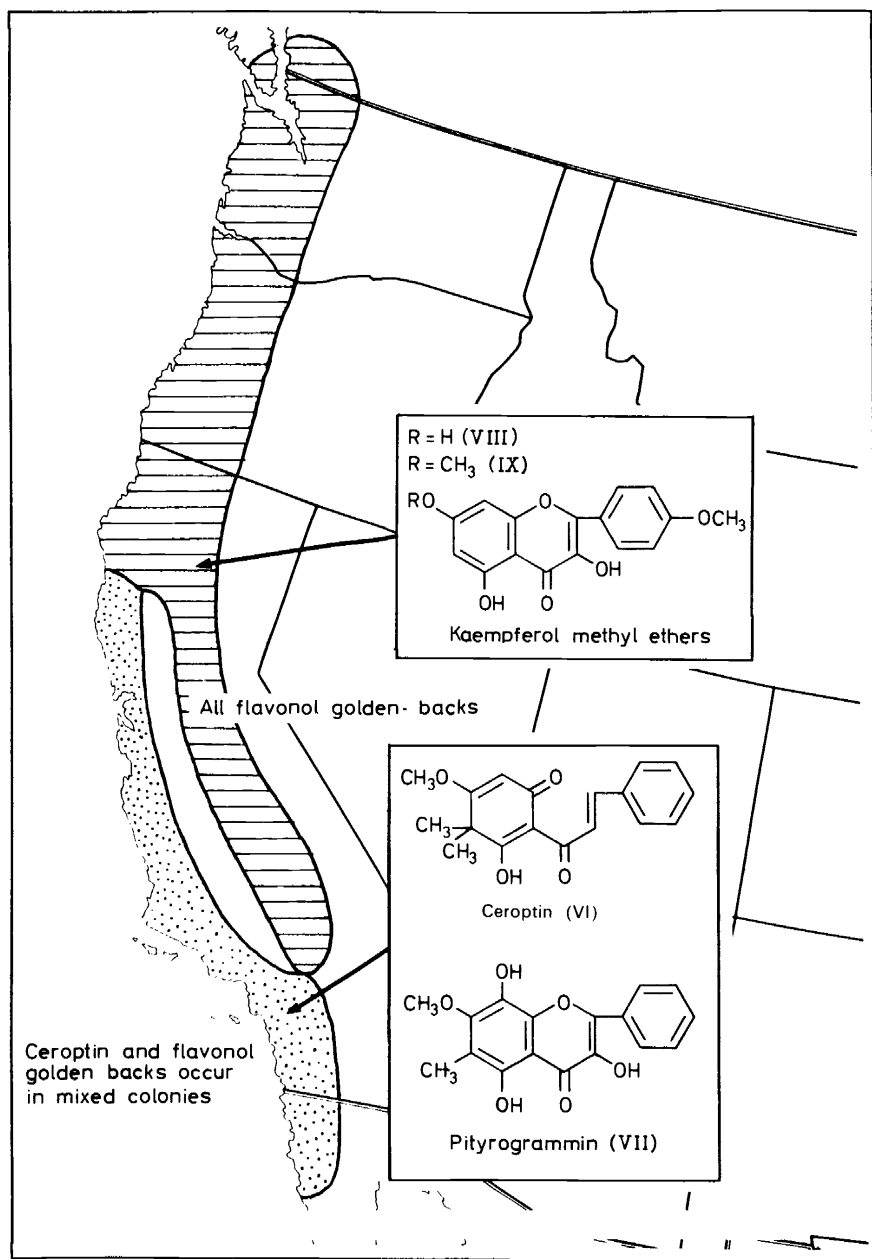
*Figure 9.* Distribution of two major chemical types of fronds exudates present in the *P. triangularis* va. *triangularis* group (from D. Smith, private communication).

Ceroptin occurs in nearly pure form on some plants while kaempferol and its methyl ethers are found in almost pure form on others. (Some white or silver backed *Pityrogramma* species exude dihydrochalcones). Some *P. triangularis* plants have similar morphology and internal flavonoids while differing only in these exudate substances; also the exudates may be similar when the internal flavonoids are different (Dale Smith, private communication).

Most workers had considered *Pityrogramma triangularis* to be a single polytypic species until 1960 when Alt and Grant[25] suggested that some of the *P. triangularis* populations which had from time to time been treated as varieties (e.g., *pallida* and *viscosa*) might well be recognized as species. In their opinion, the remaining more narrowly defined *P. triangularis* var. *triangularis* consists of at least four sympatric populations, a tetraploid ($n = 60$), two morphologically distinguishable diploids (A and B) ($n = 30$), and a triploid hybrid; they recognized three sibling species, namely, the diploids of morphological types A and B and the tetraploid. In the most recent attempt to sort out the relationship of the chemical and ploidy levels in this same *P. triangularis* var. *triangularis* complex, Smith *et al.*[24] studied the cytology and chemistry of three populations from Santa Barbara County, California. These workers also detected the three ploidy levels mentioned above (diploids, triploids and tetraploids) along with four exudate pigment chemotypes. To add to the complexity of the situation, ten of the twelve possible combinations of ploidy level and pigment types were detected in the Santa Barbara area alone.

Smith *et al.*[24] (as have most others)[27] felt that the available data still do not fully explain the complexity of the chemical and cytological variation observed in *P. triangularis.* Two alternative interpretations have been discussed, namely: (a) *P. triangularis* is one species but exists as an auto-polyploid complex and that the named and unnamed varieties are simple genetic variants which share the same basic genome. In some populations autotetraploids may have crossed with their diploid progenitors, producing sterile triploid hybrids. (b) A second interpretation is that *P. triangularis* contains more than one genome and exists as an allopolyploid complex; in this case the populations would consist of an array of several distinct biological species, rather than varieties.

### Flavonoid patterns for *Empetrum rubrum*

Moore *et al*[28] compared flavonoid patterns, fruit colours and breeding systems for 90 populations of plants belonging to the three genera of the Family Empetraceae.

Here we comment only on the leaf flavonoids of one of the species, *E. rubrum*; this species exhibited patterns which could be correlated with geographical distribution and habitat differences. The data for 18 samples which represent the range of *E. rubrum* (see *Figure 10*) indicate that the northern Chilean populations (populations 1 and 2, *Figure 10*) contain a flavonoid typical of North American taxa not found in the more Southern populations of *E. rubrum*, thus suggesting either amphitropical gene exchange or loss of synthetic ability as the species migrated southwards. A second flavonoid, which is not found in these northern populations of *E. rubrum*, is

also absent from a population which occurs 3200 km away on the Gough Island near the Tristan da Cunha group in the South Atlantic Ocean. These data suggest that this latter population may have resulted by long distance dispersal from the northern Chilean populations. Of course, the absence of this particular compound (characteristic of most southern populations of *E. rubrum*) may only indicate the loss of synthetic ability in this small, isolated
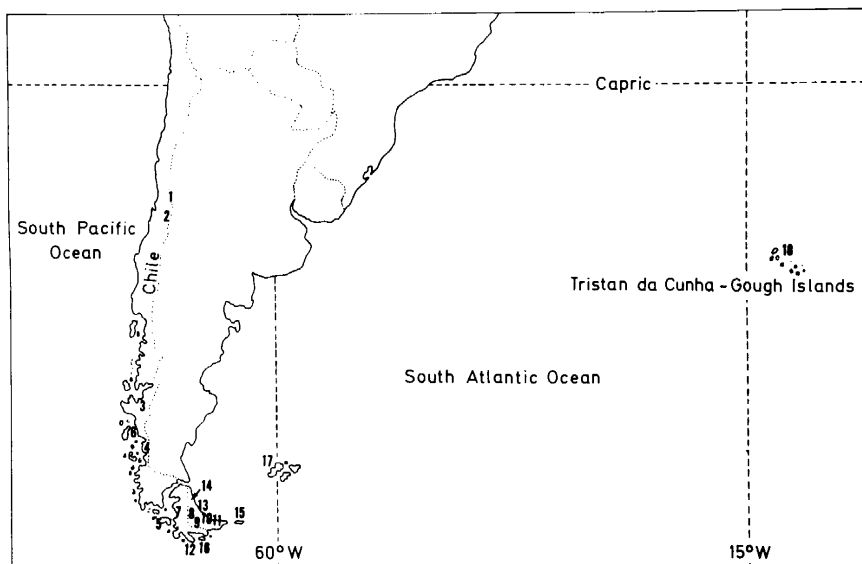


*Figure 10.* Locations of samples used in flavonoid survey of *Empetrum rubrum*.

island population. However, it is interesting to note that these isolated island populations also lack other flavonoids usually found in the Chilean populations of *E. rubrum*. In this connection one of my colleagues, Professor Don Levin, suggested that the latter phenomenon (i.e., fewer phenolics in island populations of a mainland taxon) might be typical for island populations well separated from those on the mainland because of a decrease in predators which in turn would reduce the need for defensive compounds. Alternatively, the island habitat may have required a physiologically different organism and this biochemically different organism secondarily produces fewer phenolic compounds.

## V. SESQUITERPENE LACTONES IN *AMBROSIA CHAMISSONIS*

Infraspecific variation of sesquiterpene lactones in *Ambrosia*[29–31] and other genera of the Compositae is a well established phenomenon. For example, complex chemical patterns have also been reported for such species
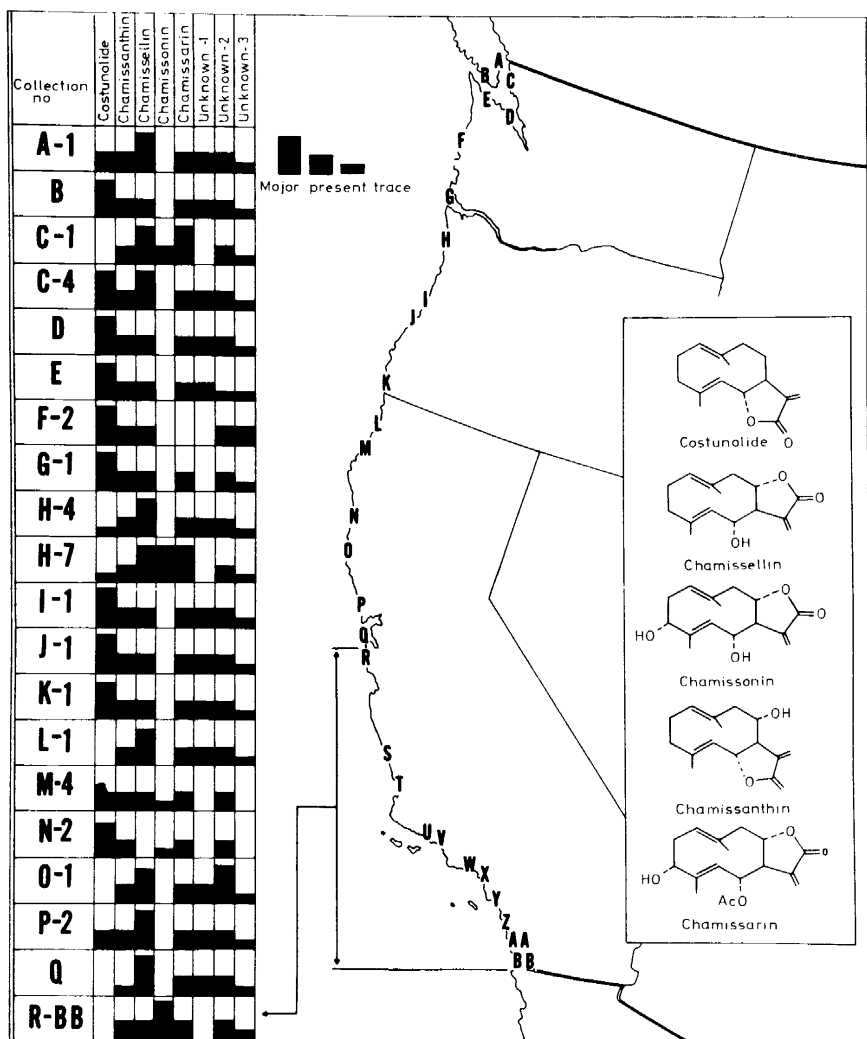
393

*Figure 11.* Sesquiterpene lactones from selected North American populations of *Ambrosia chamissonis*.[40] (The collection no. column indicates the location by letter while the sample (if more than one was collected) from that site is designated by a number; these numbers correspond to samples reported in reference 40.)

as *Gaillardia pulchella* Foug[32-34], *Helenium autumnale* L.[35] and *Cynara scolymus* L.[36].

Here we discuss in detail only the pattern of sesquiterpene lactones in populations of *Ambrosia chamissonis* in order to illustrate the way an organism with an appropriate ecological amplitude can rapidly colonize a new area.
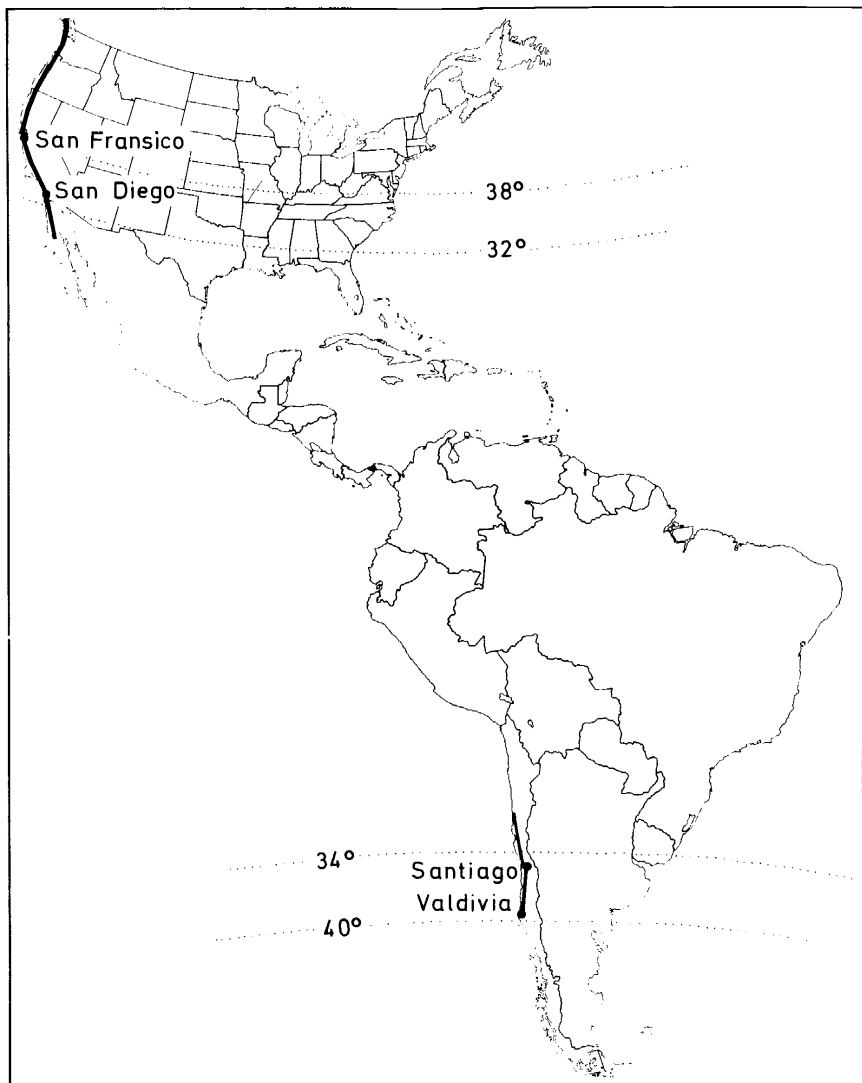
*Figure 12.* The distribution of *Ambrosia chamissonis* in North and South America.

Most *Ambrosia* species[29-31] are rich in sesquiterpene lactones and *Ambrosia chamissonis* (Less) Greene is no exception producing several structurally-related germacranolides (see *Figure 11*)[37-40]. Because *A. chamissonis* has long been renowned for its variable leaf morphology it was of interest to determine whether or not the sesquiterpene lactone chemistry correlated with the variable exomorphic features.

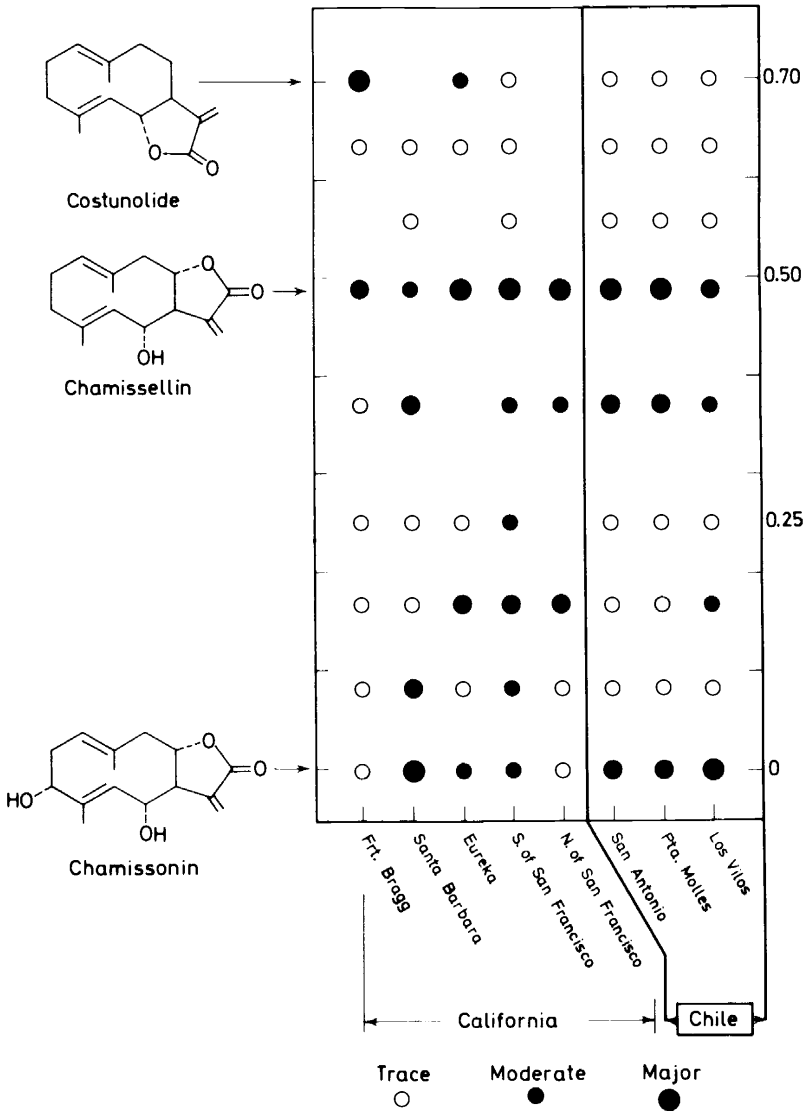*Ambrosia chamissonis* is endemic to the Pacific coast of North America

Figure 13. Data for the sesquiterpene lactones in populations of *Ambrosia chamissonis* in Chile and California indicating a consistent chemistry for all collections.

from British Columbia to Baja California and in the last 100 years it has also colonized much of the coastal region of Chile (*Figure 12*)[41, 42].

Nearly 100 North American populations of *A. chamissonis* were recently scrutinized for their morphological and chemical properties[39, 40]. Payne *et al.* recognized five leaf patterns; two unlobed 'chamissonis' forms, two pinnately dissected 'bipinnatisecta' forms and an intermediate form. In

general, their studies indicated that there is little correlation between chemistry and leaf morphology in the populations north of San Francisco.

In contrast, the populations of *A. chamissonis* which occur in a region beginning about 75 miles south of San Francisco and extending into Baja California did show some chemical and morphological uniformity. It was suggested that this uniformity in the southern California populations might have resulted from genetic drift and with the operation of the 'founder principle'[43] and 'that the populations now found south of Monterrey are developed from limited introductions from the north'. In this connection it was pointed out that for miles south of San Francisco the coastal region contains mostly steep, rugged cliffs; thus *A. chamissonis* may have reached southern California only in more recent times.

Some of our data (unpublished) for the Chilean populations of *A. chamissonis* are of interest in connection with this 'founder principle' concept for the origin of the southern California populations.

During 1970 and 1972, *A. chamissonis* was collected from throughout the several hundred mile coastal region it presently occupies in Chile; these collections exhibited a consistent sesquiterpene lactone chemistry (*Figure 13*) and leaf morphology and typify the way a weedy species can rapidly colonize a new habitat within a 100 years or so and spread one genetic line over a large region. (My colleague Calvin McMillan recently pointed out to me that the cockleburs (*Xanthium*) are presently colonizing many disturbed parts of the world, including major areas of such countries as India and Australia; the sesquiterpene lactone patterns of these introduced cockleburs are always in accord with the chemistry of their source.)

The chemistry and leaf morphology (bipinnatisecta-form) for all the Chilean populations of *A. chamissonis* were similar to those for populations from near San Francisco suggesting that these may have been the source of the genetic line introduced into Chile.

It is of interest to note that the regions occupied by *Ambrosia chamissonis* in North America (between approximately 30° and 50° north latitude) and in South America (between approximately 30° and 45° south latitude) represent the Mediterranean climate regions†in the two continents; thus the introduced genetic line in Chile could rapidly occupy much of the coast of Chile because it had the appropriate physiological amplitude for a Mediterranean climate. Since selection of a physiological different race was not required, the natural products chemistry remained unchanged.

## VI. VOLATILE TERPENE RACES

Other presentations at the Symposium including those of Zavarin and von Rudloff emphasize volatile terpene patterns in higher plants and readers are referred to their papers for detailed comments about this aspect of the chemistry of geographical races. Here we mention only one typical study

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† Mediterranean climate has these characteristics (H. Aschmann, private commun.) 1. More than 65% of the rainfall occurs in winter. 2. Precipitation is moderate (about 300 mm. to 1200 mm.) although the actual amounts vary according to temperatures. 3. The winters are mild (less than 3% of the hours per year are below freezing).

which indicates the way hundreds of plants may be sampled for these compounds utilizing GLC. Zavarin and Snajberk[44] determined the geographical variability of monoterpenes in two North American firs, *Abies balsamea* and *A. fraseri*. The former ranges from central Alberta to Labrador and Newfoundland in Canada and across the New England States into Minnesota in the United States while *A. fraseri* occurs in a much smaller region in the Appalachian mountains mostly at high elevations (4000 to 6000 ft.). Data for the cortical monoterpenes from nearly 300 trees and from 29 locations indicate a long-time separation of the western and eastern *A. balsamea* in the Rocky and Appalachian Mountains with *A. fraseri* evolving from the eastern *A. balsamea* by gene loss. The chemical patterns for the present populations of *Abies balsamea* and *A. fraseri* were correlated with what is known about the way these populations were affected by periods of glaciation.

## VII. CONCLUDING STATEMENT

As mentioned earlier, infra- and inter-specific chemical races are almost always based upon particular structural themes (which can be used as taxonomic characters for closely related taxa); the variations that do occur are usually based upon the number of compounds, the extent of oxygenation, methylation, acetylation, glycosylation and in such modifications as rearrangements and cyclizations. Natural products are known to be useful in defensive roles against microorganisms, fungi, and herbivores; as attractants for pollinators; as controls over plant population densities, and as plant hormones; yet, the data here and elsewhere suggest that of the minor chemical diversity observed for different populations of a given taxon (or closely-related taxa) results from the selection of a physiologically-distinct population with an ecological amplitude suitable for its habitat. Thus, during the course of the evolution of flowering plants geologic and climatic changes have produced regions which were colonized by mutants which met the physiological tolerances of the newly formed ecosystems. The members of these physiologically-distinct populations necessarily have some altered biochemical pathways which secondarily produce minor variations in the structural themes associated with that taxon, thereby producing many of today's chemical races. When the populations occur in ecologically similar ecosystems, there may be little or no selection for a physiologically modified organism and in these instances there may be few if any chemical changes.

Although the population must have appropriate physiological tolerances to survive in the ecosystem, its ultimate success may depend in part upon whether or not the modified secondary chemistry continues to function as defensive compounds, hormones, etc. in the same fashion as did the ancestral structures. Of course, in many instances the slightly modified chemical patterns may have been directly selected because the new chemistry represented an improved defensive system for the plant. If the effectiveness of the compounds against predators were increased, smaller amounts of the substances would be required; therefore, less of the available energy of the plant would be needed for a defensive system. Thus, a complete understanding

of the chemistry of geographical races will require detailed quantitative data to supplement the qualitative information.

It seems appropriate to close this account of the chemistry of geographical races by emphasizing that the phenomenon of chemical races permeates most if not all plant species and, although we are only now beginning to unravel the mechanisms whereby biochemical abilities are altered during the course of evolution of a taxon, it is clear that the occurrence of chemical races reflects much of the evolutionary process.

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