EVOLUTION OF FLORAL TRAITS: BIOGEOGRAPHY, POLLINATION BIOLOGY AND PHYLOGENETICS IN *MACROMERIA VIRIDIFLORA*

by

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Amy Elizabeth Boyd

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and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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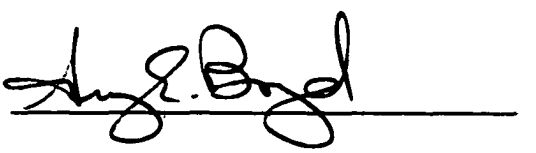
SIGNED: 

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ABSTRACT

Macromeria viridiflora is an herbaceous perennial in which floral traits vary geographically. In my dissertation research, I analyzed geographic variation in plant morphology and pollinator assemblages. I conducted experiments to determine the breeding system of the plants, and used visitation rate and pollen deposition to compare effectiveness of floral visitors as pollinators. I analyzed aspects of pollinator attractants and rewards in the flowers and placed this into the context of pollinator syndromes. In addition, I used phylogenetic analysis of the genus to determine polarity of change in corolla size within the species.

Analysis of morphometric data from eight sites across the range of the species revealed significant among-population variation in vegetative and floral traits. Flower size variation is particularly strong and follows a latitudinal cline.

Hawkmoths and hummingbirds were the main floral visitors throughout the range. The large-bodied hummingbirds visiting plants in the southern regions are not present in the northern regions, where flowers are visited by hummingbirds with barely half the body size and much shorter bills. This difference in bill size of hummingbird

pollinators mirrors the geographic variation in flower size in M. viridiflora, suggesting that pollinator-mediated selection may be acting upon the species.

Flowers of M. viridiflora have several characteristics that fit both the hummingbird and hawkmoth pollinator syndromes, namely copious sucrose-rich nectar and long floral tubes. However, they also have characteristics that correspond with a single major pollinator. This plant therefore presents a compromise floral syndrome that attracts two classes of pollinators.

Breeding system studies showed that whereas plants are self-compatible and occasionally produce seed autogamously, pollinators are important for reproductive success in the plants. Combining visitation rate and pollen deposition as measures of pollinator effectiveness, hummingbirds were found to be the most effective pollinators at both sites.

Phylogenetic analysis produced a single most parsimonious tree that supports the monophyly of the genus. Mapping of corolla size onto the phylogeny indicates that floral size has changed many times within the genus, and that very large corolla size in southern populations of Macromeria viridiflora has been derived from a smaller-flowered ancestor.

CHAPTER 1

INTRODUCTION

Variation in traits of organisms attracts the attention of biologists because of the clues that it can give us about the evolutionary process. The incredibly diverse variation in flower shape, size, color and other traits has particularly attracted the imagination of generations of biologists (Sprengel (1793) 1996, Darwin 1877, Grant 1949, Stebbins 1971). Biologists widely accept pollinator-mediated selection as an important cause of the diversity of floral forms (Stebbins 1971, Faegri and van der Pijl 1979, Crepet 1983, Feinsinger 1983, Waser 1983, Campbell et al. 1991, Inoue et al. 1996). Stebbins took this one step further in proposing the "most effective pollinator principle," which theorizes that "the characteristics of the flower will be molded by those pollinators that visit it most frequently and effectively in the region where it is evolving" (Stebbins 1974). More recent studies have questioned the universality of this idea, contending that other factors may also play an important role in the adapation of floral traits (Stephenson and Bertin 1983, Primack 1987, Willson 1991, Armbruster 1996, Mitchell et al. 1998, Galen 1999). Nevertheless, the argument is about the degree of importance of pollinator-mediated selection, and

it is still understood that divergence in floral characteristics can be the result of adaptation to pollinators.

Because available pollinators may vary spatially, the floral characteristics of a species may vary spatially as well, due to selection by different pollinators. Within-species variation in traits such as corolla length, color, scent, and nectar concentration has been linked to variation in pollinator assemblages (Grant and Grant 1965, Galen and Kevan 1980, Linhart and Feinsinger 1980, Robertson and Wyatt 1990). However, few such intraspecific studies have been conducted, and they have tended to focus either on one trait over a wide range (Miller 1981) or have been limited to only a few sites (Robertson and Wyatt 1990, Johnson 1997). These limitations restrict how much may be understood about the relationship between floral traits and pollinator assemblages.

Variation among species often leads to speculation about how that variation may have come about, but when speciation is already complete, the selective pressures that led to divergence among species may not be currently operating. Studying geographic variation within species provides an opportunity to observe divergence in action. On the other hand, looking at variation among related species can provide an historic context for the study of floral

variation. Mapping character states onto a phylogeny can provide a hypothesis for the direction of change in floral traits. In my dissertation research, I have combined these approaches by studying variation in floral characteristics both within one species and among related species.

Pollination biologists have long recognized that flowers of different species that share a pollinator "class" often have similar characteristics despite different phylogenetic ancestry. These suites of floral characteristics associated with particular pollinator groups are known as pollination syndromes (Faegri and van der Pijl 1979, Wyatt 1983). For example, the characteristics of the pollination syndrome associated with bird pollinators include bright red flowers, diurnal anthesis, deep corolla tubes or spurs, and an ample supply of nectar (Wyatt 1983). It is important not to accept these syndromes as literal prescriptions, for exceptions are common. A pollinator may visit a broader array of flowers than its syndrome may suggest; Waser (1983), for example, found that broad-tailed hummingbirds visited flowers of a variety of colors including not only red but also purple, blue, yellow and pale green. In addition, flowers that may seem to fit a certain syndrome may actually be visited by multiple types of pollinators (Chase and Raven 1975, Haber and Frankie 1989, Fishbein and Venable 1996). However, despite the

exceptions, pollinator syndromes can serve to illuminate patterns that may lead to productive studies examining the adaptive significance of certain traits.

For my dissertation research, I studied morphometrics, pollinator visitation and effectiveness, and breeding system of Macromeria viridiflora in order to elucidate the structure of geographic variation in flower size, and explore the relationship between this variation and the pollination biology of the plant. I placed the corolla size variation into a phylogenetic context to assess the polarity of the historical change in flower size within the genus. I also studied floral attractants and rewards in M. viridiflora and placed these floral traits into the context of pollinator syndromes.

Appendix A ("Morphological analysis of Sky Island populations of Macromeria viridiflora [Boraginaceae]"), a paper accepted for publication in Systematic Botany, reports the results of a morphometric analysis comparing plants from eight collection sites across the range of the species. Nine vegetative and eleven floral characters were analyzed individually and using multivariate techniques. I also present results of pollinator observations at all eight sites and suggest that geographic variation in floral characters in this species may be associated with variation in pollinator identity.

Appendix B ("Pollination biology and breeding system in Macromeria viridiflora: an example of compromise between pollinator syndromes") seeks to clarify the association between pollinator variation and flower size variation that was described in Appendix A. The following questions are addressed: (1) How consistent are floral traits with expected pollinator syndromes for the major floral visitors? (2) How important are pollinators in the plant's breeding system? (3) What is the relative pollinator effectiveness of the floral visitors? Based on the results, I suggest that this plant may represent a compromise between two pollinator syndromes, having a combination of floral traits that may ensure reproductive success if the presence of the most effective pollinator is uncertain or variable.

Appendix C ("Phylogenetic relationships and corolla size evolution among Macromeria [Boraginaceae]") reports the results of a phylogenetic analysis of the genus Macromeria based upon 35 morphological characters. In addition to determining relationships within the genus, I also examine the history of corolla size evolution in Macromeria to determine the direction of change within M. viridiflora.

CHAPTER 2

PRESENT STUDY

Macromeria viridiflora DC. (Boraginaceae) is an herbaceous perennial plant that exhibits geographic variation in floral traits. My dissertation research focused on this geographic variation within the species as well as variation in floral traits among congeners. I placed the variation into an ecological context through studies of breeding system and pollination biology. The methods, results, and conclusions of this study are presented in papers appended to this dissertation. The following is a summary of the most important findings in the appended papers.

Appendix A examines an array of morphological traits throughout the range of the species and describes geographic patterns of variation. Univariate and multivariate analysis of morphometric data from eight sites across the range of the species shows significant among-population variation in 19 of 20 vegetative and floral traits measured. Variation in corolla length is particularly strong and follows a latitudinal cline, with flowers being much larger in the southern part of the range and smaller in the northern part of the range, varying among populations from a mean of 76.9 mm to a mean of 45.0 mm. Variation in vegetative characters

is significant among populations, but this variation does not follow a latitudinal gradient. Vegetative traits are also not correlated with one another and therefore follow no geographic pattern (i.e. some traits are larger in a particular area while others may be smaller in that area).

Observations also indicate differences in floral visitors between northern and southern populations. Whereas flowers in all populations are visited by hummingbirds, the large-bodied hummingbirds visiting plants in the southern regions are not present in the northern regions, where flowers are visited by hummingbirds with barely half the body size and much shorter bills. This difference in bill size of pollinators mirrors the geographic variation in flower size in M. viridiflora, suggesting that pollinator-mediated selection may be acting upon the species.

In Appendix B, I further explore the association between variation in pollinator type and flower size variation in Macromeria viridiflora by studying the pollinator attractants and rewards presented by the plants, the breeding system of the plants, and the pollinator effectiveness of floral visitors at two sites with contrasting flower lengths. Flowers of M. viridiflora have several characteristics that fit both the hummingbird and hawkmoth pollinator syndromes, namely copious sucrose-rich nectar and long floral tubes. However, they also have

characteristics that correspond with a single major pollinator. The late afternoon anthesis, greenish-white color, and production of scent compounds fit the syndrome for hawkmoth pollination. However, unlike in most hawkmoth-pollinated flowers, scent is only present in small amounts, and the pendulous orientation of the flowers is more suited to hummingbird visitation. Further, although anthesis occurs in late afternoon, flowers remain open through the following day. This plant therefore presents a combination floral syndrome that attracts two classes of pollinators. Such a combination of characteristics may be an advantage: reproductive success may be more uncertain if the presence of one of the pollinators is uncertain or variable. Alternatively, it may be a disadvantage if it results in imperfect matches with the pollinators such that pollinator effectiveness is decreased.

Studies of the breeding system of M. viridiflora showed that the plants are self-compatible and can produce seed autogamously. However, the plants are not apomictic, and the presence of pollinators significantly increased seed set. Therefore, pollinators are important for reproductive success in the plants and there is potential for pollinator-mediated selection.

I used rate of pollinator visitation as well as amount of pollen deposited in single-pollinator visits to evaluate

pollinator effectiveness. At both sites, hummingbirds were more frequent visitors than hawkmoths. Hummingbirds also deposited more pollen than hawkmoths at both sites, though the difference was only significant at one site. Combining these into a pollination effectiveness measure (PE = mean visitation rate * mean number of pollen grains deposited), hummingbirds are shown to be the most effective pollinator at both sites.

Phylogenetic analysis of Macromeria (Appendix C) based on 35 morphological characters produced a single most parsimonious tree that supports the monophyly of the genus. The phylogeny upholds previous evaluations of relationships among species, and the resulting clades often connect sister taxa that are geographically proximate. Mapping of corolla size onto the phylogeny indicates that floral size has changed many times within the genus, and that very large corolla size in southern populations of Macromeria viridiflora has been derived from a smaller-flowered ancestor.

To sum up the conclusions of my research, I have shown that:

1. Flowers of M. viridiflora vary in size along a latitudinal gradient.

2. This geographic variation in corolla size is mirrored by geographic variation in the bill size of hummingbirds visiting the flowers.
3. Hummingbirds are more frequent visitors and more effective pollinators than hawkmoths, the other frequent visitor to the flowers.
4. The plants are self-compatible and can produce seed autogamously but seed set is increased by the presence of pollinators; therefore, there is potential for pollinator-mediated selection.
5. The flowers present a combination of traits from hawkmoth and hummingbird pollination syndromes, which may ensure reproduction if one of these pollinators is uncertain or variable.
6. Placing corolla length into an historical context, phylogenetic analysis indicates that this floral characteristic has changed many times within the genus, and that very long corollas in southern populations of Macromeria viridiflora has been derived from a smaller-flowered ancestor.

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APPENDIX A

MORPHOLOGICAL ANALYSIS OF SKY ISLAND POPULATIONS OF *MACROMERIA VIRIDIFLORA* (BORAGINACEAE)

Abstract--Macromeria viridiflora (Boraginaceae), a perennial that occurs in isolated populations on the "Sky Islands" of southwestern North America, displays geographic variation in floral morphology. This study examines an array of morphological traits and describes geographic patterns of variation throughout the range of the species. Univariate and multivariate analysis of morphometric data from eight sites across the range of the species shows significant among-population variation in 19 of 20 vegetative and floral traits measured. Flower size variation is particularly strong and follows a latitudinal cline, with flowers being much larger in the southern part of the range and smaller in the northern part of the range. Observations also indicate differences in floral visitors between northern and southern populations. Whereas flowers in all populations were visited by hummingbirds, the large-bodied hummingbirds visiting plants in the southern regions are not present in the northern regions, where flowers are visited by hummingbirds with about half the body size and much shorter bills. This difference in body size of pollinators mirrors the geographic variation in flower size in M. viridiflora, suggesting that pollinator-mediated selection may be acting upon the species.

Geographic variation in traits of organisms attracts the attention of biologists because of the clues that it can give us about the evolutionary process. Such variation may be caused by chance events such as genetic drift or founder effects, or may be the result of natural selection due to geographically patterned variation in ecological factors. Variation among species often leads to speculation about how that variation may have come about, but when speciation is already complete, the selective pressures that led to divergence among species may not be currently operating. Studying geographic variation within species, where evolutionary divergence may be in progress, provides an opportunity to observe the speciation process in action.

Within-species variation in floral traits such as tube length, color, scent and nectar concentration has been linked to variation in pollinator assemblages in a number of cases (Grant and Grant 1965; Galen and Kevan 1980; Linhart and Feinsinger 1980; Robertson and Wyatt 1990). However, few intraspecific pollinator studies have been conducted, and they have tended to focus either on one trait over a wide range (Miller 1981) or have been limited to only a few sites (Robertson and Wyatt 1990; Johnson 1997). These limitations restrict how much may be understood about the relationship between floral traits and pollinator assemblages. Examination of a variety of traits across the

range of a species provides a better overview of the structure of geographic variation.

Macromeria viridiflora DC. (Boraginaceae) is an herbaceous perennial that exhibits geographic variation in floral traits. Turner (1994) described two varieties of M. viridiflora, with M. viridiflora var. thurberi found in New Mexico and central Arizona and having smaller flowers, and M. viridiflora var. viridiflora found in southwestern Arizona and northern México and having larger flowers. In much of its range, M. viridiflora occupies forested montane habitat of the Madrean sky islands (Heald 1951, Warshall 1995), a chain of isolated mountains that reach between the southern Rocky Moutains and the northern Sierra Madre Occidental. The Madrean sky islands dot the landscape throughout southeastern Arizona and into the northern Mexican states of Sonora and Chihuahua. Composed of an array of mountaintops separated by expanses of desert and desert grasslands in the lowlands between them, the Madrean sky islands provide a unique system for studying patterns of allopatric diversification. Although a number of floristic studies have been done on the Madrean sky islands (Reeves 1976; Leithliter 1980; Bowers and McLaughlin 1987; McLaughlin and Bowers 1990; Fishbein et al. 1994), there has been very little in-depth study comparing populations of plants or animals among these sky island habitats (for

exceptions see Slentz et al. 1999; Maddison and McMahon, in press).

Here I examine an array of morphological traits in Macromeria viridiflora throughout its range and describe the geographic patterns of variation. I also present data on floral visitors and consider the correlation of pollinator and flower characteristics. I will address the following questions: (1) Are the populations morphologically distinct, and are the distinguishing characteristics floral, vegetative or both? (2) Do vegetative and floral traits show similar patterns of geographic variation? (3) Are there geographic patterns in floral visitors that might account for variation in floral traits?

METHODS

The genus Macromeria (Boraginaceae) is mostly Mexican in distribution. Nine species are found exclusively in the mountainous areas of México and one is endemic to Guatemala. The remaining species, Macromeria viridiflora, is distributed from the mountains of the northern Sierra Madre Occidental north to the Mogollon Rim of Arizona and the southern Sangre de Cristo Mountains of New Mexico, at elevations between 1500 and 3000 m (Turner 1994). This species is an herbaceous perennial found in montane pine-oak and mixed-coniferous forests (habitat types sensu Brown and

Lowe 1980). Flowers are arranged in helicoid cymose inflorescences and have long, trumpet-shaped, pale green corollas. Each flower produces up to four pearly-white ovoid nutlets.

Flowering of Macromeria viridiflora coincides with the summer wet season (June - September), and plants die back to the ground with the onset of winter. Plants are self-compatible and can produce seed autogamously, but reproductive success is enhanced by pollinator visitation (Appendix B). The seeds of M. viridiflora have no special dispersal mechanism and the nutlets fall passively from the plants when ripe.

Field sites were located from specimens housed at the University of Arizona herbarium (ARIZ). I collected plants from one area on each of eight different mountain ranges (Table 1; Fig. 1). During 1997, 1998 and 1999, I made at least one collecting trip to each site during the flowering season to gather specimens. Plants were selected by choosing the nearest plant to each of a series of random points along transects through the population. These random points were selected by measuring distance along transect based on a number taken from a random number table. Transect length was equal to the length of the area where the plants were growing in each collection site. One stem was randomly selected from each plant for collection of

leaves and flowers (again, randomness was generated using a random number table). Because population sizes are small in many of the sites, I minimized damage to plants by removing only selected leaves, bracts and flowers from these selected stems, rather than collecting entire stems or plants. I selected the most recently opened flower on the sampled stem (determined by color of flower, position on inflorescence and presence of pollen on anthers) and its subtending bract for collection. Flowers are pale green before and immediately after anthesis, and fade to a very pale yellow in the second day after anthesis; therefore, color can serve as an indicator of flower age in this plant.

In addition, measurements for two characters (corolla length and length of leaf at stem midpoint) are included from five herbarium specimens collected from a ninth site in the Sierra El Tigre region of Sonora, Mexico. The geographic gap between the Rio Durazno and Huachucas collection site is large, and the addition of this site helps to narrow this gap. Herbarium specimens were used because I was unable to collect fresh specimens from this location. The two characters were selected because comparison of fresh and herbarium specimens indicated that they are not altered significantly by pressing and drying. Other characters measured were not consistent between fresh and dried specimens, and therefore were not considered.

Morphometrics. Twenty characters were recorded, including 17 measurements and three meristics; of these, nine were vegetative and eleven were floral (Table 2). Floral characters were taken from the most recently opened flower on the stem. Bract characters were taken from the bract subtending the flower used for character analysis. Leaf characters were taken from the most basal leaf on the stem and one intermediate at ca. 1/2 of total stem height. To examine leaf and bract shape, I combined length and width measurements of individual leaves and bracts as ratios in the univariate analyses.

All measurements were log-transformed and meristic variables were square-root transformed.

Analysis. My objective in this study of quantitative morphology was to test the null hypothesis of no differences among populations. To test this null hypothesis, data were analyzed using JMP (SAS Institute Inc. 1989-1999) and SAS (SAS Institute, Inc. 1988). I used ANOVA and Tukey-Kramer Highly Significant Difference test to compare single traits among populations. Data were then combined into two multivariate data sets: one including all traits, and another of floral traits only. I used principal components analysis (PCA) to examine these data sets for distinctions among populations. Discriminate functions analysis (DFA)

was used to determine how well the morphology serves to distinguish populations from one another.

To determine whether morphological size in M. viridiflora is clinal along a latitudinal gradient, I regressed the first principal component scores for individual plants against the latitude of each population. The first principal components were used because all PC1 eigenvectors from both data sets (vegetative and floral) are positive, indicating that the first principal components are composite measures of size (Manly, 1986).

To test for correlation between geographic and phenotypic distance, I calculated Mahalanobis distances among all populations based on the 20 morphological characters. Mahalanobis distance calculates distance between multivariate populations, and takes into account the correlation between variables (Manly 1986). I then calculated the correlation between geographic distances and Mahalanobis distances using the Mantel test in the statistical program CADM (Legendre, 2001).

Floral Visitor Observations. My field assistants and I observed flower visitation by potential pollinators at groups of plants for a total of 72 hr on Mt. Lemmon and 14.5 hr at South Fork in 1998, 117 hr on Mt. Lemmon and 64 hr at South Fork in 1999. Observations included periods throughout the day and covered a variety of weather

conditions, ranging from sunny and warm to cool and rainy. No visitors were seen during observations before 0500 and after 2030; therefore, data presented here are limited to the hours between 0500 and 2030. Populations at other sites were observed for shorter periods (24.0 hr at Río Durazno, 18.0 hr at Galiuro Mts., 8.0 hr at Huachuca Mts., 21.0 hr at Santa Rita Mts., 8.0 hr at Sedona, 8.0 hr at Sangre de Cristo Mts.). At all locations, visits were recorded per group of plants being watched.

RESULTS

All floral characters and all but one vegetative character vary significantly among populations (Figs. 2 and 3), even after accounting for multiple tests using the sequential Bonferroni test (Rice 1989). Corolla overall length and lobe length, style length, sepal length and width, and anther length are generally larger in the southern populations and smaller in the northern populations. These characters are correlated with one another, and their correlation coefficients were statistically significant (Table 3).

Regression of overall floral size (as represented by PC1) vs. latitude shows a strong negative relationship ($r = 0.79$, $n = 122$, $df = 1$, $p < 0.0001$). The variation in floral size is continuous and clinal in nature, rather than

demarcating the species into morphologically discontinuous geographic groups (Fig. 2).

Corolla widths (at orifice and at ovary), corolla lobe width, and filament length and width follow the same pattern (larger in south, smaller in north; however, in these traits, the flowers from the Sangre de Cristos tend to be more similar to the flowers in the Mt. Lemmon and Galiuros populations (i.e., larger) than to those of their near neighbors in the north; and in corolla lobe width the flowers in the Santa Rita population are similar to the northern populations (i.e., smaller).

Except for number of bract veins, vegetative traits also vary significantly among populations (Fig. 3). However, linear regression does not show any relationship between vegetative size and latitude ($r = 0.0097$, $n = 102$, $df = 1$, $p = 0.9225$), and geographic variation in vegetative traits is difficult to describe. Leaves at stem midpoint and bracts are generally larger in the Río Durazno, Huachucas, Santa Ritas and Sangre de Cristos populations and smaller in the South Fork and Galiuros populations. Basal leaves are larger in the Sangre de Cristos and Sedona populations and smaller in the Huachucas populations.

Principal components analysis (PCA) of all characters does not clearly separate populations in a few dimensions (Fig. 4). The first principal component (PC1) accounts for

37.8% of the variation in the data set, and consists primarily of information from floral characters. All of these floral characters have positive eigenvectors so that this first component is largely an indication of floral size.

The second principal component has low eigenvectors for the floral characters and high eigenvectors for the vegetative characters. These vegetative characters have all positive eigenvectors for PC2 and so this PC is primarily an indicator of vegetative size. Because PC1 and PC2 are by definition not correlated (Manly 1986), this division of floral and vegetative characters between the two PCs indicates that there is little correlation between floral and vegetative characters among populations.

The PCA of floral characters alone separates populations somewhat more strongly, although there is still a large degree of overlap among populations (Fig. 5). PC1 accounts for 59.6% of the variation in the data set. All eigenvectors are positive and therefore this PC is a measure of overall floral size. PC2 accounts for an additional 10.1% of the variation in the data set, and is mostly a measure of the relationship among androecial and sepal characters. Corolla width at ovary, filament width and corolla lobe width have positive eigenvectors whereas anther length and sepal length have negative eigenvectors so that

on the positive end of the PC2 axis plants have wider corollas, filaments and corolla lobes with shorter anthers and sepals, and on the negative end they have narrower corollas, filaments and corolla lobes with longer anthers and sepals. PC3 accounts for only 6.9% of the variation and is composed of information on sepal size and flare of corolla (width of corolla at ovary vs. orifice) .

When PC1 and PC2 of floral characters (together accounting for 69.7% of the variation) are plotted for individual plants (Fig. 4), individuals from the same population tend to cluster together although there is substantial overlap among populations. However, there is no indication of plants clustering into two groups as might be expected if two varieties existed in the species.

Discriminant functions analysis (DFA) of all characters indicated that plants can be linked to their source population very accurately: 95% (87 of 92 plants) were correctly assigned (Table 6). Of the five plants misplaced, two were assigned to a nearest-neighbor population (one plant from South Fork placed in Sedona, and one plant from Sedona placed in South Fork), and two others was assigned to the population closest in latitude (two plants from Sangre de Cristos were assigned to Sedona). All of the plants from four of the eight populations were correctly assigned (Río Durazno, Huachucas, Mt. Lemmon, and Santa Ritas).

Plants can be linked to their source population fairly accurately based on floral data alone: in DFA of floral characters, 84% (80 of 95 plants) were correctly assigned (Table 7). Of the fifteen misplaced plants, six were assigned to a nearest-neighbor population, and no plants from the Río Durazno were misplaced.

The correlation between geographic distance and phenotypic distance is high (Mantel correlation coefficient = 0.52, p =0.047), indicating that in general sites that are geographically proximate to one another tend to have more similar morphologies than those that are geographically distant to one another.

Hummingbirds were the most frequent visitors, with hawkmoths (Hyles lineata) as a second frequent visitor at several sites (Table 8). Small bees and large flies occasionally visited flowers (Table 8), but did not contact the stigma. The species and size of the hummingbird visitors varied among sites. The more southern populations (Río Durazno, Santa Ritas and Mt. Lemmon) were visited by large hummingbirds with long culmens (i.e., magnificent [Eugenes fulgens] and blue-throated [Lampornis clemenciae] hummingbirds). These species have ranges that extend only as far north as southern Arizona and do not include the northern populations of Macromeria viridiflora (Johnsgard 1983). The more northern populations were visited by

smaller hummingbirds with shorter culmens (i.e., rufous [Selasphorus rufus] and broad-tailed [Selasphorus platycercus] hummingbirds). The smaller hummingbird species are present at Mt. Lemmon and did occasionally visit flowers of M. viridiflora, but were far more uncommon at that site than magnificent hummingbirds (Table 8).

DISCUSSION

The lack of correlation between vegetative and floral characters indicates that differences in floral size are not the simple result of differences in overall plant size. Vegetative characters varied among populations but did not follow a latitudinal gradient as did the floral characters.

Variation in floral traits mostly follows a latitudinal gradient in which flower size decreases as latitude increases. The hummingbirds visiting these plants also vary in size latitudinally. Northern populations of Macromeria viridiflora, where corollas are shorter, are visited by species with smaller bodies and shorter culmens (i.e., broad-tailed and rufous hummingbirds). Southern populations of M. viridiflora, where corollas are longer, are visited by species with larger bodies and longer culmens (i.e., magnificent and blue-throated hummingbirds). These larger-billed hummingbirds are restricted to the southern portion of the range of M. viridiflora, whereas the smaller-billed

hummingbirds are present throughout the plant's range (Johnsgard 1983) and occasionally visit M. viridiflora at the southern sites (Table 8). However, their visitation rate is low at sites where the larger-billed hummingbirds are present (Appendix B). The smaller-billed hummingbirds may be competitively and aggresively excluded from the flowers by larger-billed hummingbirds at these sites because dominance in hummingbirds is largely based on body size (Stiles and Wolf 1970, Cotton 1998).

This relationship between corolla size and bill size of hummingbird visitors suggests that selection by pollinators may have been an important influence in the evolution of floral variation in this species. Other environmental factors vary latitudinally, such as temperature and day length, and may play a role in natural selection on plant traits. However, because vegetative traits in this species do not vary latitudinally, it seems more likely that variation in floral morphology is due to pollinator variation.

Although hummingbirds are the most common floral visitors, hawkmoths are also common visitors. Both hummingbirds and hawkmoths have been shown to be effective pollinators of M. viridiflora (Appendix B), and therefore both must be considered in any examination of possible pollinator-mediated selection on floral traits.

Two geographically separated varieties of the species M. viridiflora were recognized by Johnston (1954) based solely upon flower size, and Turner (1994) supports this division. Both authors did recognize that intermediate populations existed in some mountains in the central parts of the species' range. Close analysis of the morphometric variation throughout the range of the species suggests that the variation in corolla size and shape that supposedly distinguish these two varieties is in fact clinal and continuous in nature. This can be seen both in the individual floral characters, which show continuous variation and do not fall into distinct population groupings (Fig. 2), and in the principal components graph (Fig. 4), which also shows no clustering of individuals or populations that could be interpreted as distinct varieties. I would therefore strongly recommend that the species not be divided into varietal groups, as this distinction is artificial: it does not match the clinal pattern of variation or recognize the significant overlap among geographic populations.

Interestingly, the flowers of M. viridiflora do not fit the traditional hummingbird pollination syndrome (Faegri and van der Pijl 1979). Flowers have the tubular shape expected in hummingbird-pollinated plants, but are greenish-white in color rather than the expected bright red or yellow, and open in the late afternoon. The color and time of anthesis

of these flowers may be adaptations for attracting moths, the second major pollinator group for the plants. On the other hand, the flowers are pendant and have no discernible odor; they therefore do not fit the traditional hawkmoth pollination syndrome either. While the concept of pollinator syndromes may illustrate trends in floral characters related to particular pollinators, in reality floral evolution in a species may be affected by more than one pollinator type, leading in some circumstances to evolution of floral morphology and/or phenology that is an intermediate compromise (for further review and commentary of pollination syndromes see Herrera 1996; Waser et al. 1996; Wilson and Thomson 1996).

Differentiation among sky islands in this species is strong in both vegetative and floral characters. Seeds are not likely to move long distances and so any gene flow among sky islands can probably be attributed to pollen dispersal. In general, the correlation between geographic distance and phenotypic (Mahalanobis) distance among populations is high. It would be interesting to know whether exceptions to this correlation are related to movement of pollinators. Unfortunately, no specific information is known about movement of individual hummingbirds and moths among sky islands.

Variation in flower length has been related to pollinator variation in other plants. Nilsson (1988) experimentally reduced the length of floral spurs in orchids (Platanthera spp.) and showed that spur length affected the likelihood of pollination by moths. When spur length was shorter than the moth's tongue length, pollen removal and receipt were both reduced. This demonstrates that pollinators have the potential to cause selection on floral tube length. Several studies have demonstrated intraspecific variation in flower length related to pollinator variation (Grant and Grant 1965; Miller 1981; Robertson and Wyatt 1990; Johnson 1994; Arroyo and Dafni 1995; Johnson 1997; Johnson and Steiner 1997). Here I have examined a variety of traits throughout the range of M. viridiflora, obtaining a broad view of geographic variation in morphological traits and pollinators of this species.

In conclusion, populations of M. viridiflora are quite distinct in both floral and vegetative traits. Floral traits show a pattern of decreasing size with increasing latitude; vegetative traits do not follow this pattern. Southern populations are visited by larger hummingbirds than northern populations, which might account for the observed pattern in floral traits. However, hawkmoths are also frequent floral visitors and should be taken into consideration as part of the plant's pollinator system.

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TABLE 1. Locations of populations of Macromeria viridiflora from which samples were taken, listed in order of latitude from south to north.

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1. Río Duraznos (DUR): Rancho El Capitán, Río Duraznos (28° 13′ 30″ N, 108° 07′ 30″ W), 2100 m elev., Chihuahua, Mexico. T. Van Devender 87-131 (ARIZ).
2. Sierra el Tigre: Río de Bavispe region, between Las Tierritas and El Tigre, Sonora, Mexico. Phillips 643 (MICH).
3. Huachuca Mountains: Miller Peak, 2880 m elev., Cochise County, Arizona, USA. A. Boyd 252 (ARIZ).
4. Santa Rita Mountains: Northeast slope of Mt. Wrightson, Bellows Spring (31° 42′N, 110° 51′W), 2545 m elev., Santa Cruz County, Arizona, USA. M. Fishbein 2118 (ARIZ).
5. Mount Lemmon: Marshall Gulch picnic area and Aspen Loop Trail, 2280 m elev., Pima County, Arizona, USA. A. Boyd 251 (ARIZ).
6. Galiuro Mountains: Ash Canyon, ca. 2.5 miles east of Upper Ash Spring (32° 30′N, 110° 14.5′ W), 1562 m elev., Graham County, Arizona, USA. M. Fishbein 2846 (ARIZ).

7. South Fork: Forested area around South Fork Campground on Little Colorado River, 2317 m elev., White Moutains, Apache County, Arizona, USA. M. Schmidt 109 (ARIZ).
8. Sedona: West Fork Canyon of Oak Creek Canyon, Coconino National Forest, 1402 m elev., Coconino County, Arizona, USA. D. Demaree 41269 (ARIZ).
9. Sangre de Cristo Mountains: Santa Fe National Forest, Gallinas Canyon, approx. 35° 43′ N, 105° 37′ W, along road about one-half mile before entrance to Evergreen Valley, San Miguel County, New Mexico, USA. Sagalyn 97 (DUKE).

TABLE 2. Morphological characters included in the study of Macromeria viridiflora. All measurements are in millimeters.

1. Length of stem base leaf
2. Width of stem base leaf
3. Number of major secondary veins on leaf at stem base
4. Length of stem midpoint leaf
5. Width of stem midpoint leaf
6. Number of major secondary veins on stem midpoint leaf
7. Bract length
8. Bract width
9. Number of major secondary veins on bract
10. Corolla length
11. Corolla width at ovary
12. Corolla width at orifice
13. Corolla lobe length
14. Corolla lobe width
15. Filament length
16. Filament width
17. Anther length
18. Style length
19. Sepal length
20. Sepal width

TABLE 3. Pearson correlation coefficients (r) among six floral characteristics measured in eight populations of Macromeria viridiflora from Arizona, New Mexico and Mexico. All correlations were significant with $p<0.0001$ (Student's t test)

Variable	Corolla length	Style length	Lobe length	Sepal length	Sepal width	Anther length
Corolla length	1.00	0.95	0.73	0.70	0.55	0.59
Style length		1.00	0.73	0.67	0.61	0.66
Lobe length			1.00	0.60	0.56	0.64
Sepal length				1.00	0.63	0.52
Sepal width					1.00	0.46
Anther length						1.00

TABLE 4. Eigenvectors and eigenvalues for the first three principal components analysis of all characters, together explaining 59.41% of the variation in the data set. Characters are listed in descending order of PC1 loadings.

Character	PC1	PC2	PC3
Style length	0.3346	-0.0629	0.0204
Corolla length	0.3280	-0.0652	0.0430
Corolla lobe length	0.3072	-0.0915	-0.0781
Corolla width at orifice	0.3035	0.0391	-0.0457
Corolla lobe width	0.2902	0.0570	0.0771
Filament length	0.2770	0.0235	-0.0492
Anther length	0.2647	-0.1173	-0.0056
Sepal length	0.2638	-0.0568	-0.1428
Sepal width	0.2601	-0.0756	0.1967
Filament width	0.2576	0.1144	0.2834
Corolla width at ovary	0.2332	0.1025	-0.0502
Bract length	0.1751	0.2905	-0.3882
Stem midpoint leaf length	0.1364	0.4136	-0.0797
Stem base leaf length	-0.1093	0.3040	-0.2504
Stem base leaf width	-0.1077	0.3407	0.0134
Stem midpoint leaf width	0.0957	0.4108	0.2796
Bract, no. of veins	-0.0939	0.1364	0.2284
Stem base leaf, no. of veins	-0.0890	0.2603	0.2285

Bract width	0.0709	0.4008	-0.3374
Stem midpoint leaf, no. of veins	-0.0037	0.2045	0.4132
Eigenvalues	7.56	2.96	1.37
% variance explained	37.78	14.78	6.85

TABLE 5. Eigenvectors and eigenvalues for the first three principal components analysis of floral characters, together explaining 76.53% of the variation in the data set. Characters are listed in descending order of PC1 loadings.

Character	PC1	PC2	PC3
Style length	0.3563	-0.1435	-0.0719
Corolla length	0.3438	-0.1531	-0.0956
Corolla lobe length	0.3274	-0.1601	-0.1755
Corolla width at orifice	0.3205	0.1242	-0.3680
Corolla lobe width	0.3105	0.3446	-0.1858
Filament length	0.3010	0.1750	-0.2861
Filament width	0.2721	0.4093	0.2528
Sepal length	0.2740	-0.4400	0.3816
Anther length	0.2748	-0.3268	-0.1636
Sepal width	0.2734	-0.2329	0.4942
Corolla width at ovary	0.2419	0.4934	0.4719
Eigenvalues	6.55	1.11	0.75
% variance explained	59.58	10.10	6.85

Table 6. Number (above) and proportion (below) of plants from source locations that were identified by Discriminant Functions Analysis of combined floral and vegetative data as from the locations listed above.

Source Location	Number and percentage of individuals classified into location:							
	Rio Durazno	Huachucas	Santa Ritas	Mt. Lemmon	Galiuros	South Fork	Sedona	Sangre de Cristos
Rio Durazno	17 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0
Huachucas	0 0.0	6 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0
Santa Ritas	0 0.0	0 0.0	12 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0
Mt. Lemmon	0 0.0	0 0.0	0 0.0	12 100.0	0 0.0	0 0.0	0 0.0	0 0.0
Galiuros	0 0.0	0 0.0	0 0.0	0 0.0	13 86.7	1 6.7	0 0.0	1 6.7
South Fork	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	11 91.7	1 8.3	0 0.0
Sedona	0 0.0	0 0.0	1 9.1	0 0.0	0 0.0	1 9.1	9 81.8	0 0.0

Sangre de Cristos	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	2 16.7	10 83.3

Table 7. Number (above) and proportion (below) of plants from source locations that were identified by Discriminant Functions Analysis of floral data as from the locations listed above.

Source Location	Number and percentage of individuals classified into location:							
	Rio Durazno	Huachucas	Santa Ritas	Mt. Lemmon	Galiuros	South Fork	Sedona	Sangre de Cristos
Rio Durazno	17 100.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0
Huachucas	1 16.7	5 83.3	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0
Santa Ritas	0 0.0	0 0.0	10 83.3	0 0.0	2 16.7	0 0.0	0 0.0	0 0.0
Mt. Lemmon	0 0.0	0 0.0	0 0.0	11 91.7	0 0.0	0 0.0	1 8.3	0 0.0
Galiuros	0 0.0	1 6.7	0 0.0	0 0.0	9 60.0	4 26.7	0 0.0	1 6.7
South Fork	0 0.0	0 0.0	1 8.3	0 0.0	1 8.3	8 66.7	2 16.7	0 0.0
Sedona	0 0.0	0 0.0	1 9.1	0 0.0	0 0.0	1 9.1	9 81.8	0 0.0

Sangre de Cristos	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	0 0.0	1 8.3	11 91.7

Table 8. Hours of observation and lists of visitors observed at M. viridiflora flowers.

Site	No. of hours of observation	No. of visits observed	Visitors
Mt. Lemmon	189	79	Eugenes fulgens (magnificent hummingbird)
		74	Hyles lineata (white-lined sphinx moth)
		4	Selasphorus sp. (rufous or broadtailed hummingbird)
		2	Small bees
South Fork	78.5	173	Selasphorus rufus (rufous hummingbird)
		16	Hyles lineata (white-lined sphinx moth)
		2	Small bees
Rio Durazno	24	2	Eugenes fulgens (magnificent hummingbird)
		2	Lampornis clemenciae (blue-throated hummingbird)
Galiuros	18	4	Selasphorus sp. (rufous or broadtailed hummingbird)
Huachucas	8	--	No visitors observed
Santa Ritas	21	9	Lampornis clemenciae (blue-throated hummingbird)
		1	Small bee
		1	Large fly

Sedona	8	4	Selasphorus sp. (rufous or broadtailed hummingbird)
Sangre de Cristos	8	4	Selasphorus sp. (rufous or broadtailed hummingbird)

Figure Legends

FIG. 1. Map showing known range (hatched area) of Macromeria viridiflora, and collection locations: (1) Río Durazno, (2) Sierra el Tigre, (3) Huachuca Mountains, (4) Santa Rita Mountains, (5) Mt. Lemmon, (6) Galiuro Mountains, (7) South Fork, (8) Sedona, (9) Sangre de Cristo Mountains.

FIG. 2. Plots for Macromeria viridiflora of 11 floral characters by the geographic locations shown in Fig. 1. Small squares indicate mean for each population, and bars show standard error. All characters measured showed statistically significant differences among populations. Horizontal bars across the top of each plot show the results of Tukey-Kramer HSD test, with each bar connecting groups that were not significantly different from one another ($p > 0.05$). Populations are arranged in the order that most clearly displays Tukey-Kramer results. For the most part this order is by decreasing means, but notable exceptions occur in the Huachucas population (#3) because of small sample size and large variation. Population #2 (Sierra El Tigre) is included in only one of the character analyses because the data from this site were from herbarium specimens and most characters are altered significantly by

pressing and drying, rendering them incomparable between herbarium and fresh specimens.

FIG. 3. Plots for Macromeria viridiflora of 9 vegetative characters by the geographic locations shown in Fig. 1. Small squares indicate mean for each population, and bars show standard error. All characters except basal leaf veins showed statistically significant differences among populations. Horizontal bars across the top of each plot show the results of Tukey-Kramer HSD test, with each bar connecting groups that were not significantly different from one another ($p > 0.05$). Populations are arranged in the order that most clearly displays Tukey-Kramer results. For the most part this order is by decreasing means, but exceptions occur in the Huachucas population (#3) because of small sample size and large variation. Population #2 (Sierra El Tigre) is included in only one of the character analyses because the data from this site were from herbarium specimens and most characters are altered significantly by pressing and drying, rendering them incomparable between herbarium and fresh specimens.

FIG. 4. First two principal components from PCA analysis of combined floral and vegetative data, plotted for individual plants. Symbols indicate populations where collections were

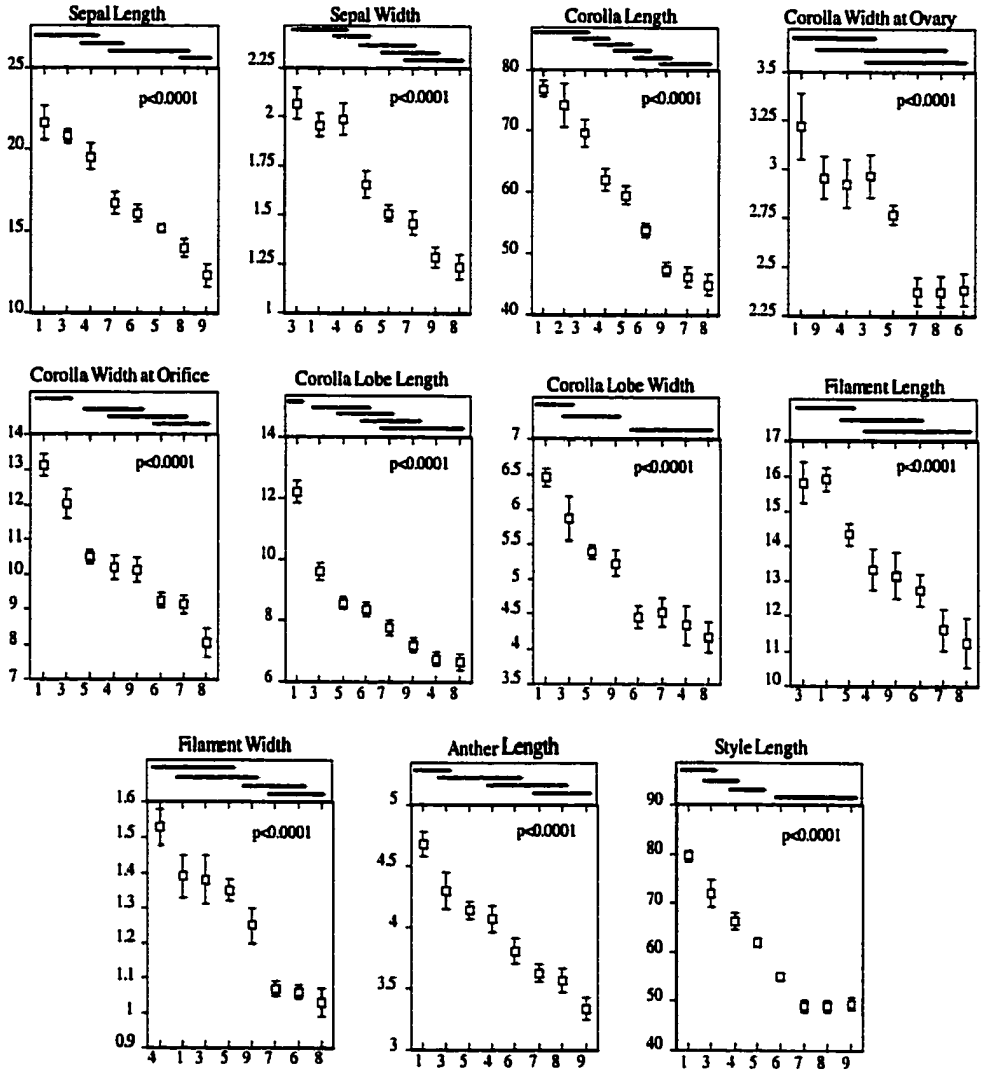
made; see Table 1 for site information. Although individuals from each population tend to cluster together, there is substantial overlap among populations.

FIG. 5. First two principal components from PCA analysis of floral data, plotted for individual plants. Symbols indicate populations where collections were made; see Table 1 for site information. Although individuals from each population tend to cluster together, there is substantial overlap among populations.

Arizona
New Mexico
UNITED STATES
MEXICO
Sonora
Chihuahua
Sinaloa
Durango
Gulf of California
1
2
3
4
5
6
7
8
9
26°
112°
200 km

Fig. 1

Fig. 2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Corolla Lobe Length
p<0.0001
Corolla Lobe Width
p<0.0001
Filament Length
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Filament Width
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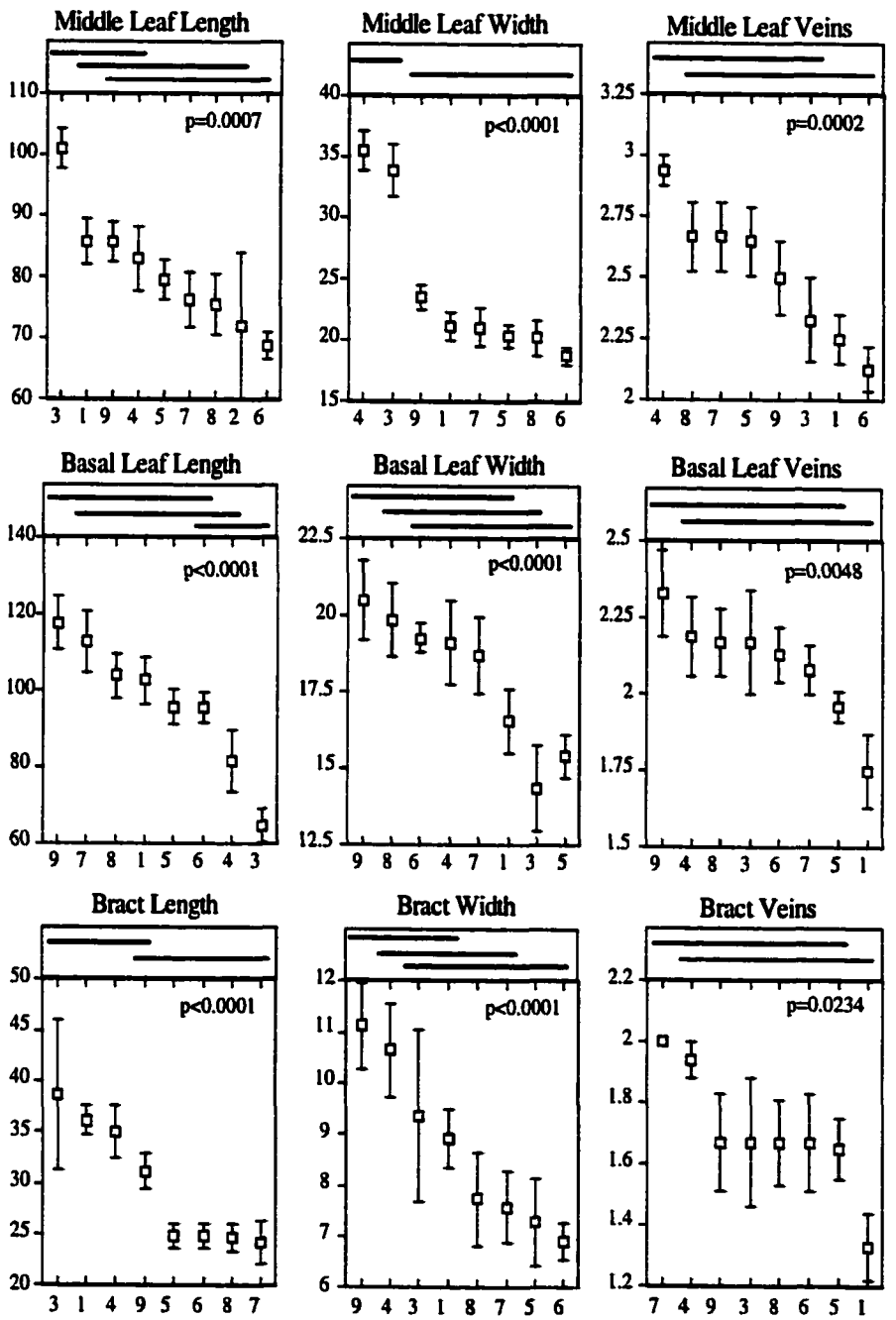
Anther Length
p<0.0001
Style Length
p<0.0001

Fig. 3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20
15
4 3 9 1 7 5 8 6
Middle Leaf Veins
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3
2.75
2.5
2.25
2
4 8 7 5 9 3 1 6
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100
80
60
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Basal Leaf Width
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20
17.5
15
12.5
9 8 6 4 7 1 3 5
Basal Leaf Veins
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2.25
2
1.75
1.5
9 4 8 3 6 7 5 1
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45
40
35
30
25
20
3 1 4 9 5 6 8 7
Bract Width
p<0.0001
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11
10
9
8
7
6
9 4 3 1 8 7 5 6
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1.6
1.4
1.2
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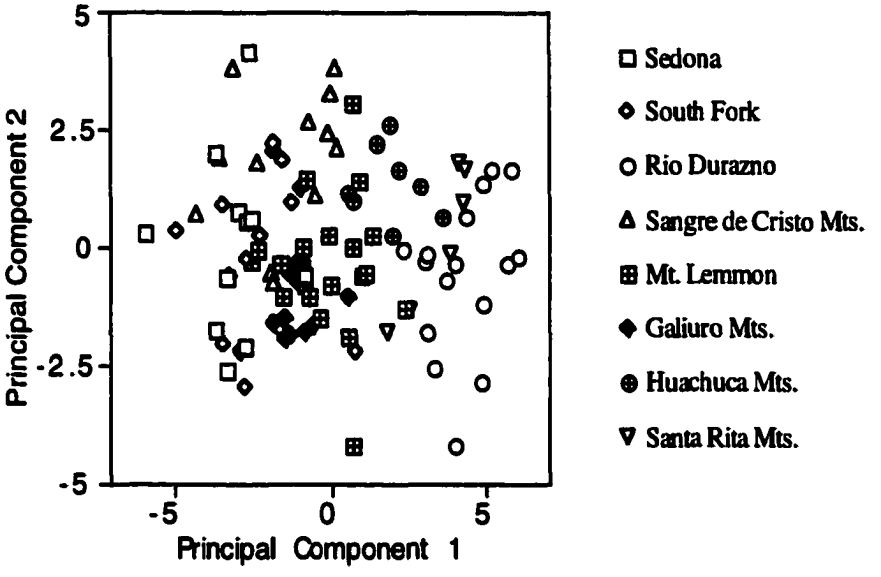
Fig. 4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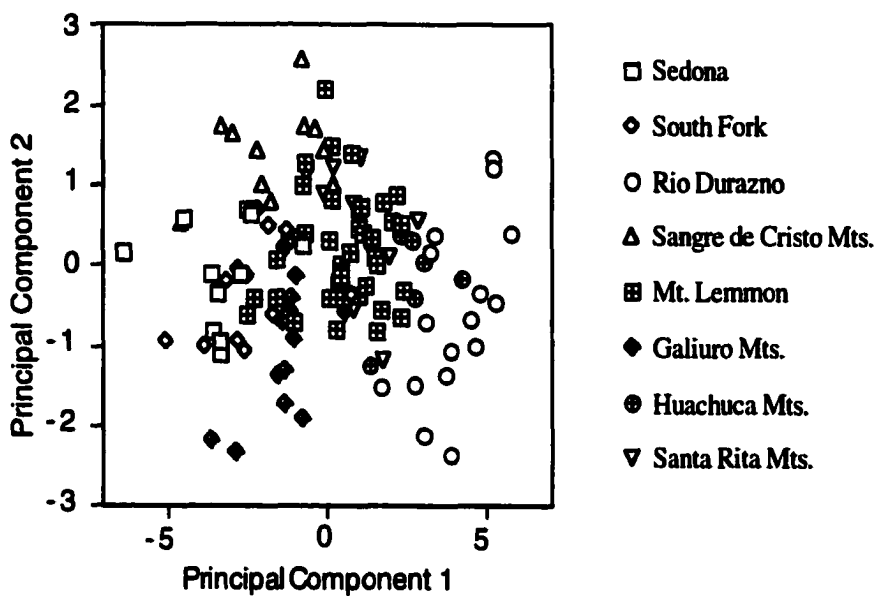
Mt. Lemmon
Galiuro Mts.
Huachuca Mts.
Santa Rita Mts.

Fig. 5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Principal Component 2
Principal Component 1
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South Fork
Rio Durazno
Sangre de Cristo Mts.
Mt. Lemmon
Galiuro Mts.
Huachuca Mts.
Santa Rita Mts.

APPENDIX B

POLLINATION BIOLOGY AND BREEDING SYSTEM IN *MACROMERIA VIRIDIFLORA*: AN EXAMPLE OF A COMBINATION POLLINATOR SYNDROME

Abstract--This study explores the association between variation in pollinator type and flower size variation in Macromeria viridiflora (Boraginaceae) by studying the pollinator attractants and rewards presented by the plants, plant breeding system, and pollinator effectiveness of floral visitors. Studies were conducted at two sites where plants differ in flower size and floral visitors. Flowers of M. viridiflora have two characteristics that fit both the hummingbird and hawkmoth pollinator syndromes, copious sucrose-rich nectar and long floral tubes. However, they also have characteristics that each correspond with a single major pollinator. This plant therefore presents a combination floral syndrome that attracts two classes of pollinators.

Breeding system studies showed that while plants are self-compatible and occasionally produce seed autogamously, pollinators are important for reproductive success in the plants. Combining visitation rate and pollen deposition as measures of pollinator effectiveness, I found hummingbirds to be the most effective pollinators at both sites. Although hawkmoths also pollinate the flowers, they visit the flowers less frequently and, at one of the two sites, deposit less pollen.

Biologists have long invoked evolution by natural selection to explain relationships between the traits of pollinating animals and those of the flowers they visit. Attracting and positioning pollinators so that effective pollen transfer occurs are necessary for the reproductive success of plants that depend on animal pollinators. Floral tube length and spur length may directly affect the physical transfer of pollen from anther to pollinator and/or from pollinator to stigma. Therefore, there is great potential for pollinator-mediated selection on these traits. Tube length and spur length have been the traits most frequently identified in intraspecific studies as exhibiting geographic variation associated with pollinator identity (Grant & Grant 1965, Miller 1981, Robertson & Wyatt 1990, Johnson 1994, Arroyo & Dafni 1995, Johnson 1997, Johnson & Steiner 1997; but see Herrera 1996 for evidence to the contrary).

Geographic variation in floral and vegetative traits of Macromeria viridiflora (Boraginaceae) has been documented in a study of the morphometrics of populations across the species' range, from southern Chihuahua, Mexico, north to the Sangre de Cristo Mountains of New Mexico (Appendix A). Although variation in vegetative traits follows no perceptible latitudinal pattern, variation in flower size follows a latitudinal gradient: corollas are shortest in the northernmost populations (ranging from 4.2 to 5.2 cm) and

longest in the southernmost populations (ranging from 6.7 to 8.5 cm). The populations with the shorter-flowered plants are visited by hummingbirds with short culmens, and the populations with longer-flowered plants are visited by hummingbirds with longer culmens (Appendix A), suggesting that this geographic variation in flower size may be associated with hummingbird pollinator-mediated selection.

Three issues must be addressed before assuming that variation in corolla length may be driven by variation in bill size of hummingbird pollinators. First, it must be shown that plants depend on visitors for reproduction; the plant's breeding system has not been studied previously. Second, hummingbirds are the most important pollinators; these plants do not have entirely typical hummingbird-pollinated flowers, and in the two populations where extensive observations have been conducted the flowers are also commonly visited by hawkmoths (Appendix A). Third, it must be shown that floral visitors are in fact pollinators; information is needed on the effectiveness of its floral visitors, and the potential for either hummingbirds or hawkmoths to be agents of selection on its floral traits.

The present study seeks to clarify the association between variation in pollinator type and flower size variation in Macromeria viridiflora. The following three questions are addressed: (1) How important are pollinators

in the plant's breeding system? (2) How consistent are floral traits with expected pollinator syndromes for hawkmoths and hummingbirds? (3) What is the relative pollinator effectiveness of the floral visitors?

To elucidate the role of pollinators in reproductive success, it is essential to know the breeding system of the plant. In experiments at two sites where plants differ in flower size and floral visitors, I tested for self-compatibility, autogamy, and the potential for pollinator visits to increase seed set.

Hummingbird- and hawkmoth-pollinated flowers are expected to be similar in having long slender floral tubes and copious sucrose-rich nectar (Wyatt, 1983), but are expected to differ in time of anthesis (diurnal for hummingbirds vs. nocturnal or crepuscular for hawkmoths), fragrance (none for hummingbirds vs. sweet scent at night for hawkmoths), and color/spectral properties (bright red or contrasting bright colors for hummingbirds vs. white or pale green or yellow for hawkmoths). I examined the pollinator attractants (spectral reflectance of flowers and floral scent) and rewards (nectar volume, concentration, and sugar composition) presented by the flowers of M. viridiflora. I discuss the implications of this information in the context of the concept of pollinator syndromes (Faegri and van der Pijl 1979, Wyatt 1983) within this plant/pollinator system.

It is also necessary to know the pollination effectiveness of common visitors in order to evaluate the potential of these visitors as agents of selection. I compared pollination effectiveness among different visitors within and between sites by analyzing frequency, duration and pollen deposition rates of floral visits.

METHODS

Macromeria (Boraginaceae) is a small genus composed of 11 species found throughout Mexico, with one species extending into southwestern United States and another endemic to Guatemala (Turner, 1994). Macromeria viridiflora is an herbaceous perennial found in montane pine-oak and mixed-coniferous forests. It is distributed from the mountains of the northern Sierra Madre Occidental north to the Mogollon Rim of Arizona and the southern Sangre de Cristo Mountains of New Mexico, at elevations between 1500 and 3000 m (Turner 1994). Flowers are arranged in helicoid cymes and have long (3.5-8.5 cm), trumpet-shaped, pale green corollas that fade to pale yellow with age. Flowering of Macromeria viridiflora coincides with the summer wet season (June - September), and plants die back to the ground with the onset of winter. Each flower produces up to four pearly-white, ovoid nutlets. These nutlets have no special

dispersal mechanism and fall passively from the plants when ripe.

Field work was conducted at two sites: Mt. Lemmon, in the Santa Catalina Mountains of southeastern Arizona (32° 25′ N ,110° 45′ W), and the South Fork of the Little Colorado River in the White Mountains of east-central Arizona (34° 05′ N, 109° 25′ W). There were two locations used on Mt. Lemmon. One location was in Marshall Gulch, 1 km south of Summerhaven; plants used in the study were located either within the picnic area in Marshall Gulch or just beyond the head of the Marshall Gulch Trail. The second location was along the Mt. Lemmon trail #5. The South Fork study site was located on the floodplain and surrounding slopes of South Fork creek, with plants located within a mile upstream and downstream from the South Fork campground.

Unless otherwise noted below, all statistical analyses were performed in JMP (1989-1999).

Breeding system. At each site, five plants with more than six flowering stems each were selected for the study; plants of this size produce sufficient flowers to permit all treatments to be conducted simultaneously. On each selected plant, one stem was chosen at random for each of six

treatments. The five most mature buds on each stem were tagged for use in the study, for a total of five flowers per plant assigned to each treatment. The six treatments were:

(1) unbagged control;

(2) bagged, emasculated;

(3) bagged, emasculated, self-pollinated by hand;

(4) bagged, emasculated, outcross-pollinated by hand;

(5) bagged, not emasculated.

For self-pollination by hand, pollen was taken from the anthers of the flower being pollinated. For outcross-pollination by hand, pollen was taken from a flower from a plant in the population that was not otherwise being used in the breeding system study. To transfer pollen, a single anther was removed from the donor flower using a pair of forceps, and the pollen was then applied directly from this anther to the recipient flower's stigma. Hand pollinations were performed the first day that the flower was open.

Visitors were excluded from the flowers in bagged treatments 2 through 5 by covering the flowers with fine-mesh cloth bags secured with twist-ties. Bags were placed over flowers before anthesis and were removed after flowers wilted or fell from the plants. Because anthers dehisce prior to anthesis, flowers in treatments 2 through 4 were emasculated in the bud before anther dehiscence. After 21-

28 days, treated flowers were assessed for fruit and seed set.

Results of treatments were compared using one-way ANOVA. Three a priori contrasts were made comparing the following treatments:

--(2) and (5), to determine if plants produce seed through autogamy;

--(3) and (4), to determine if plants are self-compatible;

--(1) and (5), to determine if seed set is increased by the presence of pollinators.

Variance associated with the results for treatment 2 differed sharply from that for the other treatments. Because ANOVA assumes homogeneity of variance, treatment 2 was omitted from the ANOVA. I used Welch's approximate t-test (Zar 1984) for the first of these comparisons because it does not assume equal variances. The other two comparisons were made using ANOVA.

Timing of floral anthesis. Observations of the timing of floral anthesis were conducted on Mt. Lemmon in July 1997 and at South Fork in July 2000. At each site, 40 flower buds were marked for observation. Flower buds were selected if they appeared to be fully developed (i.e., approximately the size of open flowers) and were adjacent to an open

flower on the inflorescence, indicating that they would be the next bud to open. These buds were marked at 14:30 and observed hourly from that time until 19:30 the first day and from 5:30 to 17:30 the following day. Time of opening was recorded for each bud.

Nectar concentration and composition. At each site, 20 newly opened flowers were bagged to allow accumulation of nectar in the absence of pollinators. After 12 hours, nectar was collected from each flower using a 5-microliter glass pipette inserted down the throat of the corolla to the base. Concentration of sugars in nectar was measured using a hand-held refractometer; when necessary, nectar from several flowers was pooled to amass adequate volume for measuring with the refractometer. Nectar concentration between sites and between morning and afternoon collections were compared using Student's t-test.

Nectar from three to four flowers from each of five plants on Mt. Lemmon was collected in the same manner as above for analysis of sugar composition. Sugar composition was analyzed using methods described in Adams et al. (1992). The nectar was stored in Eppendorf tubes in the freezer until it could be diluted and analyzed. The analysis of sugars was performed using a Dionex (Sunnyvale, CA) HPLC system in conjunction with a Dionex Carbopac PA1 column with

150 mM NaOH. Carbohydrate detection was by pulsed amperometric detection (Dionex Cadvanced PAD) at 35°C. Detection limits for each sugar were at least 10 pmoles/50 µL sample. Nectar composition within and among plants was compared using ANOVA.

Floral scent. To determine whether flowers of M. viridiflora produce scent compounds that might attract pollinators, floral fragrance was collected in the field using the dynamic head-space collection method described by Raguso and Pellmyr (1998) and references therein. Simultaneous collections of ambient and vegetative volatiles were used to distinguish between true floral compounds, compounds emitted from the vegetation, and ambient contaminants. Because most flowers that produce scent compounds to attract moths release their scent at night, scent collections commenced at dusk (ca. 19:30) and continued for 12 hrs. Scent was not collected during the day because diurnal visitors were mostly hummingbirds, which do not use odor as a means to locate flowers (Proctor et al. 1996). Fragrance was collected once from each of two individuals, and the number of open flowers included for each fragrance collection was noted.

After the 12 hr collection period, cartridges were wrapped in aluminum foil and kept chilled. Cartridges were

then eluted with 3 ml of hexane, and the eluate was stored frozen in glass vials until they were analyzed using gas chromatograph/mass spectrometer following the methods of Raguso and Pellmyr (1998). Compounds were tentatively identified using computerized mass spectral libraries (Wiley and NIST libraries [>120,000 mass spectra]). The identity of many compounds was also verified using retention times of known standards.

Spectral reflectance of flowers. Hawkmoth retinas contain 3 classes of photoreceptors sensitive in the green, violet, and ultraviolet regions of the spectrum (Bennett and Brown 1985). Because these pollinators have ultraviolet-sensitive photoreceptors, flower patterns reflecting UV wavelengths (not visible to humans) might be significant to hawkmoth feeding behavior. Light from the moon is similar to sunlight in spectral content (Stair and Johnston 1953) and so UV reflectance patterns may be perceived by nocturnal insects with UV-sensitive receptors. However, previous study of color discrimination in Manduca sexta indicated that these moths avoided artificial flowers transmitting ultraviolet wavelengths, and the authors suggested that activation of the UV-sensitive photoreceptors may interfere with feeding behavior (White et al. 1994). In the same

study, flowers of 10 hawkmoth-pollinated species in Costa Rica were found to have no ultraviolet reflectance.

To check for the presence of UV reflectance in flowers of M. viridiflora, I analyzed the spectral reflectance of ten fresh flowers. Because flowers change color from greenish-white in the first day of anthesis to pale yellow in the second day, five of the flowers collected were in the greenish-white phase and five were in the pale yellow phase in order to compare the reflectance between phases.

Spectral reflectance of the corollas was measured using a Spectral Instruments (Tucson, AZ) SI-440 CCD array UV-VIS Spectrophotometer. This instrument was connected by a 400 µm fiber optic probe to a Labsphere, Inc. (North Sutton, NH) 9 cm ID integration sphere. Using a 10 W tungsten light source, I collected reflectance data from 350 nm (UV) to 980 nm (IR) wavelengths. The integration sphere, fitted with a 1.5 cm diameter sampling port, was placed over flowers with a black felt cloth as background. Reflectance of the exterior and interior of the corolla for each color phase was measured. Data were collected as percent transmission (1/absorbance) in comparison to a white pigment (Duraflect™) standard. This standard reflected evenly in all non-UV wavelengths, with a 5% drop-off from 400 to 350 nm (Labsphere, Inc. product literature). The reflectance of

the black cloth background was negligible (0.5-1% of standard) for all wavelengths tested.

Pollinator effectiveness. I used two measures of pollinator effectiveness: rate and duration of visits, and amount of pollen deposited onto stigmas. Visitation rate gives a measure of pollinator quantity, whereas pollen deposition gives a measure of pollinator quality (Herrera 1987). These measures have been combined in previous studies to quantify pollinator effectiveness (Thomson et al. 1982, Kearns & Inouye, 1994, Fishbein & Venable 1996).

I observed groups of plants for flower visitation for a total of 64 h at South Fork and 117 h on Mt. Lemmon in 1999. Observations included periods throughout the day from 0500 to 2030 and covered a variety of weather conditions, ranging from sunny and warm to very cool and rainy. I watched flowers for nocturnal visitors during periods throughout several nights as well, but because no visitors were seen at night I limited my observations to the times above. During each observation period, I recorded number of flowers open in the observed area, duration of each visit, identity of the visitor, number of flowers visited, and any movement of the visitor between plant species. I tested for differences among visitor taxa in visitation rate (number of visits per flower per 1-hour observation period) and mean visit

duration. A Kruskal-Wallis test was used to compare frequency of visits to patches; Welch's ANOVA and Tukey-Kramer tests were used to compare visit duration among taxa.

To test the pollen deposition rate for each visitor species, I emasculated flowers in the bud before anther dehiscence (a total of 77 buds on Mt. Lemmon and 74 buds in the White Mountains, both in the summer of 1999) and then enclosed them in fine-mesh bags to exclude pollinators. After anthesis, I removed the bag and watched the flower until one visitor probed the flower. I recorded the visitor taxon, and then removed the stigma and distal 3-4 mm of the style and mounted it in melted bee jelly (Kearns & Inouye 1993) on a glass slide. This method preserved the stigma and pollen that had been placed there until they could be transported to the lab, where pollen grains present on each slide were counted under a microscope.

Because distributions were not normal, pollen deposition was compared between visitor species within each site using the Wilcoxon 2-sample test. I also compared the pollen deposition by one visitor (hawkmoths) between the two sites using the same test.

To create a composite measure of pollinator effectiveness, I multiplied mean pollen deposition by mean visit duration for each floral visitor species. I used means despite the non-parametric nature of the data because

the medians of zero for visitation rates by hawkmoths would result in pollinator effectiveness quotients of zero. This would not reflect the fact that while these pollinators only visit for a short while at dusk, they are effective pollinators during that time, visiting many flowers and depositing plenty of pollen.

RESULTS

Breeding system. Flowers in the unbagged control treatments had the highest mean seed set (Mt. Lemmon: mean = 1.6 ± 0.91 nutlets; South Fork: mean = 2.6 ± 0.91 nutlets; Table 1). The bagged, emasculated flowers had the lowest mean seed set (Table 1) and in fact only two of 23 flowers at South Fork produced one seed each (out of four possible seeds maximum per flower; mean = 0.16 ± 0.47). No seeds were produced by emasculated, bagged flowers on Mt. Lemmon, which was expected because the treatment was intended to prevent any pollen from reaching the stigma. The low level of seed set that did occur in bagged, emasculated flowers at South Fork could be due to a low frequency of agamospermy or may be due to experimental error. The latter seems more likely, especially considering that no seeds were produced by this treatment on Mt. Lemmon.

The overall treatment effect was strong at both sites (Tables 2 and 3), but it is the paired comparisons that give insight into the mating system of the species. There was no difference in seed set between the self-pollinated and outcross-pollinated treatments at either site (Tables 2 and 3). At South Fork, non-emasculated bagged flowers produced significantly more seeds than emasculated bagged flowers (Welch's approximate t-test: $t = 3.51$, $p = 0.0014$), whereas at Mt. Lemmon seed set from non-emasculated bagged flowers was very low such that there was no difference in seed set between these treatments (Welch's approximate t-test: $t = 1.26$, $p = 0.21$). This lack of difference is surprising given that at Mt. Lemmon seed set in emasculated bagged flowers was zero. Unbagged control plants produced significantly more seeds than bagged, non-emasculated flowers at both sites (Tables 2 and 3).

Timing of floral anthesis. At both sites, floral anthesis was concentrated in the afternoon between 14:30 and 17:30 (Fig. 1). Forty-two to 50 percent of flowers that opened during a 24-hour period opened between these hours. The other 50-58% of flowers opened primarily during earlier daylight hours; about 10% opened during the night.

Nectar concentration and composition.

Sugar concentration of nectars did not vary between sites (Mt. Lemmon: mean = 24.0% ± 1.76 s.d.; South Fork: mean = 23.3% ± 2.83 s.d.; $t = 0.664$, $p = 0.64$).

All nectar samples analyzed contained large amounts of sucrose and small amounts of fructose and glucose (Fig. 2). The proportions of each of these sugars were remarkably consistent among flowers from the same plant, but varied significantly among plants; percent sucrose varied from a mean of 60% to a mean of 87%. Much of this variation is due to plant 3, which had a substantially lower percentage of sucrose than other plants; a larger sample size would provide a better understanding of the pattern of variation among plants. Nevertheless, all plants produced sucrose-dominated nectars.

Floral scent. The flowers of Macromeria viridiflora have no scent that is obvious to humans. However, floral fragrance collection and analysis revealed that small quantities of fragrance are emitted by the flowers. Most of the fragrance compounds found in the floral samples (i.e., benzaldehyde, caryophyllene, acetophenone) were present in similar amounts in the ambient and/or vegetative samples (Fig. 3). However, ocimene was present in much greater amounts in the floral samples and germicrene was only found in the floral samples (Fig. 3), suggesting that these compounds are being produced

by the flowers. Linalool was not present in the atmospheric samples, and was present in a greater amount in the vegetative samples than in the floral samples (Fig. 3), suggesting that it may be a product of the vegetation.

Spectral reflectance. Spectral reflectance of the inner and outer surfaces of flowers in their green and yellow phases is shown in Fig. 4. Most of the reflectance of the flowers is in the 525 - 625 nm range (i.e., the range of green to yellow in the visible spectrum). Yellow-phase flowers reflected more light within this range than did the green-phase flowers. There was little or no reflectance of UV wavelengths (300 - 400 nm) by the flowers. These flowers therefore would trigger the green-range photoreceptors in hawkmoth retinas and would not trigger the UV photoreceptors. This result agrees with the observations of hawkmoth flower reflectance by White et al. (1994), and if UV reflectance inhibits hawkmoth feeding behavior (White et al. 1994) the lack of reflectance at UV wavelengths may be an important factor for hawkmoth pollination.

Pollinator effectiveness.

At Mt. Lemmon, magnificent hummingbirds (Eugenes fulgens) and hawkmoths (white-lined sphinx moths, Hyles lineata) were common visitors. Smaller hummingbirds

(Selasphorus sp.) and small bees were rare visitors to flowers. At South Fork, rufous hummingbirds (Selasphorus rufus) and hawkmoths (white-lined sphinx moths, Hyles lineata) were common floral visitors, and small bees were rare visitors. Because rare visitors were seen only a few times, the analyses presented here will include only the common visitors at each site.

Mean duration of visits by hawkmoths on Mt. Lemmon was longer than any other pollinator at either sites (mean = 6.22 sec ± 3.4 s.d.; Welch's ANOVA: $F = 11.32$, $p < 0.0001$, Table 4). There was no difference in visit duration among other pollinators.

Visitation rates (measured by number of visits per flower per hour) were mostly quite low, with only rufous hummingbirds at South Fork being active fairly consistently throughout the day (Figure 5). Rufous hummingbirds had much higher visitation rates at South Fork than did hawkmoths (Table 5; Kruskal-Wallis test: $\chi^2 = 27.44$, $p < 0.0001$), and magnificent hummingbirds had higher visitation rates than hawkmoths at Mt. Lemmon (Table 5; Kruskal-Wallis test, $\chi^2 = 16.51$, $p = 0.0001$). However, between the hours of 1900 and 2100, hawkmoths were more frequent visitors than magnificent hummingbirds ($\chi^2 = 4.69$, $p = 0.0302$). At South Fork there

was no difference between visitation rates of hummingbirds and hawkmoths between the hours of 1900 and 2100 (χ^2 = 2.72, p = 0.0989).

For viistation rates and amounts of pollen deposited by pollinator classes, I reported medians and interquartile ranges because of very skewed distribution of data. However, although hawkmoths at South Fork have an interquartile range of 0.00 - 0.00, the frequency of visits during the hours when they did visit flowers were quite a bit higher than the highest values for any other pollinators (highest value = 3.9 visits/flower/hr; highest value for other pollinators = 1.78 visits/flower/hr). Thus, the median and interquartile values for hawkmoths at this site reflects the fact that these pollinators are only present for a few hours during the course of the day, but when they are present they visit flowers at a high rate.

Hawkmoths at Mt. Lemmon deposited fewer pollen grains per visit than did hummingbirds (Table 5; χ^2 = 5.54, p = 0.0186). At South Fork, there was no significant difference in pollen deposition among pollinators (Table 5; χ^2 = 2.04, p = 0.1531). Hawkmoths at South Fork deposited significantly more pollen than did their conspecifics at Mt. Lemmon (Table 5; χ^2 = 12.43, p = 0.0004).

Combining pollen deposition and visitation rate into a pollination effectiveness (PE) measure (Table 5), hummingbirds emerge as much more effective pollinators than hawkmoths, with rufous hummingbirds at South Fork being the most effective pollinators. The marked difference between rufous hummingbirds (PE = 516.8) and magnificent hummingbirds (PE = 86.5) is due to its much higher visitation rate, whereas the very low value for hawkmoths at Mt. Lemmon (PE = 12.4) relative to its conspecifics at South Fork (PE = 27.1) is due to a much lower pollen deposition rate at the former site.

DISCUSSION

Flowers of Macromeria viridiflora usually first open in the late afternoon between the hours of 14:30 and 17:30. They produce nectar with sugar concentrations around 23-24%, and the sugar content of the nectar is strongly sucrose-dominated. The flowers do not have a strong scent but do produce small amounts of scent compounds. Spectral reflectance of the flowers is within the yellow to green range of visible light (525 - 625 nm), with little or no reflectance of ultraviolet wavelengths (300 - 400 nm).

Flowers of M. viridiflora do not fit any one pollinator syndrome perfectly, but instead have some traits that fit each of their two major classes of pollinators, hummingbirds

and hawkmoths. Both of these pollinators tend to visit flowers with long floral tubes, and both tend to prefer large amounts of dilute nectar that is high in sucrose (Wyatt 1983, Faegri and Van der Pijl 1989). M. viridiflora fits these two criteria. Flowers are consistent with the syndrome for hummingbird pollination (Wyatt 1983) except for the greenish-white color and late-afternoon anthesis, which are more commonly associated with moth-pollinated flowers. Flowers are consistent with the moth pollination syndrome (Wyatt 1983) except that there is only a small amount of scent produced and the flowers are pendulous, which has been shown to make hawkmoth pollination difficult or even impossible (Fulton & Hodges 1999). However, observation of visitation behavior and successful pollen deposition by hawkmoths in single-pollinator visits to M. viridiflora (Table 5) indicate that the moths are clearly able to probe and pollinate these pendulous flowers.

The pollination syndrome concept has come under considerable criticism in recent years. Waser et al. (1996) contended that interactions between plants and pollinators are more likely to be generalized than specialized. Several studies have tested the predictability of pollinator type based on pollination syndrome and have produced contradictory evidence; Momose et al. (1998) found that pollinator syndromes successfully predicted pollinators in a

Malaysian dipterocarp forest, whereas Hingston and McQuillan (2000) found syndromes to be unreliable predictors of pollinators in Tasmania. The utility of pollination syndromes has been questioned because insects from more than one order may pollinate a particular plant (Herrera 1996), plants with very different floral morphologies blooming together may share the same visitors (Herrera 1988), and insect taxa may exhibit different flower color preferences in different habitats habitats (McCall and Primack 1992). In addition, insect visitors to a single plant species may vary geographically at the level of order (Rozzi et al. 1997), and the most effective pollinator may not be the one that would be predicted by pollination syndromes (Fishbein and Venable 1996).

Certainly, generalization and specialization in plant-pollinator interactions are ends of a continuum rather than a dichotomy (Waser et al. 1996). One alternative within that continuum is combined pollination systems (Baker 1961), in which plants are adapted to visitation by more than one class of pollinator and flowers display a combination of characteristics from two or more pollination syndromes (Grant and Grant 1965). Combined pollination systems have been studied in numerous plant species, including Lonicera japonica, pollinated nocturnally by hawkmoths and diurnally by bees (Miyake and Yahara 1998), Asclepias tuberosa,

pollinated by bees and butterflies (Fishbein and Venable 1996), and Polemonium viscosum, pollinated by bumblebees and hawkmoths (Galen and Kevan 1980; Galen 1996). Even Faegri and Van der Pijl (1979), who formalized the idea of pollination syndromes, recognized that intermediates exist such as Caesalpinia pulcherrima in which flowers display a combination of characteristics from the bird-pollination syndrome and the butterfly-pollination syndrome.

Having floral characteristics that draw in several classes of floral visitors may increase reproductive success for the plant if it increases visitation or ensures pollination if the presence of one of the pollinators is uncertain or variable. Alternatively, such a combination of characteristics may decrease reproductive success if it creates an imperfect match to either pollinator and consequently decreases effectiveness of both pollinators. The combination pollinator syndrome seems to works well in the case of M. viridiflora because both hummingbirds and hawkmoths are effective pollinators.

If floral visitors are not important in the reproduction of a plant, they are unlikely to influence evolution of floral traits and therefore the flowers may not fit the expected pollinator syndromes. To confirm the role of pollinators in reproductive success, it is essential to

know the breeding system of the plant. Seed set from self-pollinated and outcross-pollinated M. viridiflora flowers is equal, showing that these plants are highly self-compatible; seed set was not dependent on pollen source. It is possible that some degree of inbreeding depression would emerge in seed germination rates or other measures of offspring fitness. It would be useful to test this, but I have to date been unsuccessful at inducing M. viridiflora seeds to germinate.

Autogamy can occur in these plants, as demonstrated by seed set in bagged flowers with intact anthers (treatment 5) as compared with emasculated, bagged flowers (treatment 2) at South Fork. Therefore, not only are plants self-compatible but they are also capable of producing seed in the absence of pollinators. Autogamous seed production occurred in the plants on Mt. Lemmon as well, but at a very low level.

The fact that these plants are self-compatible and are somewhat autogamous raises the question as to whether or not attracting pollinators is important to this species. Pollinators can be an important contributor to reproductive success even in self-compatible, autogamous plants if reproductive success is significantly increased by pollinator visitation. In M. viridiflora, seed set was increased at both sites by the presence of pollinators, as

shown by the comparison between unbagged and bagged flowers with intact anthers (treatments 1 and 5). Therefore, even against the background of a low rate of autogamy, pollinators have the potential to apply selective forces by increasing seed set.

To evaluate the potential of floral visitors as agents of selection, it is also necessary to know the pollination effectiveness of common visitors. At both sites, hummingbirds were more frequent visitors than hawkmoths. At Mt. Lemmon, hummingbirds also deposited significantly more pollen per visit than hawkmoths, and at South Fork they deposited more pollen although the difference was not significant. Combining these two measures, hummingbirds are apparently more effective pollinators of Macromeria viridiflora than are hawkmoths. Even at South Fork, where pollen deposition by hummingbirds was not significantly greater, the large difference in visitation rate would mean that hummingbirds were responsible for more seed production than hawkmoths.

The hawkmoths at Mt. Lemmon deposited less pollen per visit than their conspecifics at South Fork. The difference may be due to the difference in flower size (i.e., plants at South Fork have much shorter corollas than plants on Mt. Lemmon; see Appendix A). These hawkmoths may be too small to use the larger flowers at Mt. Lemmon as effectively as

the smaller ones at South Fork. Tongue lengths of Hyles lineata averaged around 4 cm in a study by Miller (1981), which is close to the average corolla tube length at South Fork (mean = 3.8 cm +/- 0.51 s.d.) but shorter than the average corolla tube length at Mt. Lemmon (mean = 4.8 cm +/- 0.60 s.d.).

Hummingbirds at both sites were frequent and effective pollinators. The difference in bill length between the two species mirrors the difference in corolla length of the flowers at the two sites (Appendix A); this difference probably allows the birds to be effective pollinators at their respective sites. In addition, the difference in hummingbird bill size may have resulted in selective pressure for geographic differentiation in corolla size in this species. To test this hypothesis, one could design experiments to determine (a) if hummingbirds differentially select for flowers of a certain size, and (b) if flower size affects pollen deposition per visit by hummingbirds. However, even if experiments showed no effect on both of these counts, pollinator-mediated selection on floral size could not be ruled out, as the selective pressures that led to divergence in the past may not be currently operating.

Pollen deposition is not a direct measure of reproductive success; differences in pollen deposition may not translate into differences in seed set (McDade and

Davidar 1984). However, the amount of pollen a visitor delivers onto the stigmatic surface can be an important aspect of pollinator effectiveness (Silander & Primack 1978, Snow 1982). Because of the destructive method used to measure pollen deposition, it was impossible to also measure seed set in these single-pollinator visitation experiments. It would be useful in future experiments to track seed set from visits by various pollinators to ensure that differences in pollen deposition translate into differential reproductive success.

To conclude, I have shown that pollinators have the potential to increase reproductive success in M. viridiflora. Therefore, the potential for pollinator-mediated selection exists in this system. Flowers of M. viridiflora vary in size along a latitudinal gradient, and this geographic variation in corolla size is mirrored by geographic variation in the bill size of the major hummingbird species visiting the flowers (Appendix A). Although hawkmoths also pollinate the flowers, they are less effective pollinators, visiting the flowers less frequently and, and one of the two sites, depositing less pollen. It is therefore possible that the geographic variation in corolla size may be the result of selection by different hummingbird species. Even so, the flowers of M. viridiflora have characteristics that draw in several different classes

of pollinators, which may ensure pollination if the presence of one of the pollinators is uncertain or variable.

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Table 1. Mean seed set for flowers in each treatment at each study site.

	White Mountains			Mt. Lemmon		
		Mean			Mean	
Treatment	N	Seed Set	SE	N	Seed Set	SE
1: unbagged control	25	2.600	0.183	15	1.600	0.235
2: bagged emasculated	25	0.160	0.095	25	0.000	0.000
3: bagged, emasculated, self-pollinated	25	2.120	0.226	25	0.960	0.268
4: bagged, emasculated, outcross-pollinated	25	1.640	0.251	25	1.360	0.299
5: bagged, not emasculated	25	1.080	0.244	15	0.267	0.206

Table 2. Summary table from ANOVA with a priori contrasts comparing treatments in controlled breeding experiment on Macromeria viridiflora at South Fork (excluding treatment 2 due to unequal variance)

Source of variation	SS	df	MS	F	p
Treatments	42.75	4	10.69	9.15	<0.0001
A priori contrasts:					
3 vs. 4	2.88	1	2.88	2.46	0.1191
1 vs. 5	28.88	1	28.88	24.71	<0.0001
Error	140.24	120	1.17		
Total	182.99	124			

Table 3. Summary table from ANOVA with a priori contrasts comparing treatments in controlled breeding experiment on Macromeria viridiflora at Mt. Lemmon (excluding treatment 2 due to unequal variance)

Source of variation	SS	df	MS	F	p
Treatments	16.55	4	4.14	2.65	0.0377
A priori contrasts:					
3 vs. 4	2.00	1	2.00	1.59	0.2100
1 vs. 5	13.33	1	13.33	10.58	0.0015
Error	156.21	100	1.66		
Total	172.76	104			

Table 4. Mean duration of pollinator visits to Macromeria viridiflora at each study site, by pollinator taxa. Means with the same letter are not significantly different ($p > 0.05$, Tukey-Kramer test).

Site	Pollinator	Mean visit duration (sec)		SE
Mt. Lemmon	hawkmoths	6.22	a	0.55
	magnificent hummingbirds	2.81	b	0.17
South Fork	hawkmoths	2.66	b	0.98
	rufous hummingbirds	3.09	b	0.22

Table 5. Visitation rates and pollen deposition amounts for pollinators of Macromeria viridiflora at two sites. Pollinator effectiveness is a composite value calculated by multiplying visitation rate by amount of pollen deposited for each pollinator.

	Mt. Lemmon		South Fork	
	hawkmoths	Magnificent hummingbirds	hawkmoths	rufous hummingbirds
Visitation rate (visits/flower/hr)				
Median	0.00	0.22	0.00	0.30
Interquartile range	0.00 - 0.06	0.01 - 0.79	0.00 - 0.00	0.16 - 0.44
Pollen deposition (pollen grains/visit)				
Median	19	69	75	91
Interquartile range	7 - 35.5	9 - 231	18 - 197.5	36.5 - 341.5
Pollinator effectiveness (pollen grains/hr)	12.4	86.5	27.1	516.8

FIGURE LEGENDS

Fig. 1. Anthesis of flowers of Macromeria viridiflora at two study sites, graphed as cumulative percentage of flowers open.

Fig. 2. Mean percent of sugar components in nectar samples for each of five individual plants sampled at Mt. Lemmon, Santa Catalina Mountains, Arizona.

Fig. 3. Chromatogram peak areas for scent compounds present in two scent samples taken from flowers of Macromeria viridiflora (floral sample 1: n = 11 flowers; floral sample 2: n = 16 flowers). Results from vegetative and ambient samples are included for comparison.

Fig. 4. Spectral reflectance (% transmission) of corolla surfaces in Macromeria viridiflora. Green phase and yellow phase flowers, and inner and outer surfaces of corollas, were tested separately.

Fig. 5. Mean number of visits per hour by common floral visitors to Macromeria viridiflora at two study sites.

Fig. 1

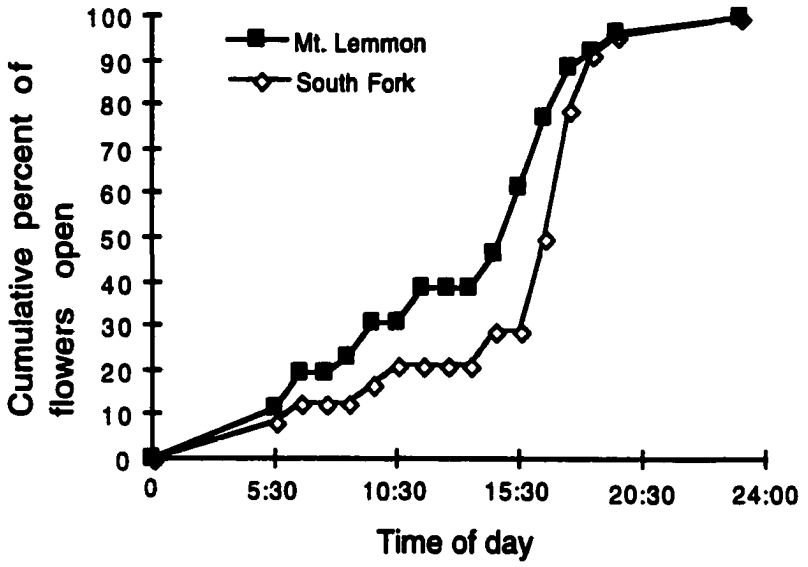

Cumulative percent of flowers open
100
90
80
70
60
50
40
30
20
10
0
Mt. Lemmon
South Fork
0
5:30
10:30
15:30
20:30
24:00
Time of day

Fig. 2

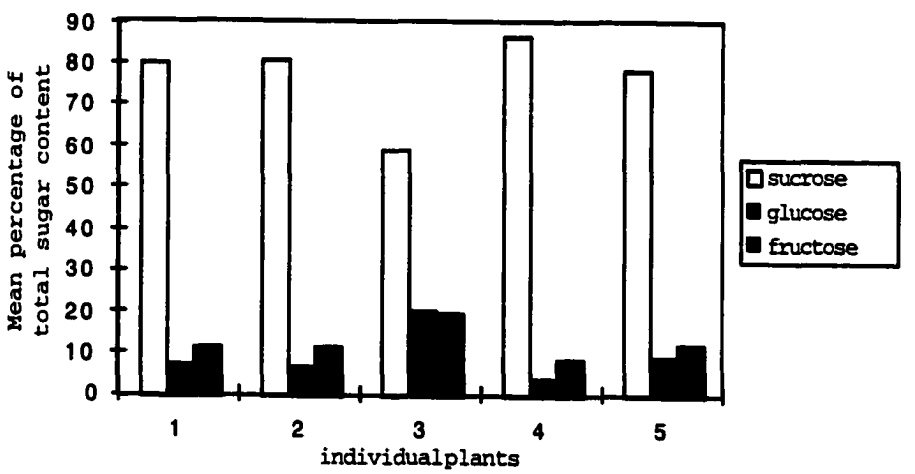

Mean percentage of total sugar content
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
individualplants
sucrose
glucose
fructose

Fig. 3

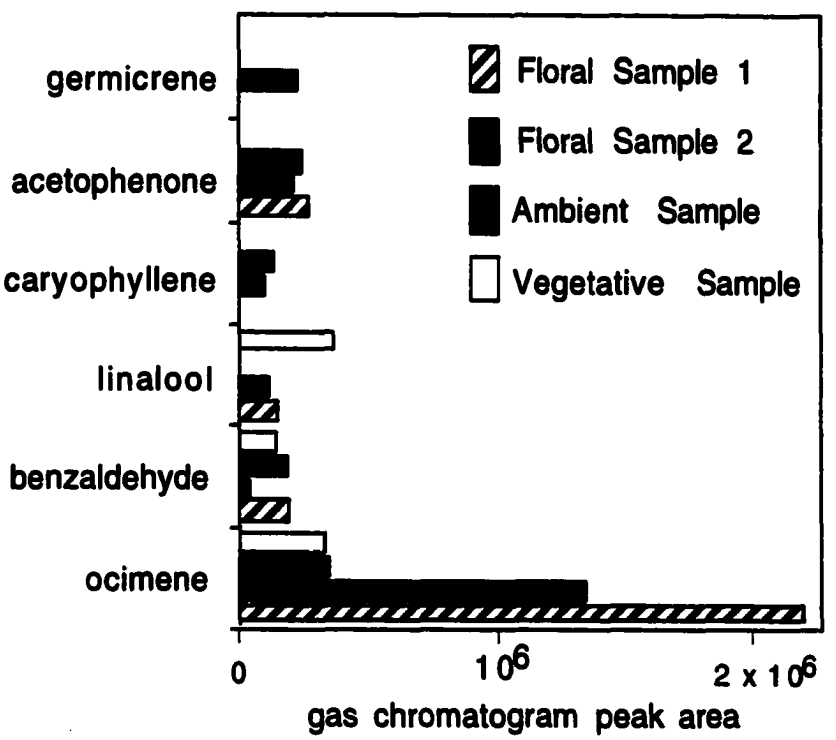

germicrene
acetophenone
caryophyllene
linalool
benzaldehyde
ocimene
Floral Sample 1
Floral Sample 2
Ambient Sample
Vegetative Sample
0
10^6
2 x 10^6
gas chromatogram peak area

Fig. 4

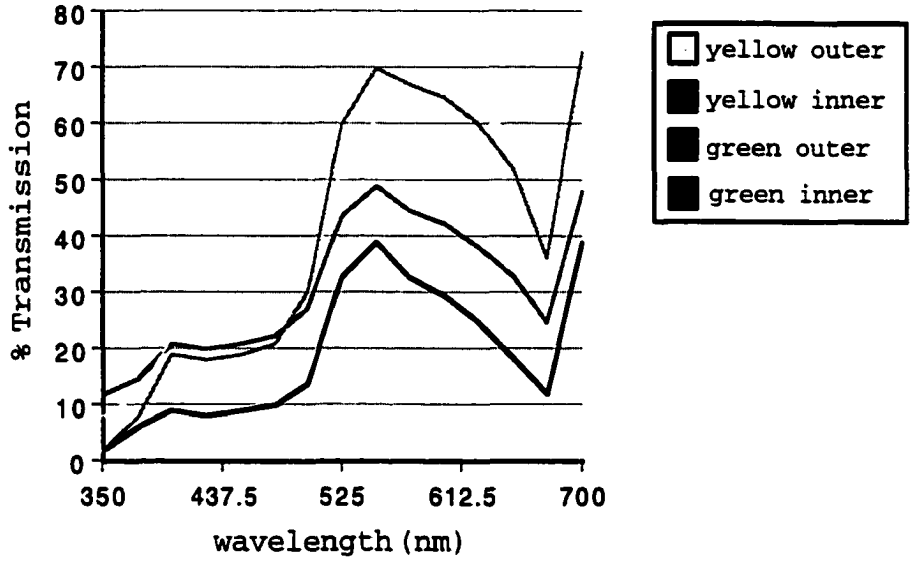
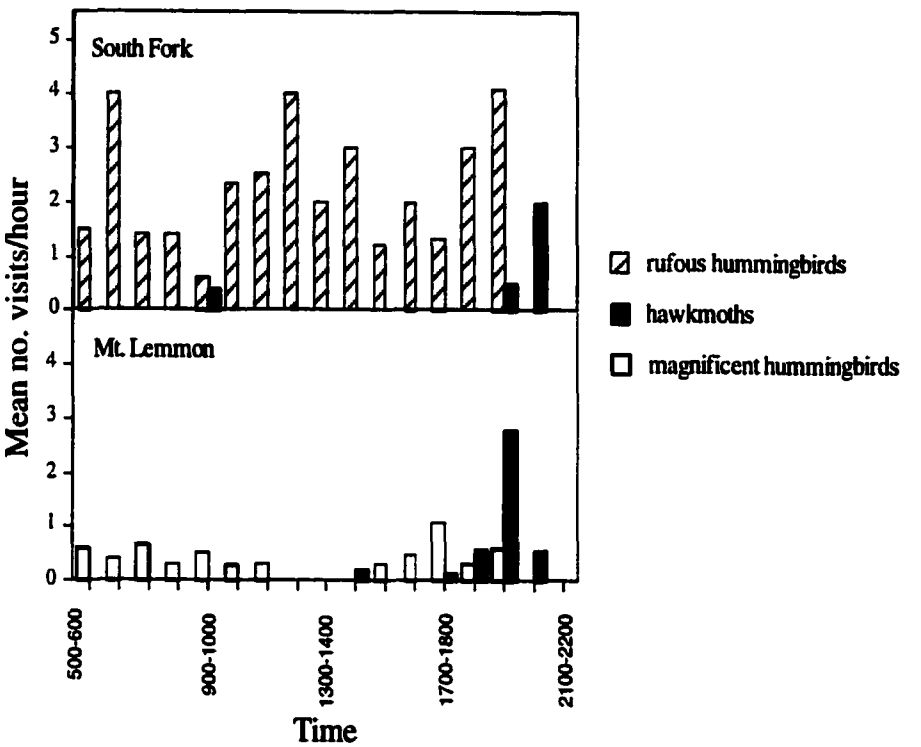

80
70
60
50
40
30
20
10
0
% Transmission
350
437.5
525
612.5
700
wavelength (nm)
yellow outer
yellow inner
green outer
green inner

Fig. 5


South Fork
Mt. Lemmon
Mean no. visits/hour
0
1
2
3
4
5
500-600
900-1000
1300-1400
1700-1800
2100-2200
Time
rufous hummingbirds
hawkmoths
magnificent hummingbirds

APPENDIX C

PHYLOGENETIC RELATIONSHIPS AND COROLLA SIZE EVOLUTION AMONG SPECIES OF *MACROMERIA* (BORAGINACEAE)

(Abstract)

Phylogenetic analysis of Macromeria based on 35 morphological characters produced a single most parsimonious tree that supports the monophyly of the genus. The phylogeny supports previous evaluations of relationships among species, and the resulting clades often connect sister taxa that are geographically proximate. Mapping of corolla size onto the phylogeny indicates that floral size has changed many times within the genus, and that the very large corolla size in southern populations of Macromeria viridiflora has been derived from a smaller-flowered ancestor.

The genus Macromeria D. Don consists of eleven species of perennial herbs or small shrubs distributed in Mexico, southwestern United States and Guatemala (Fig. 1). Plants in this genus occur mainly in woodlands between 1500 and 3500 m elevation. The flowers are long (3.5 - 9 cm) and tubular; most are pale yellow or pale greenish yellow. Size of flowers varies widely among species, though all are larger than typical flowers in Boraginaceae, and one species, M. exserta (Don), has the largest flowers in the family (Johnston 1954).

Macromeria is a member of tribe Lithospermeae within Boraginoideae (Johnston 1954). No formal phylogenetic study of Boraginaceae has been completed and therefore little is known about relationships of groups within the family. The closest relative is most likely Onosmodium (Michx.), due to their strong similarity in vegetative and some floral characters (Turner 1994). Macromeria is delimited from Onosmodium by its much longer stamens well exserted from the corolla, as well as corolla size and shape. Johnston (1954) provided a revision of the Macromeria, including eight species. Turner (1994) provided an updated overview of the genus with three additional species, and included a key to species and distributional maps.

I present here an explicit phylogenetic study of the genus Macromeria based on morphological characters. In addition to determining relationships within the genus, I also examine corolla size evolution in Macromeria as indicated by the phylogeny, with the goal of placing intraspecific variation in one spcies into an historical context. Previous studies (Johnston 1954; Turner 1994; Appendix A) have described intraspecific variation in flower size. I analyzed morphological variation among populations of Macromeria viridiflora (Appendix A) and found that geographic variation in corolla size corresponded to geographic variation in pollinator relationships. In the southern part of the range, flowers were much longer and were visited by species of hummingbirds with longer bills. The present study provides an hypothesis for the direction of change in floral size within M. viridiflora.

MATERIALS AND METHODS

Taxa. All eleven species of Macromeria were included in the study. Specimens of M. viridiflora were placed into two geographically delimited groups in order to evaluate the direction of change in corolla length within the species. These groups correspond to the varieties described by Johnston (1954), but because variation among populations is

clinal rather than categorical (Appendix A), I treat this as one species with no infraspecific taxa. I used two species of Onosmodium Michx. (O. bejariense and O. virginianum) as outgroups.

Characters. Thirty-five morphological characters were included in the analysis (Table 1). Character states were taken from personal observations of fresh material and from study of more than 250 herbarium specimens from seven herbaria (Table 2). A dissecting microscope was used when needed to examine small characteristics (e.g., trichomes).

Twenty-two of the characters were qualitative in nature. Of the 13 quantitative characters, nine were easily divided into discrete character states. The remaining four quantitative characters were ratios designed to describe shape of leaves and flowers. Due to overlapping values among taxa, I used Archie's (1985) generalized gap-coding method to delimit character states. Archie's method ranks taxa according to their character mean, then identifies subsets that include all taxa whose means differ from each other by less than a chosen discriminant criterion (a constant multiplied by an estimate of the standard deviation of the population).

HABIT (character 1). Most species of Macromeria are herbaceous, as are all species of Onosmodium. In contrast,

individuals of M. guatemalensis (I. M. Johnst.) are small, woody shrubs, and M. hintoniorum (B. L. Turner) can be woody and shrub-like.

VEGETATION (characters 2 - 10). Plants in this genus produce long stalks from a perennial rootstock that may branch several times. The most distinctive characteristic of vegetative structures of these species is pubescence. This is typical of Boraginaceae; plants belonging to this family are often covered in dense, coarse trichomes (Cronquist 1981; Heywood 1993) There are differences among Macromeria species in the shape, density and orientation of the trichomes on leaves, bracts, and stems.

LEAF SHAPE (character 11). Leaves are simple with entire margins, elliptical to almost linear in shape, with acute apices and narrowly cuneate bases; they are sessile or have extremely short petioles. In order to quantify leaf shape, I measured length and width of a leaf midway between the stem base and apex. Character states were determined and coded using the generalized gap-coding method (Archie 1985).

INFLORESCENCES (character 12). Plants of this genus produce flowers in helicoid cymes that terminate stems and/or branches. These cymes may be terminal only or may be found on terminal and axillary branches.

CALYX. As with vegetative structures, the most distinctive characteristic of the calyces is the pubescence.

In this case, trichomes vary little among species in shape or orientation, but do show variation in density (character 13).

COROLLA (characters 14 - 24). Most species in the genus, and in Boraginaceae in general, have regular corollas. Plants of M. exserta, however, have zygomorphic corollas with very irregular flower buds; M. hispida (Mart. & Gal.) has slightly zygomorphic corollas (character 14).

All but one species of Macromeria have pale-colored flowers (white to pale yellow or pale greenish-yellow), as do the outgroup species; only M. longiflora (Sessé & Moçiño ex D. Don) departs from this, with corollas that are orange to orange-yellow. Corolla color, therefore, is an autapomorphy for this species and, though included in the analysis (character 15), is not phylogenetically informative.

In nine species of Macromeria, as well as in one of the the outgroup species, the corolla mouth bears glandular trichomes (simple, short-stalked glands ≈ 0.1 mm in length; character 16). In three of these species, the glands also extend onto the corolla lobes (character 17).

Lobes of the corolla may be ascending or erect from the corolla throat, or may be at a 90 degree angle or reflexed from the corolla throat (character 18). This variation was

expressed as only two character states because of the presence of intermediates within species (e.g., lobes between a 90 degree angle and reflexed) and because of the difficulty of determining the exact position of lobes from herbarium specimens.

Corolla length (character 19) varies widely among species but also distinguishes the genus from its relatives. This quantitative character was divided into three states based upon gaps between the ranges of species. Because one of the purposes of the study was to evaluate the evolution of corolla length in the group, the phylogenetic analysis was performed both with and without this character to avoid bias (de Queiroz 1996).

Three quantitative measures of corolla shape were used in the analysis. The ratio of corolla width at mouth to corolla width at ovary describes how broadly the corolla flares (character 20). Ratio of corolla lobe length to corolla length is a measure of how much of the total corolla length is taken up by the lobes of the corolla versus the sympetalous tube (character 21). The ratio of corolla width at mouth to corolla length (character 22) describes the shape of the corolla itself (i.e., long and narrow versus short and broad). Character states were determined and coded using the generalized gap-coding method (Archie 1985).

The corollas in all species are covered in trichomes. Shape and density of these hairs vary among species (characters 23 - 24).

STAMENS (characters 25 - 31). One of the distinctions between Macromeria and its relatives is in the degree of exertion of the stamens from the corolla throat (character 26). To some extent, of course, this is related to filament length; species of Onosmodium have minute (<1 mm) filaments and therefore have stamens that are completely included within the corolla throat. However, filament length (character 25) does provide added information to distinguish species of Macromeria and therefore it was included as a separate character.

The anthers of some species bear sterile appendages on the distal end (as described by Johnston [1954]; character 28 here). Plants of M. viridiflora are distinct in having trichomes on the dorsal surface of the anthers (character 31). Anthers within the genus vary in color (character 30) and may be yellow, black/purple or brown, although the pollen is always yellow.

POLLEN (characters 32 - 35). Characters for the four pollen traits were taken from scanning electron microscopy (SEM) photographs. Whole anthers were removed from herbarium specimens, rehydrated in a weak soap solution to return them to their original shape, and then dehydrated

using a series of ethanol baths followed by critical point drying. Comparison with pollen taken from fresh specimens showed that pollen from herbarium specimens reclaims the shape of fresh pollen grains when treated in this fashion. Pollen from the anthers was then placed onto metal SEM stubs and coated with 30-60 nm of gold-palladium and viewed using an I.S.I. DS-130 scanning electron microscope. Several photographs were taken of pollen from at least one specimen per species. In species for which I had access to more than one specimen with intact anthers, I photographed pollen from three specimens from different parts of the geographic range to check for variation within species.

Pollen length (character 35) was measured along the polar axis. Pollen shape (character 34) was expressed as the P/E ratio (i.e., the ratio between length of polar axis and length of equatorial axis; Erdtman 1943). In addition, some species have pollen that is constricted at the equatorial axis, creating an hourglass shape (Figs. 3, 5). Pores (character 32) may be positioned at the midpoint of the polar axis length (Figs. 2, 4), slightly off midpoint (Fig. 6), or far to one end of the polar axis length (Figs. 3, 5). Verrucae (character 33) may be limited to the colpi (Figs. 2, 4) or may extend onto the sexine surface (Figs. 3, 5-7).

GYNOECIUM. All species examined had simple, narrow, unbranched styles that are exserted from the corolla throat. The style emerges from the base of four carpels that develop into four white, almost spherical, pearl-like nutlets that may be slightly keeled. No gynoecial characters were used in this analysis because no clear variation was found among species. There may be variation in fruit traits, but I did not have access to fruits for several species (no collections available); the variation I saw among the species I did examine was subtle at best. Therefore, fruit traits were not included in the analysis.

Data Analysis. MacClade 4.0fc4 (Maddison and Maddison 2000) was used to edit the data set (Table 3). Phylogenetic analysis was performed with the aid of PAUP* version 4.0b4a (Swofford 2000). Branch-and-bound search technique was employed using maximum parsimony. Eleven characters were ordered because they described continuous variation with intermediate values (Table 3); all other characters were unordered.

The generalized gap-coding used for quantitative ratio data resulted in a large number of character states for these traits. To avoid undue influence of these characters on the phylogeny, these characters were weighted 0.5 relative to other characters.

The analysis was repeated with character 19, corolla length, excluded to test for the effect of this character on the topology of the resulting tree. Support for individual clades was estimated in two ways. Two hundred bootstrap replicates (Felsenstein 1985) were executed using branch-and-bound search algorithm. Decay indices were generated using constraint trees analyzed with ten replicates of random addition sequence.

Corolla length was mapped onto the resulting phylogeny as a continous character using both linear and squared change parsimony in MacClade (Maddison and Maddison 2000).

RESULTS

Character states of taxa are listed in Table 3. Only 0.4% of the data matrix cells were scored as missing data. Generalized gap-coding with a constant of 1 produced states for ratio characters that were rather finely parsed (characters 11 and 20 - 22). To check the effect of this fine parsing on the phylogenetic results, I also tried using a constant of 2 to create less finely parsed character states. Resulting changes in the phylogeny were in branches that are only weakly supported, and therefore the results are not included here.

Pollen of Macromeria has not previously been described or illustrated. Here I provide representative photographs (Figs. 2-7) and a description of pollen in the genus. The pollen ranges in length from 12.9 to 24.0 μm, and in shape from spheroidal to prolate (P/E ratios from 0.88 to 2.00). The pollen in some species is constricted at the equatorial axis, creating an hourglass shape (Figs. 3, 5). Pollen in all species of the genus appear to have 8 colpi, each with a single pore. All species have abundant verrucae within the colpi and, in some species, verrucae are also present on the sexine surface. Four pollen characters had distinctive variation among species and were used in the phylogenetic analysis.

The phylogenetic analysis resulted in a single most-parsimonious tree of 119 steps, with a consistency index of 0.48 and a retention index of 0.47 (Fig. 8). There was extensive homoplasy, and thus bootstrap and decay indices (Fig. 8) indicate weak support for most branches. Character state changes are mapped onto the phylogeny in Fig. 9.

The genus Macromeria is well-defined as a monophyletic clade, with eight synapomorphies supporting its distinction from Onosmodium. These synapomorphies include lengthened anthers, stamens exserted from the corolla throat, and longer corollas with greater flare of corolla tube from

ovary to throat. In addition, this clade is supported by two pollen characters: longer pollen grains with sparser verrucae on sexine surface compared to Onosmodium.

M. guatemalensis and M. hintoniorum are placed as sister taxa at the base of the genus. These species share the shrubby habit as a synapomorphy, as well as several other homoplasious characteristics related to pollen shape and trichomes on the stems. These two species are each known from only three collections, with M. guatemalensis found in the southwest corner of Guatemala near the Mexican border, and M. hintoniorum found in the southern Mexican states of Oaxaca and Guerrero. They therefore have geographically proximate but allopatric distributions.

M. exserta and M. hispida are placed as sister taxa at the next most basal position in the genus. In addition to sharing broadly flaring, zygomorphic corollas with reflexed lobes, these species have geographic ranges that overlap in the Mexican states of Michoacán, Jalisco and Nayarit.

M. notata (I. M. Johnst.) and M. alba (Nesom) are placed as sister taxa in the next most basal position in the phylogeny. These two species share the characteristic of glands in the corolla throat extending onto the inner surface of the corolla lobes. The two species are both restricted to the mountains of northeastern Mexico. M. alba

is endemic to the Sierra Guatemala of west-central Tamaulipas; M. notata is found in the high mountains of southeastern Coahuila and west-central Nuevo León (Fig. 1).

The two groups of M. viridiflora are strongly supported as a clade, with a bootstrap value of 99 and decay index of 6.5. These plants share the synapomorphies of very dense trichomes on the corolla, trichomes present on the dorsal surface of anthers, and short corolla lobes in relation to corolla length, plus many homoplasious characters. The closest relative to M. viridiflora appears to be M. longiflora, with which it shares very similar pollen characters. In both species, the pollen is constricted at the equatorial axis, creating an hourglass shape. The pores are located far to one polar end of the pollen grains in both species, and the verrucae extend onto the sexine surface (the latter character is shared homoplasiously with several other species as well).

M. barbigera (I. M. Johnst.) is sister to the clade consisting of M. viridiflora + M. longiflora. These three species share similar flower and leaf shapes, as indicated by the ratio of leaf length to leaf width and the ratio of corolla lobe length to corolla length.

The results of the analysis place M. leonotis (I. M. Johnst.) as sister to the clade described above, with M.

pringlei (Greenm.) placed next below that. The clades created by these two placements are supported by only a few homoplasious characters describing trichome length and orientation and corolla shape.

When corolla length is removed from the data set, the same single most parsimonious tree is found, with a consistency index of 0.48 as before. When corolla length is mapped onto the phylogeny as a continuous character with squared-change parsimony, there is considerable homoplasy; corolla length changes many times throughout the phylogeny (Fig. 10). Linear parsimony produces very similar results (results not shown); there is somewhat less homoplasy but corolla length still changes many times throughout the phylogeny. All species of Macromeria have longer corollas than the outgroup (> 3.5 cm). Very long corollas have apparently evolved three times within the genus, once for each of the three species with this trait.

DISCUSSION

The monophyly of Macromeria is strongly supported by the analysis, with eight synapomorphies distinguishing it from the outgroup Onosmodium. To test this hypothesis thoroughly would require inclusion of many more species of Onosmodium and other related taxa, a task beyond the scope

of this study. Only two species of Onosmodium have been examined, and so their ability to thoroughly represent the genus merits further examination in a larger scale generic-level study.

Many of the relationships defined by the results of the phylogenetic analysis have been recognized in previous studies. The close relationship of M. guatemalensis and M. hintoniorum is in accord with Turner (1994), who considered these to be each other's closest relative due to their shrubby habit, coarse pubescence and corolla shape. Johnston (1954) believed that M. exserta and M. hispida were closest relatives because of their zygomorphic corollas. In the analysis I present here, these two species are placed as sister taxa, supporting Johnston's evaluation. In his original description of M. alba, Nesom (1989) considered M. notata to be its closest relative, based upon the erect or ascending corolla lobes and the glands extending from the corolla throat onto the lobes. This sister relationship is also supported by the analysis presented here.

The species of Macromeria are quite distinct from one another morphologically. In my examination of specimens for this study, I encountered no specimens that were difficult to assign to species, or any strong evidence for hybridization. There are, however, several species that are

only known from a few specimens. In the case of M. alba, the few specimens are all from the same location. In the case of M. guatemalensis and M. hintoniorum, there are several known locations but very few collections per site available. While each of these species appears to be distinct from the other species in the genus, this evaluation is based on limited material; further collecting and field study are warranted. Corolla size, particularly length, has apparently increased several times in the evolution of Macromeria (Fig. 10). The closest relatives of the genus have smaller flowers (as do most species in Boraginaceae), whereas flowers of Macromeria are longer. In addition, three of the taxa included in this study have extremely long corollas: M. exserta (66.8 +/- 17 mm [mean +/- 1 s.d.]), M. leonotis (62.2 +/- 10.4 mm), and M. viridiflora, southern group (52.3 +/- 13.8 mm). According to the phylogeny presented here, very long corollas have evolved independently three times, because each of these three species have closest relatives with shorter corollas.

Therefore, the most parsimonious reconstruction indicates that the very long corollas in the southern populations of M. viridiflora are derived from a shorter-flowered ancestor. The question follows as to what force(s) may have selected for longer flowers in these populations.

Because flowers in these populations are visited by longer-billed hummingbirds than those in the northern populations (Appendix A), the hypothesis that pollinator-mediated selection has caused the evolution of longer flowers is consistent with these results. However, without manipulative experiments to demonstrate pollinator-mediated selection, we cannot know for sure. Because of the clinal nature of the variation (Appendix A), it is impossible at this point to rule out environmental factors (besides pollinators), especially those that run along a latitudinal gradient.

Corolla color changes dramatically only once in the phylogeny: M. longiflora has reddish-orange corollas, whereas all other species have pale white, yellow, or greenish flowers. There may be other more subtle corolla color changes within the genus, but color is difficult to discern from the herbarium specimens available for the study, and collector's notes may not be sufficient for determining subtler differences in color.

Nothing is known about the pollinators of Macromeria, with the exception of M. viridiflora, which is pollinated by hummingbirds and hawkmoths (Appendix B). Data on pollinator relationships for other species in the genus are necessary to understand the relationship between pollinators and corolla color, size and shape in Macromeria.

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Table 1. List of characters used in phylogenetic analysis of Macromeria

1. Habit
   0 Woody
   1 Herbaceous

Stem:

2. Shape of stem trichomes
   0 Long (0.75 - 1 mm), thin
   1 Long (0.75 - 1 mm), broad
   2 Short (0.25 - 0.5 mm), thin

3. Density of stem trichomes
   0 Medium-dense (some trichomes overlap)
   1 Dense (trichomes overlap but leaf surface is visible)
   2 Very dense (only small patches of leaf surface is visible through trichomes)

4. Orientation of stem trichomes
   0 45° angle or greater from stem
   1 Less than 45° degree angle from stem

Leaves:

5. Shape of trichomes on leaf upper surface
   0 None
   1 Long (0.75 - 1 mm), thin
   2 Long (0.75 - 1 mm) and short (0.25 - 0.50 mm) trichomes (dimorphic)

6. Density of trichomes on leaf upper surface
   0 Trichomes sparse, not overlapping
   1 Trichomes dense, overlapping

7. Orientation of trichomes on leaf upper surface
   0 Approx. 45° angle
   1 Less than 30° angle
   2 Arching

8. Length of trichomes on leaf lower surface
   0 Long trichomes (0.75 - 1.0 mm) only
   1 Long (0.75 - 1 mm) and short (0.25 - 0.50 mm) trichomes (dimorphic)

9. Density of trichomes on leaf lower surface
   0 Trichomes sparse, not overlapping
   1 Trichomes dense, overlapping

10. Orientation of trichomes on leaf lower surface
   0 Approx. 45° angle
   1 <30° angle
   2 Arching

11. Ratio of leaf length to leaf width (values below indicate ranges of sample means)
   0 2.91 - 3.34
   1 3.50 - 3.61
   2 3.67 - 3.88
   3 3.90 - 4.00
   4 4.61 - 4.72
   5 5.10 - 5.15
   6 5.18 - 5.22

Inflorescences:

12. Cymes, position
   0 Terminal only
   1 Terminal and axillary

Calyx:

13. Calyx trichomes, density
   0 Medium-dense (some trichomes overlap)
   1 Dense (trichomes overlap but leaf surface is visible)
   2 Very dense (only small patches of leaf surface is visible through trichomes)

Corolla:

14. Corolla symmetry
   0 Zygomorphic
   1 Regular

15. Corolla color
   0 White to greenish yellow
   1 Orange to orange yellow

16. Mouth of corolla tube, vestiture
   0 Glanduliferous
   1 Glabrous

17. Glands within corolla, position
0 Confined to corolla tube and mouth
1 Extending onto lobes

18. Corolla lobes, orientation at maturity
0 Ascending/erect
1 90 degrees or reflexed

19. Corolla, length, mean +/- 1 s.d.
0 10 - 35 mm
1 35 - 55 mm
2 55 - 90 mm

20. ratio of corolla width at mouth to corolla width at ovary (values below indicate ranges of sample means)
0 1.65 - 2.00
1 3.26 - 3.60
2 3.93 - 4.07
3 4.09 - 4.95

21. ratio of corolla lobe length to corolla length (values below indicate ranges of sample means)
0 0.110 - 0.120
1 0.126 - 0.133
2 0.143 - 0.194
3 0.196 - 0.198
4 0.216 - 0.218
5 0.249 - 0.259

22. ratio of corolla width at mouth to corolla length (values below indicate ranges of sample means)
0 0.140 - 0.158
1 0.163 - 0.196
2 0.200 - 0.220
3 0.270 - 0.330

23. Corolla trichomes, shape
0 Long (0.75 - 1 mm), wispy
1 Short (0.25 - 0.50 mm), thin
2 Long (0.75 - 1 mm), stiff

24. Corolla trichomes, density
0 Medium-dense (some trichomes overlap)
1 Dense (trichomes overlap but leaf surface is visible)
2 Very dense (only small patches of leaf surface is visible through trichomes)

Androecium:

25. Filament, length
    0 < 1 mm
    1 4 - 20 mm
    2 > 30 mm

26. Stamens, position
    0 Exserted from corolla throat
    1 Included within corolla throat

27. Filament attachment
    0 Filaments attached near midpoint of anther length
    1 Filaments attached toward basal end of anther

28. Sterile appendage on anther
    0 Present
    1 Absent

29. Anthers, length
    0 2.0 - 2.8 mm
    1 > 3.0 mm

30. Anthers, color
    0 Yellow
    1 Black/purple
    2 Brown

31. Trichomes on dorsal surface of anthers
    0 Present
    1 Absent

32. Pollen pores, position
    0 approx. midpoint of polar axis length
    1 slightly off midpoint of polar axis length
    2 far to one end of polar axis length

33. Verrucae on pollen grain, position
    0 Limited to colpi
    1 Not limited to colpi; extending onto the sexine surface

34. Pollen shape
    0 spheroidal to prolate-spheroidal (P/E ratio = 0.88 - 1.14), not constricted at equatorial axis
    1 prolate (P/E ratio = 1.33 - 2.00), not constricted at equatorial axis
    2 prolate (P/E ratio = 1.33 - 2.00), constricted at equatorial axis

35. Pollen, length
    0 8.5 - 11.9 µm
    1 12.9 - 19.1 µm
    2 19.6 - 24.0 µm

Table 2. Specimens studied of Macromeria and outgroup (Onosmodium).

Macromeria (D. Don)

M. alba Nesom: Richardson 366, 1263, 1367 (TEX).

M. barbigera I. M. Johnst.: Hinton 21351, 22117, 22912, 24906, 25283 (TEX), Mayfield 2089 (TEX), Mueller 174, 287, 563, 741, 2871 (TEX), Smith M191 (TEX).

M. exserta Don: Alexander 2171 (MI), Alexander XA160 (MI), Anderson 4818 (DUKE, MI), Breedlove 12206 (MI, TEX), Breedlove 15823 (MI), Fuentes 601 (MI), Gentry 9483 (ARIZ), Gentry 12089 (TEX), Hinton 13107 (MI, TEX), Hinton 14809 (RSA), Jack 087 (TEX), Kenoyer A287 (ARIZ), Marcks 1027 (TEX), McVaugh 14183, 12945, 19887 (MI), Mendoza 4014 (RSA), Northington 1154 (TEX), Roe 1643, 1682 (MI), Rowell 3040 (MI), Rzedowski 1643, 1682 (MI), Rzedowski 18512 (DUKE, MI), Rzedowski 20759 (MI, TEX), Santos 3209 (MI), Stevens 1424 (DUKE), Torres 6940, 7049 (TEX), Wilbur 2264 (MI).

M. guatemalensis I.M. Johnst.: Beaman 3852 (DUKE), Cosminsky (F), Steyermark 35898, 50069 (F).

M. hintoniorum B.L. Turner: Hinton 14439 (NY, F).

M. hispida Mart. & Gal.: Diguet s.n. (MI), Kenoyer A287 (F, MI), Gregory 208 (NY, MI), Hartweg 372 (NY), Huerta 805 (TEX), LaSalle 810709-3 (ARIZ), McVaugh 16577 (MI), Rzedowski 27472 (MI), Sessé 5217 (F), Torke 286 (NY, TEX).

M. leonotis I.M. Johnst.: Hernandez 2724 (TEX), Hinton 17412 (MI), Hinton 17566, 18523, 19375, 21026 (TEX), Hinton 22277 (ARIZ, TEX), Hinton 23060 (ARIZ, MI, TEX), Nesom 4772 (ARIZ, TEX), Nesom 6013 (TEX), Stanford 660 (ARIZ).

M. longiflora Sessé & Moçiño ex D. Don: Anderson 4741, 5124 (DUKE), Boege 571 (DUKE), Gentry 20320 (ARIZ), Goldsmith 65 (RSA), Hernandez 7830 (RSA), Hinton 8257 (TEX), Hinton 15274 (ARIZ, RSA, TEX), Hinton 15981 (RSA), Iltis 471 (TEX), Leavenworth 286 (ARIZ), Maysilles 8203 (DUKE), McVaugh 13539 (MI), Rzedowski s.n. (TEX), Rzedowski 18513 (DUKE), Rzedowski 26198 (MI), Rzedowski 27516, 28272 (TEX), Straw 1089 (RSA), Tillett 637-139 (RSA), Warnock 2177 (TEX).

M. notata I.M. Johnst: Dorr 2272 (TEX), Ferguson 1246 (ARIZ), Henrickson 16129 (TEX), Hinton 18287 (TEX), Mueller 830 (MI, TEX), Mueller 2238 (MI), Westlund 5.23.88.24 (TEX).

M. pringlei Greenm.: Cota 3008 (TEX), Moore 3105 (MI), Hernández 123 (TEX), Hinton 14439 (RSA), García 975 (TEX), Pringle 6949 (RSA), Pringle 11044 (TEX), Rzedowski 26767 (MI).

M. viridiflora DC. var. thurberi (A. Gray) I. M. Johnst.: Baker 10111 (RSA), Barr 60-331, 68-332 (ARIZ), Castetter 5084, 10705 (UNM), Darrow s.n. (ARIZ), David s.n. (RSA), Deaver 6370 (ARIZ), Deaver s.n. (RSA). Demaree 41269 (ARIZ, RSA), Demlong 25 (RSA), Dunbar 411 (UNM), Ferris 10099 (RSA), Fisher 17593 (DUKE), H. S. Gentry 4556 (RSA), J. L. Gentry 2258 (ARIZ, RSA), J. L. Gentry 2280 (RSA), Gooding 1200 (ARIZ), Gould 5037 (ARIZ), Granfelt 69-204 (ARIZ), Hoggren 849 (UNM), Hutchins 1629 (UNM, RSA), Hutchins 2164 (RSA), Hogne 194 (RSA), Johnson s.n. (ARIZ), Kearney 12255, 13987 (ARIZ), Knight 1609 (UNM), Kuntze s.n. (ARIZ), MacDougal 323 (ARIZ), Morefield 2773 (RSA), McLaughlin 783 (ARIZ), Munz 1174 (RSA), Nelson 1975 (UNM), Phillips 3406 (RSA), Sagalyn 97 (DUKE), Schmidt 75, 109, 132 (ARIZ), Sivinski 1743 (UNM), Studhalter 1832 (MI), Wagner s.n. (UNM), Windham 81-232 (ARIZ).

M. viridiflora DC. var. viridiflora I. M. Johnst.: Barr 62-418,419,420 (ARIZ), Benson 8863 (RSA), Bloomer s.n., 1817

(ARIZ), Blumer 3623 (ARIZ), Bowers R372, R285 (ARIZ), Bye 7839 (TEX), Correll 20130, 23040 (TEX), Darrow 7106 (ARIZ), Diaz 251 (TEX), Ferris s.n. (MI), Fishbein 2118, 2846 (ARIZ), Goodding 173 (ARIZ), Imdorf 1332 (ARIZ), Johnson s.n. (ARIZ), Knobloch 1826 (MI), Lane 2253 (TEX), LeSueur 895 (TEX), Loomis 2244 (ARIZ), Martin s.n. (ARIZ), Maysilles 7027, 7363, 7515, 8347 (MI), McLaughlin 6419 (ARIZ), Niering s.n. (ARIZ), Parker 5847 (ARIZ), Pennington 691 (TEX), Pringle s.n. (MI), Rickert 104 (ARIZ), Rusby s.n. (MI), Straw 1696, 1921 (MI, RSA), Tenorio 6555 (RSA), Thornber 4474 (ARIZ), Townsend s.n. (TEX), Van Devender 87-131 (ARIZ), Wilson 8520 (TEX).

Onosmodium Michx.:

O. bejariense A.DC.: Bennett 380 (ARIZ), Boivin 14019 (ARIZ), Bondy 889 (ARIZ), Carr 7275, 12110 (TEX), Chase 13502 (TEX), Correll 23988, 25268, 27055, 32494 (TEX), Crawford 1429 (TEX), Demaree 31381 (TEX), Eggert s.n. (TEX), Fryxell 3744 (TEX), Gentry 2941 (ARIZ), Hapeman 7113 (ARIZ), Lindheimer 1023, 1024 (ARIZ), Lundell 10610 (ARIZ), Lundell 13750 (ARIZ, TEX), Lundell 13774 (TEX), McVaugh 7137 (TEX), Moldenke 27415 (TEX), Simpson 1113 (TEX), Tharp s.n. (ARIZ, TEX), Thornber s.n. (ARIZ), Traverse 1481 (TEX), Turrell s.n. (ARIZ), Small s.n. (ARIZ), Wallis 7386 (TEX).

Q. virginianum (L.) A.DC.: Allen 2183 (TEX), Bozeman 9147 (TEX), Duncan 14339 (TEX), Fisher s.n. (ARIZ), Fleming 1745 (ARIZ), Godfrey 57012 (TEX), Holder 4536 (TEX), Kral 59578 (TEX), Moldenke 26540, 26581 (TEX), Phillips 1838 (ARIZ), Radford 11386 (ARIZ).

TABLE 3. Character by taxon matrix used for phylogenetic analysis of Macromeria. See Table 1 for character list. Asterisk beside character number indicates characters that were ordered in the analysis. Hyphen indicates character not applicable to the taxon.

	Character:																		
Taxon	1	2	3*	4	5	6	7	8	9	10	11*	12	13*	14	15	16	17	18	19*
M. alba	1	0	0	0	1	0	1	0	0	1	4	1	0	1	0	0	1	0	1
M. barbigera	1	0	1	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1
M. exserta	1	0	1	0	1	1	1	0	1	0	0	1	0	0	0	01	0	1	2
M. guatemalensis	0	0	0	1	1	1	1	0	1	1	5	1	0	1	0	1	0	0	1
M. hintoniorum	01	0	0	0	1	0	0	0	0	1	1	1	1	1	0	1	0	0	1
M. hispida	1	2	1	0	1	1	1	0	1	1	6	1	0	0	0	1	0	1	1
M. leonotis	1	1	1	1	1	1	0	1	1	1	1	0	0	1	0	0	0	1	2
M. longiflora	1	0	2	0	0	-	-	1	1	1	0	1	1	1	1	0	0	0	1
M. notata	1	0	1	1	1	0	2	0	0	2	2	0	0	1	0	0	1	0	1
M. pringlei	1	0	0	0	1	0	0	1	1	1	6	1	2	1	0	0	0	0	1
M. viridiflora var. viridiflora	1	0	1	1	2	1	0	1	1	1	0	1	0	1	0	0	0	0	2

M. viridiflora var. thurberi	1	0	1	1	2	1	0	0	1	1	2	1	0	1	0	0	0	0	1
O. bejariense	1	0	1	1	2	0	0	1	1	1	3	1	2	1	0	01	0	0	0
O. virginianum	1	2	2	0	1	1	1	0	0	1	4	1	0	1	0	1	0	0	0

Table 3. Continued.

Taxon	Character: 20*	21*	22*	23	24*	25*	26	27	28	29	30	31	32*	33	34	35*
M. alba	3	3	1	0	3	1	0	0	1	0	0	1	0	0	0	2
M. barbigera	6	1	2	2	2	1	0	0	1	0	2	1	0	0	0	1
M. exserta	5	6	0	0	4	2	0	0	1	1	0	1	1	0	1	2
M. guatemalensis	4	3	2	0	2	1	0	0	0	0	0	1	0	1	1	1
M. hintoniorum	3	5	2	0	2	1	0	0	1	0	0	1	0	1	1	1
M. hispida	4	2	1	0	3	1	0	0	1	0	1	1	1	0	0	12
M. leonotis	4	2	1	1	3	1	0	0	0	0	0	1	0	0	0	1
M. longiflora	5	4	1	1	3	1	0	0	0	0	2	1	2	1	2	1
M. notata	2	1	1	0	3	1	0	0	1	0	0	1	0	0	0	1
M. pringlei	4	2	1	1	3	1	0	0	1	0	0	1	0	0	1	1
M. viridiflora var. viridiflora	4	0	0	2	4	1	0	1	1	1	0	0	2	1	2	2
M. viridiflora var. thurberi	4	1	0	2	4	1	0	1	1	1	0	0	2	1	2	2
O. bejariense	0	5	4	1	0	0	1	1	0	0	0	1	1	1	0	0

O. virginianum	0	6	3	1	1	0	1	1	1	0	0	1	1	1	0	0

FIGURE LEGENDS

FIG. 1. Map showing geographic distribution of Macromeria (after Turner, 1994).

FIGS. 2-7. Representative SEM photographs of pollen in Macromeria and Onosmodium. 2. M. leonotis. 3. M. viridiflora. 4. M. barbigera. 5. M. longiflora. 6. O. virginianum. 7. Detail of colpus in M. viridiflora pollen showing verrucae within colpus and on sexine surface. Scale bars in 3 - 7 = 2.34 µm ; scale bar in 8 = 0.86 µm.

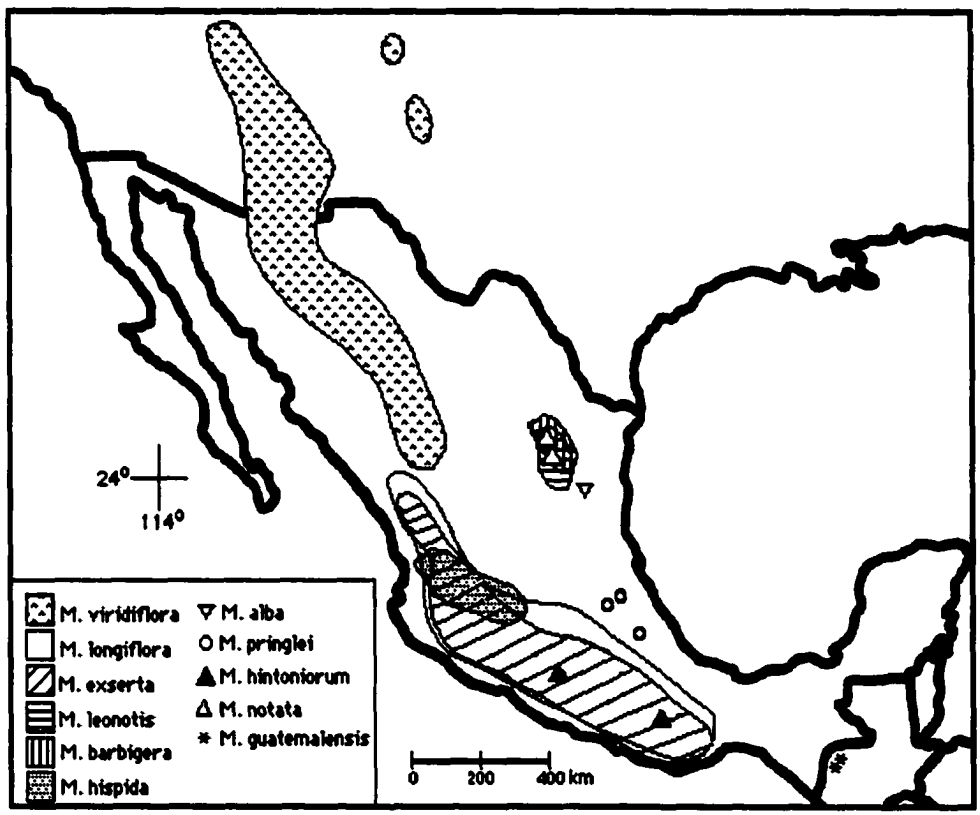
FIG. 8. Single most parsimonious tree describing phylogenetic relationships within the genus Macromeria, based on parsimony analysis of morphological data (consistency index = 0.48; retention index = 0.47). Bootstrap support values (%) shown above branches, with values above 70 in bold and values below 70 in italics. Decay indices shown below branches.

FIG. 9. Single most parsimonious tree describing phylogenetic relationships within the genus Macromeria, showing state changes for all characters. Solid, striped, and open rectangles represent unique gains, homoplasious

gains, and reversals, respectively. Numbers below rectangles indicate character numbers as listed in table 1; subscripts are character states as listed in table 1.

FIG. 10. Single most parsimonious tree describing phylogenetic relationships within the genus Macromeria, with corolla length mapped on as a continuous character using squared-change parsimony. Numbers above branches are corolla lengths in millimeters. Up arrows indicate increases in corolla length along branches; down arrows indicate decreases in corolla length along branches.

Fig. 1


24°
114°
M. viridiflora
M. longiflora
M. exserta
M. leonotis
M. barbigera
M. hispida
M. alba
M. pringlei
M. hintoniorum
M. notata
M. guatemalensis
0
200
400 km

Figs. 2-7

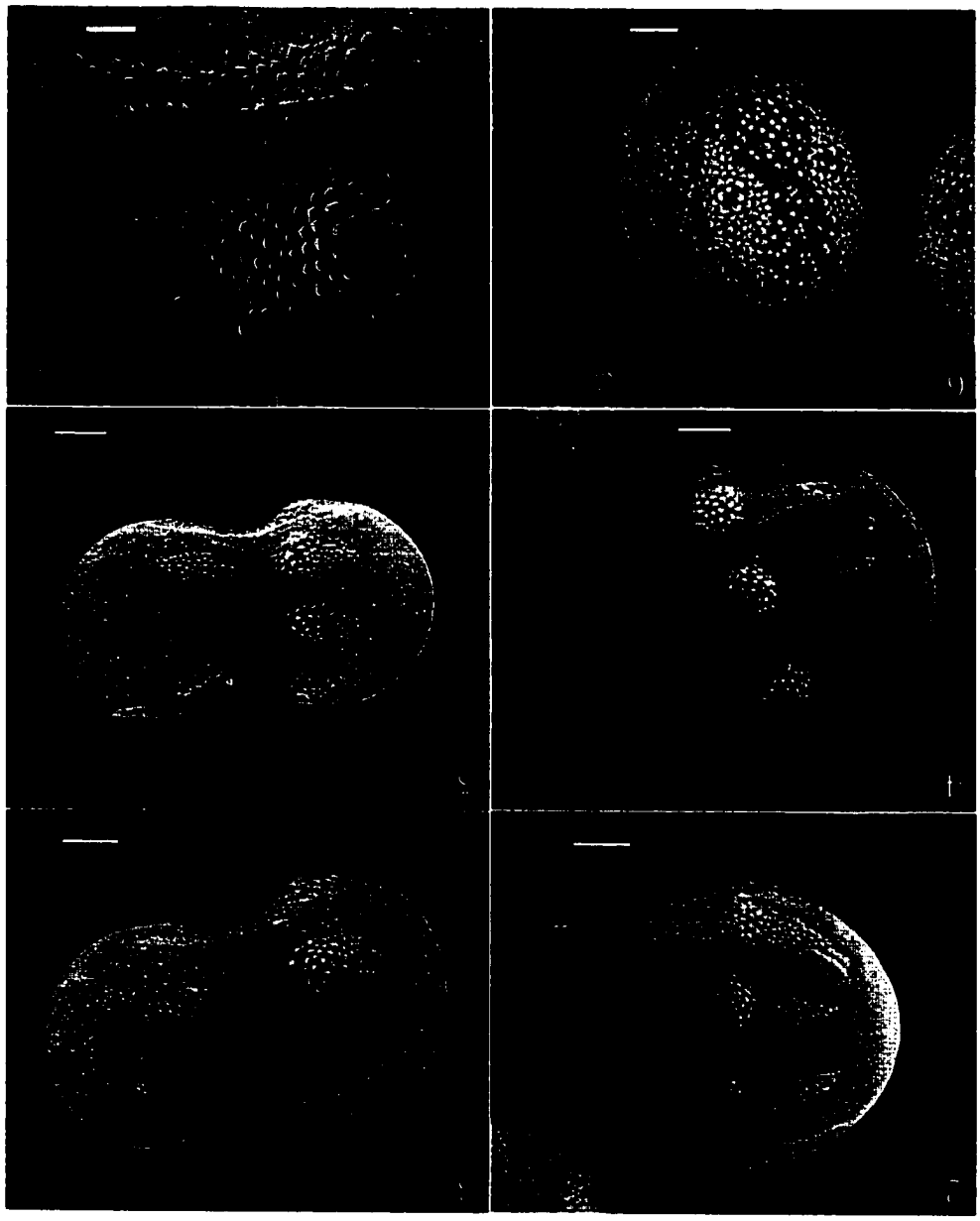


Fig. 8

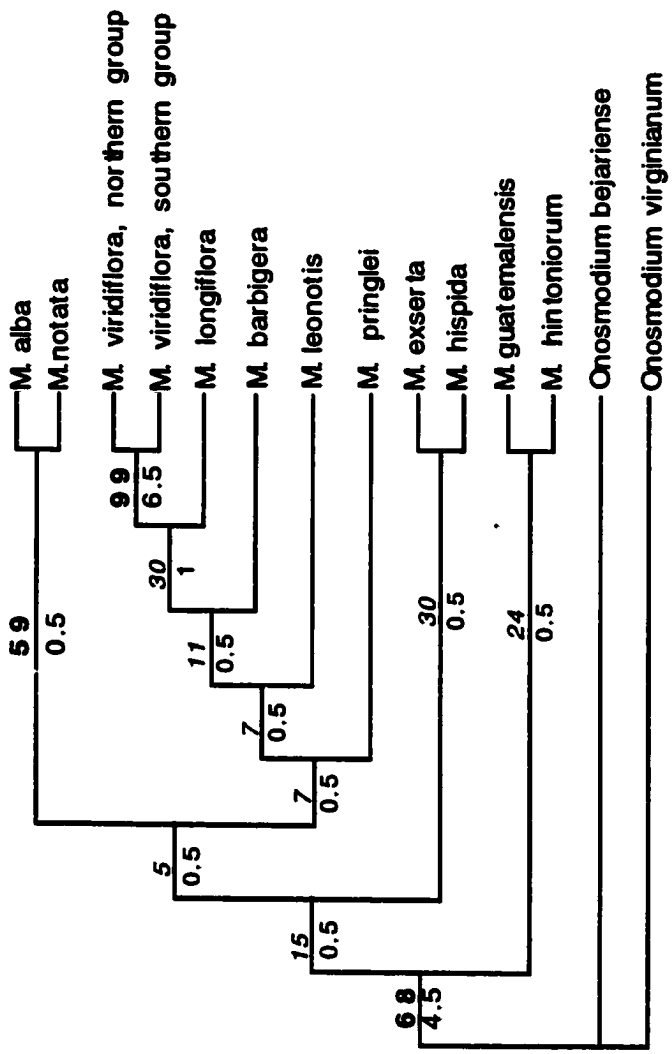

M. alba
M.notata
M. viridiflora, northern group
M. viridiflora, southern group
M. longiflora
M. barbigera
M. leonotis
M. pringlei
M. exserta
M. hispida
M. guatemalensis
M. hintoniorum
Onosmodium bejariense
Onosmodium virginianum
59
0.5
99
6.5
30
1
11
0.5
7
0.5
7
0.5
5
0.5
15
0.5
30
0.5
24
0.5
68
4.5

Fig. 9

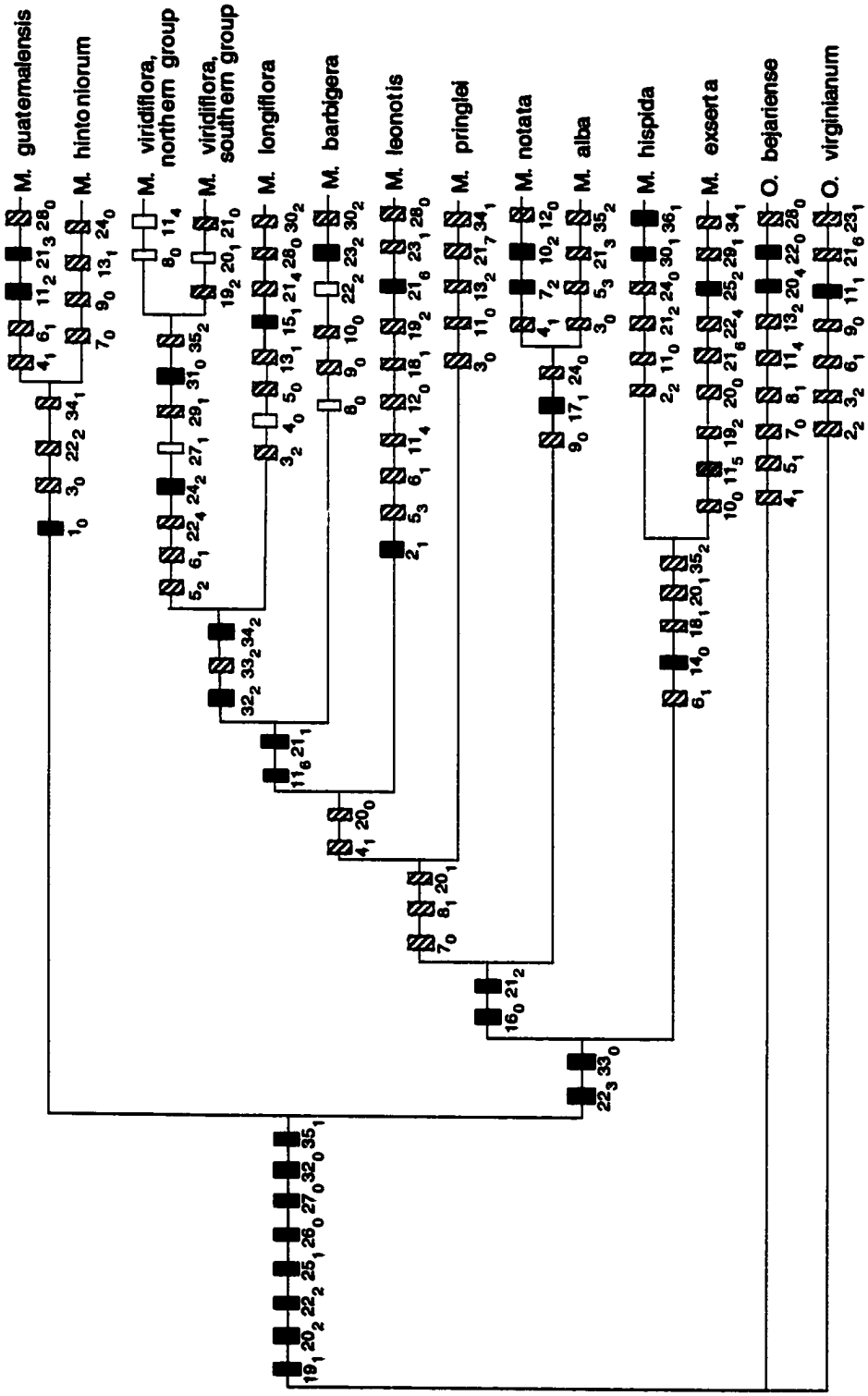
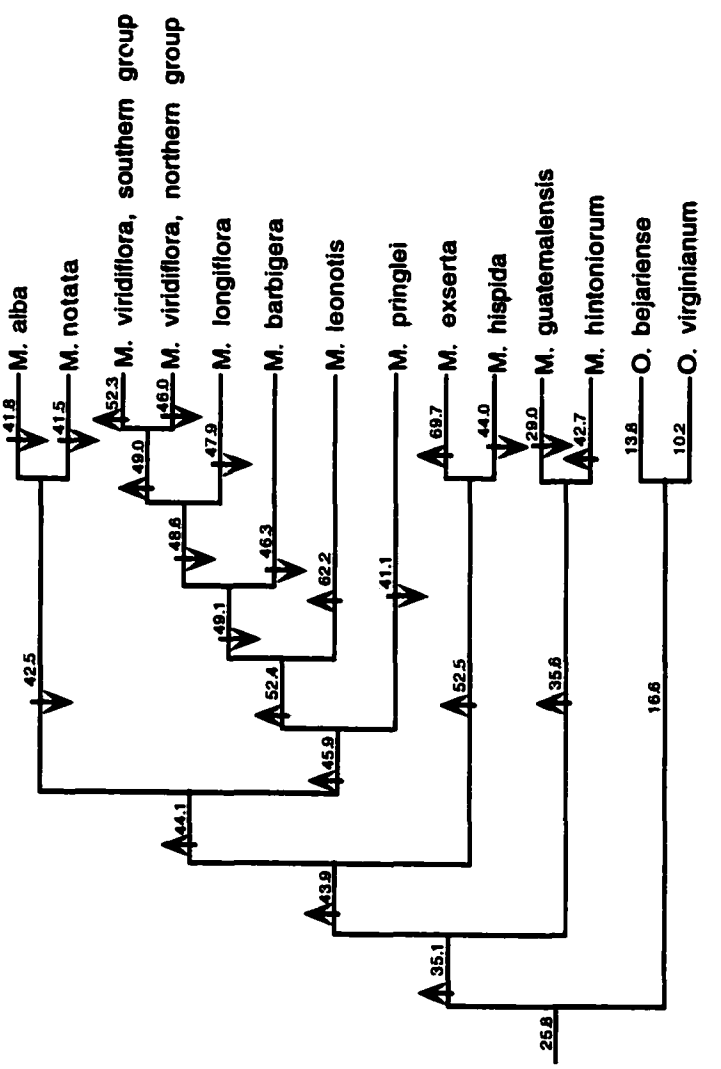

M. guatemalensis
M. hintoniorum
M. viridiflora, northern group
M. viridiflora, southern group
M. longiflora
M. barbigera
M. leonotis
M. pringlei
M. notata
M. alba
M. hispida
M. exserta
O. bejariense
O. virginianum

Fig. 10


M. alba
M. notata
M. viridiflora, southern group
M. viridiflora, northern group
M. longiflora
M. barbigera
M. leonotis
M. pringlei
M. exserta
M. hispida
M. guatemalensis
M. hintoniorum
O. bejariense
O. virginianum
41.8
41.5
52.3
46.0
47.9
49.0
48.6
46.3
62.2
49.1
41.1
52.4
42.5
45.9
69.7
44.0
29.0
42.7
13.8
10.2
44.1
52.5
35.6
16.6
43.9
35.1
25.8