To the Graduate Council:

I am submitting herewith a thesis written by Loren Rieseberg entitled "Floral Ultraviolet Patterns and Flavonoid Pigments for Six Taxa of Viguiera series Viguiera." I have examined this final copy of the thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree if Master of Science, with a major in Botany.

Edward E. Schilling, Major Professor

We have read this thesis
and recommend its acceptance:

David K. Smith

Accepted for the Council:

The Graduate School

FLORAL ULTRAVIOLET PATTERNS AND FLAVONOID PIGMENTS FOR SIX TAXA OF *VIGUIERA* SERIES *VIGUIERA*

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Loren Rieseberg

June 1984

DEDICATION

This work is dedicated

to my mother

Marilyn Morgan

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The author would like to thank the following people for their assistance and encouragement during this study:

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ABSTRACT

Floral ultraviolet (UV) patterns and the distribution of flavonoid pigments were studied in relation to ligule anatomy for six taxa of Viguiera series Viguiera. This investigation used data obtained from UV photography, flavonoid chemistry, scanning electron microscopy and light microscopy.

UV photographs showed three distinct UV absorption-reflection patterns to be present on the ray florets of this series. Ligules for three taxa, V. deltoidea var. deltoidea, V. deltoidea var. parishii, and V. dentata had UV-absorbance restricted to the proximal portion of the ligule while the distal region was UV-reflecting. The ligules of two taxa, V. laciniata and V. reticulata, were completely UV-absorbing. The ligules of V. potosina were unique for this group, being entirely UV-reflecting, suggesting that V. potosina may not belong in this series.

Four general classes of compounds were found to be present in the ray flowers of Viguiera: flavonoid aglycones, flavone glycosides, flavonol glycosides, and anthochlor pigments. The glycosides and anthochlors are responsible for UV "nectar guide" formation. Variation for floral flavonoids divided this series into two groups. One group consisting of V. deltoidea, V. laciniata, and V. reticulata was characterized by the presence of quercetin 3-O-methyl ether compounds, the chalcone/aurone pair, coreopsin/sulfurein, and the presence of flavonoid aglycones. The other group, composed of V. potosina and V.

dentata, lacked quercetin 3-O-methyl ether marker compounds and flavonoid aglycones. In addition, the ligules of V. dentata contained two aurone/chalcone pairs, while the ligules of V. potosina lacked anthochlors. Variation for flavonol glycosides was also detected for V. deltoidea, suggesting that the taxonomy of this species needs to be re-evaluated.

Investigation of flavonoid distribution in relation to the functional anatomy of ligules disclosed the presence of adaptive features responsible for UV-patterning in Viguiera. Flavonoid glycosides and anthochlor pigments were confined to papillate epidermal cells occurring in the solution of the vacuole. Flavonoid aglycones, when present, were localized in glandular trichomes on the abaxial ligule surface and did not contribute to UV patterning. SEM confirmed the presence of glandular trichomes and papillate cells on the ligule epidermis and disclosed other epidermal features associated with the absorption and reflection of light. Structural aspects of the ligule, in conjunction with floral flavonoid distribution, play an important role in the formation of UV "nectar guides" in Viguiera. It is discussed that UV-absorbance patterns in Viguiera may be important for reducing UV-B radiation to the reproductive organs, and may also serve as recognition cues for flower-constant pollinating insects, thus reducing cross-pollination between visibly similar species.

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CHAPTER I

INTRODUCTION

The genus Viguiera contains a diverse array of about 150 species, with relationships extending to such well-known genera as Helianthus and Tithonia. Members of this genus range from Nevada to Argentina, with large aggregates of species in Mexico and northern South America. The last taxonomic revision of Viguiera (Blake, 1918) was based on morphological data. Since that time, Vigiuera has received little attention, and the cytology and phytochemistry of this genus are poorly understood. Because of a lack of distinctive morphological characters to delimit species and genera in the Asteraceae, the affinities of Viguiera with other genera remain obscure, and its species ill-defined.

The present project was an investigation of six of the thirteen taxa in series Viguiera (series Dentatae Blake). The distribution of these taxa is shown in Figure I-1. Viguiera dentata is widespread, ranging from Southern Texas to Southern Mexico, Viguiera potosina is endemic to San Louis Potosi State in Central Mexico, and V. reticulata occurs in the Mojave Desert. The other three taxa are restricted to the Upper Sonoran Desert of Baja California and Southern California.

Because Blake's revision of Viguiera was based primarily on floral characters, some members have been incorrectly placed in this series. Specifically, V. dentata and V. potosina have distinctive chromosome numbers of n=17 in contrast to n=18 reported for the majority of taxa in this series. In addition, each has a unique complement of floral

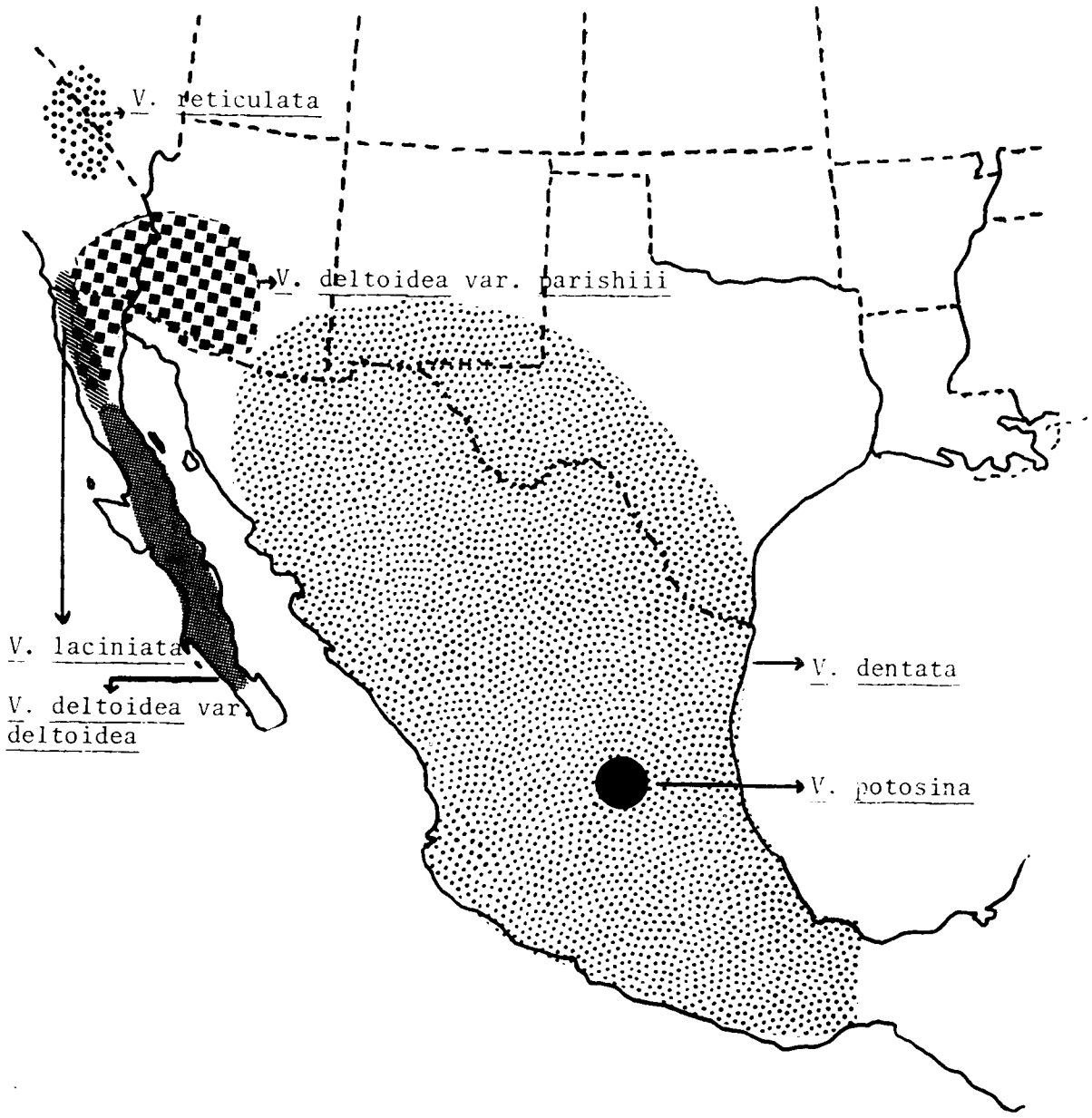


Figure I-1. Generalized Geographical Distribution of Six Taxa of Viguiera series Viguiera.

flavonoids, and V. potosina lacks the floral anthochlors responsible for UV-absorption that are characteristic of the other members of this series.

This study focused upon two objectives. The first was to make a systematic study of floral UV patterns in six taxa of Viguiera series Viguiera using UV photography and comparative floral flavonoid analysis. When floral UV patterns are integrated with visible coloration, the spectral patterns formed may be of possible orientation value to pollinating insects. In addition, interspecific polymorphism for UV patterns found in this series, as well as variation in floral flavonoid compounds, provides taxonomically significant data for this group. These data were used to assess species relationships in this series. For example, the species with chromosome numbers of n=17, V. dentata and V. potosina, differed with regard to floral flavonoids when compared to those species with chromosome numbers of n=18. Also, variation in UV patterns and floral flavonoids has been found for varieties of V. deltoidea, described by Blake (1918) as the most variable species in Viguiera.

The second objective was to analyze the distribution of flavonoid pigments in order to speculate on the functional anatomy of ligules. Flavonoid distribution was determined by microscopic examination of fresh ligule sections. Scanning electron micrographs disclosed the structural features of the ligule epidermis which are associated with UV-absorption. These facts contributed toward understanding the role of flavonoids in creating spectral patterns in Viguiera in addition to

clarifying the contribution of ligule epidermal features to ligule color and pollination ecology.

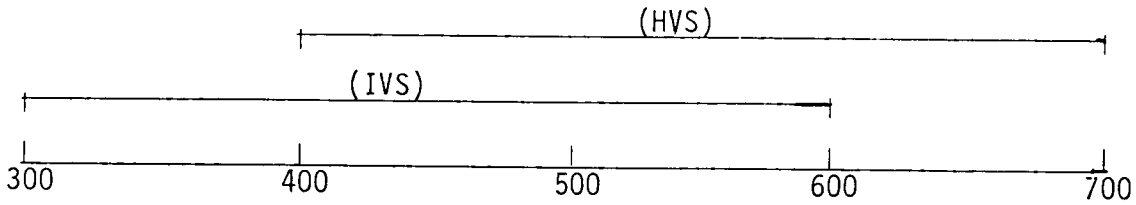
CHAPTER II

ULTRAVIOLET FLORAL PATTERNS

It has been widely established that the colors and patterns of insect-pollinated flowers have both visible and UV-components, and that insect pollinators are sensitive to both types of floral coloration (Figure II-1). Interest in the relationship between floral coloration and pollination biology was stimulated by Eisner, Silberglied, Aneshanseley, Carrel, and Howland (1969) who developed simple photographic methods, using a UV-sensitive television camera, for visualizing UV floral features. Later, Eisner, Eisner, Hyypio, Aneshanseley, and Silberglied (1973) described simple techniques for black and white photography of UV patterns using a single-lens reflex camera with a UV-transmitting filter. However, these methods considered only the UV component of floral coloration which was portrayed as dark (UV-absorptive) versus light (UV-reflective). These visualizations ignored the visible wavelengths that are also perceived by pollinators and contribute to the summary color pattern of the floral signal.

To clarify the role of floral colors in attracting insects, Kevan (1978, 1979) has designed a colorimetric scheme that defines floral color with respect to the daylight spectrum and the insect visual system (IVS). Although this scheme deals effectively with the trichromatic nature of insect vision, actually visualizing floral colors in the (IVS) entails some difficulties.

Recently, McCrea and Levy (1983) described a photographic method



(HVS) Human Visual System
(IVS) Insect Visual System

Figure II-1. Comparison of Insect and Human Visual Systems.

which integrated visible and UV wavelengths. This technique emphasized the importance of considering the entire spectrum of floral colors relevant to insect pollinators.

Visible nectar guides and their importance to pollination biology have been recognized for many years (Clements and Long, 1923; Levin and Kerster, 1967). However, the contribution of UV-absorbing/reflecting areas has only recently been studied.

Floral UV patterns may differ between flowers of different species or genotypes, and may be important adaptive and taxonomic characters. For example, Jones (1978) ostensibly demonstrated that pollinator fidelity to floral UV patterns could be effective as pre-pollination isolating mechanisms between closely related, sympatric species which differ only in UV nectar guide formation. Flint and Caldwell (1983) suggested that UV-absorption in the corolla may reduce UV-B radiation (280 to 300 nanometers) to the reproductive organs. Other studies concerning floral coloration have indicated the possible taxonomic significance of floral coloration patterns, especially the UV component (Eisner et al., 1973; Scogin 1976, 1978; Abrahamson and McCrea, 1977; Cunningham, 1983).

In the present study, the objective was to make a sytematic study of the floral coloration patterns in the six taxa of Viguiera series Viguiera with special emphasis on the UV component. Black and white and color UV photography were used to show the relationship between UV nectar guides and the summary floral signal seen by insect pollinators.

Materials and Methods

Floral UV patterns were investigated by viewing pressed herbarium specimens under UV light and documented with UV photography. Voucher specimens are listed in Table II-1.

Black and white photographs were taken using a 35 mm single-lens reflex camera equipped with a UV-transmitting filter (Kodak Wratten 18A) that effectively transmits light only between about 300 and 400 nanometers. Ultraviolet light was provided by two black-light flourescent lamps mounted at a distance ca. 50 centimeters (cm) from the subjects. Photographic images were recorded on Kodak Tri-X (ASA 400) film.

Color photographs were taken using the same camera without the UV filter. As outlined by McCrea and Levy (1983), black-light and daylight fluorescent lamps were used to provide near-UV and visible illumination, respectively, for photography. All fluorescent lamps were mounted at a distance ca. 50 cm from the subjects. The daylight lamps were filtered through blue and green cellophane to attenuate red illumination. Photographs were taken with Kodak Ektachrome (ASA 400) color film.

Results and Discussion

Photographs of the six taxa of *Viguiera* series *Viguiera* are displayed in Figures II-2 through II-5. Black and white photographs depicting only the UV component of floral coloration are shown in Figure II-2. Color photographs, which provide a means of visualizing the summary features of floral coloration as they appear to pollinating

Table II-1. Populations of *Viguiera* series *Viguiera* used for Photography

Species	Voucher[a]
V. deltoidea var. *deltoidea*	Mexico Baja California: Rieseberg 8A
V. deltoidea var. *parishii*	Cal. San Diego County: Rieseberg 3
V. laciniata	Mexico Baja California: Rieseberg 8A
V. reticulata	Cal. Inyo County: Rieseberg 25

[a]Specimens are deposited in The University of Tennessee Herbarium (Tenn).

Figure II-2. Black and white photographs of Pressed Flower Heads as seen under UV Light.

A. *V. deltoidea* var. *deltoidea*

B. *V. deltoidea* var. *parishii*

C. *V. dentata*

D. *V. laciniata*

E. *V. potosina*

F. *V. reticulata*

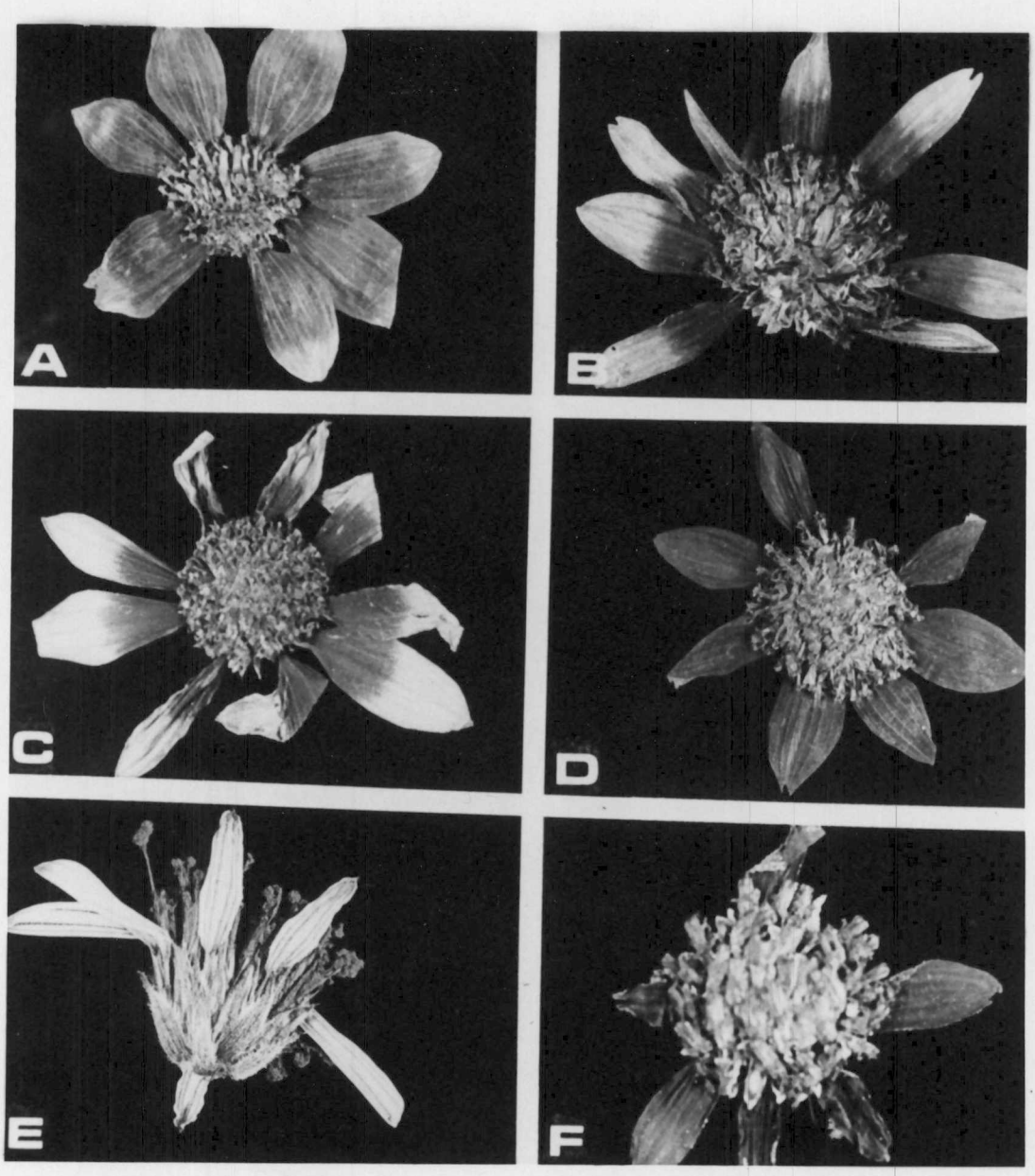


Figure II-2

Figure II-3. Color Photographs of Pressed Flower Heads as seen under UV and Visible Light.

A. *V. deltoidea* var. *deltoidea*

B. *V. deltoidea* var. *parishii*

C. *V. dentata*

D. *V. laciniata*

E. *V. potosina*

F. *V. reticulata*

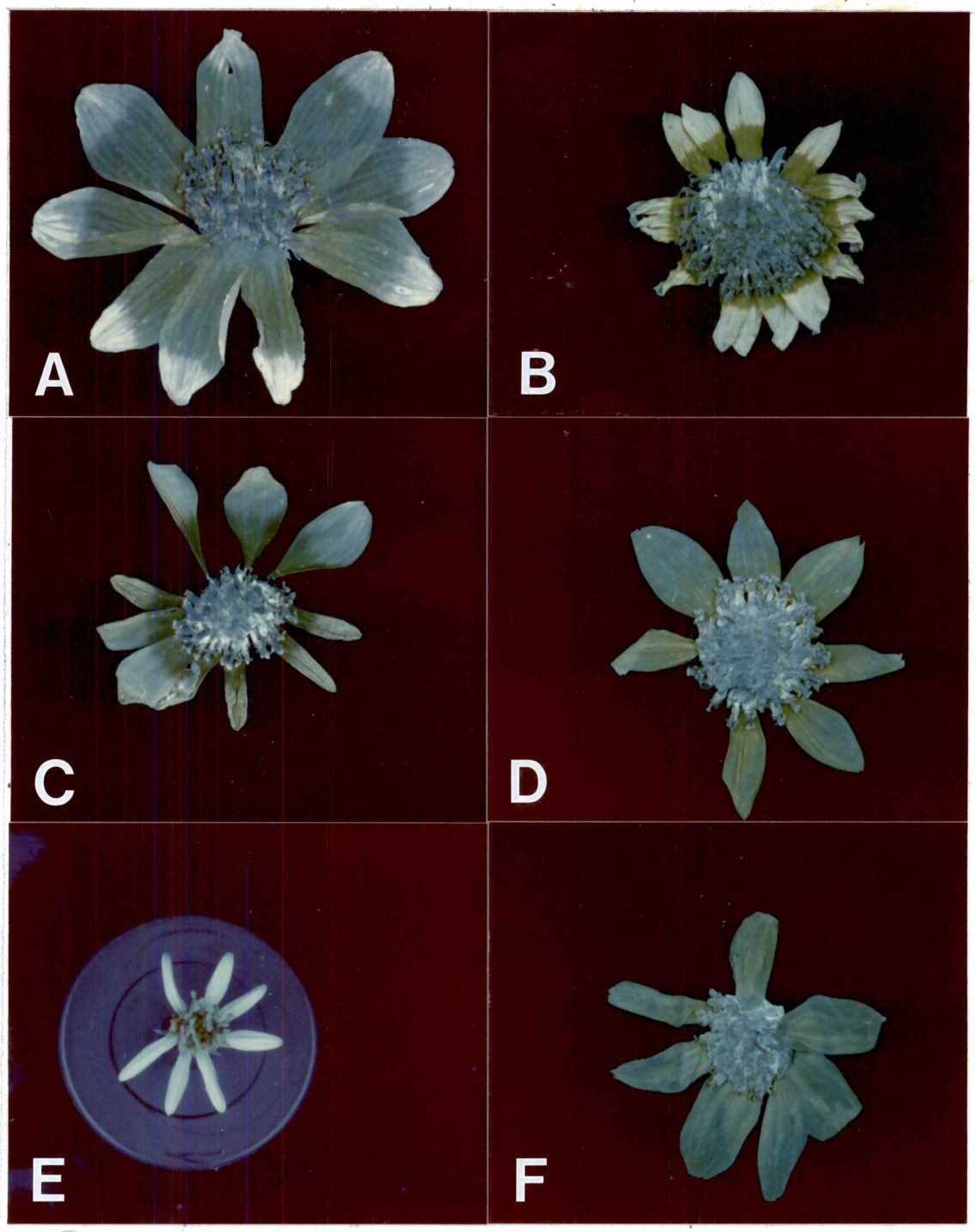


Figure II-3

Figure II-4. Color Photographs of Pressed Flower Heads as seen under Visible Light.

A. *V. deltoidea* var. *deltoidea*

B. *V. deltoidea* var. *parishii*

C. *V. dentata*

D. *V. laciniata*

E. *V. potosina*

F. *V. reticulata*

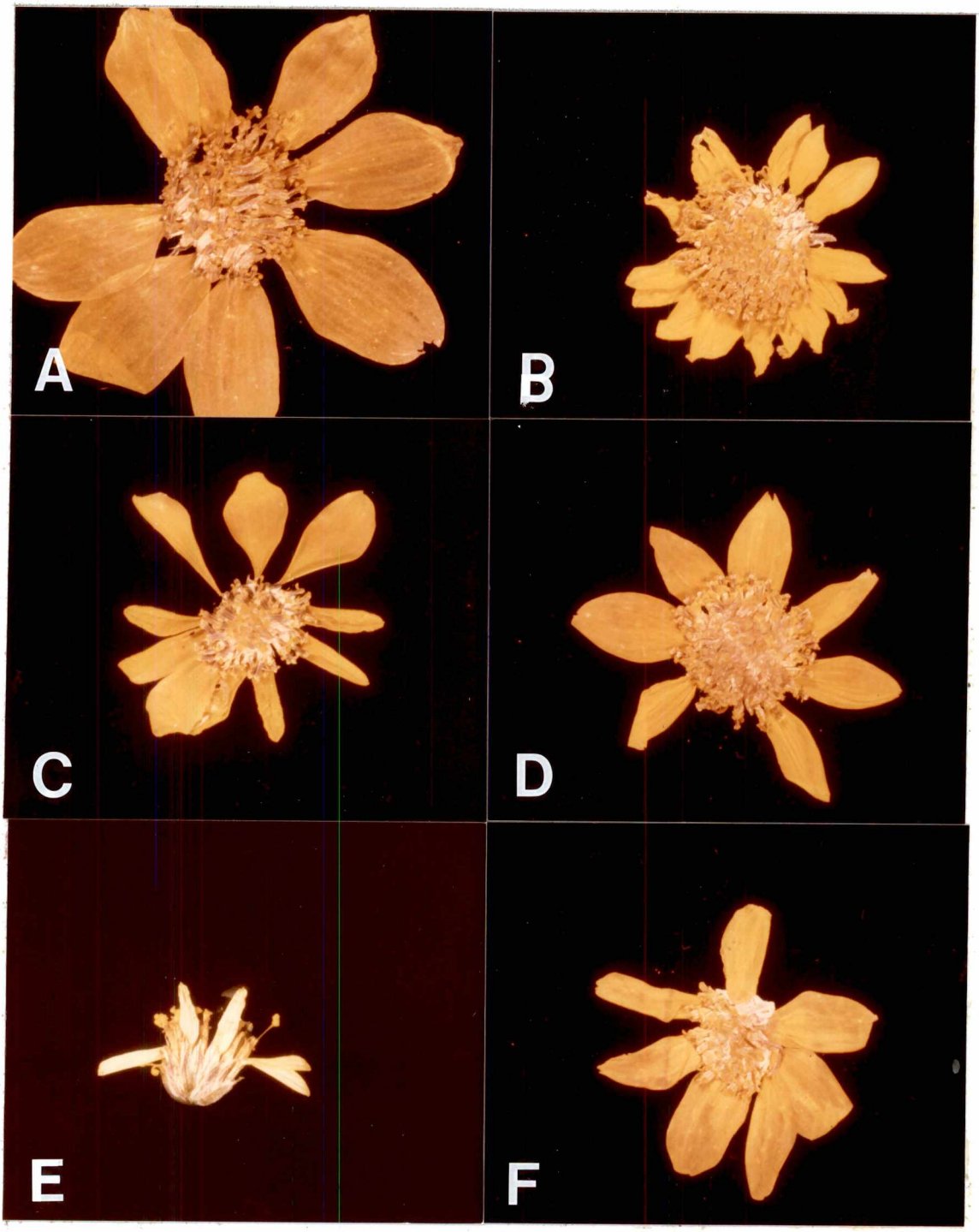


Figure II-4

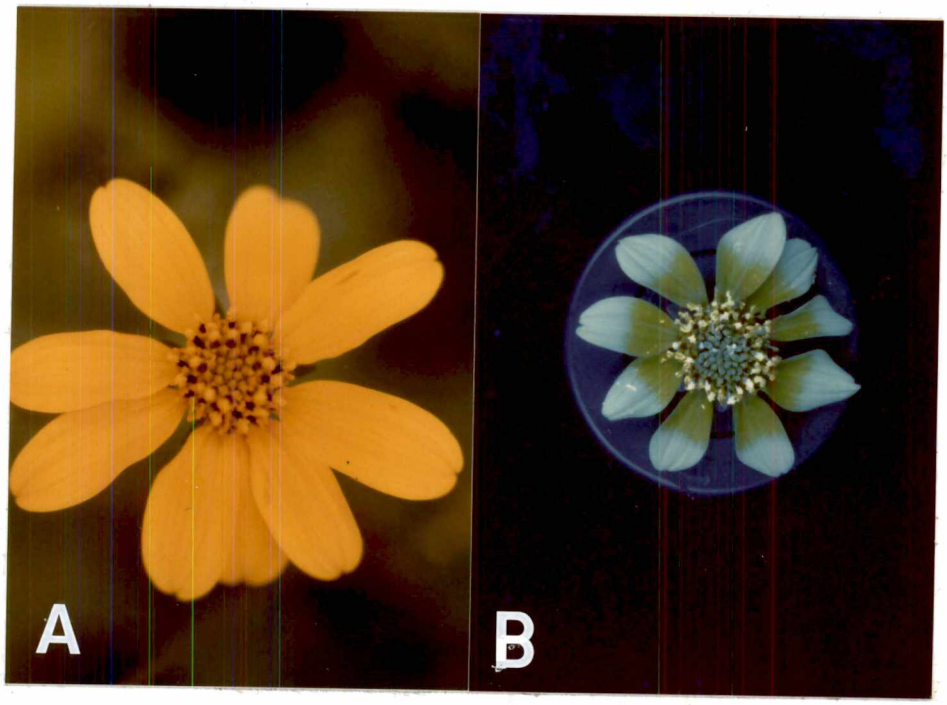


Figure II-5. Color Photographs of Fresh Flower Heads of V. dentata as seen under UV and Visible Light.

insects, are illustrated in Figure II-3. Photographs of all six taxa taken under visible light are displayed in Figure II-4. Photographs of fresh specimens of V. dentata are illustrated in Figure II-5 to show that fresh specimens provide the best basis for comparison of (IVS) and (HVS) visualizations of floral coloration.

The three types of photographs (Figures II-2, II-3, II-4) illustrate that the nectar guides in Viguiera are more complex than UV features alone (Figure II-2) indicate, and are the result of integrated visible and UV coloration. Unfortunately, the term UV nectar guide is sometimes interpreted to mean that the UV-absorbance alone constitutes the pollinator's orientation cue to food rewards. Certainly the UV components of floral color contribute to pollination (Jones, 1978), but it is unlikely that pollinators functionally dissect the spectral components of coloration during their foraging behavior. It is more biologically realistic to consider that a combination of UV and visible components produce an integrated color cue for pollinators (Kevan, 1976). Although the UV contribution to floral color will be stressed in this chapter, it should be understood that the visible component is also important.

Three distinct floral UV absorption-reflection patterns were detected for the ray florets of the six taxa studied. Ligules of two species, V. laciniata and V. reticulata, were completely UV-absorbing (Figure II-1 D,F). Ligules of V. potosina were entirely UV-reflecting (Figure II-1 E). Ligules of the other three taxa, V. deltoidea var. deltoidea var. deltoidea, V. deltoidea var. parishii, and V. dentata ,

were UV-absorbing only in the proximal portion of the ligule, while the distal region was UV-reflecting (Figure II-2 A,B,C). These results agree with those of Scogin (1978), who reported on the floral UV patterns of four of the six taxa being investigated.

Systematic study of floral UV patterns in Viguiera is of taxonomic value. For example, ligules of V. potosina are completely UV-reflecting (Figure II-2 C), while ligules of the other members of this series are at least partially UV-absorbing. In addition, V. potosina is endemic to central Mexico, and is isolated from other members of this series, most of which are endemic to the Baja California peninsula of Mexico. Differences with respect to UV patterning, chromosome number, and range, suggest that V. potosina may not be as closely related to other members of this series as has been proposed (Blake, 1918).

Another taxonomic question concerns the status of V. deltoidea, described by Blake (1918) as the most variable species of Viguiera. The two varieties of V. deltoidea investigated in this study, V. deltoidea and V. deltoidea var. parishii differ with regard to UV-absorbance patterns. Specifically, the ligules of V. deltoidea var. deltoidea are UV-absorbing up to 3/4 of the total ligule length (Figure II-2 D). In the ligules of V. deltoidea var. parishii UV-absorbance is restricted to the basal 1/3 of the ligule (Figure II-2 E). In addition, V. deltoidea var. deltoidea recently has been shown to be a hexaploid, and V. deltoidea var. parishii to be a diploid (Schilling, unpublished results). Differences in UV patterns as well as ploidy level provide evidence for elevating V. deltoidea var. parishii to the species rank.

There are several possible functions for floral UV patterns, although this study did not attempt to analyze them. For example, radiation in the UV-B waveband has been shown to damage pollen (Pfahler, 1981). After anthesis, but before removal by a pollinating agent, pollen may be subjected to both incident solar UV and solar UV reflected from adjacent surfaces such as the inner surface of the corolla (Flint and Caldwell, 1983). The UV-absorbing basal region of the ligules in Viguiera may reduce the UV-B radiation load on the pollen. However, it is speculated that, in Viguiera, UV patterns probably play a minor role, if any, in reducing UV-B radiation to the reproductive organs.

UV nectar guides in Viguiera may also serve as recognition features for flower-constant pollinating insects, allowing the pollinator to feed more efficiently. This behavior may result in a reduction of cross-pollination between species. A plausible example of pollinator partitioning in Viguiera is the sympatric occurrence of V. laciniata and V. deltoidea in the Vizcaino Desert of Baja California. In visible light, ligules of both species look very similar (Figure II-4 A,D) but differ with regard to UV patterns (Figure II-2 A,D). Other visibly similar, simultaneously flowering Composites, particularly members of the genus Encelia, have been observed to occupy large areas of sympatry with many members of this series. Differences in UV nectar guides may be important to pollinator partitioning between these species, thus enhancing the likelihood of pollination for each species, and possibly reducing cross-pollination between them.

CHAPTER III

FLAVONOID CHEMISTRY

The value of flavonoid compounds as taxonomic characters has been thoroughly demonstrated (Harborne, Mabry and Mabry, 1975). In particular, flavonoid chemistry has proved to be a valuable tool for systematic studies of the Compositae (Heywood, Harborne and Turner, 1977). Although there is a paucity of published data concerning the flavonoid chemistry of Viguiera, flavonoid studies of such closely related taxa as Helianthus (Schilling and Mabry, 1981; Schilling, 1983), indicate the probable value of flavonoid chemistry to understanding the taxonomy of Viguiera.

Flavonoid studies of Helianthus have shown three types of compounds to be present: flavone aglycones found mainly in glandular trichomes on the lower-leaf surface; flavonol glycosides and anthochlors that are present in leaf and floral tissues (Schilling and Mabry, 1981; Schilling, 1983; Gershenzon, Mabry and Kreitner, 1981). Preliminary studies by Schilling (Personal Communication) report the presence of the same three classes of compounds observed in Helianthus: flavone aglycones, flavonol glycosides, and anthochlors. Because this investigation concerns both floral UV coloration and the taxonomy of Viguiera series Viguiera, flavonoid pigments occurring in the floral tissues were chosen for study.

Floral flavonoids may have important biological functions in addition to their possible usefulness as taxonomic characters. These functions are discussed in Chapters II and IV.

Investigation of floral pigments in the Heliantheae has shown that floral flavonoids, especially anthochlor pigments, are often responsible for UV nectar guides (Harbourne and Smith, 1978; Schilling, 1983; Cunningham, 1983). In Viguiera a complete chemical analysis has been done for only one species, V. multiflora, and it was reported that two chalcone/aurone pairs were present: coreopsin/sulfurein; and marein/maritimein (Shikoriyama and Geissman, 1963).

In this study, floral flavonoids were investigated for six taxa of Viguiera series Viguiera. The objective was to determine the identity and characteristics of the flavonoids responsible for floral UV-absorption patterns in Viguiera and to assess the floral flavonoids for taxonomic significance in this series.

Materials and Methods

Floral tissues were collected in Southwestern U.S. and Baja California, Mexico. Voucher specimens are listed in Table III-2. Approximately 25 g (dry weight) of flower heads was extracted twice with 85% methanol for each taxon. The methanol was evaporated under vacuum in a flash evaporator, leaving only a water extract. This solution was extracted three times with methylene chloride to separate flavonoid aglycones from the flavonol glycosides still dissolved in water. The final aqueous fraction was then dried by flash evaporation, re-dissolved in 100% methanol (MeOH), streaked onto Whatman 3MM chromatography paper, and developed in one-dimension using 15% acetic acid as the solvent. Flavonoid compounds, appearing as UV-absorbing strips

on the dried chromatogram, were cut from the paper and eluted in 100% MeOH. Further purification was done using column chromatography with Sephadex LH-20 in 85% and 100% MeOH. Identification of the flavonoid compounds was based on their color under UV light, Rf values, UV spectral data, and co-chromatography with "authentic" compounds. Sugars were identified after acid hydrolysis (6N HCL, 40 minutes over steam) by co-chromatography with known standards on cellulose TLC plates.

Intrapopulational and interpopulational variation for floral flavonoids was investigated in Viguiera by extensively sampling different populations of collected material. Voucher specimens are listed in Table III-1. Flower heads from 2 to 10 individuals per population were extracted in 85% MeOH. Compounds were separated using two-dimensional paper chromatography on Whatman 3MM paper, employing tertiary butanol: glacial acetic acid: water (3:1:1) as the first solvent and 15% acetic acid as the second. Variation was detected by comparison of color characteristics and Rf values with "authentic compounds" previously isolated and identified for the taxon under investigation.

Distribution of anthochlor pigments was investigated by placing air-dried ligules in an alkaline solution such as ammonium hydroxide (NH_4OH). Ionization of the anthochlor compounds produces a color change. Distribution of anthochlors was determined by observing the ligule color changes.

Results and Discussion

Floral flavonoids isolated and identified for Viguiera series Viguiera can be grouped into four general classes: flavonoid aglycones;

Table III-1. Specimens of *Viguiera* series *Viguiera* examined for Flavonoids.

Species	Voucher[a]
V. deltoidea var. *deltoidea*	Mexico Baja Cal.: Rieseberg 8A
	Mexico Baja Cal.: Rieseberg 8B
	Mexico Baja Cal.: Rieseberg 8C
	Mexico Baja Cal.: Rieseberg 8D
	Mexico Baja Cal.: Rieseberg 8E
	Mexico Baja Cal.: Rieseberg 8F
	Mexico Baja Cal.: Rieseberg 8G
	Mexico Baja Cal.: Rieseberg 8H
	Mexico Baja Cal.: Rieseberg 8I
	Mexico Baja Cal.: Rieseberg 17C
V. deltoidea var. *parishii*	Cal. San Diego Co.: Rieseberg 3
	Cal. Imperial Co.: Rieseberg 4
	Cal. Imperial Co.: Rieseberg 6A
	Cal. Imperial Co.: Rieseberg 6B
	Cal. Imperial Co.: Rieseberg 6C
	Cal. Imperial Co.: Rieseberg 6D
	Cal. Imperial Co.: Rieseberg 6E
V. laciniata	Cal. San Diego Co.: Rieseberg 1
	Cal. San Diego Co.: Rieseberg 2
	Mexico Baja Cal.: Rieseberg 7
	Mexico Baja Cal.: Rieseberg 7A
	Mexico Baja Cal.: Rieseberg 7B
	Mexico Baja Cal.: Rieseberg 7C
	Mexico Baja Cal.: Rieseberg 7D
	Mexico Baja Cal.: Rieseberg 7E
	Mexico Baja Cal.: Rieseberg 7F
	Mexico Baja Cal.: Rieseberg 7G
	Mexico Baja Cal.: Rieseberg 7H
	Mexico Baja Cal.: Rieseberg 11
	Mexico Baja Cal.: Rieseberg 12
	Mexico Baja Cal.: Rieseberg 16B
	Mexico Baja Cal.: Rieseberg 19
	Mexico Baja Cal.: Rieseberg 20
	Mexico Baja Cal.: Rieseberg 21
	Mexico Baja Cal.: Rieseberg 22
	Mexico Baja Cal.: Rieseberg 23
	Mexico Baja Cal.: Rieseberg 24
V. reticulata	Cal. Inyo Co.: Rieseberg 27
	Cal. Inyo Co.: Rieseberg 28
	Cal. Inyo Co.: Rieseberg 29

Table III-1 (Continued)

Species	Voucher[a]
V. reticulata	Cal. Inyo Co.: Rieseberg 31
	Cal. Inyo Co.: Rieseberg 31A
	Cal. Inyo Co.: Rieseberg 31B
	Cal. Inyo Co.: Rieseberg 31C
	Cal. Inyo Co.: Rieseberg 31D
	Cal. Inyo Co.: Rieseberg 31E

[a]Specimens are deposited in the University of Tennessee Herbarium (Tenn).

flavone glycosides; flavonol glycosides; and anthochlors. The generalized structures of these compounds are illustrated in Figure III-1.

After flavonoid identification intrapopulational and interpopulational variation for floral flavonoids was investigated. Extensive interpopulational variation was found for some compounds (Table III-2). Variation for floral flavonoids is not unusual, and has been reported for other taxa (Harborne et al., 1975).

Flavonoid aglycones were localized in glandular trichomes on the abaxial ligule surface of four of the six taxa. The lack of aglycones in V. potosina and V. dentata correlates with the lack of glandular trichomes.

Only one flavone glycoside, luteolin 7-O-xyloside, was identified for Viguiera (Table III-2). It was present in significant quantities in only one species, V. laciniata. Traces of this compound were also observed for some populations of V. deltoidea var. deltoidea.

Six flavonol glycosides were isolated, five of which were identified (Table III-2). Ray flowers of four taxa, V. deltoidea var. deltoidea, V. deltoidea var. parishii, V. laciniata, and V. reticulata, contained two compounds based on quercetin 3-O-methyl ether, and quercetin 7-O-glucoside. The ligules of V. laciniata contained an additonal quercetin 3-O-methyl ether glycoside. This was in contrast to V. potosina ligules, which lacked flavonol glycosides, and to V. dentata ligules, for which only one flavonol glycoside, quercetin 3-O-glucoside, was observed.

Anthochlor pigments were present in both ray and disc flowers of

Figure III-1 Skeletons of the Classes of Compounds included in Table III-2.

A. Flavone

B. Flavonol

C. Chalcone

D. Aurone

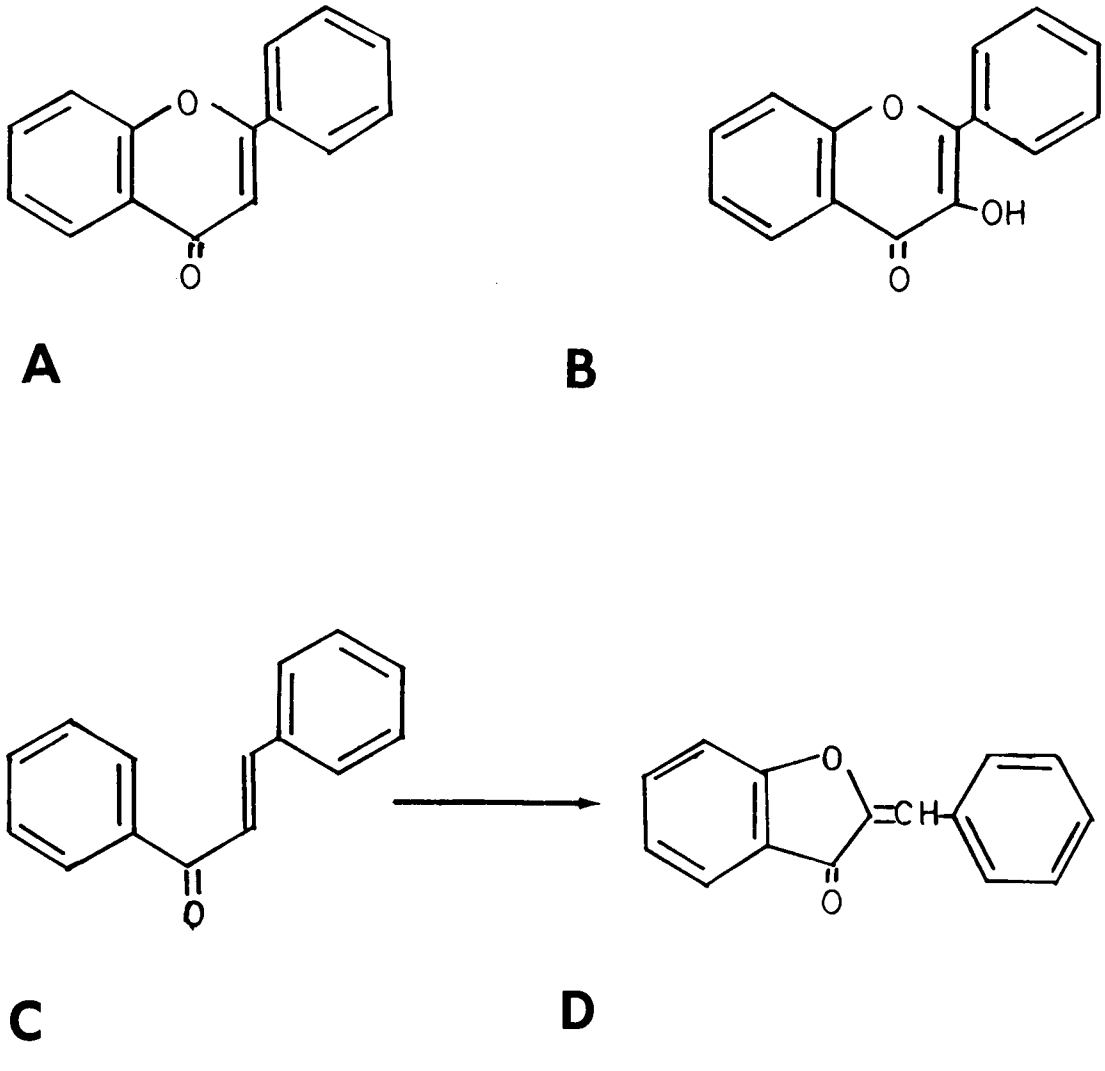


Figure III-1

Table III-2. Distribution of Floral Flavonoids for Six Taxa of *Viguiera* series *Viguiera*

TAXA	Coreopsin	Sulfurein	Quercetin 3-OMe 7-O-glucoside	Quercetin 3-OMe 7-O-glucuronide	Quercetin 3-OMe 7-O-glycoside	Flavonol (unknown)	Luteolin 7-O-xyloside	unknown	Quercetin 7-O-glucoside	Chalcone (unknown)	Marein	Maritimein	Quercetin 3-O-glucoside
V. deltoidea var. *deltoidea*	+	+	+	±		±		±	±				
V. deltoidea var. *parishii*	+	+	+	±					±				
V. dentata	+	+									+	+	+
V. laciniata	+	+	+	+	±	±	+	±	+	±			
V. potosina	+	+											
V. reticulata	+	+	+	±				±	+				

OMe O-methyl ether
\+ present in all populations examined
± variable between populations examined

five of the six taxa. In V. potosina, anthochlors were present only in the disc flowers. These findings correlated nicely with preliminary studies showing V. potosina ligules to be entirely UV-reflecting while at least portions of the ligules for the other five taxa were UV-absorbing. Analysis of the ligules of V. deltoidea var. deltoidea, V. deltoidea var. parishii, V. laciniata, and V. reticulata showed only one chalcone/aurone pair, coreopsin/sulfurein, to be present. In contrast, two anthochlor pairs, coreopsin/sulfurein and marein/maritimein, were identified for V. dentata.

Variation in floral flavonoids in Viguiera series Viguiera is of taxonomic importance (Table III-2). Specifically, V. dentata and V. potosina lack glandular trichomes containing flavonoid aglycones. Both species lack the quercetin 3-O-methyl ether glycosides which may be marker compounds for series Vigiuera. In addition, V. potosina and V. dentata differ from the other members of this series with regard to anthochlor pigmentation. V. potosina ligules lack anthochlors, while V. dentata ligules contain two chalcone/aurone pairs. This is in contrast to ligules of the other four taxa which contain only one chalcone/aurone pair, coreopsin/sulfurein. These data indicate that V. dentata and V. potosina are not closely related to the other members of this series. In addition, differences in floral flavonoids between these two species suggest that V. potosina and V. dentata are not closely related and could eventually be placed into different series (or sections) of Viguiera.

Another taxonomic consideration concerns the status of V. deltoidea.

Flavonoid studies show that V. deltoidea var. parishii lacks two unidentified floral flavonoids found to be present in V. deltoidea var. deltoidea. Further chemical analysis of the V. deltoidea complex will be important to understanding the taxonomy of this species.

Floral flavonoids may also be of biological importance in Viguiera. Using the "anthochlor test" it was shown that the distribution of anthochlor pigments correlated with the presence of UV nectar guides. The possible biological functions of floral flavonoids in relation to floral UV patterns are discussed in detail in chapters II and IV.

CHAPTER IV

FLAVONOID DISTRIBUTION AND LIGULE ANATOMY

Although the taxonomic distribution and chemistry of floral flavonoid pigments have been intensively studied (Harborne et al., 1975), there remains a surprising lack of knowledge concerning the basic biology of flavonoids. Such aspects as the localization of flavonoids in cells and tissues, and their functional significance, remain largely unexplored. Determining the relationships between ligule anatomy and flavonoid distribution may be of importance to understanding the function of floral flavonoids.

An early study of floral flavonoid localization was performed by Brehm and Krell (1975), who analyzed flavonoid distribution in the petals of several angiosperm species and observed that UV-absorbing flavonoids were localized in the tips of specialized epidermal papillae. An extensive survey of 201 species from sixty angiosperm families by Kay and Daoud (1981) confirmed the epidermal localization of flavonoid pigments. They reported the flavonoids to be present in solution, and to be evenly dispersed throughout the vacuoles of epidermal cells, rather than confined to the tips of epidermal cells as reported previously.

Published information concerning petal structure is also rather scarce. Probably the most extensive source of data on the structure of petals is the survey made by Schubert (1925), who investigated more than 330 species from 51 angiosperm families. Recently, the distinctive

surface structures of the outer cell walls of the epidermis of petals have been investigated for some major plant families including the Asteraceae (Baagoe, 1977). Kay and Daoud (1981) described the internal and external structure of petals for over 200 species and attempted to correlate petal anatomy with the mechanisms by which petals absorb and reflect light.

The most obvious function of floral flavonoids is their role as pigments (McClure, 1975), where they may provide recognition cues for pollinating insects. Richtmeyer (1923) and Lutz (1924) were among the first to report UV floral patterns in North American wild flowers, but offered no explanation for their presence. Daumer (1958) established that insects are sensitive to wavelengths of light from 300-600 nanometers. Later, Thompson, Meinwald, Aneshansley and Eisner (1972) were able to provide a chemical basis for these spectral patterns. Using Rudbeckia hirta ligules, they clearly demonstrated that the strong UV-absorbance at the basal portion of the ligule was due to the occurrence of flavonol 3-O-glycosides which have their maximum spectral absorption in the UV region (340-380 nm). Brehm and Krell (1975) reported the epidermal localization of flavonoids, and the papillate epidermal structure of many petals. Kay and Daoud (1981) proposed that the papillate epidermis acts as a light trap to guide incident light through flavonoid pigments in the epidermal cells. This work combined to create a strong chemical and structural basis for these invisible nectar guides.

Other scientists have investigated if UV patterns actually do

constitute a major orientation cue to pollinators. Evidence for this idea came from studies of pollinator behavior on flowers with nectar guides. Jones and Buchmann (1974) demonstrated bee orientation to the UV absorbing portion (banner petal and stamens) of two legume species. Jones (1978) demonstrated that pollinator fidelity to UV patterns could be effective as pre-pollination isolating mechanisms between two closely related sympatric species which differ only in nectar guide formation. In other instances UV patterns were shown to increase pollinator efficiency because UV patterns tended to orient the pollinator toward the nectar source (Waser and Price, 1983). These efforts have combined to form a strong case for the role of floral flavonoids in creating UV patterns which serve as orientation cues for pollinators.

Although the contribution of flavonoids to floral color is obvious, there is a lack of understanding regarding the relationships between petal structure, pigment distribution, and the way in which petals absorb and reflect light and thus acquire characteristic color patterns, brightness and texture.

In the present study, ligules of Viguiera were investigated with SEM and light microscopy to determine internal and external ligule structure and floral flavonoid distribution. These data have led to a better understanding of petal structure, pigmentation, function and appearance in this genus.

Materials and Methods

The localization of flavonoid pigments was investigated using fully opened heads of V. dentata. Hand-sectioned fresh ligules were

examined. Most UV-absorbing flavonoids became visible as yellow-orange pigments when immersed in alkaline solution causing bathochromic shifts in their absorption spectrum (Kay and Daoud, 1981). Flavonoid localization was determined by irrigating ligule sections with potassium hydroxide solution and observing color change. Results were documented with an Olympus Vanox Universal Research Microscope fitted with an Olympus automatic photography system. Photographic images were recorded on Kodak Ektachrome film (ASA 160).

Ligules were also dissected to separate their UV-reflecting distal portion of the ligule from the UV-absorbing proximal portion. Flavonoids from each region were extracted and tentatively identified by co-chromatography on TLC plates with previously identified standards.

Dried ligules from herbarium specimens of all six taxa were examined by scanning electron microscopy (SEM) to determine ligule anatomy. Voucher specimens are listed in Table IV-1. Ligules were attached to standard 3/4-inch aluminum stubs with double-faced cellophane tape. The specimens were coated with about 30 nanometers gold:palladium (60:40) in a Denton DV-515 vacuum evaporator and viewed with an ETEC Autoscan scanning electron microscope operating at 20 KV. Photographs were taken using Polaroid Type 655 Positive/Negative film.

Results and Discussion

UV-absorbing flavonoids were found to be localized in the vacuoles of the adaxial epidermal cells of *V. dentata* ligules (Figure IV-1 A). Comparison of the UV-absorbing proximal portion of the ligules with the reflecting distal region showed that the color and concentration

Table IV-1. Populations of *Viguiera* series *Viguiera* examined with the Scanning Electron Microscope.

Species	Subject	Voucher [a]
V. deltoidea var. *deltoidea*	ligule	Mexico Baja California: Rieseberg 8A
V. deltoidea var. *parishii*	ligule	Cal. San Diego County: Rieseberg 3
V. laciniata	ligule	Mexico Baja California: Rieseberg 7A
V. reticulata	ligule	Cal. Inyo County: Rieseberg 25

[a]Specimens are deposited in The University of Tennessee Herbarium (Tenn).

Figure IV-1. Light Micrographs of *V. dentata* Ligules.

A. *V. dentata*, proximal portion of ligule, X.S., 40X.

B. *V. dentata*, proximal portion of ligule, X.S., 10X.

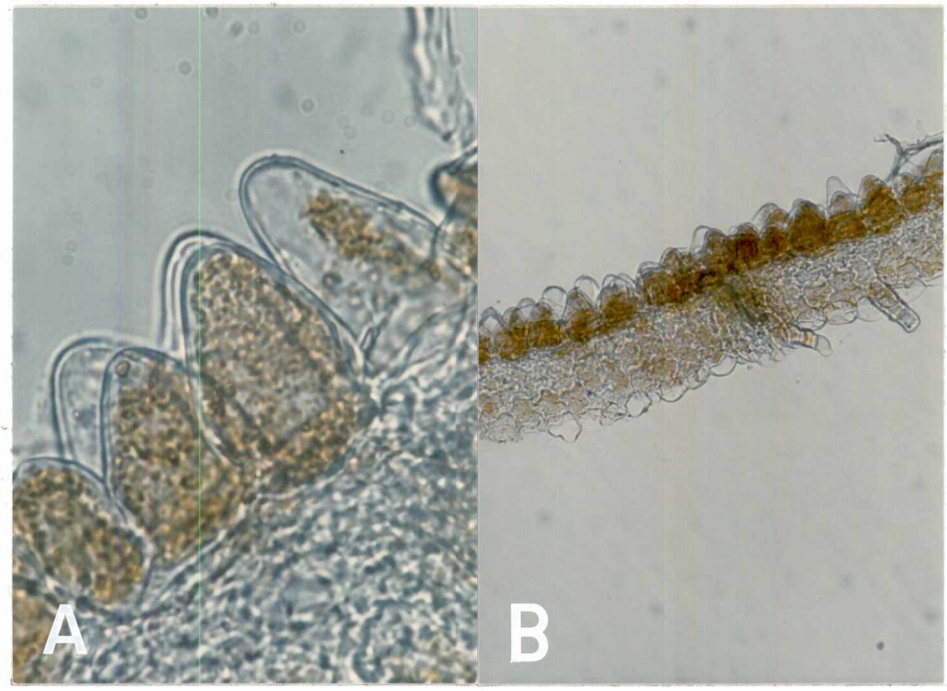


Figure IV-1

of chromoplastic material differed between the two regions. The distal cells contained loosely packed, light yellow chromoplasts, while the proximal cells were densely packed with yellow-orange chromoplasts (Figure IV-1 B). Application of an alkaline reagent to the ligule cells caused the proximal cells to stain a deep orange (Figure IV-1 B), while the distal cells stained very little or not at all. I was unable to observe any apical concentrations of UV-absorbing pigment granules in living cells of the type reported in freeze-dried petals by Brehm and Krell (1975). Baagoe (1977) suggested that this stratification is an artifact produced during the freeze-drying process. Correlation of flavonoids with UV patterns in ligules of Viguiera suggests that the high concentration and epidermal localization of UV-absorbing flavonoids in the basal portion of the ligule are important for the formation of UV nectar guides.

The ligules of V. dentata contained three flavonoid pigments: coreopsin, marein, and quercetin 3-O-glucoside. These compounds, which show intense absorption at 300 to 400 nanometers, are concentrated in the proximal portion of the ligule, which is UV-absorbing as a result. Two of these compounds, marein, and quercetin 3-O-glucoside, are restricted to the proximal portion of the ligule, whereas coreopsin was found primarily in the proximal portion of the ligule with traces also present in the reflecting distal portion of the ligule. These data imply that UV-absorbing flavonoids from the UV nectar guides found in Viguiera.

All Viguiera specimens examined had an adaxial ligule structure

consisting of papillose, nearly isodiametric cells, with very pronounced surface striations (Figure IV-2 A,B). This is in direct contrast to the flat, uniform abaxial ligule surface (Figure IV-2 D). These observations are similar to those reported by Baagøe (1977) for other members of the Heliantheae.

The papillate petal epidermis is thought to function as a light trap and, in conjunction with the reflective mesophyll, to guide incident light through the pigments contained in the epidermal cells and to return it to the exterior by a combination of external reflection, refraction and internal reflection (Kay and Daoud, 1981). Striations on the epidermal papillae increase the refractive properties of the epidermis, giving the ligules a saturated hue, which, according to Kugler (1955), is preferred by bumblebees.

Another micromorphological feature of the ligules is the presence of hairs on the abaxial ligule surface for all six taxa (Figure IV-2 D). Glandular trichomes (Figure IV-2 C) containing flavonoid aglycones were present in four of the six taxa, V. deltoidea var. deltoidea, V. deltoidea var. parishii, V. laciniata, and V. reticulata, but did not contribute to nectar guide formation. Glandular trichomes have previously been reported for the genus Helianthus (Schilling, 1983), which is closely related to Viguiera. This observation may be of taxonomic value and serves to separate further V. potosina and V. dentata from the other four taxa in which glandular trichomes were reported.

Figure IV-2. Scanning Electron Micrographs of the Ligules of *Viguiera*

A. *V. laciniata*, adaxial surface, epidermal papillae, 200X.

B. *V. laciniata*, adaxial surface, epidermal papillae, 800X.

C. *V. reticulata*, abaxial surface, glandular trichome, 1000X.

D. *V. dentata*, abaxial surface, 200X.

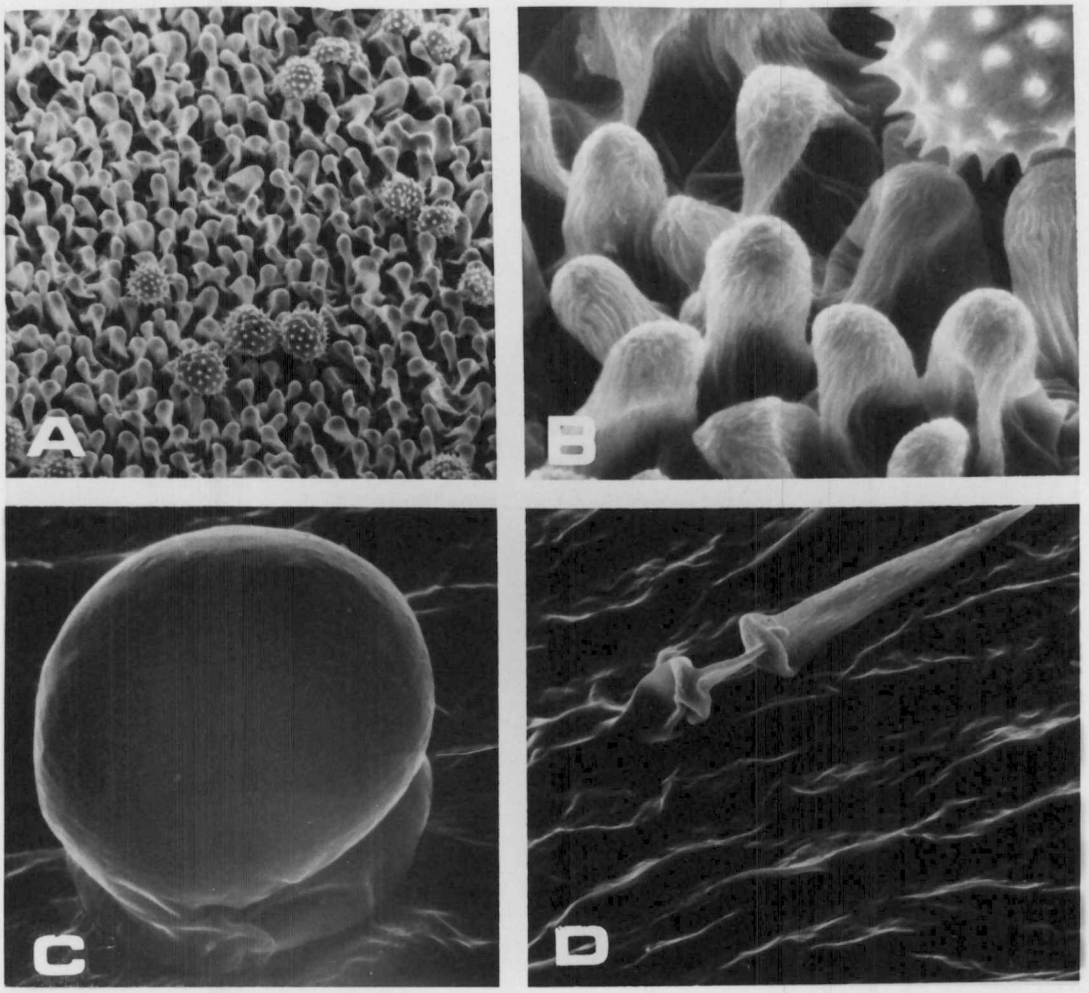


Figure IV-2

CHAPTER V

CONCLUSIONS

This study accomplished two objectives. The first objective was to make a systematic study of floral UV patterns in six taxa of Viguiera series Viguiera using UV photography and comparative floral flavonoid analysis. The second objective was to analyze the distribution of flavonoid pigments in relation to the functional anatomy of ligules using light microscopy and scanning electron microscopy.

Three distinct floral UV absorption-reflection patterns were detected for the ray florets of this series. Ligules of two taxa, V. laciniata and V. reticulata were completely UV-absorbing. Ligules of V. potosina were entirely UV-reflecting. Ligules of the other three taxa, V. deltoidea var. deltoidea, V. deltoidea var. parishii, and V. dentata, had UV-absorbance restricted to the proximal portion of the ligule, while the distal region was UV-reflecting. Analysis of the UV-absorbing flavonoids thought to be responsible for these patterns showed four general classes of compounds to be present: flavonoid aglycones, flavone glycosides, flavonol glycosides, and anthochlor pigments.

Variation for UV patterns and floral flavonoids was of taxonomic importance in this series. Specifically, V. dentata and V. potosina differed from the majority of taxa in this series in three ways: both lacked flavonoid aglycones and glandular trichomes; both lacked quercetin 3-O-methyl ether marker compounds; and both differed with respect

to anthochlor pigmentation. In addition, the ligules of V. potosina were entirely UV-reflecting while the ligules of the other members of this series were at least partially UV-absorbing. These data suggest that V. dentata and V. potosina, which have chromosome numbers of n=17 in contrast to n=18 reported for the majority of taxa in this series, may not belong in this group. A second taxonomic consideration is the status of V. deltoidea. Variation for floral flavonoids in this species suggests that the taxonomy of V. deltoidea needs to be re-evaluated.

Analysis of the distribution of floral flavonoids in relation to ligule anatomy in Viguiera showed that flavonoid aglycones were located in glandular trichomes on the abaxial ligule surface, and that the flavonol glycosides and anthochlor pigments were confined to papillate epidermal cells occurring in the solution of the vacuoles. Scanning electron micrographs confirmed the presence of papillate cells and glandular trichomes on the ligule epidermis, and disclosed other epidermal features associated with the absorption and reflection of light. Structural features of the ligule, in conjunction with floral flavonoid distribution, were shown to play an important role in forming UV patterns on the ligulate corolla of Viguiera. These UV patterns may play a minor role in reducing UV-B radiation to the reproductive organs. In addition, UV patterns may be important as recognition cues for flower-constant pollinating insects, thus reducing cross-pollination between visibly similar species. Determining the relationships between floral flavonoids, UV patterning, ligule anatomy, and pollinator behavior may be important to understanding the reproductive biology of Viguiera.

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VITA

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In 1979 Loren graduated from Little Creek Academy in Knoxville, Tennessee, and subsequently found himself at Southern Missionary College. Two years later he received a B.A. in Biology. After spending half a year in Australia he accepted a teaching assistantship at The University of Tennessee, Knoxville and began studying toward a Master of Science degree with a major in Botany. This degree was awarded in June 1984.

Loren has accepted a research assistantship at Washington State University this fall, and will begin studying toward a Doctor of Philosophy degree with a major in botany.